Arctic health: Investigating the gut microbiota and parasite diversity of Arctic species

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy

By

Sophie Elizabeth Watson (BSc, MRes)

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"The unique nature of the Arctic is not just an asset for us to use. It is also a source of wonder, enjoyment and inspiration to people living in the Arctic and across the globe. It has intrinsic values that cannot be measured."

Evgeny Syroechkovskiy and Mark Marissink
2013 Arctic Biodiversity Assessment

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Statement of contributions

Where colleagues and students have assisted with work presented in this thesis, their contributions are explicitly detailed below:

Chapter 2. **Sharing isn't always caring: A host-parasite sharing network of the Arctic -** Undergraduate students Guy Oldrieve, Daniel Geerah and Stephen Cheung contributed towards the collation of data used within the whole Arctic host-parasite sharing network. Supervision of project students, collation of additional data, analysis of data in R and write up was conducted myself.

Chapter 3. Parasites of an Arctic scavenger; the wolverine (*Gulo gulo*) -
Indexing and library preparation was conducted by the team at Génome Québec, Montreal, Canada. Molecular identification used to verify parasite species extracted via traditional count methods was conducted by Pratap Kafle and Rajnish Sharma of the Jenkins Lab, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan. Wolverine dissections, helminth recovery, sample collection, DNA extraction/optimisation, primer testing, PCR optimisation, bioinformatic analysis, statistical analysis in R and write up was conducted myself.

Chapter 4. The gut biome of a scavenger; the wolverine (*Gulo gulo*) -
Indexing and library preparation was conducted by the team at Génome Québec, Montreal, Canada. Molecular identification used to verify parasite species extracted via traditional count methods was conducted by Pratap Kafle and Rajnish Sharma of the Jenkins Lab, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan. Stable isotope analysis was conducted by students of Professor Nicolas Lecomte, Université de Moncton, Canada. Wolverine dissections, helminth recovery, sample collection, DNA extraction/optimisation, primer testing, PCR optimisation, bioinformatic analysis, statistical analysis in R and write up was conducted myself.

Chapter 5. Global change-driven use of onshore habitat impacts polar bear faecal microbiota -
Dr Matthew Bull contributed towards the bioinformatic analyses of 16S microbial data. Indexing and library preparation was conducted by Dr Massimo Pindo and his team at the Next Generation Sequencing Platform, Fondazione Edmund Mach in collaboration with the Core Facility, CIBIO, University of Trento, Italy. Additional bioinformatic analysis, analysis of data in R and write up was conducted myself.

Chapter 6. Diet-driven mercury contamination is associated with polar bear gut microbiota -
Dr Matthew Bull contributed towards the bioinformatic analyses of 16S microbial data. Indexing and library preparation was conducted by Dr Massimo Pindo and his team at the Next Generation Sequencing Platform, Fondazione Edmund Mach in collaboration with the Core Facility, CIBIO, University of Trento, Italy. All diet and contaminant data was provided by Dr Melissa McKinney of McGill University. Additional bioinformatic analysis, analysis of data in R and write up was conducted myself.

List of Figures

List of Tables

Table of Contents

Summary

The Arctic faces some of the most rapid and extreme climatic changes on earth, and within forthcoming years these changes are expected only to accelerate. The evolution of Arctic species has been shaped by a turbulent climatic history encompassing multiple cycles of glacial advance and retreat, and associated changes in sea-ice extent. As such, many Arctic species are well adapted to climatic extremes, but remain poorly adapted to secondary ecological stressors such as competition, contaminants, parasites and disease, leaving them vulnerable. Despite this, there remains a paucity of data addressing two major influencers of health, i) the composition of host gut microbiota and ii) parasitic infection. Here, these shortcomings are addressed using a whole-Arctic approach and two model apex species; the polar bear (*Ursus maritimus*) and the wolverine (*Gulo gulo*). Within this thesis, a combination of data mining, high-throughput sequencing, and traditional parasite count approaches are used to i) establish the parasite diversity and data deficient species across the whole Arctic, and ii) to determine the gut bacterial communities and parasite diversity of polar bears and wolverines in association with contaminant profiles, changes in land use and diet.

Firstly, this thesis shows that humans and domestic dogs are hubs for parasites within the Arctic, while other species (even those which are ambassadors of the Arctic, e.g. polar bears) remain poorly studied. Secondly, this thesis is the first to conduct a full assessment of the gastrointestinal parasite diversity of Arctic wolverines, finding that the distribution of their predominant helminth infection, *Baylisascaris devosi*, although limited by latitude, is present in individuals from the Arctic tundra, which was previously unrecorded. Furthermore, we describe how the composition of bacterial communities within the gut of wolverines may reflect their ability to scavenge a wide variety of prey and tissue types. In polar bears, we show that the gut microbiota differs significantly in individuals using onshore coastal regions, compared to those that exhibit their typical behaviour of remaining offshore year-round. Finally, we demonstrate that differences in diet-driven mercury levels linked with on- versus offshore behaviour are associated with significant changes to polar bear microbiota.

Composition and diversity of gut bacteria is deeply rooted in the evolution of the host. As such, although little is known of the gut microbiota of free ranging species, it is apparent that changes to the gut microbiota may influence health and ultimately survival. By establishing what constitutes typical polar bear and wolverine gut microbiota, this thesis provides a biomarker against which to measure the effects of current and future environmental and anthropogenic stressors. Furthermore, this thesis bridges the gaps in knowledge regarding the parasite diversity of this fragile ecosystem.

1

General Introduction

The Arctic: current threats

The Arctic is home to some of the most extreme environments on Earth, with the evolution of Arctic species having been closely shaped by a climatic history of over 20 cycles of glacial advance and retreat and associated changes in sea-ice extent (Callaghan et al. 2004; Meltofte et al. 2013). As such, many Arctic species are well adapted to climatic extremes, but remain poorly adapted to secondary ecological stressors such as competition, parasites and disease (Callaghan et al. 2004; Meltofte et al. 2013), making them especially vulnerable.

Global mean temperatures are predicted to increase worldwide by 2.6°C to 4.8°C, with parallel increases in precipitation and humidity by an average of 7% before the year 2100 (IPCC 2014). It has been forecast that these increases in temperature will be most dramatic at high latitudes (Dobson et al. 2015) and already the Arctic has experienced the 10 warmest years in the past 2 millennia (Kaufman et al. 2009). As well as directly impacting Arctic species by means of habitat loss and degradation, climate change prompts a surplus of indirect impacts to host health and survival. Rapid reductions in the extent and duration of sea ice habitat not only alters sea ice dynamics, but also the biodiversity and productivity of marine ecosystems of the Arctic shelves, impacting prey abundance and distribution (Simmonds and Isaac 2007; Nøttestad et al. 2015). Such changes in prey availability alter trophic interactions and lead to shifts in host distributions and habitat use, forcing species in to new, less productive areas (Simmonds and Isaac 2007; Laidre et al. 2008). In addition, warming temperatures lead to a northward shift in the distribution of more southern dwelling Arctic or boreal species (Parmesan and Yohe 2003; Pearson et al. 2013; Tape et al. 2016), having the potential to outcompete and displace unique Arctic species assemblages that are already at the most northern limit of their distribution (Davidson et al. 2011). Terrestrial Arctic species are confined to the north by marine territories and to the south by the temperature constraints associated with

snow and ice dependencies (Virkkala et al. 2008; Copeland et al. 2010; Bilodeau et al. 2013; Elmhagen et al. 2017). In some cases, with shifts in host species distributions, comes the range expansion of associated parasites (Kutz et al. 2005; Laaksonen et al. 2010; Davidson et al. 2011; Kutz et al. 2013; Kutz et al. 2014) (here 'parasites' are defined as helminths, protozoa, bacteria and viruses). Invasion by parasites in northern areas is greatly increased by warming temperatures which benefit the survival and reproduction success of parasites adapted to more temperate regions (Kutz et al. 2013; Kutz et al. 2014). Range expansion can in turn lead to the spillover of parasites in to naive Arctic species (Ims et al. 2013).

In addition to ecosystem effects, warming temperatures also make the Arctic more accessible to human exploration (Davidson et al. 2011). Arctic habitats used to be among the least anthropologically disturbed on Earth (Meltofte et al. 2013). However, in recent years there have been dramatic rates of increase in mining and petroleum exploration and development, commercial wildlife uses, ship traffic, subsistence harvesting and long-range pollution, all of which are potential drivers of change in the population, distribution, and health of many species in the Arctic (Huntington et al. 2007; Meltofte et al. 2013). Pollution in the Arctic is a major conservation concern. Persistent organic pollutants (POPs) and heavy metals accumulate in the Arctic through air and ocean currents after long-range travel from industrial and agricultural areas located within the lower latitudes (Barrie et al. 1992). Many contaminants accumulate in species that are low in the trophic web and magnify as they move up each trophic level (Alexander 1995; Hoekstra et al. 2003; Borgå et al. 2004). This means long-lived, high trophic feeders, such as pinnipeds and polar bears (*Ursus maritimus*), bioaccumulate the highest levels of contaminants and since the middle of the 20th century, a number of pollutants have been detected in Arctic predators at levels that threaten health (Dietz et al. 2006; Basu et al. 2009; Letcher et al. 2010; Andvik et al. 2020). However, a deficiency of data (in part due to the logistics of sampling the Arctic) may mask the true extent to which contaminants impact the health and long term survival of Arctic species. Consequently, the monitoring of wildlife plays an important role in identifying change in populations and habitat such that actions can be taken to mitigate or minimize pressure.

The gut microbiota and health

Signatures of global change driven shifts in land use, diet, parasite profiles and contaminant levels may also be detectable in a more inconspicuous environment; the host

gut microbiota. The gut microbiota is an abundant and highly diverse community of bacteria which resides within the gastrointestinal tract, the composition of which has evolved to execute vital nutritional and physiological roles within the host (Diaz Heijtz et al. 2011; Yeoman et al. 2011; Maynard et al. 2012; Xing et al. 2013). In effect, the regular intestinal development and function of a host is attributed to a complex web of specific bacterial groups or species. For example, most complex carbohydrates and plant polysaccharides cannot be broken down by human enzymes, instead we depend upon a community of bacteria which reside within the colon to break down such molecules (Hooper et al., 2002). Similarly, the gut microbiota has been shown to play an important role in the facilitation of fat storage. For example, germ-free mice demonstrated a 60% increase in body fat when colonized with a normal gut microbiota, even with a 30% reduction in food intake (Bäckhed et al., 2004). The metabolic products of gut microbes are thought to act as signalling molecules, inducing changes in host metabolism (Tremaroli and Bäckhed, 2012). As a result, the gut microbiota of a species is a reflection of its environment.

One of the largest drivers of change to the gut microbiota is diet (Muegge et al. 2011; David et al. 2013; Carmody et al. 2015). Gut microbiota analysis of >50 mammalian species indicates that bacterial diversity increases from carnivory to omnivory to herbivory (Ley et al. 2008a). In herbivores, gut microbial communities allowed for the evolution of a polysaccharide-rich diet (Ley et al. 2008b), while in carnivores a high Firmicutes-Bacteroidetes ratio is typical, attributed to the efficient extraction of energy from the diet (Ley et al., 2006; Turnbaugh et al., 2006; Cheng et al. 2015). In addition to diet, a number of other factors are known to impact the diversity and composition of host microbiota, including contaminant levels. For example, isopods (*Porcellio scaber*) from mercury polluted environments were found to have a significantly lower bacterial species richness compared to individuals from unpolluted areas. In laboratory mice, the abundance of a number of bacterial taxa were significantly altered in treatment groups exposed to high mercury levels (Ruan et al. 2019). It is thought that gut bacterial communities are an important mediator for heavy metal toxicity (Breton et al. 2013; Claus et al. 2016). In laboratory mice and rats, a depleted gut microbiota is associated with poorer excretion of mercury and subsequent increased accumulation of mercury in host tissues, ultimately influencing host health (Nakamura et al. 1977; Rowland et al. 1980; Seko et al. 1981). The protective properties of the gut microbiota to host health are also seen in its ability to inhibit certain parasitic infections within the intestine, although this

is a two-way street. The gut microbiota's responsiveness to the presence of gastrointestinal parasites (Kreisinger et al. 2015) is likely owed to their close temporal and spatial evolutionary history (Waterfield et al., 2004; Glendinning et al., 2014). On one hand, the gut microbiota is an important defence against parasitic infection. For example, in laboratory mice, a normal, healthy gut microbiota was associated with reduced susceptibility to infection by the protozoan parasite *Cryptosporidium parvum* when compared to individuals with experimentally deficient gut bacteria (Harp et al., 1992). However, on the other hand there are examples where parasite survival and infection success is reliant on a rich bacterial resource within the host gut. For example, five specific bacterial strains of the intestinal tract are required for the successful embryonation of *Trichuris muris* eggs (Hayes et al., 2010). The survival of gastrointestinal parasites is likely dependent on their ability to harvest energy extracted during nutrient metabolism by resident bacteria (Sekirov et al., 2010).

Composition and diversity of gut bacteria is deeply rooted in the evolution of the host (Ley et al. 2008a). As such, although little is known of the gut microbiota of free ranging species (Pascoe et al. 2017), it is apparent that changes to the gut microbiota may influence health and ultimately survival. The gut microbiota has been shown to respond to environmental fluctuations experienced by the host. For example, a significant difference in gut microbiota composition was seen between laboratory mice acclimated to cold temperatures compared to those raised at higher temperatures (Chevalier et al. 2015). Similarly, small temperature increases of 2–3 °C were associated with a dramatic loss of bacterial diversity in the common lizard (*Zootoca vivipara*)(Bestion et al. 2017). It is therefore possible that the effects of global change on a given species may be more far reaching that previously thought. This is an especially important consideration in species of conservation concern, such as those in rapidly changing environments, including the Arctic.

Broad objective

Despite its importance to host health and nutrition, the gut microbiota of wild species remains poorly understood (Pascoe et al. 2017), as does the diversity and distribution of parasites in Arctic species (Davidson et al. 2011). A deeper understanding of factors that influence the intestinal health of Arctic species will feed into the development of future conservation strategies aiming to improve their persistence and resilience. This thesis therefore investigates the macro- and microbiota of two Arctic species, polar bears (*Ursus*

maritimus) and wolverines (*Gulo gulo*), in response to climatic and anthropogenic driven shifts in land use, diet and contaminant exposure.

Polar bears

Based on current and projected population statistics, polar bears are listed as 'vulnerable' under the IUCN Red List, with it being specifically noted that, in light of potential climatic- and anthropogenic-driven increases in parasite exposures, detection and continued monitoring of disease within polar bear populations should be addressed within future conservation efforts (Fagre et al. 2015; Patyk et al. 2015; Wiig et al., 2015). Our understanding of the conservation status of polar bears is currently hindered by incomplete and inconsistent monitoring across their range.

As a wide-ranging, low-density apex predator, polar bears (*Ursus maritimus*) are widely acknowledged as important indicators of Arctic ecosystem health (Amstrup et al., 2010; Kirk et al., 2010). For over 20 years, polar bears have been considered an ideal species through which to study the effects of climatic and anthropogenic stressors on Arctic environments (Stirling and Derocher, 1993; Parmesan, 2006; Simmonds and Isaac, 2007) and so have become a flagship species within environmental change discussions (Fagre et al., 2015). A total of 19 subpopulations of polar bear reside within the circumpolar Arctic, one subpopulation (M'Clintock Channel) has increased in size, six are stable (Davis Strait, Foxe Basin, Gulf of Boothia, Northern Beaufort Sea, Southern Hudson Bay, and Western Hudson Bay), three have declined (Baffin Bay, Kane Basin, and Southern Beaufort Sea) and, insufficient data is available for the remaining subpopulations to determine their population trend (Arctic Basin, Barents Sea, Chukchi Sea, East Greenland, Kara Sea, Lancaster Sound, Laptev Sea, Norwegian Bay, and Viscount Melville Sound) (Wiig et al. 2015; Figure 1). A continuing decline of mature individuals has also been recorded (Wiig et al. 2015). Based on a range of factors (subpopulation size, amount of continental shelf habitat, prey diversity and changing ice conditions) it is thought that the most vulnerable subpopulations are the southern Beaufort Sea, northern Beaufort Sea and Arctic Basin subpopulations (Hamilton and Derocher 2019).

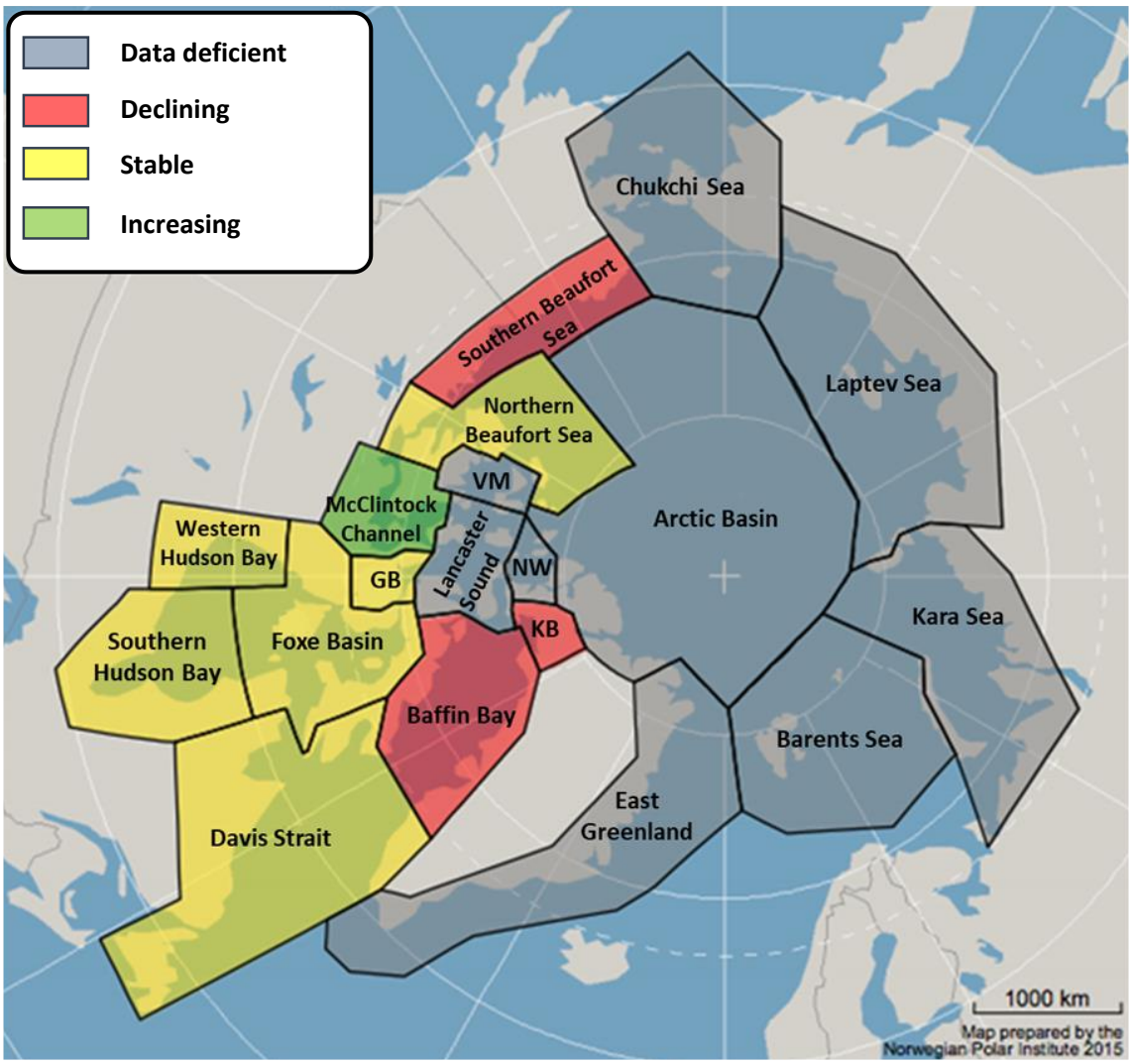


Figure 1. All nineteen polar bear subpopulations within the circumpolar arctic and their population status (increasing, stable, decreasing or data deficient); GB (Gulf of Boothia), KB (Kane Basin), NW (Norwegian Bay), VM (Viscount Meville). Adapted from Norwegian Polar Institute (2015), based on the IUCN Polar Bear Specialist Group report (2014)

Typically, open basin subpopulations of polar bears (e.g. southern Beaufort Sea, Chukchi Sea, Barents Sea, Laptev Sea, Franz Joseph, Svalbard and East Greenland subpopulations) remain on the sea ice year-round. While on the sea ice, polar bears consume a typical diet of ringed seal (*Pusa hispida*), bearded seal (*Erignathus barbatus*) and occasionally beluga whale (*Delphinapterus leucas*) (Herreman and Peacock 2013). However, an increasing proportion of polar bears are being forced onto land during the autumn open-water period in response to long-term reductions in the duration and extent of sea ice which extends beyond the biologically productive continental shelf (Schliebe et al., 2008). For example, there have been numerous reports of southern Beaufort Sea polar bear sightings in the vicinity of Prudhoe Bay oil field and the surrounding remote

northern communities of North Slope, Alaska (Burek et al. 2008; Schliebe et al., 2008; Herreman and Peacock, 2013). In the 1990s, less than six percent of southern Beaufort Sea polar bears spent significant time onshore (Atwood et al. 2016). However, since then, the open-water season has expanded by approximately 36 days, and now approximately a fifth of southern Beaufort Sea polar bears utilize onshore habitat (Atwood et al. 2016). Furthermore, individuals that come onshore spend more than a month longer onshore than they did in the 1990s (Atwood et al. 2016). While on land, southern Beaufort Sea polar bears have been observed scavenging on non-typical prey items such as bird eggs, plants and bone piles which are left behind by the Inupiat hunters of northern communities (Schliebe et al., 2008; Miller et al., 2015). Bone piles consist of the remains of harvested bowhead whale (*Balaena mysticetus*), caribou (*Rangifer tarandus*), birds and fish (Herreman and Peacock 2013). While onshore, polar bears share territory and beach-cast food resources with other species with which they would not historically interact, including humans, their domestic animals, Arctic fox (*Vulpes lagopus*), birds and barren ground grizzly bears (*Ursus arctos*) (Miller et al. 2015). Space and resource sharing increases the potential for microbiota and pathogen spillover to immunologically naive polar bears from terrestrial species. Furthermore, beach-cast bones piles increase contact rates between polar bears (a typically solitary species), with a single pile attracting as many as 65 polar bears (Miller et al. 2006) (Figure 2).

a)

b)

Figure 2a. Southern Beaufort Sea polar bears sharing bone piles with gulls (photo credit: Michelle Theall, Alaska magazine) **b.** Numerous polar bears congregate around a beach-cast bowhead whale left by Inupiat hunters on the North Slope of Alaska. Photo credit: Loren Holmes, Alaska Dispatch

Within this thesis, samples used are from southern Beaufort Sea polar bears, collected between 2008-2013 by the United States Geological Survey (Figure 3). The sampling area ranged approximately from Utqiagvik, Alaska (156°W) in the west to Demarcation Point (140°W) at the US-Canada border in the east, extending outwards approximately 135 km north from the shoreline. Sample number and type differ between chapters.

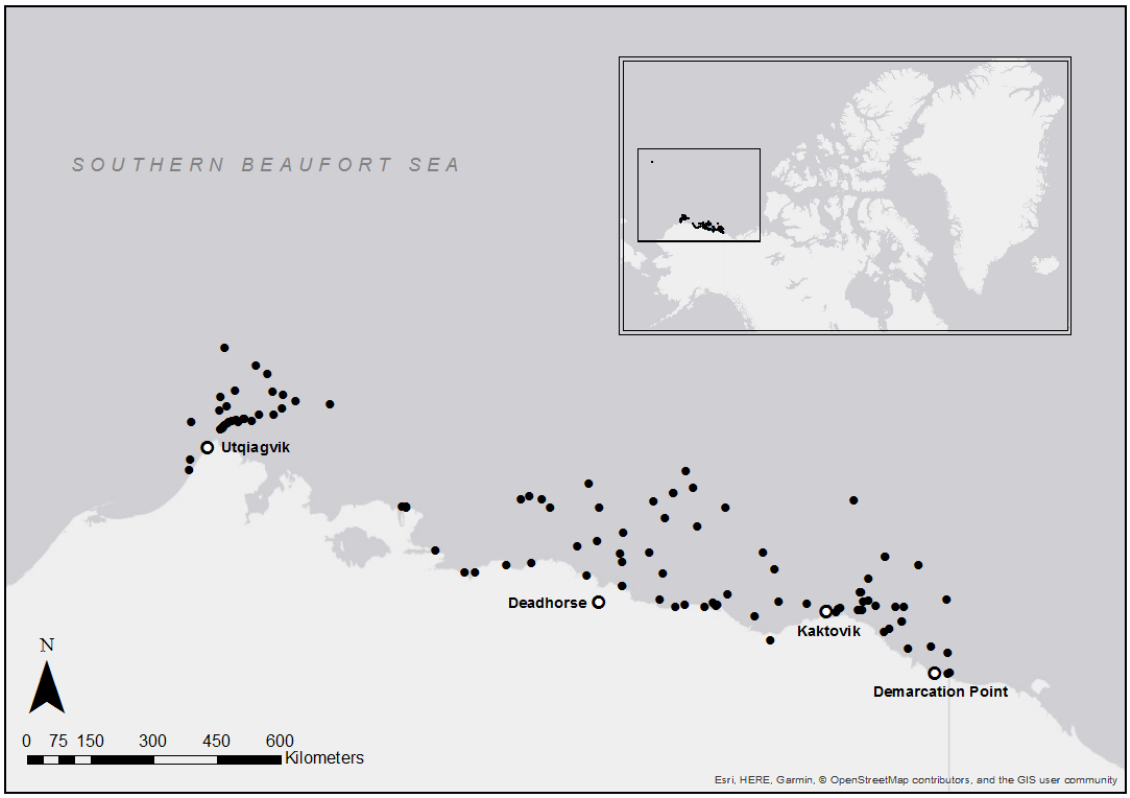


Figure 3. Map of polar bear sampling area used within this thesis, showing the North Slope of Alaska. Inset map shows the broader location of the study area

Wolverines

Wolverines (*Gulo gulo*) occupy large home ranges (Copeland et al. 2010; Dawson et al. 2010) across a broad geographic area, including Arctic/sub-Arctic regions and boreal forests, yet despite their global distribution, populations of wolverines are continuing to decline (IUCN 2016). This is, in part, due to the strong dependency of wolverines on snow cover and availability (Magoun and Copeland 1998; Aubry et al. 2007; Copeland et al. 2010), which is continuously depleting due to climatic changes. In addition to providing suitable denning habitat (Magoun and Copeland 1998), wolverines commonly cache their food in cold, structured microsites, such as around boulders with low ambient temperatures and persistent spring snow cover (Inman et al. 2012). The behaviour of using cool microsites to preserve food items, referred to as the "refrigeration-zone hypothesis",

eases decomposition rate, which not only inhibits the loss of valuable prey biomass but is also thought to limit competition from insects, other scavengers, and bacteria (Inman et al. 2012). Bacteria rapidly begin to decompose their hosts after death and, in doing so, excrete toxic metabolites which render carcasses a hazardous food source for many animals (Vass 2001), but not for wolverines. As facultative scavengers, wolverines consume a broad range of species across their geographic ranges, including moose (*Alces alces*), caribou (*Rangifer tarandus*), muskoxen (*Ovibos moschatus*), hare (*Lepus* sp.), Arctic ground squirrels (*Spermophilus parryii*), voles and lemmings (Muridae), ptarmigan (*Lagopus* sp.), seal (*Phoca* sp.) and migratory bird species (Lofroth et al. 2007; Koskela et al. 2013; L'Hérault et al. 2018b)(Figure 4). In addition to consuming a broad range of species, wolverines also consume a variety of typically unpalatable tissue types. Unusually, the species is one of very few known to consume bone, hide and feathers (van Dijk et al. 2007).

Figure 4. Wolverine feeding on a caribou carcass in the Arctic. Photo credit: Peter Mather

Considering their vast, heterogeneous geographic range, broad dietary preference and their caching behaviour, it might be expected that wolverines host a unique gut bacterial community, as well as an expansive diversity of parasites. Hosts with a broad diet are potentially exposed to a larger variety of trophically transmitted parasites (Vitone et al. 2004; Anderson and Sukhdeo 2011; Aponte et al. 2014) and the generalist diet of wolverines may therefore equate to high parasite exposure. An extensive survey of helminths belonging to wolverines of Alaska and the Northwest Territories of Canada was conducted over 40 years ago (Rausch 1954; Addison and Boles 1978) but, since then, parasite surveillance in wolverines has been scarce. Not only is parasite research in wolverines limited, but there appears to be a paucity of information related to how parasitic infections of wolverines may be associated with host and geographic metadata. Typically, due to ecological conditions, parasite species richness in carnivores decreases on a latitudinal gradient from south to north (Lindenfors et al. 2007), though in Arctic hosts there is a lack of comprehensive baselines for parasite diversity to assess this (Meltofte et al. 2013). The absence of parasite data for wolverines is likely due to difficulties in sampling logistics. Sampling wolverines is notoriously problematic, owed to their low densities, wide-ranging behaviour and highly elusive nature and, as such, data related to their parasite ecology is limited, while data associated with the diversity and composition of their gut microbiota is completely absent. However, subsistence harvesting of this furbearer is common in Nunavut communities, providing a unique opportunity for sample collection and ecological monitoring.

Within this thesis we aim to investigate the gut microbiota and parasite diversity of wolverines. As part of a wolverine carcass collection programme initiated by the Government of Nunavut Department of Environment, samples used within this thesis are from wolverines inhabiting an area between Kugluktuk (115°W) and Repulse Bay (82°W), Nunavut, Canada (Figure 5).

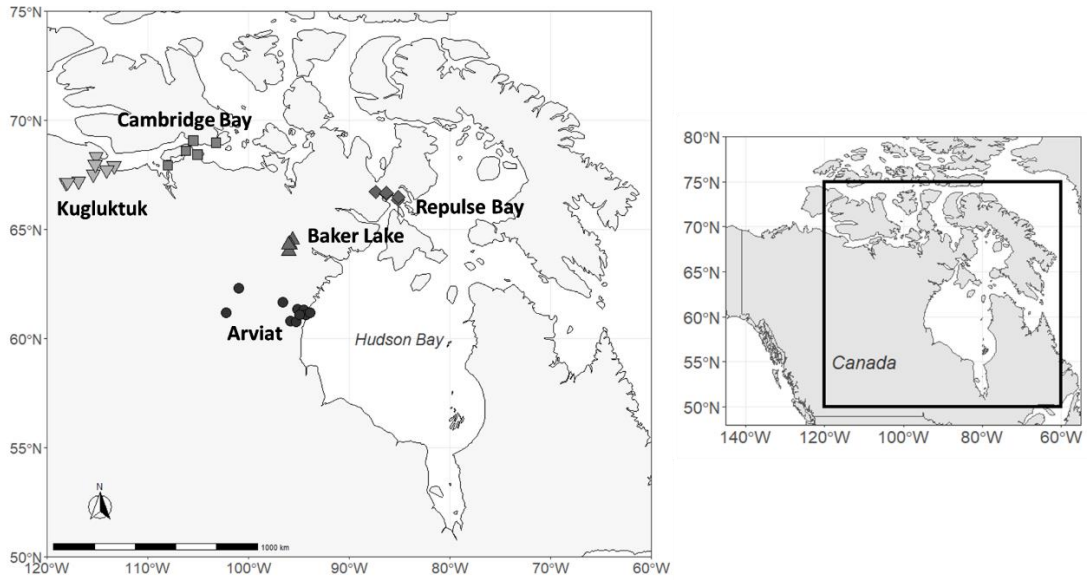


Figure 5. Map of individual wolverine hunting locations close by Inuit communities (circles= Arviat, triangles= Baker Lake, diamonds= Repulse Bay (Naujaat), squares= Cambridge Bay, inverse triangles= Kugluktuk) in Nunavut, Canada, 2010-2013. Inset shows sampling location (black outline) on a map of North America

Specific objectives

Investigating changes in the parasite diversity and gut microbiota of Arctic wildlife presents a number of challenges, the greatest of which is the absence of baseline data for the most remote, northern dwelling species (Kutz et al. 2004). In the absence of baseline information, there is a lack of comparative context by which to interpret 'new' findings. This thesis investigates the macro- and microbiota of Arctic species in response to climatic and anthropogenic driven shifts in land use, diet and contaminant exposure. Chapter 2 quantifies a whole-ecosystem host-parasite sharing network for the Arctic, and subsequent chapters will focus on the gut biomes of polar bears (*Ursus maritimus*) and wolverines (*Gulo gulo*) of the high Arctic, due to their high spatial requirement and overall continued population declines due to human stressors or land-use change. Specifically, within this thesis I aim to:

i) Determine the diversity and connectedness of host-parasite networks across the Arctic ecosystem as a whole (Chapter 2)

ii) Combine the use of traditional parasite count methods and high-throughput sequencing to determine the total parasite diversity associated with wolverines and to additionally investigate associations with host metadata (sex and age) or geographic metadata (Chapter 3)

iii) Investigate the gut microbiota diversity and composition associated with a high Arctic, bone-eating scavenger; the wolverine (Chapter 4)

iv) Establish the diversity and composition of the polar bear gut microbiota using high-throughput sequencing and to determine whether host metadata (age, sex, body condition) or climate driven changes in land use influence gut microbiota communities (Chapter 5)

v) Determine the impacts of mercury level and dietary items on polar bear gut microbiota diversity and composition (Chapter 6)

Explanation of diversity measures used within this thesis

Shannon diversity index: A diversity metric that gives more emphasis to species richness and rare taxa in its calculation of diversity (Shannon 1948).

Inverse Simpson diversity index: Simpson's diversity index gives more weight to species evenness and common taxa in its calculation of diversity (Simpson 1949). The higher the Simpson's diversity index, the lower the diversity. To conform with other diversity measures used within this thesis, here we use Inverse Simpson (i.e. 1/D, where D = the Simpson's diversity score), so that the higher the score, the higher the diversity.

Chao1 diversity index: An abundance-based estimator of species richness (Chao 1984).

Faith's phylogenetic distance: A diversity metric that characterises only the relatedness or distinctness of species and works under the assumption that different species make unequal contributions to diversity (Faith 1992).

2

Sharing isn't always caring: A host-parasite sharing network of the Arctic

Abstract

The Arctic is displaying a strong response to climate warming, with temperatures increasing at least twice as fast as the global average. Such temperature increases may have profound effects on host-parasite interactions, altering parasite transmission dynamics and disease emergence in both wildlife and humans. However, the total diversity and pervasiveness of parasites throughout the Arctic ecosystem remains unquantified, meaning we currently have no benchmark against which to compare and predict future change. Further, as a number of Arctic hosts have a distribution that falls both in- and outside of the Arctic boundary, our appreciation as to the extent of host-parasite sharing may be impacted by sampling effort within the Arctic boundary. Here we aimed to address these shortcomings by investigating i) how many host-parasite interactions there are within the Arctic (i.e. the 'potential' network) and how much of the data is confirmed from species sampled within the Arctic (i.e. the 'realised' network), ii) how important introduced species are to the connectivity and parasite diversity within the network, iii) which hosts act as major parasite reservoirs and which host traits are associated with inter-specific parasite sharing, iv) how pervasive parasitism is across the Arctic and v) how many parasites are zoonotic. We used open-source databases to construct a host-parasite sharing network of 172 Arctic mammals and birds, harbouring a total 2618 parasite species between them (3943 host-parasite interactions total), including associated host and parasite metadata. Within the potential network the mean number of shared parasite links between hosts (degree) was 38.2 (ranging from 1 to 116; SD = 27.6), compared to the realised network, which had a mean degree of 12.0 (ranging from 1 to 45; SD = 10.0); three times the number of connections recorded within the realised network. Introduced species, predominantly humans (*Homo sapiens*) and domestic dogs (*Canis lupus familiaris*) were highly connected within the network, (adjusted degree = 12.6 and 9.4, respectively), harboured the highest diversity of parasites (n = 454 and 80, respectively) and were identified as key nodes in bridging subgroups of

hosts according to their 'importance ratio', i.e. betweenness centrality to degree ratio (importance Ratio = 1.2 and 0.8, respectively). The most pervasive of each parasite type recorded (i.e. those that were shared widely) were all zoonotic species, namely Influenza A (virus), *Toxoplasma gondii* (protozoa), *Trichinella nativa* (macroparasite), *Brucella* sp. (bacteria) and *Encephalitozoon cuniculi* (fungi). Zoonotic parasites were predominantly shared between carnivore species, in particular domestic dogs, domestic cats and wolves (adjusted degree = 26, 23, 22, respectively). These species also harboured the highest diversity of zoonotic parasites (adjusted parasite diversity = 80, 48 and 24, respectively). Overall, we highlight the diversity and connected nature of parasites within the Arctic, and quantify the vulnerability of Arctic ecosystems to emerging parasites.

Background

The Arctic faces multiple pressures which have substantial potential to affect wildlife and the ecosystems that support these species. As such, the Arctic serves as a sentinel for assessing the cascading ecological effects of global change (Grebmeier et al. 2006; Moore and Huntington 2008; Kutz et al. 2009; Mueller et al. 2009; Post et al. 2009). A rapid rate of change is observed and projected in the Arctic, which is largely due to warming being considerably higher than the global average (IPCC 2014). Such changes have the ability to rapidly alter parasite transmission dynamics and disease emergence in humans, domestic animals and wildlife, which inhabit Arctic regions (Kutz et al. 2005). Increases in temperature drive the range expansion of host species and their associated parasites, in turn altering the geographic distribution of parasites and facilitating the spill-over of non-indigenous parasites into naive fauna (Hoberg et al. 2008). For example, two species of protostrongylid nematodes (*Umingmakstrongylus pallikuukensis* and *Varestrongylus* sp.) emerged and persisted for the first time in muskoxen (*Ovibos moschatus*) and caribou (*Rangifer tarandus*) populations in the western Canadian Arctic Archipelago, a spillover event that was thought to be initiated by a mainland-to-Archipelago caribou migration, a route that was historically inhibited by climatic conditions (Kutz et al. 2013). In addition, warming temperatures have reduced parasite generation times, boosted development rates and augmented seasonal windows for transmission (Hoberg et al. 2008). For example, a more permissible climate has shortened the life cycles and increased longevity of two novel lungworms (protostrongylid; nematoda) within muskoxen (Kutz et al. 2013). Similarly, small temperature shifts have been shown to incur substantial effects on the

transmission dynamics of protostrongylid lungworms and muscleworms such as *Parelaphostrongylus*, *Protostrongylus* and *Umingmakstrongylus* (Hoberg et al. 2008).

In addition to climatic influences, the conversion of land-use for human development and/or resource extraction within the Arctic has the ability to impact host-parasite dynamics (Patz et al. 2000; Salb et al. 2008; Kutz et al. 2009; Thompson 2013; Atwood et al. 2017). Such developments constrict wildlife into smaller and more fragmented pockets of land, often increasing wildlife-human and wildlife-domestic animal interactions, posing a risk to wildlife health. For example, as the result of environmental contamination from humans or domestic animals, *Giardia* is now maintained in a sylvatic cycle in muskoxen in the Canadian Arctic (Kutz et al. 2008). Similarly, wolves (*Canis lupus*) within Canada are commonly found to be infected with zoonotic species of *Giardia*, with it being suggested that domestic dogs (*Canis lupus familiaris*) may serve as the initial infection source (Bryan et al. 2011). Domestic dogs were also suggested as the infection source for the introduction of a 'European strain' of *Echinococcus multilocularis* in to its non-endemic range of Canada (Jenkins et al. 2012) and were the spillover source for canine distemper virus (CDV) in to the Arctic seal population (Härkönen et al. 2006) as well as to other species of major conservation concern, such as grey wolves (*Canis lupus*), and polar bears (*Ursus maritimus*) (Tryland et al. 2005; Bryan et al. 2011). Currently, 11 species of Arctic fauna (canidae, ursidae phocidae and mustelidae) are known to be competent reservoirs of canine distemper virus (Dalerum et al. 2005; Tryland et al. 2005; Härkönen et al. 2006; Bryan et al. 2011). Not only is there a risk of parasite spillover from humans and their domestic pets into Arctic wildlife, but urbanisation poses the risk of spillover from wildlife to humans; i.e. zoonotic emergence. In the Arctic, where tradition and subsistence life style are commonplace and meat inspection services are rare, the potential for zoonotic infections in people is elevated, and poses a food security risk (Davidson et al. 2011).

With rapid rates of climatic and anthropogenic change underway (IPCC 2014), the Arctic is a frontier for exploration of emerging infectious diseases, hosting endemic species of conservation concern and wildlife species that serve as a food source; yet our knowledge of host-parasite associations at this whole ecosystem level remains poorly understood. An absence of baseline data regarding parasite diversity within the Arctic could pose a significant challenge to understanding the effects of environmental change on Arctic host-parasite systems (Hoberg et al. 2003). One useful approach for understanding parasite

diversity and connectivity on a broad scale is through the use of network theory (Poulin 1999; Krasnov et al. 2012; Luis et al. 2015; Dallas et al. 2019; Gibb et al. 2020). Parasites, that coinfect multiple species form inter-species connections, which can be used to construct an ecological network, the structure of which can be used to identify host-parasite interactions within the Arctic. The Arctic has a comparatively low biodiversity of host species and minimal anthropological influence (in comparison to temperate or tropical climates); an ideal ecosystem for exploring interactions between hosts, parasites and the environment (Burek et al. 2008; Kutz et al. 2013). By quantifying key transmission pathways of parasites between species we can enable informed decisions on how best to conserve or protect any given species, community or ecosystem. The understanding of underlying parasite transmission within the network can benefit conservation (species or ecosystem level), promote biodiversity and increase food security for humans.

Here, we data-mine existing open source databases and the published literature to create a database of host-parasite diversity in the Arctic from which we construct a network of host-parasite interactions. Using this network, we ask **'how many host-parasite interactions there are within the Arctic (i.e. the 'potential' network) and how much of the data is confirmed from species sampled within the Arctic (i.e. the 'realised' network).** Additionally, we ask, **'how important are introduced species to the connectivity and parasite diversity within the network?'** and **'which host species are major reservoirs for pathogens, and which host traits are associated with inter-specific parasite sharing?'**. We also examine whether 'hub' host species exist; those that bridge smaller subgroups via shared parasites**.** Finally, from a parasite perspective we ask **'how pervasive is parasitism in the Arctic and how many of the parasites are zoonotic?'**

Methods

Creating an Arctic host-parasite database

We used the International Union for the Conservation of Nature's (IUCN) Arctic Biodiversity Assessment to identify 172 host species (94 bird species and 78 mammal species) with their distribution within the Arctic (See Supplementary Table 1. for a full list). Additionally, we included humans (*Homo sapiens*), domestic dogs (*Canis lupus familiaris*), and domestic cats (*Felis catus*) as they are all established species within the

Arctic (Salb et al. 2008; Messier et al. 2009; Schurer et al. 2012) but not listed in the Arctic Biodiversity Assessment. Open-sourced databases, i.e. the Enhanced Infectious Disease Database (Wardeh et al. 2015) (a global parasite diversity database for both human and non-human animals) and the Global Mammal Parasite Database (Stephens et al. 2017), alongside the published literature were used to identify parasites (defined as any bacteria, virus, protozoa, fungi and macroparasites) living on or in any of our identified Arctic hosts. Web of Science keyword searches spanning 1900 to July 2020 used the following terms: '*infec*', 'transmiss*', 'diseas*'*, '*parasit*'* and *'patho*'*, alongside taxonomic and common name(s) for each identified Arctic mammalian species. For example, '*Alces alces' OR 'Moose', 'Eurasion moose', 'European Elk', 'Eurasian Elk', 'Siberian Elk'.* Captive population data was excluded.

Creation of such datasets can lead to sample bias, due to uneven research efforts on given species (Farine and and Whitehead 2015). As such a proxy for sampling effort was created using the number of citations in Web of Science (http://thomsonreuters.com/products_services/science/science_products/az/web_of_science/) against the search terms detailed above for each given species (after Altizer *et al* 2011). This sampling effort provided a weighting for each host species, and hence the likelihood of finding a parasite associated with that hosts species, (i.e. degree / sampling effort = adjusted degree, where degree is the number shared links with other hosts).

Host-Parasite traits

We assigned host conservation status according to the International Union for Conservation of Nature (IUCN) as critically endangered, endangered, vulnerable etc. for each of the 172 host species. Other host traits, including host class (Aves versus Mammalia), associated biome (marine, terrestrial, freshwater, terrestrial-marine or terrestrial-marine-freshwater), feeding strategy (herbivorous, carnivorous or omnivorous) and migratory status was determined using the Encyclopedia of Life (EoL) (Parr et al. 2014). Parasite taxonomy and life-history (i.e. parasite type; bacteria, virus, protozoa, fungi or macroparasite) was obtained using open access databases: Tree Of Life Web Project (http://tolweb.org), WormBase (http://parasite.wormbase.org), Parasites World (http://parasites-world.com/parasite-database), FungiDB (http://fungidb.org), Mycobank (http://mycobank.org, International Mycological Association) and Natural History Museum Host-Parasite Database (http://nhm.ac.uk). Zoonotic parasites were identified

using the Projecting Responses of Ecological Diversity in Changing Terrestrial Systems (PREDICTS) biodiversity database (Hudson et al. 2017).

Arctic host-parasite sharing network

Of the 1086 known Arctic host species (Arctic Biodiversity Assessment), the Enhanced Infectious Disease Database had parasitic data for 127 species. After supplementation from the Web of Science and Global Mammal Parasite Database, a total of 3943 parasite-host relationships were identified for 172 host species (78 mammal species with 2318 parasite interactions and 94 avian species with 300 parasite interactions). Using our created database, we produced a network of parasite sharing for all Arctic species. In the network each node represents a host species and the edges between two nodes represent the presence of a parasite in both species, so indicating parasite generalism. Some of the host species included within the analyses have a distribution that occurs both within and outside of the Arctic, for example moose (*Alces alces*). As such, the database may include parasites that can infect these hosts, but the current known distribution of the parasite falls outside of the Arctic for that host. Therefore, we created a 'potential' network, which represents host-parasite interactions that have not been confirmed within the Arctic in accordance with the sampling location provided by the database or publication. Secondly, a 'realised' network was constructed, which represented host-parasite interactions that have been detected within the Arctic boundary according to the sampling location provided by the database or publication. We compare the parasite diversity and number of connections between the 'potential' and 'realised' networks, to investigate the total number parasite-host interactions that could potentially make up the Arctic host network, and highlight areas that are data deficient. We use the 'realised' network to investigate our aims. Here, we used the Arctic boundary definition detailed by the Conservation of Arctic Flora and Fauna (CAFF), whereby boundaries are defined by the 10°C isotherm line, habitat, latitude or geopolitical zones (Figure 6).

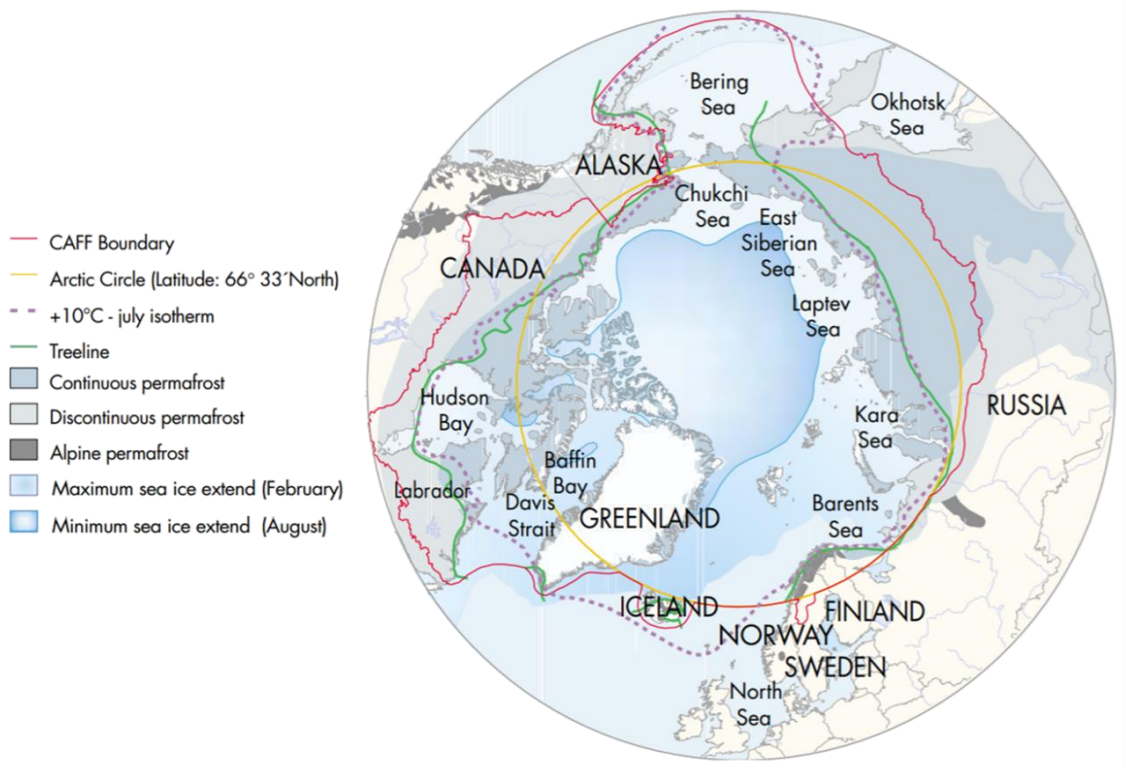


Figure 6. Arctic and Subarctic boundaries, defined by isotherms, habitat, latitude or geopolitical zones as described by the Programme for the Conservation of Arctic Flora and Fauna (CAFF). Source map made available by CAFF

Host-centric network interactions

To identify key hosts in Arctic host-parasite associations we calculated, four parameters; i) *Degree* - the number of connections (shared parasites) between host species ii) *Betweenness centrality* - the number of shortest paths to go through a node (an index for parasite flow or potential reach of a host's parasite community through the network) iii) *Closeness centrality* - the shortest path (number of links) needed to reach all individuals in network from a given individual (an index for the ease/speed of parasite spread). Key host species were identified using a *betweenness centrality to degree* ratio (Topirceanu et al. 2018), herein deemed iv) the '*Importance Ratio'*. A high Importance Ratio denotes a species that bridge subgroups of species in the Arctic, and so represent hubs.

Parasite-centric interactions

To identify which parasite were most pervasive throughout the network, the most abundant species of each parasite type (bacteria, virus, protozoa, fungi and macroparasite) with the highest degree were plotted in a network. Within the parasite-centric network, edges between hosts pass through a shared parasite.

Statistical Analysis

We performed all statistical analyses using R Studio (version 3.5.2) (R Development Core Team 2015) with use of the '*iGraph'* (Csárdi and Nepusz 2006), the '*ggnet2*' visualisation function of '*ggplot2*' (Tyner et al. 2017) and '*statnet'* (Handcock et al. 2008) packages. A Generalised Linear Mixed-effects Model using a Markov chain Monte Carlo (MCMCglmm) approach was run using the '*MCMCglmm*' package (Hadfield 2010) in R to determine which host traits (host class, feeding status and migratory status) correlated to connectedness (degree) in the network. MCMCglmms were run using 13,000 iterations, with a sample size of 1000 and thinning interval of 10.

Results

Potential network

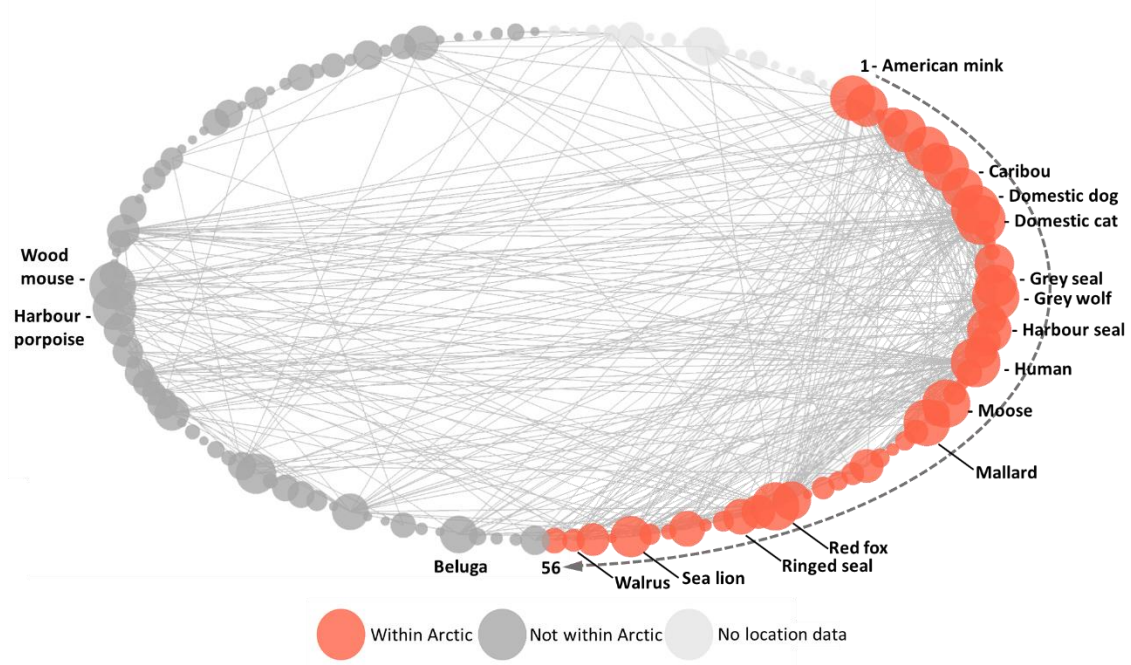
In total, 172 hosts were included in the 'potential' network (78; Mammalia, 94, Aves), encompassing 2618 parasite species with 3943 host-parasite interactions. Hosts which have been introduced to the Arctic, i.e. humans, domestic dogs, domestic cats and the American mink, demonstrated i) the highest number of shared parasites (edges) within the network and ii) the highest level of betweenness (Figure 7a); humans (degree = 116, betweenness = 954), domestic dog (degree = 111, betweenness = 669), domestic cat (degree = 97, betweenness = 299), American mink (degree = 92, betweenness = 314) (Figure 7a). The five most parasite rich species within the network were humans (*Homo sapiens*; n = 1628), domestic dogs (*Canis lupus familiaris*; n = 340), domestic cats (*Felis catus*; n = 203), red foxes (*Vulpes vulpes*; n = 82) and mallards (*Anas platyrhynchos*; n = 78) (Figure 7a). Excluding species which have been introduced, the top five mammalian hosts with the highest number of recorded parasite species are; red foxes (*Vulpes vulpes*; parasite species = 82), grey wolves (*Canis lupus*; parasite species = 77), wood mice (*Apodemus sylvaticus*; parasite species = 58), moose (*Alces alces*; parasite species = 57) and caribou (*Rangifer tarandus*; parasite species = 54), all of which are terrestrial species (Figure 7a). Of the marine mammals, harbour seals (*Phoca vitulina*) had the highest number of recorded parasites (n = 42), followed by harbour porpoises (*Phocoena phocoena*; n = 37), grey seals (*Halichoerus grypus*; n = 30), Steller sea lions (*Eumetopias jubatus*; n = 30) and beluga whales (*Delphinapterus leucas*; n = 25) (Figure 7a). In addition to their high parasite diversity, harbour seals and grey seals were the most connected marine mammal hosts (degree = 88 and 86, respectively), followed by walruses (*Odobenus rosmarus*; degree = 81), ringed seals (*Pusa hispida*; degree = 73) and hooded

seals (*Cystophora cristata*; degree = 71) (Figure 7a). Overall, within the potential network, the mean number of shared parasite links between hosts (degree) was 38.2 (ranging from 1 to 116; SD = 27.6). Mean betweenness within the potential network was 43.85 (range = 0 to 954.36, SD = 111.95) and mean importance ratio was 0.86 (range = 0 to 14.13, SD = 1.68).

Realised network

Of the 2618 parasite species (and 3943 host-parasite interactions) included in the 'potential' network (Figure 7a), 1954 parasites and 2497 host-parasite interactions were from hosts sampled within the Arctic and as such, make up the 'realised' network (Figure 7b). The 'realised' network consists of 79 hosts, of which 27 were birds and 52 mammals. For all other data points, either sampling location was missing (as was the case for 252 parasites making up 365 host-parasite interactions) or the distribution of the host species sampled fell outside of the Arctic boundary (as was the case for 690 parasites making up 1081 host-parasite interactions). A total of 23 hosts did not share parasites with any other species, leaving 56 connected hosts (Figure 7b and Table 1). Within the realised network, the mean number of shared parasite links between hosts (degree) was 12.0 (ranging from 1 to 45; SD = 10.0); three times fewer connections than recorded in the potential network. Mean betweenness within the realised network was 13.19 (range = 0 to 246.88, SD = 37.52) and mean importance ratio was 0.54 (range = 0 to 5.49, SD = 1.07).

a)



b)

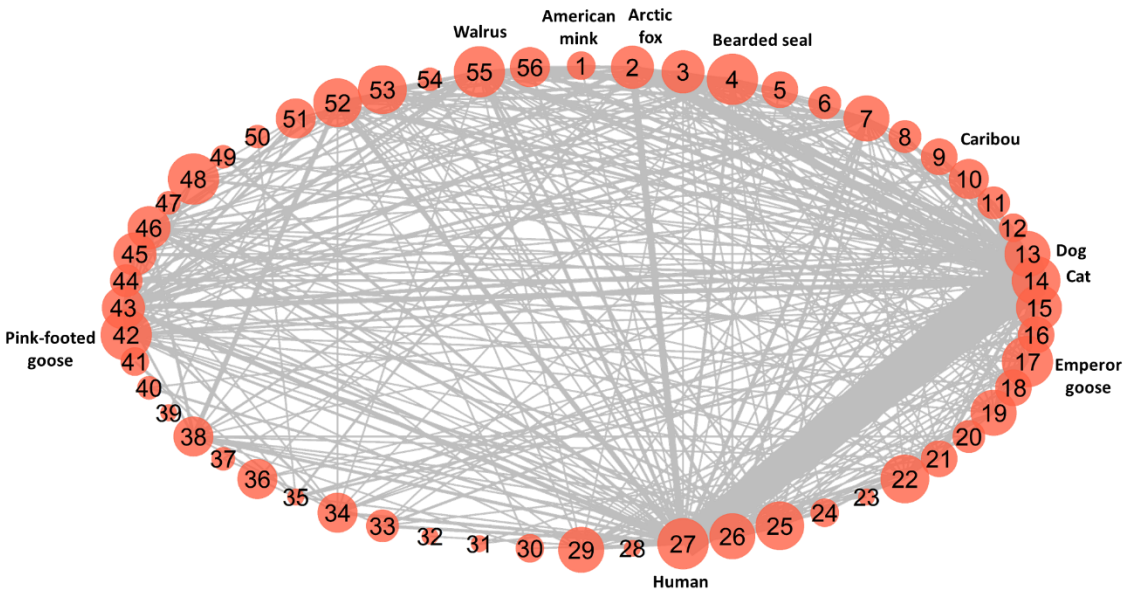


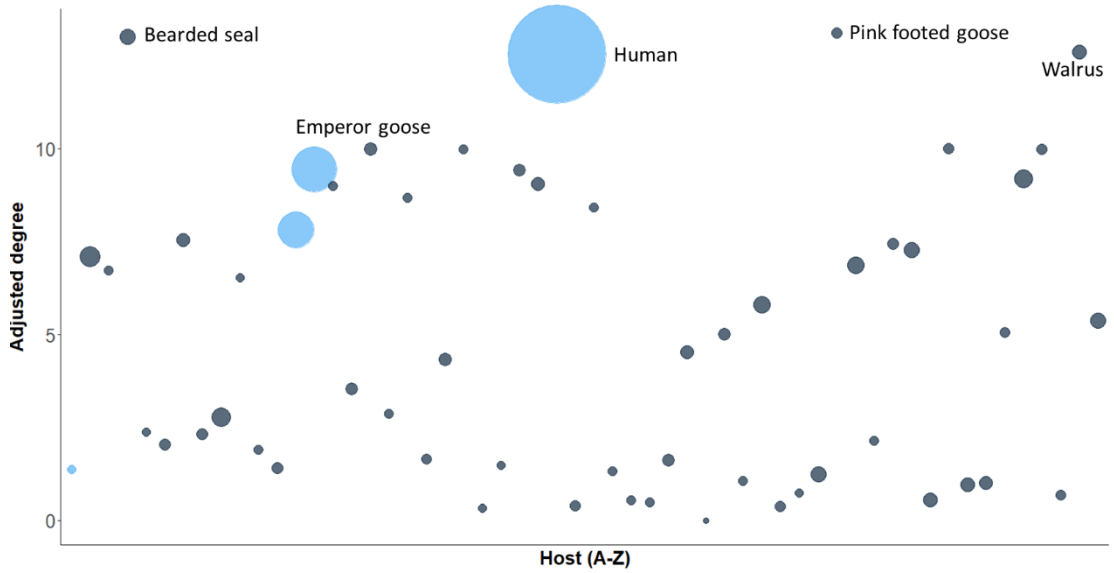
Figure 7a. Potential network with realised network included, of 144 Arctic species joined by 2618 shared parasites (edges), using circle format. Host species lacking a shared parasite with another host (n = 28) were excluded. To improve clarity of the network, only edges that have weight higher than the mean for the network (n = 2.8) were included. Nodes are coloured by whether their parasitic infections are confirmed (i.e. 'realised') in the Arctic (orange; full list of 56 hosts shown in Table 1) or not (dark grey), or whether there was an absence of location data (light grey) and are weighted by adjusted degree **b.** Realised Arctic host parasite sharing network using a circle format containing only those 56 hosts which were sampled within the Arctic (listed within Table 1). Edges are weighted by number of shared parasites and nodes are weighted by adjusted degree. Host species lacking a shared parasite with another host (n = 23) were excluded

Table 1. List of 56 host species that make up the realised host-parasite sharing network of the Arctic, excluding host species lacking a shared parasite with another host. The number assigned to each host name corresponds to the number labels in Figure 7a and b

Number	Host	Number	Host
1	American mink	**29**	Lesser black-backed gull
2	Arctic fox	**30**	Long-finned pilot whale
3	Baikal teal	**31**	Mallard
4	Bearded seal	**32**	Minke whale
5	Black bear	**33**	Moose
6	Bowhead whale	**34**	Muskox
7	Brown bear	**35**	Nearctic brown lemming
8	Canadian lynx	**36**	Nearctic collared lemming
9	Caribou	**37**	North Atlantic right whale
10	Common murre	**38**	Northern fur seal
11	Coyote	**39**	Northern pintail
12	Dall's sheep	**40**	Northern red-backed vole
13	Domestic cat	**41**	Norway lemming
14	Domestic dog	**42**	Pink-footed goose
15	Dunlin	**43**	Polar bear
16	East European vole	**44**	Red fox
17	Emperor goose	**45**	Ribbon seal
18	Eurasian lynx	**46**	Ringed seal
19	Glaucous gull	**47**	Rock ptarmigan
20	Gray seal	**48**	Ross's goose
21	Gray wolf	**49**	Sea otter
22	Great black-backed gull	**50**	Siberian brown lemming
23	Greylag goose	**51**	Snow goose
24	Harbor seal	**52**	Steller's sea lion
25	Harp seal	**53**	Thick-billed murre
26	Hooded seal	**54**	Tundra vole
27	Human	**55**	Walrus
28	Killer whale	**56**	Wolverine

The top five most connected hosts within the realised network were pink-footed goose (*Anser brachyrhynchus*, adjusted degree = 13.1), bearded seal (*Erignathus barbatus*, adjusted degree = 13.0), walrus (*Odobenus rosmarus*, adjusted degree = 12.6), humans (*Homo sapiens*, adjusted degree = 12.6) and emperor goose (*Anser canagicus*, adjusted degree = 10.0) (Figure 8). Other than bearded seals, the most connected species within the realised network are all migratory species. Domestic dogs (*Canis lupus familiaris*), an introduced species, were within the top ten most connected species (adjusted degree = 9.4). Even when accounting for sampling bias, humans harboured the highest number of recorded parasites (n = 454), followed by domestic dogs (n = 80), domestic cats (*Felis catus*, n = 48), Arctic fox (*Vulpes lagopus*, n = 11) and caribou (*Rangifer tarandus*, n = 9). How connected hosts were within the network (degree) was significantly associated with host feeding status, whereby herbivores were associated with a significantly lower degree (mean = 3.2; P = 0.026) compared to carnivores and omnivores, and were therefore less connected within the network. Host class and migratory status were not significantly associated with differences in degree.

a)



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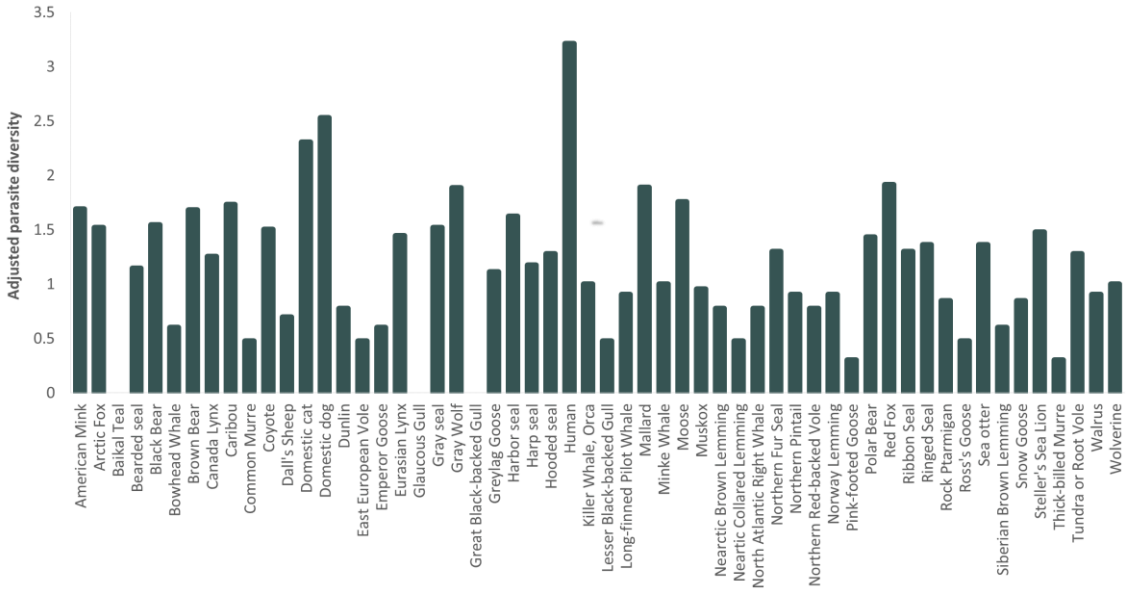

Figure 8a. Adjusted degree (i.e. degree adjusted by sampling effort) for host species and their total recorded parasites within the realised Arctic network, highlighting species that are highly connected, i.e. share parasite with the most other host species. Node size represents bias-adjusted parasite diversity. Node colour denotes whether a host species was an introduced species (light blue) or not (grey), where introduced species were; American mink, domestic cat, domestic dog and human (from left to right). Host species are displayed alphabetically from left to right. **b.** Adjusted parasite diversity (i.e. parasite diversity adjusted by sampling effort) for host species within the realised Arctic network. Adjusted parasite diversity has been log transformed for clarity

Identifying key nodes and subgroups within the realised network

On average, mammals were the most highly connected (mean degree = 12.0, mean betweenness = 16.0), compared to birds (mean degree = 11.7, mean betweenness = 5.3) and therefore displayed high importance to network cohesion (i.e. are important in connecting the clusters of the network, representing a potential pathway for parasite sharing between host groups) (Table 2). Mammals also demonstrated the lowest closeness (mean = 5.3; Table 2) compared to birds (mean = 7.3), and therefore had the shortest path (number of links) needed to reach all individuals in network.

Table 2. Network statistics for the 'realised' Arctic host-parasite sharing network

Metric	Arctic Network	Mammals	Aves
Network dimensions:			
No. of host species	79	52	27
No. of parasite species	1954	1921	33
Bacteria	950	949	1
Fungi	302	299	3
Macroparasite	307	289	18
Protozoa	131	125	6
Virus	264	259	5
No. of parasite-host interaction	2497	2450	47
Network statistics:			
Mean parasites per host species	31.6	47.1	1.7
Mean betweenness centrality	13.2	16.0	5.3
Mean degree	12.0	12.0	11.7
Mean closeness ($ex10^{-5}$)	5.8	5.3	7.3

Key host species linking subgroups via shared parasites were calculated using the Importance Ratio (Importance ratio = betweenness centrality / degree). The five highest-ranking host species were predominantly mammals; human (*Homo sapien*, Importance Ratio = 1.2), domestic dog (*Canis lupus familiaris*, Importance Ratio = 0.8), snow goose (*Anser caerulescens*, Importance Ratio = 0.8), nearctic collared lemming (*Dicrostonyx groenlandicus*, Importance Ratio = 0.6) and domestic cat (*Felis catus*, Importance ratio = 0.5) (Figure 9).

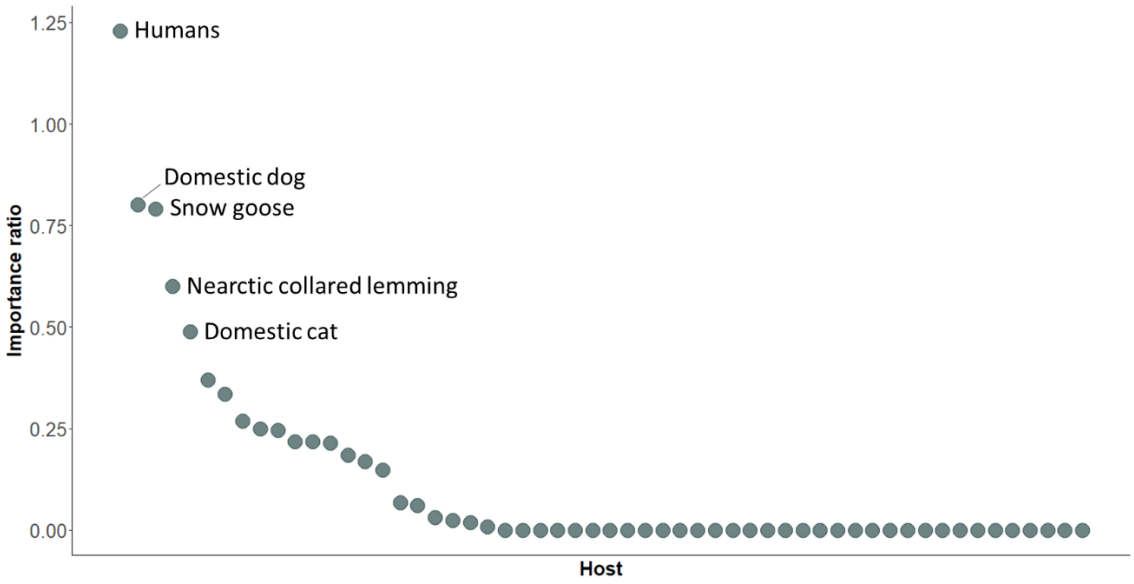

Figure 9. Importance Ratio distribution for host species in the 'realised' network, whereby Importance Ratio = betweenness centrality / degree. Higher value denotes greater importance of species joining disparate subgroups to the network

Most pervasive parasites in the realised network

The predominant parasite type recorded were bacteria, with a total of n = 950, followed by fungi (n = 302), macroparasites (n = 307), protozoa (n = 131) and viruses (n = 264). Bacteria was also the most common parasite type amongst mammals (n = 949 species), while macroparasites were most common in birds (n = 18 species). The most common parasite species of each parasite type were Influenza A (virus, n = 17), *Toxoplasma gondii* (protozoa, n = 11), *Trichinella nativa* (macroparasite, n = 11), *Brucella* sp. (bacteria, n = 11) and *Encephalitozoon cuniculi* (fungi, n = 5) (Figure 10). The most pervasive parasites within the network link a broad range of host taxa across both marine and terrestrial environments (Figure 10).

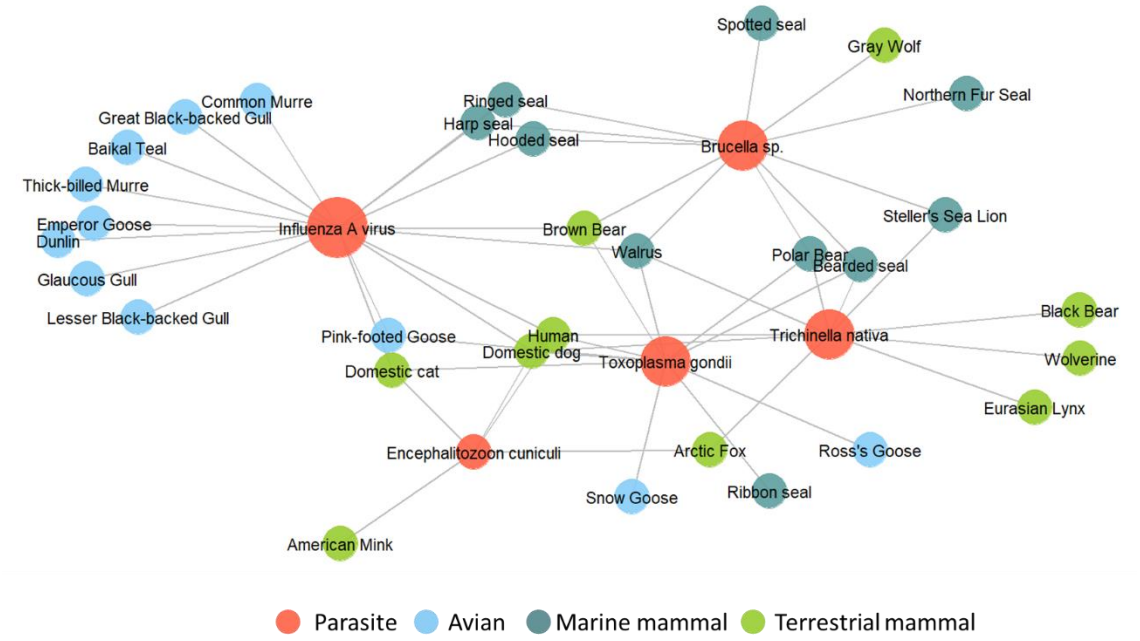

Figure 10. Realised network of the most commonly occurring parasites of each parasite type within the Arctic; virus (Influenza A), macroparasite (*Trichinella nativa*), fungi (*Encephalitozoon cuniculi*), protozoa (*Toxoplama gondii*) or bacteria (*Brucella* sp.). The network is made up of 31 Arctic species. Nodes are weighted by degree and coloured by whether they are a parasite (orange), Avian (light blue), marine mammal (dark blue) or terrestrial mammal (green)

Zoonotic parasites of the realised network

In total, 81 zoonotic parasite species were present in 21 non-human hosts (encompassing 150 host-parasite interactions; Figure 11). The majority of hosts harbouring zoonotic parasites were mammals (n = 18) compared to birds (n = 3), with the majority of the network made up of carnivores (n = 13). Domestic dogs (*Canis lupus familiaris*) harboured the most zoonotic parasites (adjusted parasite diversity = 80), followed by domestic cats (*Felis catus*, adjusted parasite diversity = 48), gray wolves (*Canis lupus*, adjusted parasite diversity = 24), red foxes (*Vulpes vulpes*, adjusted parasite diversity = 22) and caribou (*Rangifer tarandus*, adjusted parasite diversity = 17). Domestic dogs, domestic cats and gray wolves were highly connected within the network (adjusted degree = 26, 23, 22, respectively), but the most connected species within the network were both birds; pink footed goose (*Anser brachyrhynchus*, adjusted degree = 47) and Ross's gull (*Anser rossii*, adjusted degree = 36). Most zoonotic parasites present in the 'realised' network were bacteria (n = 43), followed by macroparasites (n = 17), fungi (n = 9), protozoa (n = 8), and viruses (n = 4).

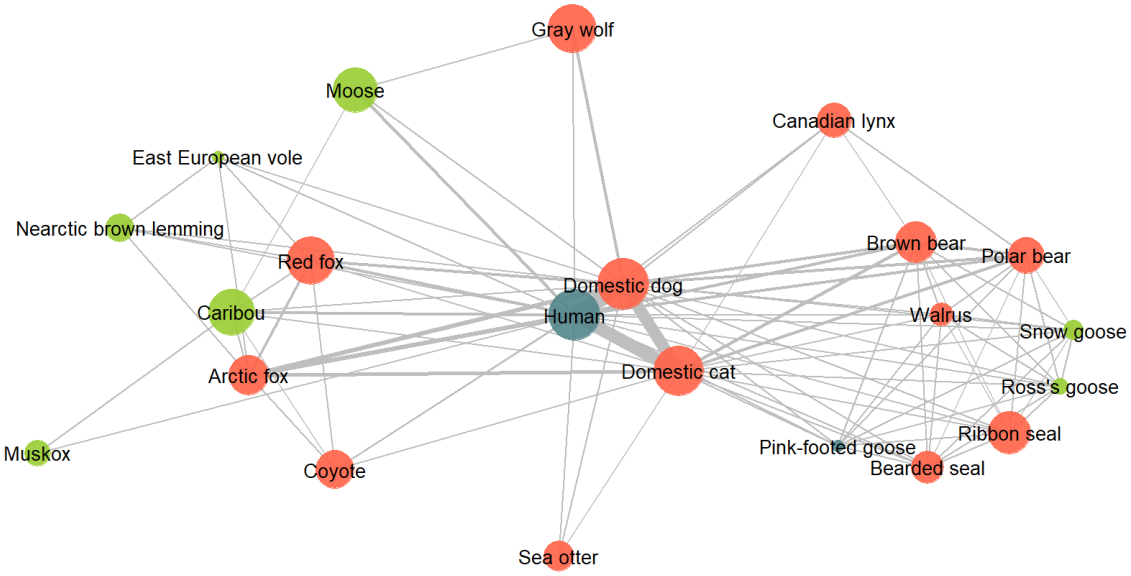

Figure 11. Realised network of 21 Arctic species joined by 81 shared zoonotic parasites (edges). Nodes are weighted by adjusted parasite diversity and coloured by their feeding status; herbivore (green), omnivore (grey) and carnivore (red). Edges are weighted by number of shared parasites

Discussion

Here, for the first time, we have quantified the known parasite diversity across the Arctic and have demonstrated the significant contribution to the Arctic parasitome by introduced host species (namely humans and their domestic pets). We show that humans and domestics dogs harbour a high diversity of parasites and are highly connected within the Arctic network. We also demonstrate that the most pervasive of each parasite type within the network (Influenza A, *Toxoplasma gondii*, *Trichinella nativa*, *Brucella* sp. and *Encephalitozoon cuniculi*) all have zoonotic potential, which is a concern for the northern communities that rely upon Arctic species for subsistence hunting. Our work indicates which Arctic hosts are most likely to harbour zoonotic parasite species, therefore providing an inventory against which surveillance can be executed.

Within this study we found that only ~45% of hosts have been sampled within the Arctic (and are therefore grounded within the network). The potential network contained three times more connections than the realised network, as well as demonstrating a higher mean betweenness and mean importance ratio. By constructing a 'potential' and a 'realised' network of parasite sharing in the Arctic, we have highlighted the substantial variation in sampling effort, or true absence of data, across taxa and geography. Of the 2618 parasite species (and 3943 host-parasite interactions) included in the 'potential' network, 1954

parasites and 2497 host-parasite interactions were from hosts sampled within the Arctic. Given species, for example the red fox (*Vulpes vulpes*) had a high parasite diversity, but parasites recorded from individuals sampled within the Arctic were relatively sparse. As such, we suggest low sampling effort in the Arctic leads to an underestimate of the full extent of the parasite diversity. A deficiency of data may, in part, be owed to the logistics of sampling in the Arctic and the expense of sampling remote and endangered species, which typically makes data difficult to obtain. Attempts to quantify total parasite diversity and pervasiveness is often challenged by uneven sampling effort across host taxa (Cooper and Nunn 2013; Stephens et al. 2016). As such, for all of our analyses, sampling effort was important – the more a species was studied, the more parasites, the higher the degree and the higher the betweenness. For this reason, we used number of citations on Web of Science to adjust for sampling effort (e.g. Nunn et al. 2003; Luis et al. 2015).

When taking in to account sampling bias, some of the most connected species (i.e. those with the highest degree) within the realised network were migratory species, such as pink-footed goose (*Anser brachyrhynchus*), walrus (*Odobenus rosmarus*) and emperor goose (*Anser canagicus*). Migratory animals are known to have an extensive role as parasite sources and transmission pathways, likely owing to their long-distance movements and often exposure to a diversity of habitat and dietary types (Altizer et al. 2011). The most highly connected species, were also species that congregate in large numbers (Hupp et al. 2007; Jensen et al. 2016; Øren et al. 2018). Walruses, for example, are highly social and assemble on land or ice in densely crowded groups of often >100 individuals (referred to as haul-out areas) (Gjertz et al. 2001; Øren et al. 2018). Similarly, emperor geese and pink-footed geese congregate in large numbers during moulting events, migrations and stopovers (Hupp et al. 2007; Jensen et al. 2016). High host densities are known to promote high contact rates and parasite transmission (Calisher et al. 2006; Ryder et al. 2007; Johnson et al. 2011; Luis et al. 2015). However, bearded seals (*Erignathus barbatus*) were also highly connected within the realised network, a species that is non-migratory and that congregate in relatively low densities ($\leq$ 19 individuals km^{-2}) (Bengtson et al. 2005). The high connectivity of bearded seals within the network may instead reflect their high trophic position and varied diet (Finley and Evans 1983; Crawford et al. 2015) (the same as walruses (Sheffield and Grebmeier 2009)), exposing them to a higher diversity of parasites shared with other high trophic marine feeders (Marcogliese 2002; Chen et al. 2008; Lagrue et al. 2011).

Introduced species, predominantly humans and domestic dogs were also highly connected –additionally they were found to harbour the highest frequency of parasites within the network and were identified as key nodes in bridging subgroups to the network (i.e. had the highest Importance Ratio). This is concerning considering the risk of parasite spillover between humans, domestic pets and wildlife. Domestic dogs are thought to be the source of a debilitating canine distemper outbreak among lions (*Panthera leo*) in the Serengeti (Cleaveland et al. 2000) and have been implicated as the source of infection for epizootic outbreaks such as the 1998 to 2002 phocine distemper virus (PDV) outbreak which killed approximately 40,000 seals in the UK (Härkönen *et al.,* 2006). Unlike humans and domestic dogs, the American mink (*Neovison vison*), another introduced species to the Arctic, demonstrated a low parasite diversity and low connectivity within the network. Introduced species are sometimes known to evade their parasites during the initial invasion wave, and are sometimes demonstrated to be less parasite species rich than in their native range (Torchin et al. 2002; Torchin et al. 2003; Marr et al. 2008). An introduced species that leaves behind its parasites can experience a demographic release, facilitating its establishment in its new environment and allowing it to become a pest to wildlife communities (Torchin et al. 2003).

In addition to posing as a spillover risk to wildlife, domestic dogs also host a number of parasites that are zoonotic. For example, domestic dogs, along with other canids, are known to harbour *Echinococcus multilocularis* (Gottstein et al. 2001; Jenkins et al. 2012), a metacestode which causes Alveolar hydatid disease (AHD), a lethal helminthic disease in humans (Gottstein and and Felleisen 1995). Domestic dogs also harbour *Brucella canis*, a bacterial species which sustains a broad spectrum of symptoms in human hosts that can lead to hospitalisation (Marzetti et al. 2013). In fact, domestic dogs have served as a spillover link among wildlife and humans throughout the history of their domestication (Macpherson 2005) and, in the Arctic, zoonotic parasites in dogs have long been recognised (Saunders 1949; Unruh et al. 1973; Salb et al. 2008). Minimal veterinary care is available for domestic dogs in remote northern communities and so routine preventative health measures, such as deworming and vaccination are lacking (Salb et al. 2008). For this reason, the diversity of parasites within dogs is potentially high (as we see within our analyses), while our knowledge of parasite interactions between dogs, humans and wildlife is limited. The results we present here demonstrate how important it is to bridge that knowledge gap, as dogs may act as parasite bridges between wildlife and humans (Salb et al. 2008).

Free ranging wildlife are also recognised as important sources and reservoirs of zoonotic parasites (Daszak 2000; Polley 2005). Within the networks we analysed, zoonotic parasites were predominantly shared between carnivore species, in particular domestic dogs, domestic cats, wolves and red foxes. These species also harboured the highest frequency of zoonotic parasites and carnivores were found to have a significantly higher degree compared to herbivores. An estimated 43% of zoonotic infections are thought to originate from carnivore hosts (Cleaveland et al. 2001) and a study analysing the parasites of 1,345 mammalian wildlife species demonstrated that carnivores are significantly more likely to share parasites with humans compared to omnivores (Wells et al. 2018). The same study demonstrated that red foxes (*Vulpes vulpes*) and grey wolves (*Canis lupus*) were found to harbour the highest numbers of zoonotic parasites (Wells et al. 2018), which matches what we see in our zoonotic network (after humans and domestic cats and dogs). Wild canids are known to harbour potential sources of human infection such as *Echinococcus* species (Eckert and Deplazes 2004; Xiao et al. 2005) and *Toxocara canis* (Luty 2001). We did, however, also detect a high frequency of zoonotic parasites in the commonly consumed herbivores species, caribou and moose. In northern communities, subsistence hunting and the consumption of country foods is a significant cultural activity (Berman and Kofinas 2004). Of the zoonotic agents that humans can attain, viruses and bacteria are most intensively studied (Polley 2005), which explains our finding that bacteria were the most prominent parasite type within the zoonotic network. Interestingly, the most common species of each parasite type that we detected within the network (Influenza A (virus), *Toxoplasma gondii* (protozoa), *Trichinella nativa* (macroparasite), *Brucella* sp. (bacteria) and *Encephalitozoon cuniculi* (fungi)) are all zoonotic parasites. These pervasive species were shown to link a broad range of host taxa across both marine and terrestrial environments.

The work presented here highlights the interface between domestic animals, wildlife, and humans in the Arctic. From our analyses it is clear that there remains a deficiency of parasite data for Arctic species, and that a number of parasite species are shared with humans, posing a substantial risk to health. In light of rapid climatic and anthropogenic changes in the Arctic, there is a desperate need for baseline data against which to measure and predict changes in parasite and disease dynamics. A paucity of data may mask the true extent to which climate change is impacting the health and long term survival of Arctic species. As such, we posit that further work needs to be carried out in order to fill the knowledge gaps within the Arctic parasite network. We have also demonstrated the

interconnectedness of Arctic species with respect to parasite sharing and have highlighted the benefits of parasite surveillance in a community level context. The work presented here may help guide future management for optimal prevention of emerging zoonoses and anthroponoses within the Arctic.

3

Parasites of an Arctic scavenger; the wolverine (*Gulo gulo*)

Abstract

Parasites are fundamental components within all ecosystems, shaping interaction webs as well as host population dynamics and behaviour. Despite this, baseline data is lacking to understand the parasite ecology of many Arctic species, including the wolverine (*Gulo gulo*), a top Arctic predator and scavenger. Here, we combined traditional count methods (i.e. adult helminth recovery based on gross examination of intestinal contents) with 18S rRNA high-throughput sequencing to document the wolverine parasite community. Further, we investigated whether the abundance of parasite species detected using traditional methods were associated with wolverine sex, age class, body length, carcass mass, latitude, and longitude (ranging from the northern limit of the boreal forest to the low Arctic and Arctic tundra). In total, we analysed 54 wolverine carcasses collected from Inuit hunters across Nunavut, Canada. Traditional count methods on the small and large intestinal contents identified two parasite species; *Baylisascaris devosi* and *Taenia* spp. (including *T. twitchelli*) at a prevalence of 72% (n = 39) and 22% (n =12), respectively. In addition, 18S rRNA high-throughput sequencing on DNA extracted from faeces detected additional helminth and protozoan parasites, including a pseudophyllid cestode (*Diplogonoporus* spp. or *Diphyllobothrium* spp.), two metastrongyloid lungworms (*Angiostrongylus* spp. or *Aelurostrongylus* spp., and *Crenosoma* spp.), an ascarid nematode (*Ascaris* spp. or *Toxocara* spp.), a *Trichinella* spp. nematode, and the protozoan *Sarcocystis* spp., though each at a prevalence level less than 13% (n = 7) and taxonomy varied by reference database. The abundance of *B. devosi* significantly decreased with latitude (slope = -0.68; R^2 = 0.17; P = 0.004), suggesting a northerly limit in its distribution. We describe *B. devosi* and *Taenia* spp. in Canadian wolverines for the first time since 1978, and extend the recorded geographic distribution of these parasites ca 2,000 km to the East and into the tundra. To this end, our findings illustrate the value of molecular methods in support of traditional methods, encouraging additional work to improve the advancement of molecular screening methods for parasites.

Background

Representing over 50% of all organisms on Earth, parasites are a fundamental component within all ecosystems –shaping interaction webs as well as host population dynamics and behaviour (Marcogliese 2001; Hudson et al. 2006; Dobson et al. 2008). Even at high latitudes where biological diversity is purported to be generally low (such as the Arctic), parasite communities can be diverse and often more species-rich than those of their vertebrate hosts (Hoberg et al. 2008; Kutz et al. 2009). Over 60 species of parasite, for example, are described in four ungulate species of High Arctic Greenland and North America (Kutz et al. 2012). Such descriptions highlight the extensive distribution of parasites throughout Arctic hosts.

The Arctic is undergoing some of the most rapid rates of climate change and is therefore at high risk of parasite emergence, which may shift baseline data (Brooks and Hoberg 2007; Kutz et al. 2009; McLaughlin 2011). Before the year 2100, global average air temperatures are predicted to increase worldwide by 2.6 °C to 4.8 °C, with parallel increases in precipitation and humidity by an average of 7% (IPCC 2014). It has been forecast that these increases in air temperature will be most dramatic at high latitudes (Dobson et al. 2015) and already the Arctic has experienced the 10 warmest years in the past 2 millennia (Kaufman et al. 2009; IPCC 2018). One expected consequence of warming air temperatures and increased precipitation in the northern hemisphere is a significant increase in the northern expansion of parasites and their hosts into regions that were previously inhospitable to them (Brooks and Hoberg 2007; Kutz et al. 2009; McLaughlin 2011). Consequently, the monitoring of wildlife plays an important role in identifying changes such that actions can be taken to mitigate or minimize pressure. Despite this, there remains a lack of baseline data regarding the parasite ecology for many Arctic species, including the wolverine (*Gulo gulo*), a top Arctic predator and scavenger.

Helminths have previously been recovered from wolverines of Alaska and the Northwest Territories of Canada, including; *Alaria* sp., *Taenia martis*, *T. twitchelli*, *Diphyllobothrium* sp., *Physaloptera* sp., *Baylisascaris devosi*, *Mesocestoides kirbyi* and *Molineus patens* (Rausch 1954; Addison and Boles 1978), though, to the best of our knowledge, these parasites have not been reported or surveyed in wolverines in over 40 years. Sequences for *B. devosi* from Canadian wolverines are, however, present on Genbank, as uploaded by Gesy et al. in 2015 (Accession number: KM216978 to 985). Additionally, *Trichinella* infection has been widely reported in wolverines across their entire range (Reichard et al. 2008b; Sharma et al. 2019c; Sharma et al. 2020) with the

highest prevalence (88%) being reported in 41 wolverines from Nunavut (Reichard et al. 2008b), and two species of Apicomplexa, *Sarcocystis* have been reported in wolverines from Nunavut, Canada (Dubey et al. 2010); sarcocysts were recovered from 33 of 41 (80%) wolverines screened (Dubey et al. 2010). Additionally, *Toxoplasma gondii* infection has been documented in wolverines from the Northwest Territories, the Yukon, Nunavut and British Columbia (Philippa et al. 2004; Reichard et al. 2008a; Sharma et al. 2019a; Sharma et al. 2019b). Not only is parasite surveillance in wolverines limited, but there appears to be a paucity of information related to how parasitic infections of wolverines may be associated with host and geographic metadata.

The wolverine occupies a heterogeneous geographic range that covers northern Arctic tundra, taiga, mountain, and boreal forest ecosystems (Copeland et al. 2010; Dawson et al. 2010). Typically, due to ecological conditions, parasite species richness in carnivores decreases on a latitudinal gradient from south to north (Lindenfors et al. 2007), though there is a lack of comprehensive baselines for parasite diversity in Arctic hosts (Meltofte et al. 2013). It might therefore be expected that wolverines occupying a more northerly region of their geographic range may host a diversity of parasites that is less species rich compared to more southerly inhabitants. Across the varied landscapes they occupy, wolverines can travel huge distances each year (Copeland et al. 2010; Dawson et al. 2010), with male wolverines (which are the larger sex (Banci 1994)) occupying a larger home range compared to females (Pasitschniak-Arts and Larivière 1995; Bischof et al. 2016). Across multiple mammalian taxa, where sexual size dimorphism is male-biased, so too is parasitism (reviewed in Poulin and Morand 2000; Moore and Wilson 2002). Similarly, male bias in home range size can also lead to disparity in infection between sexes (reviewed in Poulin and Morand 2000). A large home range equates to an increased overlap with other host species and environmental conditions, which can expose a host to a broad diversity of parasites (Poulin and Morand 2000; Leung and Koprivnikar 2016; Becker et al. 2018). As such, prevalence and intensity of parasitic infection is commonly, but not exclusively, higher in male mammalian hosts compared to females (Poulin 1996; Zuk and McKean 1996). Similarly, prevalence and intensity of parasitic infection typically differs between adult compared to juvenile hosts, though this varies depending on host and the conditions of infection (Woolhouse 1998). Across their large home range, wolverines consume a broad range of prey items. As facultative scavengers, wolverines consume a range of species, including moose (*Alces alces*), caribou (*Rangifer tarandus*), muskoxen (*Ovibos moschatus*), hare (*Lepus* sp.), Arctic ground squirrels (*Spermophilus*

parryii), voles and lemmings (Muridae), ptarmigan (*Lagopus* sp.), seal (*Phoca* sp.) and migratory bird species (Lofroth et al. 2007; Koskela et al. 2013; L'Hérault et al. 2018b). Hosts with a broad diet are potentially exposed to a larger variety of trophically transmitted parasites (Vitone et al. 2004; Anderson and Sukhdeo 2011; Aponte et al. 2014). A generalist diet in wolverines may therefore equate to high parasite exposure.

Here we use both a traditional parasite count method (i.e. adult helminth recovery based on gross examination of intestinal contents) and 18S rRNA high-throughput sequencing to characterise the parasite community of wolverines from Nunavut, Canada. Secondly, we investigate whether the abundance of the most common parasite; *Baylisascaris devosi* was associated with the following metadata: wolverine sex, age class, body length, carcass mass, latitude, and longitude (ranging from the tree line to low Arctic and Arctic tundra). We additionally determine whether a coinfection with both *B. devosi* and *Taenia* spp. (i.e. 0, 1, or 2 parasite species present) is associated with our wolverine metadata.

Methods

Wolverine sampling

As part of a wolverine carcass collection programme initiated by the Government of Nunavut Department of Environment, 54 skinned wolverine carcasses (legally harvested for purposes other than research) were obtained from local Inuit hunters with the assistance of Hunters and Trappers Organizations (HTOs) of Nunavut, Canada. Wolverines were harvested between November 1st and April 30th from 2010 through 2013 at five distinct geographical locations representing different Inuit communities; Arviat (61 °10'N / 94 °06'W; n = 17), Baker Lake (64 °31'N / 96 °02'W; n = 9), Repulse Bay (Naujaat) (66 °52'N / 82 °24'W; n = 7), Kugluktuk (67 °82'N / 115 °09'W; n = 11), and Cambridge Bay (69 °11'N / 105 °05'W; n = 10) (Figure 12). The skinned wolverines were delivered to community wildlife offices where carcasses were frozen at -20 °C or below and the following information was recorded: kill date, location, sex (male, 37; female, 17) and age (yearlings, 18; juveniles, 16; adults, 20). To determine the age, a lower canine from each individual was submitted to Matson's Laboratory LLC (Milltown, MT, USA) for analysis. Following Banci and Harestad (1988) and Vangen et al. (2001) individuals were then grouped into three age classes: juvenile (0-1 year, date of birth is set to March 1st), yearling (1-2 years) and adult (≥ 2 years). Necropsies were performed

to collect the gastrointestinal tracts used within this study. All gastrointestinal tracts were shipped to Université de Moncton, January 2018.

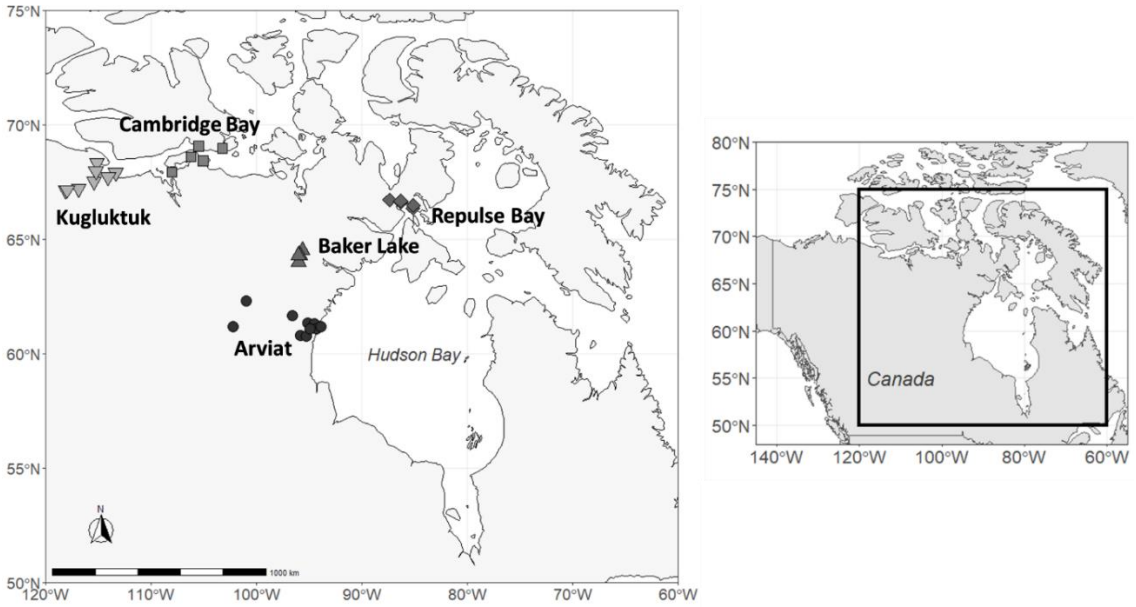

Figure 12. Map of individual wolverine hunting locations close by Inuit communities (circles= Arviat, triangles= Baker Lake, diamonds= Repulse Bay (Naujaat), squares= Cambridge Bay, inverse triangles= Kugluktuk) in Nunavut, Canada, 2010-2013. Inset shows sampling location (black outline) on a map of North America

Processing/dissection of gastrointestinal tracts

Dissections were conducted on gastrointestinal tracts of 54 wolverines (pylorus to anus, excluding the stomach) at the Université de Moncton (February 2018). Each intestinal tract was partially defrosted until pliable. A faecal sample and a small intestine sample were collected from near the rectum of each individual (54 faecal and 54 small intestine samples; 108 samples total) and immediately frozen at -20 °C for later 18S parasite profiling. Starting from the stomach end, the intestinal wall of the tract was then systematically cut open, washed through a series of sieves (minimum mesh size of 0.01 mm), and examined for intestinal helminths by naked eye and then using a 40 X hand lens. The mucosa was scraped with a spatula into a petri dish and also examined. Any helminths discovered were removed, counted, and stored in 70% ethanol at -20 °C until further analysis.

Identification of adult parasites retrieved via traditional count methods

Recovered adult helminths were identified at the Jenkins Lab, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, via molecular methods. DNA of 44 worms from 34 hosts was extracted individually using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, Canada) following manufacturer instructions. The primer set COX-1F (5′— TTTTTTGGGCATCCTGAGGTTTAT—3') and COX-1R (5′— TAAAGAAAGAACATAATGAAAATGY —3') (Bowles et al. 1992) was used to target a ~366 base pair region of the cytochrome *c* oxidase subunit 1 (COX1) mitochondrial gene following methods detailed in Lavikainen *et al.* (2003). For each sample, 25 µL reaction mix was prepared by mixing 12.5 µL 2× Taq FroggaMix (FroggaBio, Toronto, Canada), 1 µL forward and reverse primer mixture (10 µM of each primer), 3 µL template cDNA and 7.5 µL nuclease-free water. The thermal conditions used were: preheating at 94 °C for 3 mins followed by 30 cycles of denaturation at 94 °C (30 s), annealing at 55 °C (30 s) and extension at 72 °C (1 min). This was followed by a 10 min final extension at 72 °C. PCR products were separated by gel electrophoresis on 1% agarose gels in TAE buffer. PCR products of 15 samples, representing samples which yeilded bands at different positions, were purified and sent for sequencing at Macrogen, South Korea. All nucleotide sequences were compared to sequences from the NCBI GenBank database using BLAST.

18S parasite profiling using high-throughput sequencing

Total genomic DNA was extracted from 54 faecal samples using a QIAamp DNA Stool Mini Kit (Qiagen, Toronto, Canada). Five of the samples were extracted and processed a second time, to check for metabarcoding consistency. Methods followed the manufacturer's instructions for pathogen detection, with the addition of a 6 min homogenisation step to enhance bacterial cell lysis after the addition of buffer ASL (Step 2 in manufacturer handbook). Homogenization was achieved using a TissueLyser II (Qiagen) for 6 min at 5.5 Hz with the following combination of glass beads per tube: 0.3 g of 106 µm beads, 0.5 g of 425-600µm beads, and x1 3mm bead (Sigma-Aldrich, Ontario, Canada).

For parasite detection within faeces and small intestine samples, the V9 fragment of the 18S rRNA gene was amplified using the primer set Euk_1391f (5′— CTCAAAGATTAAGCCATGC —3') and EukBr (5′— TTTACGGTCAGAACTAGGG

—3') (Amaral-Zettler et al. 2009) in conjunction with the mammal blocking primer: GCCCGTCGCTACTACCGATTGGIIIIITTAGTGAGGCCCT-(C3 Spacer) (Vestheim and Jarman 2008) following methods described in the Earth Microbiome Project (http://www.earthmicrobiome.org/). In brief, targeted PCR reactions were used whereby a sequence tag was added to the 5′ end of each primer. The tag sequence was used to bind primers in a second PCR reaction during which individual sample barcodes and Illumina adapters were annealed. All barcoded products were run on 2% agarose gels. DNA concentrations of all samples were measured using PicoGreen, allowing pooling of samples at equimolar amounts. The pool (library) was cleaned using AMPure® beads. The library was then quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Illumina MiSeq PE250 high-throughput sequencing of 18S libraries was conducted with the MiSeq Reagent Kit v2 (500 cycles) at Génome Québec, Montréal, Canada.

Bioinformatic analysis

All bioinformatic analyses were conducted using QIIME2, version 2018.11 (Bolyen et al. 2018). Briefly, paired-end reads were joined using VSEARCH (Rognes et al. 2016) and quality filtered using the default settings of q-score-joined (Bokulich et al. 2013). Data reads were denoised using DADA2 (Callahan et al. 2016) with a minimum phred quality score of 28 (below which data quality tailed off). Taxonomic assignments of representative sequences from each Amplicon Sequence Variant (ASV) were performed using the SILVA (release132) reference database (Quast et al. 2013) at 99% identity so as to minimise potential identification mismatch. Taxonomic assignment was conducted in conjunction with the alignment-based taxonomy consensus classifier, BLAST+ (Camacho et al. 2009). To additionally evaluate taxonomic assignment, the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) was used to compare representative sequences of parasite taxa against the NCBI database. As lower taxonomic identifications are less certain, parasite identifications are reported at genus level.

Statistical analysis

All statistical analyses were conducted in R version 3.5.0 (R Core Team 2018). Generalized Linear Models (GLMs) were used to investigate whether i) total *B. devosi*

abundance and ii) coinfection of *B. devosi* and *Taenia* spp. (i.e. 0, 1, 2 parasite species present) were associated with sex, age class, body length, carcass mass, latitude, and longitude. An outlier, identified via a Cook's distance residuals versus leverage plot, was omitted from the models. The coinfection model did not include parasite species detected using molecular methods owing to the low prevalence identified and difficulty in distinguishing true wolverine infections from secondary parasite detection from infected individuals. To follow GLM assumption, a negative binomial error family with a log link function was used for *B. devosi* abundance. A Poisson family and identity link function was used for coinfection of *B. devosi* and *Taenia* spp., as based on residual plots. No interacting terms were included due to small sample size limiting the numbers of individuals represented in each group. For 18S data, a parasitic infection was considered present if a parasite was detected in either the faecal or small intestine sample. Due to the low prevalence of detection and because the range of dietary items consumed by wolverines makes it difficult to discern whether parasite detection indicated a true infection of wolverines or is instead a secondary detection from an infected prey item, parasites detected through 18S rRNA high-throughput sequencing were not included in the statistical models.

Results

Parasite diversity and abundance

Based on adult helminth recovery from intestines, 83% of wolverines (n = 45) were parasitised; nine individuals had no visible parasite infection, 34 were infected with one parasite species, and 11 were infected with two. *Baylisascaris devosi* (Nematoda) was found in 72% (n = 39) of individuals at an average of 3.6 worms per individual (SD: ± 4.6, abundance range: 0-21; Figure 13a), and *Taenia* spp. (Cestoda, O. Cyclophyllidea) were found in 22% (n = 12) individuals at an average of 1.8 worms per individual (SD: ± 6.5, abundance range: 0-45). Based on COX 1 primers, two taeniid cestodes from two hosts were identified as *T. twitchelli* (99% similarity to Genbank accession number EU544598) and seven nematodes from seven hosts were identified as *B. devosi* (99% similarity to Genbank accession number KM216978). When using 18S rRNA high-throughput sequencing, six parasite genera were detected, at a low overall prevalence (22% total, n = 12; ≤ 13% prevalence for any parasite). The prevalence of each parasite genus against associated host metadata can be found in Supplementary Table 2. Although a higher parasite diversity was detected using molecular methods, it is uncertain whether

or not the parasites are secondary detections from infected prey items as opposed to true infections of wolverines and, in some instances, there was no consensus in taxonomic identification between the two reference databases used, and likely identifications for wolverine parasites are seldom present in these databases. For example, a metastrongyloid lungworm (detected at a prevalence of 2%; n = 1) was identified as *Angiostrongylus* sp. in accordance with the SILVA reference database (99% similarity) but *Aelurostrongylus* sp. when using the NCBI database (99.4% similarity). Similarly, an ascarid nematode was detected in 6 small intestinal and 1 faecal sample at a prevalence of 13% (n = 7), and identified as an *Ascaris* sp. in the SILVA database (99% similarity) but as *Toxocara* sp. in the NCBI database (99.4% similarity). A pseudophyllid cestode (Subclass: Eucestoda) which was identified as *Diplogonoporus* sp. according to the SILVA database (99%) or *Diphyllobothrium* sp. according to NCBI (99.4%) was detected at a prevalence of 2% (n = 1). Further, the following parasitic genera were each detected in 2% (n = 1), the identification of which were consistent between the SILVA and the NCBI databases (≥99% similarity); *Crenosoma* spp., *Trichinella* spp. (Nematoda) and *Sarcocystis* spp. (Protozoa). In addition to these 6 parasites for which wolverine could serve as potential definitive hosts, two parasites were detected which are likely parasites detected from infected prey items. For example, *Gregarina* sp. (detected in both the SILVA and NCBI databases; 99% and 96.5% respectively), a parasite of insects, was recovered at a prevalence of 7% (n = 4). Similarly, *Bodonidae* sp. (detected in both the SILVA and NCBI databases; ≥99%), an ectoparasite of fish, was recovered at a prevalence of 4% (n = 2). The *Baylisascaris* sp. and *Taenia* sp. detected using traditional methods were not detected by 18S rRNA high throughput sequencing. When using 18S rRNA sequencing, a greater diversity of parasites was recovered from faecal samples (n = 8) as opposed to small intestine samples (n = 2). However, a greater number of *Ascaris* sp. detections were recorded in small intestine samples (n = 6) compared to faecal samples (n = 1). Only *Ascaris* sp. and *Bodonidae* sp. were detected in both a faecal and a small intestine sample.

Parasitism and host metadata

No association was found between *B. devosi* abundance and wolverine sex, age class, body length, carcass mass or longitude, but a significant decrease in *B. devosi* abundance was associated with an increase in latitude (slope = -0.68; R^2 = 0.17; P = 0.004). Specifically, a total of 52 *B. devosi* nematodes (mean; 5.8) were recovered at 61 °N,

compared to a total of 5 (mean; 1.6) at 69 °N, a mean decrease of 0.5 per degree North. The geographic region of detection for *B. devosi* recorded here extends the known distribution recorded from previous research and demonstrates the presence of this species within the Arctic tundra (Figure 13a). Due to the low number of individuals infected with *Taenia* spp. (22%, n = 12), it was not possible to compare abundance of this species alone with any host metadata. However, *Taenia* spp. is present in wolverines in a region where it was previously unrecorded, and also demonstrates the presence of this species within the Arctic tundra (Figure 13b).

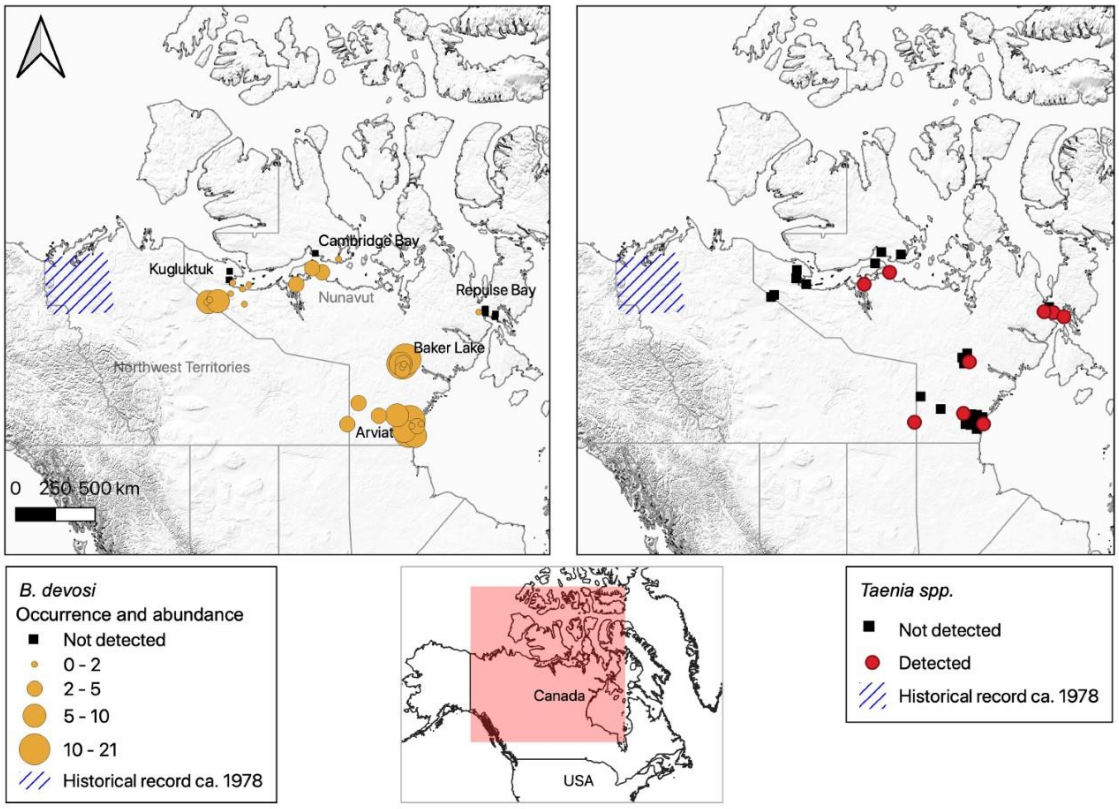

Figure 13a. Records of *Baylisascaris devosi* in Nunavut, Canada detected within this paper (yellow), where each point is weighted by parasite abundance. Black squares represent wolverines in which *B. devosi* was not detected. Blue hashed line area indicates the previously known records of *B. devosi* reported by Addison and Bole (1978) **b.** Records of *Taenia* spp. in Nunavut, Canada detected within this paper (red). Previously known records of *Taenia* spp. (*T. twitchelli* and *T. martis*) reported by Addison and Bole (1978) are indicated by the blue hashed line area. Black squares represent wolverines in which *Taenia* spp. was not detected

No association was found between the presence of a coinfection (0, 1 or 2 parasite species present) and sex, age class, body length, carcass mass, longitude or latitude ($P > 0.05$).

Discussion

Within this study, we combined adult helminth recovery (where taxonomy was confirmed by molecular identification using the COX1 mtDNA locus) and 18S rRNA high-throughput sequencing of DNA extracted from faecal and small intestinal samples to provide comprehensive insight into the gastrointestinal parasite community of wolverines. Two parasite species were detected using traditional methods, *Baylisascaris devosi* and *Taenia* spp. (including *T. twitchelli*), both of which have been recovered in wolverines previously, although the latest published study to do so is over 40 years old (Addison and Boles 1978). Sequences for *B. devosi* from morphologically confirmed specimens from Canadian wolverines are, however, present in Genbank (Accession numbers KM216978-985). In addition, 18S rRNA targeted high-throughput sequencing detected threefold the diversity of parasite species (n = 6) compared to gross adult parasite recovery (n = 2), suggesting that combined molecular and adult parasite recovery represents an important tool for characterising the parasite community of a given host. Comparative studies in other species, including wild rats (*Rattus norvegicus* and *R. rattus*), found 18S rRNA targeted high-throughput sequencing to be at least as sensitive as traditional count methods (Tanaka et al. 2014; Hino et al. 2016), and detects a broad diversity of intestinal eukaryotes in long-tailed macaques (*Macaca fascicularis*) and spotted hyenas (*Crocuta crocuta*) (Heitlinger et al. 2017; Wilcox and Hollocher 2018).

Although the methods used within this study offer an insight into the diversity of parasites in wolverines, it is important to note that the results may not reflect the total diversity, especially since we did not search for parasites in extra gastrointestinal locations that may be shed in faeces. All sequencing primer sets have some level of bias and the small amount of sample from which DNA is extracted for PCR may simply not contain eggs or DNA of all parasites present (Pompanon et al. 2012; Elbrecht and Leese 2015; Pawluczyk et al. 2015). This may explain why the 18S rRNA targeted high-throughput sequencing approach did not detect *Taenia* spp.; as well, taeniid egg shedding is sporadic, often shed in segments, and eggs are notoriously difficult to open up for DNA extraction (Hidalgo et al. 2018). The same is true of *Baylisascaris* spp.; *Baylisascaris* eggs have thick walls, again making it difficult to liberate the DNA (Dangoudoubiyam et al. 2009; Testini et al.

2011). Taxonomic resolution from metabarcoding is limited by the availability of data in reference databases, which is often lacking for wildlife parasites. For example, unless the *Ascaris* sp. detected was a misidentification of *B. devosi* (which is possible considering that the 18S gene is highly conserved), the molecular methods used within this study failed to detect the species recovered by traditional methods. Additionally, because the 18S gene is highly conserved, it is important to interpret the metabarcoding results with caution. We report parasite taxonomy at genus level, as lower taxonomic classification is uncertain, but even so there are genera that may be misidentifications. For example, we detected a metastrongyloid lungworm that could be *Angiostrongylus gubernaculatus* or *Aelurostrongylus pridhami,* both previously identified in mustelids in North America (Dougherty 1946; Anderson 1963; Faulkner et al. 2001), or a closely related genus not represented in the reference databases we used. Further, it is important to note that the parasites detected here through 18S rRNA high-throughput sequencing may not be parasites of wolverines, but instead may be a parasite of an infected prey item (Sheppard et al. 2005; reviewed in Pompanon et al. 2012).

The molecular methods used here allow us to comprehensively characterise the broader parasite diversity in wolverines. However, due to the lower prevalence of parasites detected using molecular methods (22% total; no more than 13% prevalence for any given parasite) and because of the added benefit of abundance data associated with traditional methods, only the data obtained from traditional techniques was used when running our models. As reported previously (Addison and Boles 1978), adult helminths *Baylisascaris devosi* and *Taenia* spp. (including *T. twitchelli*) dominated the gastrointestinal tract helminth fauna of wolverines, present in 72% and 22% of wolverines in the current study, respectively, compared to 74% and 11%, respectively, in Addison and Boles (1978). We found a lower prevalence of *Sarcocystis* and *Trichinella* (2%, n = 1 for both) compared to other studies (which found a prevalence of 80% and 88%, respectively) (Reichard et al. 2008b; Dubey et al. 2010), although the last was based on larval recovery from muscle rather than intestinal based methods (Reichard et al. 2008b). It is likely that the DNA we detected for *Trichinella* is either from larvae ingested from a prey item, or from a transient adult nematode. The high parasite prevalence detected in our wolverine samples using count methods may reflect the fact that wolverines travel huge distances across a heterogeneous geographic range, from boreal forests to the Arctic tundra (Copeland et al. 2010; Dawson et al. 2010), which may lead to high exposure to parasites. It is more likely, however, that diet drives the high parasite prevalence detected. Wolverines consume an

intensely varied diet of live prey and carcasses (Lofroth et al. 2007; Koskela et al. 2013), which may lead to high infection rates from trophically transferred parasites, such as *B. devosi* (transmitted directly and through paratenic hosts) and *T. twitchelli* (transmitted through consumption of intermediate hosts, including ground squirrels (family: Sciuridae), lemmings (family: Cricetidae), voles (family: Cricetidae), muskrats (*Ondatra zibethicus*), and porcupine (*Erethizon dorsatum*)) (Rausch 1959).

In total, 10 species of *Baylisascaris* exist worldwide, most of which utilise carnivorous mammals as definitive host, with a smaller prey host serving as a paratenic host (Sapp et al. 2017). Some species of *Baylisascaris* incur detrimental health effects on their paratenic hosts; the raccoon roundworm, *B. procyonis*, for example, causes severe or even fatal neurological disease in humans and wildlife, yet little or no clinical disease in raccoon definitive hosts (Sapp et al. 2017). The effect that *B. devosi* infection has on wolverine health remains unknown; however, as they serve as definitive hosts, it is likely to be minimal. The common occurrence of *Baylisascaris* in species of the lower Arctic is attributed to the parasite's ability to persist in the external environment. It would appear, however, there is perhaps a northern limit of *B. devosi*, indicative from our finding that *B. devosi* abundance decreases with latitude. This finding resembles what is seen in some ascarid nematodes, such as *Toxocara canis* which does poorly above 60°N (reviewed by Jenkins et al. 2011), but not others, such as *Toxascaris leonina* which is found all the way up in to the high Arctic (Kapel and Nansen 1996; Andreassen et al. 2017). Lindenfors *et al.* (2007) found latitude to be a primary predictor of parasite species richness in carnivores. *Taenia* spp. have a broad host range in mammalian definitive hosts that occupy northern territories, including brown bears (*Ursus arctos*), wolves (*Canis lupis*), reindeer (*Rangifer tarandus*) and arctic foxes (*Vulpes lagopus*) (Kapel and Nansen 1996; Lavikainen et al. 2011). The prevalence of *Taenia* spp. in northern environments is again likely owed to the ability of *Taenia* eggs and gravid proglottids to survive for months in the external environment (Ilsøe et al. 1990), and the ability to transmit between predator-prey cycles. Likely intermediate hosts for *T. twitchelli* consumed by wolverine in Nunavut include ground squirrels, lemmings, voles, muskrats, and porcupine (*Erethizon dorsatum;* Rausch 1954; Dalerum et al. 2009; Kukka and Jung 2015).

Our findings showed that the abundance of *B. devosi* and co-infection of *Baylisascaris* and *Taenia* (i.e. 0, 1, 2 parasite species present) did not differ with sex or age class, a finding that mirrors what is seen with *Sarcocystis* and *T. gondii* infections in wolverines (Reichard et al. 2008a; Dubey et al. 2010). The lack of sex bias, however, challenges what

might be expected, as male wolverines occupy a larger home range compared to females (Pasitschniak-Arts and Larivière 1995) which may increase their exposure to parasites. Geographical range size is also considered an important determinant of parasite infection in various other carnivores (Lindenfors et al. 2007). Additionally, male wolverines are larger in both size and mass compared to females (Banci 1994; Awan and Szor 2012); larger-bodied organisms require a greater resource intake, potentially increasing exposure to trophically transmitted parasites (reviewed in Morand and Poulin 1998). Alternatively, the increased parasite abundance typically found in males compared to females may be attributable to immunological differences that exist between sexes, which may in turn influence the susceptibility of male hosts (reviewed in Klein 2004).

Wolverines are a culturally important species to northern communities and, as such, it is important to address parasite species that are of concern to human health. It is unlikely that the parasites detected within this paper are of risk to trappers and hunters handling wolverine carcasses. *Trichinella* spp. are zoonotic, but it is important to note that the *Trichinella spp.* detected within our study may be from an ingested prey item, rather than being a true parasite of wolverines, and so may not pose a risk.

Monitoring wildlife plays an important role in identifying changes such that actions can be taken to mitigate or minimize pressure. Here we have filled a knowledge gap in parasite community data and have shown, through the use of combined adult parasite recovery and molecular methods, that the parasite diversity of wolverines is greater than previously observed. To this end, we recognise the value of molecular methods to aid adult parasite recovery, especially in remote species. We encourage more work be done to improve the advancement of molecular screening methods for parasites, including broader databases with sequences from morphologically confirmed specimens, and interpretation of findings in light of the best available understanding of parasite life cycles and known host and geographic distributions.

4

The gut biome of a scavenger; the wolverine (*Gulo gulo*)

Abstract

Wolverines (*Gulo gulo*) inhabit taiga and tundra regions across the Holarctic region and are facultative scavengers of a broad range of terrestrial and marine prey. Unusually, across the animal kingdom, the species is one of very few known to digest bone. Prey items are often cached during times of low availability, enabling wolverines to exploit a cold, low-productivity niche. Such a feeding strategy, however, may expose wolverines to unique microbial communities, and potentially bacterial pathogens associated with decomposed carrion. In addition, the tight evolutionary coupling of hosts to their gastrointestinal bacteria (gut microbiota) may explain how scavengers, such as the wolverine, are able to consume carrion and bone. Here, we use 16S rRNA targeted high-throughput sequencing and stable isotope analysis, respectively, to characterise the gut microbiota and dietary niche of wolverines from Nunavut, Canada, in an area ranging from boreal forest treeline to arctic tundra. In addition, we investigate associations between both the gut microbiota and dietary niche, with geographic location and host metadata, including the abundance of gastrointestinal helminths. We find a relatively high proportion of the bacterial phylum Tenericutes (the third most abundant phylum in our data), which is typically associated with marine organisms, and a low proportion of potentially pathogenic bacteria (0.7% of total reads). No association was found between gut microbial diversity and host sex or age class. Similarly, no association was found between gut microbial diversity and location, despite known differences in dietary niche. Our study gives the first insight in to the gut bacterial communities associated with a free-ranging, Arctic scavenger; the wolverine. A deeper understanding of factors that influence wolverine intestinal health will feed into the development of future conservation strategies aiming to improve the persistence and resilience of this Arctic carnivore.

Background

After death, bacteria rapidly begin to decompose their hosts (Vass 2001) and, in doing so, excrete toxic metabolites, which render carcasses a hazardous food source for many animals (Macfarlane and Macfarlane 1997; Macfarlane and Macfarlane 2012). Carcasses harbour bacteria that form part of the complex temporal succession of epinecrotic and thanatomicrobiome communities involved in carrion decomposition (i.e. the bacteria found on a carcass's external surface and internal organs/cavities, respectively) —known collectively as the 'necrobiome' (Pechal et al. 2013; Pechal et al. 2014; Javan et al. 2016; Pascual et al. 2017). The necrobiome typically encompasses bacteria such as *Clostridium perfringens, Escherichia coli, Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi*, and *Bacillus stearothermophilus*, all of which produce toxins during the decomposition process (Janzen 1977; Vass 2001) and so contribute to the generally unpalatable nature of decomposing animals.

Despite the extensive bacterial diversity of the necrobiome (Janzen 1977; Vass 2001), for species utilising a scavenging niche, the consumption of carrion is possible, and occurs without the onset of apparent adverse effects (Roggenbuck et al. 2014; Wang and Rozen 2017). This is, in part, likely owed to a low pH gastric acid acting as a strong filter against pathogenic bacteria (Giannella et al. 1972; Martinsen et al. 2005; Tennant et al. 2008), which may explain the low pH gastric acids of scavenger species such as possums (*Trichosurus vulpecula*; pH = 1.5), ferrets (*Mustela putorius furo;* pH = 1.5) and white-backed vultures (*Gyps africanus*; pH = 1.2) (Houston and Cooper 1975; Fox et al. 1991; Skinner et al. 2005), compared with much less acidic gastric acids of non-scavenging species, e.g. sheep (*Ovis aries*; pH = 4.7), horses (*Equus ferus caballus*; pH = 4.4) or wild mice (*Mus masculus*; pH = 3.8) (see Beasley et al. 2015). However, it is possible that gastric acid only plays a partial role in antimicrobial filtering. Another mechanism that may facilitate digestion of carrion and prevent potential infection from pathogenic bacteria in the necrobiome is the gut microbiota (i.e. the complex community of bacteria within the gut). A high bacterial diversity within the gut is typically associated with increased resilience against potentially pathogenic bacteria (Van Der Waaij et al. 1971; Girvan et al. 2005) and could therefore protect the host from pathogen invasion. However, a low, specialised bacterial community has also been demonstrated as beneficial to the host pathogen resistance (Roggenbuck et al. 2014).

Previous studies on the microbiota of scavengers are uncommon but those that exist present contrasting results; some detect high, and others low, bacterial diversity within the scavenging host. For example, a low, conserved diversity of gut bacteria, was found in two species of New World vultures (black vulture; *Coragyps atratus*, and turkey vulture; *Cathartes aura*) (Roggenbuck et al. 2014). In contrast, Tasmanian devils (*Sarcophilus harrisii*), which are predominantly scavengers of carrion (Brown 2006), instead demonstrate a high diversity of gut bacteria (Cheng et al. 2015). Given the lack of congruence on microbial diversity it may be a scavenger's gut microbiota composition rather than diversity that plays an important role in their ability to consume decomposed vertebrate matter (Roggenbuck et al. 2014; Wang and Rozen 2017).

In addition to their potential role in host defence against pathogens within the ingested necrobiome, a given bacterial composition may facilitate scavengers to digest bone. The bone-eating polychaete worm (*Osedax sp.*), for example, hosts a diverse and abundant population of symbiotic bacteria, dominated by Epsilonproteobacteria, which is thought to contribute to the digestion of bone (Goffredi et al. 2007). The rectal microbiota of wild spotted hyenas (*Crocuta crocuta*), a bone consuming species (Tanner et al. 2010), is predominantly made up of the bacterial families *Clostridiales XI* (phylum: Firmicutes), *Corynebacteriaceae* (phylum: *Actinobacteria*), unclassified *Clostridia* (phylum: *Firmicutes*) *and Bacteroidaceae* (phylum: Bacteroidetes) (Rojas et al. 2020). In human remains and pig cadavers, bacteria-mediated bone decomposition is well characterised due to interest as a branch of forensic science. From this work, it is thought that the principal forms of bioerosion of bone originate from the host's intrinsic gut microbiota that escape after death (White and Booth 2014; Damann et al. 2015). However, it remains poorly understood how scavengers at large are able to digest bone as a key component of their diet and whether microbiota composition of scavengers is unique.

Here, we contribute new knowledge to the existing literature on the gut biome of scavengers, by characterising the gut microbiota of wolverines from the Canadian Arctic. The species is a scavenger that utilises a broad diet that includes terrestrial and marine sources, and frequently caches food for consumption during winter (Dalerum et al. 2009; Copeland et al. 2010; L'Hérault et al. 2018b; L'Hérault et al. 2018a). Our sampling area ranges from the boreal treeline into Arctic tundra, and encompasses both coastal and terrestrial regions. Our analysis includes spatial data (harvest community) and broad information on dietary niche from stable isotope analysis. The latter was used to assess

whether patterns in microbiota diversity and composition are linked to harvest community or scavenging types, e.g. marine versus terrestrial scavenging (where increased $\delta^{13}C$ is indicative of a marine-based diet), or trophic level (where increased $\delta^{15}N$ is typically indicative of an increasingly high trophic position diet). To assess the whole gut biome we also quantify helminth intensity due to their coupled evolutionary history with microbiota and the potential mechanistic interactions between the two components (Waterfield et al. 2004; Koch and Schmid-Hempel 2011; Glendinning et al. 2014; Kreisinger et al. 2015).

Methods

Wolverine sampling

As part of a wolverine carcass collection programme initiated by the Government of Nunavut Department of Environment, 49 wolverine carcasses were donated by local Inuit hunters from Nunavut, Canada. Wolverines were harvested between November 1st and April 30th from 2010 through 2013 at five distinct geographical locations; Arviat (61°10'N / 94°06'W), Baker Lake (64°31'N / 96°02'W), Repulse Bay (Naujaat) (66°52'N / 82°24'W), Kugluktuk (67°82'N / 115°09'W), and Cambridge Bay (69°11'N / 105°05'W) (Figure 14). From here on in, these geographical locations are referred to as 'harvest community'. Harvested wolverines were delivered to community conservation offices where carcasses were frozen at -20°C. Due to regional winter temperatures averaging -35°C, carcasses remained frozen throughout transit. The following information was recorded for each wolverine: kill date, location, sex (male, 35; female, 14), and age (yearlings, 8; juveniles, 11; adults, 13; age not recorded, 17). Necropsies were performed to collect the gastrointestinal tracts used within this study. All gastrointestinal tracts were shipped to Université de Moncton in January 2018.

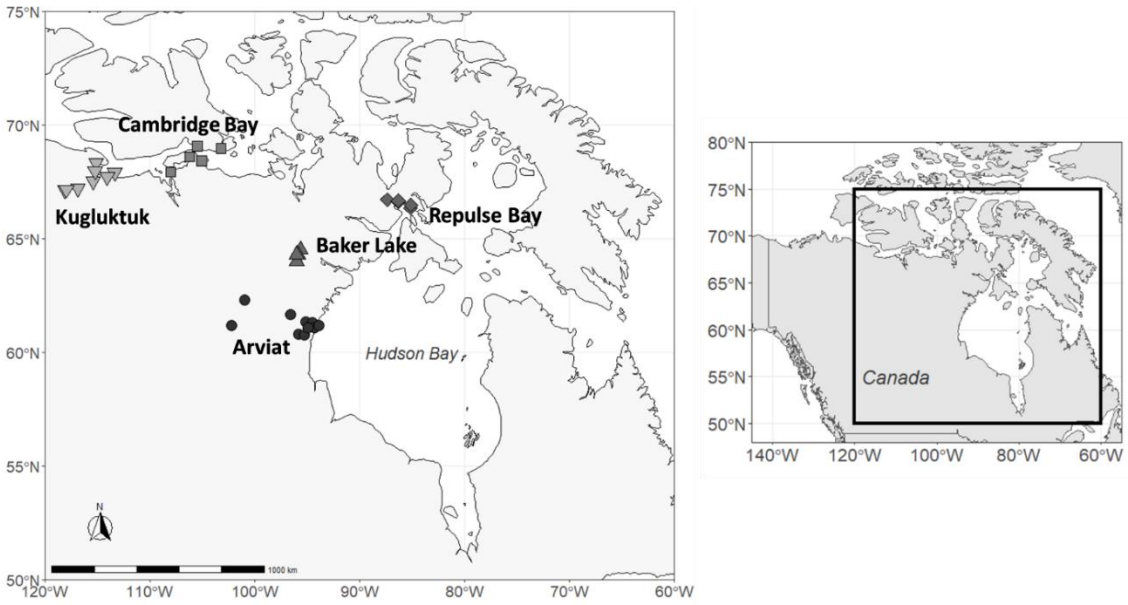

Figure 14. Individual wolverine harvest locations in Nunavut, Canada (n = 49, 2009-2010). Wolverine locations are associated with the Inuit community where the samples have been gathered (circles= Arviat, triangles= Baker Lake, diamonds= Repulse Bay (Naujaat), squares= Cambridge Bay, inverse triangles= Kugluktuk). Inset shows sampling area on a map of Canada

Wolverine gastrointestinal tract dissections

Dissections were conducted on a total of 49 wolverine gastrointestinal tracts at the Université de Moncton (February 2018). Each intestinal tract was partially defrosted until pliable. A faecal sample was collected from near the rectum of each individual and immediately frozen at -20°C for later 16S microbial profiling. Starting from the stomach end, the intestinal wall of the tract was systematically cut open, washed through a series of sieves (minimum mesh size of 0.01 mm) and examined for intestinal helminths. Any helminths discovered were removed, counted and stored in 70% ethanol at -20°C.

Identification of adult parasites retrieved via traditional count methods

Recovered adult helminths were identified at the Jenkins Lab, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, via molecular methods. DNA of 44 worms from 34 hosts was extracted individually using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, Canada) following manufacturer instructions. The primer set COX-1F (5′— TTTTTTGGGCATCCTGAGGTTTAT—3’) and COX-1R (5′— TAAAGAAAGAACATAATGAAAATGY —3’) was used to target a ~366 base pair region of the cytochrome *c* oxidase subunit 1 (COX1) mitochondrial

gene following methods detailed in Lavikainen *et al.* (2003). For each sample, 25 µL reaction mix was prepared by mixing 12.5 µL 2× Taq FroggaMix (FroggaBio, Toronto, Canada), 1 µL forward and reverse primer mixture (10 µM of each primer), 3 µL template cDNA and 7.5 µL nuclease-free water. The thermal conditions used were: preheating at 94 °C for 3 mins followed by 30 cycles of denaturation at 94 °C (30 s), annealing at 55 °C (30 s) and extension at 72 °C (1 min). This was followed by a 10 min final extension at 72 °C. PCR products were separated by gel electrophoresis on 1% agarose gels in TAE buffer. PCR products of 15 samples, representing samples which yeilded bands at different positions, were purified and sent for sequencing at Macrogen, South Korea. All nucleotide sequences were compared to sequences from the NCBI GenBank database using BLAST.

Stable Isotope Analysis

Analysis followed methods detailed in L'Hérault *et al.* (2018). In brief, liver samples were fragmented, stored at −80°C for 24 h, desiccated by vacuum lyophilization, and reduced to powder using a grindmill (Retsch©, Eragny sur Oise, France). Samples were then combusted in a Carlo Erba NC2500 (ThermoFinnigan, Bremen, Germany) connected via continuous flow to a Finnigan Mat Delta Plus isotope-ratio mass spectrometer (ThermoFinnigan), where isotopic discrimination of carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) were quantified. Elevated levels of $\delta^{13}C$ are indicative of a marine-based diet, while elevated levels of $\delta^{15}N$ are indicative of increased trophic level consumption (Schulting 1998).

DNA Extraction

Total genomic DNA was extracted from 49 faecal samples using a QIAamp DNA Stool Mini Kit (Qiagen, Toronto, Canada). Five of the samples were extracted and processed a second time, to check for metabarcoding consistency. Methods followed the manufacturer's instructions for pathogen detection, with the addition of a 6 min homogenisation step to enhance bacterial cell lysis after the addition of buffer ASL (Step 2 in manufacturer handbook). Homogenization was achieved using a TissueLyser II (Qiagen) for 6 min at 5.5 Hz with the following combination of glass beads per tube: 0.3 g of 106 µm beads, 0.5 g of 425–600 µm beads and one 3 mm bead (Sigma-Aldrich, Ontario, Canada).

High-throughput Sequencing of 16S rRNA

In order to establish the abundance and composition of bacterial communities within faecal samples, the primer set 341F (5′-CCTACGG GNGGCWGCAG-3′) and 806R (5′-GACTACNVGGGTWTCTAATCC-3′) (Yu et al. 2005) was used to target a ~460 base pair fragment within the V3/V4 region of the 16S rRNA gene. In brief, targeted PCR reactions were conducted using HiFi polymerase, including a sequence tag on the 5' end of each primer. This tag was used to bind primers in a second PCR reaction, during which individual sample barcodes and Illumina adapters were annealed. PCR products were run on 2% agarose gels to check for successful amplification. DNA concentrations of all samples were measured using PicoGreen, allowing pooling of samples at equimolar amounts. The pool (library) was cleaned using AMPure® beads. The library was then quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Illumina MiSeq PE250 high-throughput sequencing of 16S libraries was conducted with the MiSeq Reagent Kit v2 (500-cycles) at Génome Québec, Montreal, Canada. For quality control and standardization of the sequencing run, Genomic DNA from Mock Microbial Community B (Staggered, Low Concentration), v55.2L, for 16S rRNA Gene Sequencing, HM-783D was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project.

Bioinformatic analysis

All bioinformatic analyses were conducted using QIIME2, version 2018.11 (Bolyen et al. 2018). Briefly, paired-end reads were joined using VSEARCH (Rognes et al. 2016) and quality filtered using the default settings of q-score-joined (Bokulich et al. 2013). Reads were denoised and dereplicated using Deblur (Amir et al. 2017) using a trim length of 400 bp, with a minimum phred quality score of 28. Taxonomic assignments of representative sequences from each Amplicon Sequence Variant (ASV) were performed using the SILVA 132 classifier, with 99% similarity. Taxonomic assignment was conducted in conjunction with Scikit-learn version 0.21 (Pedregosa et al. 2011). PICRUSt (Phylogenetic Investigations of Communities by Reconstruction of Unobserved States) version 1.1.0 (Douglas et al. 2018) was used to infer functional predictions of bacterial communities, following the ‘Metagenome Prediction Tutorial’ pipeline.

Statistical analysis

All statistical analyses were conducted in R version 3.5.0 (R Core Team 2018). Following initial processing of 16S data, all samples with fewer than 5000 reads were removed using the '*phyloseq*' package (McMurdie and Holmes 2013) in R, leaving a total of 545,474 reads across 49 samples. All samples were rarefied to an equal depth within 90% of the minimum observed sample size (specifically 4968 reads per sample).

Generalized Linear Models (GLMs) with a Gamma family and log link function were used to investigate whether alpha diversity of the gut microbiota (measure using Shannon and Faith's Phylogenetic Distance) was associated with; sex, age class, harvest community, parasite abundance or $\delta^{13}C$ and $\delta^{15}N$ in liver, which were included in global models. One data point was omitted when modelling Shannon measure of alpha diversity, and three data points were omitted when modelling Faith's Phylogenetic Distance, as their values exceeded three standard deviations from the mean. Generalized Linear Models (GLMs) were also used to investigate whether the abundance of the top five most abundant bacterial phyla (Firmicutes, Proteobacteria, Tenericutes, Actinobacteria and Bacteroidetes) was associated with; sex, age class, harvest community, parasite abundance or $\delta^{13}C$ and $\delta^{15}N$. A negative binomial and log link function was used to model Proteobacteria, Tenericutes, Actinobacteria and Bacteroidetes, while an inverse Gaussian family and inverse link function were used to model Firmcutes. All top five bacterial phyla were square root transformed prior to modelling for better residual fit and data points were omitted where they exceeded three standard deviations from the mean; two data points were omitted for Actinobacteria, one data point was omitted for Tenericutes and Bacteroidetes, no data points were omitted for Firmicutes and Proteobacteria. Model selection was based on fit of residual plots (Bolker et al. 2009) and subsequent multi-model inference and model averaging of the global model using the R package '*MuMIn*' (Barton 2015). The dredge function was used to generate all possible model combinations, using the variables detailed in the global model, which were then ranked by Akaike's Information Criterion (AIC) values. Models between which there was a delta AIC of < 2 were used to create the average model and conditional average values were used to infer significant factors. Models were run both with and without omitted data points; residual fit benefitted from omission of data points, but results did not differ in either case. Inter-sample bacterial beta diversity was investigated using Non-Metric Multidimensional Scaling (NMDS) plots for both Bray-Curtis and unweighted UniFrac indices, with 95% Confidence Interval (CI) ellipses for appropriate variables plotted on

the same axes. Where 95% CIs were overlapping, the faecal microbiota communities were not considered significantly different.

To investigate whether associations between bacterial diversity and host metadata mirror any differences detected in stable isotope data, Generalized Linear Models (GLMs) were also used to investigate whether $\delta^{13}C$ and $\delta^{15}N$ in the liver were associated with wolverine sex, age class and harvest community, using the same model selection methods detailed above. A Gaussian error family and identity link function were used for modelling $\delta^{13}C$, while a log link function was used to model $\delta^{15}N$.

Results

Microbiota diversity and composition

In total, the faecal microbiota of all 49 wolverines was composed of 1607 amplicon sequence variants (ASVs) encompassing 20 bacterial phyla, with prevalence and abundance of specific phyla differing among individuals (Figure 15a). Across sampled wolverines, the most abundant phylum was Firmicutes, which constituted 75% of the total reads, and was the only phylum present in all 49 individuals (Figure 15a). Within the phylum Firmicutes, 87% (159,278) reads belonged to the class Clostridia, order Clostridiales (Figure 15b), which was present in all 49 individuals. The next most abundant phylum was Proteobacteria (9% of reads), followed by Tenericutes (6% of reads), of which all reads belonged to the class Mollicutes, order Mycoplasmatales (Figure 15b). Actinobacteria and Bacteroidetes made up a much lower percentage of the reads 3% and 1% of reads respectively (indicating a high Firmicutes-Bacteroidetes ratio), while all other phyla each made up <1% of reads.

Pathogenic bacteria within microbiota

The order Chlamydiales was present in 21% (n = 10) of individuals at a low read count (2332 reads total); 99% of these reads (2325 reads total) belonged to the potentially pathogenic family Chlamydiaceae, present in 9 of the 10 individuals. The predominantly pathogenic bacterial class, Campylobacteria, was present in 37% of individuals (n = 18) but at a low level (1600 reads total). The genus *Staphylococcus* was present in 14 individuals, again with a low total read count (782 reads), while the *Escherichia* genus was present in a higher read abundance of 9584 reads and a higher prevalence (present in 32 individuals). Functional analyses of bacteria through PICRUSt demonstrated that

bacteria contributing to disease made up a relatively small proportion of reads (0.7%), and instead bacteria which contribute to metabolism dominated (45% of bacteria present). Further to this, 20% of the encountered bacteria play a role in genetic information processing and 16% contributed towards environmental processing. The remaining bacteria contribute towards cellular processes (4%), organismal processes (0.6%) or an unclassified process (14%). Notably, the antibiotic resistant genus *Mycoplasma* was detected in four individuals, though the read count was low (27 reads total).

Bacteria associated with bone digestion, decomposition and pH tolerance

A higher abundance of Gammaproteobacteria relative to Alphaproteobacteria was detected (total reads = 16,166 and 3634, respectively), and a high abundance of Clostridiaceae (total reads = 69,669; Figure 15) was also found. However, a low abundance of the families Pseudomonadaceae and Sphingobacteriaceae was detected (total reads = 2,342 and 228, respectively), while the families Tissierellaceae and Caulobacteracea were entirely absent. Similarly, low abundance of the families Wohlfahrtiimonadaceae and Corynebacteriaceae were detected (total reads = 82 and 76, respectively) along with the genus *Psychrobacter*, which was also present in a low read abundance (total reads = 402) and prevalence (n = 11; mean abundance = 8; SD = 26). Instead, Peptostreptococcaceae was the most abundant bacterial family (total reads = 83,988) and was present in all 49 wolverines (mean abundance = 1714 reads; SD = 1345). The pH tolerant bacterial order Lactobacillales was both highly abundant within the microbiota (total reads = 19,649; Mean = 401; SD = 777; Figure 15b) and highly prevalent (n = 43; 88%).

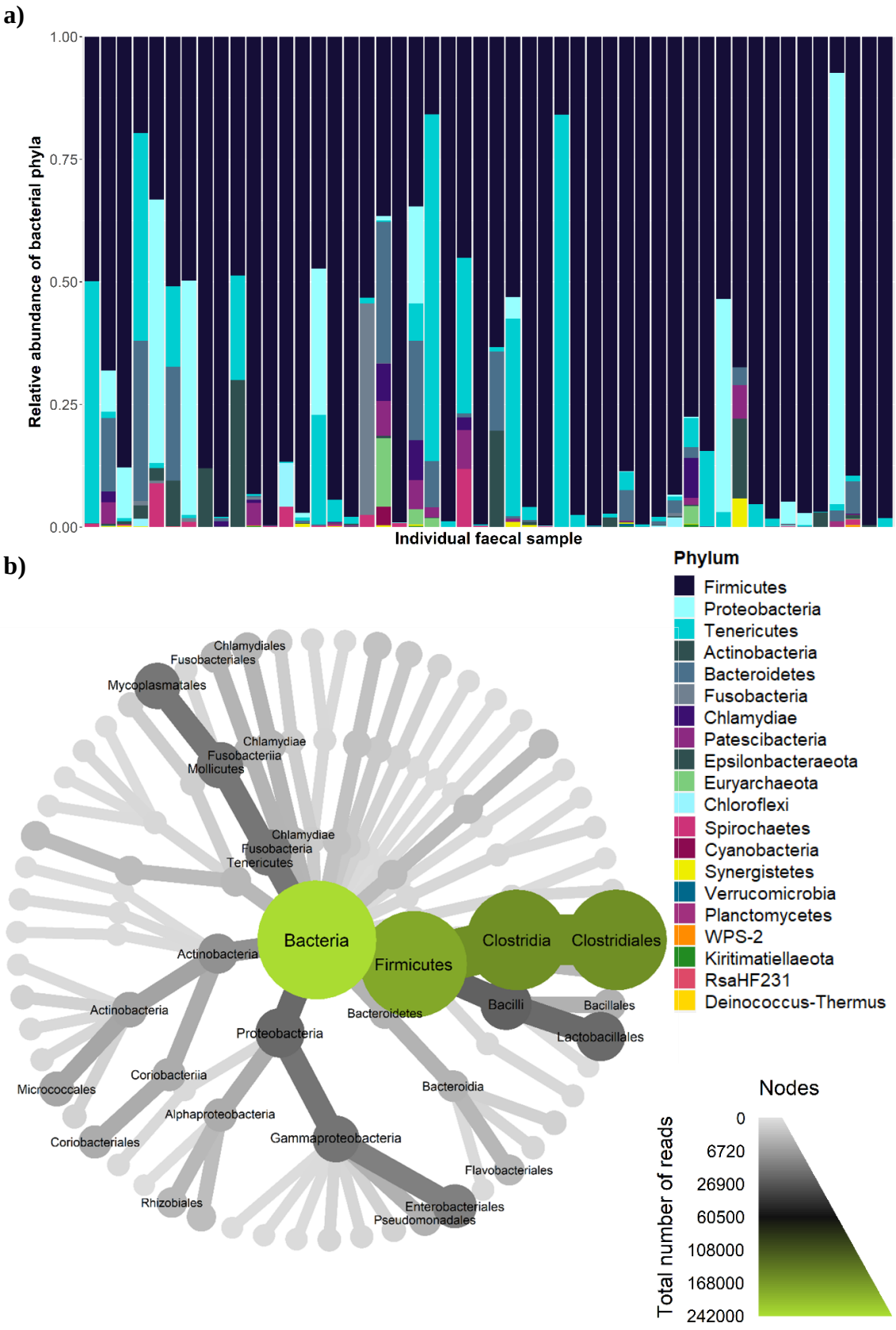

Figure 15a. Relative abundance of 20 bacterial phyla in the faecal microbiota of 49 wolverines from Nunavut, Canada. Phyla in the legend are listed in order of decreasing abundance **b.** Metacoder heat tree plotted to order level: each node (circle) moving from the centre outwards represents a different taxonomic rank, whereby kingdom is in the centre of the network and nodes representing order appear on the outer edges. The tree is weighted and coloured by read abundance

Interactions between gut microbiota and host metadata

The Shannon alpha diversity was 2.5 on average (SD = 0.7) and Faith's phylogenetic distance was 6.3 on average (SD = 4.5). There was no association between bacterial alpha diversity (both Shannon and Faith's phylogenetic distance) and sex, harvest community, parasite abundance, $\delta^{13}C$, $\delta^{13}N$ or age class. Although no significant difference in diversity was seen with age class or harvest community, the abundance of the phylum Tenericutes was ca 21 times higher in juveniles (Total reads = 9848; Mean = 657; SD = 969) compared to adults (Total reads = 448; Mean = 35; SD = 79; adjusted $R^2 = 0.99$, $F_{1,34} = 8.00$, P = 0.016) and significantly lower in Repulse Bay (Naujaat) and Cambridge Bay compared to other harvest communities (P = 0.007 and P = 0.039, respectively). No significant difference was found between the abundance of Tenericutes in yearlings compared to either juveniles or adults (Mean = 610 reads; SD = 966). A strong association was also found between the abundance of Tenericutes and $\delta^{13}C$, though the association was not significant (P = 0.078). In contrast, Bacteroidetes was significantly decreased in juveniles (Total reads = 47; Mean = 3; SD = 6) compared to adults (Total reads = 1376; Mean = 106; SD = 237) (P = 0.047). No other of the top five most abundant bacterial phyla (Firmicutes, Proteobaceria and Actinobacteria) were associated with sex, harvest community, parasite abundance, $\delta^{13}C$, $\delta^{13}N$ or age class. Bacterial beta diversity did not differ with sex, age class, parasite abundance or harvest community when using a Bray-Curtis and unweighted UniFrac measure of diversity (Supplementary Figure 1).

A significantly higher $\delta^{13}C$ in liver was detected in the harvest community of Kugluktuk (P = 0.0086), despite this not being reflected in changes to the diversity or composition of the gut microbiota. No associations were found between $\delta^{13}C$ and $\delta^{13}N$ with sex, harvest and parasite abundance, and or between $\delta^{13}N$ with harvest community.

Discussion

It has been suggested that the tight evolutionary coupling of hosts to their gastrointestinal bacteria (gut microbiota) may explain how scavengers can safely consume decomposed carrion and digest bone. Our study gives the first insight into the gut bacterial communities associated with a free-ranging, Arctic scavenger; the wolverine. In total, 20 bacterial phyla were detected in the faeces of wolverines. Similar numbers of phyla have been quantified in other carnivorous and omnivorous species, such as polar bears (*Ursus maritimus*) and brown bears (*Ursus arctos*), with 25 and 24 bacterial phyla respectively

(Sommer et al. 2016; Watson et al. 2019). The high Firmicutes-Bacteroidetes ratio we detected in our samples may be attributed to the efficient extraction of energy from the diet (Ley et al., 2006; Turnbaugh et al., 2006) and is thought to play an important role where food sources are occasionally limited (Cheng et al. 2015). However, a high ratio of Firmicutes-Bacteroidetes has also been linked to immune and gastrointestinal diseases as well as diarrhoea and obesity (Turnbaugh et al. 2009; De Filippo et al. 2010). Although the high Firmicutes-Bacteroidetes ratio seen in wolverine microbiota reflects that seen in other carnivorous species (Cheng et al. 2015), the high abundance of Tenericutes found within wolverines (i.e. the third most abundant bacterial phyla detected within this study, at 6%) is unusual when compared to other mammals. Typically, Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria and are the most abundant bacterial phyla within mammals (Ley et al. 2008a). The high abundance of Tenericutes found within wolverine faeces may be the result of a marine diet, as Tenericutes is typically found in the gut microbiota of predominantly aquatic species (Romero and Ringø 2014; Bik et al. 2016; Llewellyn et al. 2016). It is important to note, however, that Tenericutes are not found in such high abundance in all marine consumers—for example polar bears, where Tenericutes are present in <1% total reads (Chapter 5; Watson et al. 2019).

Interestingly, we found no association between bacterial diversity, or the abundance of the top five bacterial phyla, and parasitic abundance. This contrasts what is seen in other systems, where gastrointestinal parasites are associated with increased bacterial diversity within the gut (Waterfield et al. 2004; Koch and Schmid-Hempel 2011; Glendinning et al. 2014; Kreisinger et al. 2015). Only a few potentially pathogenic bacteria were detected at low read counts within the wolverine faeces; Campylobacteria (1600 reads), Chlamydiaceae (2325 reads) and *Staphylococcus* (782 reads), with bacteria contributing to disease constituting 0.7% of the wolverine gut microbiota. *Staphylococcus* can induce a wide variety of diseases in humans, with staphylococcal toxins being a common cause of food poisoning (Hennekinne et al. 2012). Whether or not these bacteria are pathogenic to wolverines (i.e. induce disease) is, to the best of our knowledge, unknown. A low abundance of *Mycoplasma* was also detected, a genus which is known to have a strong tendency for causing chronic infections in humans and other vertebrates (Razin et al. 1998; Krasteva et al. 2014). The *Escherichia* genus was present at a higher read abundance of 9584 reads and a higher prevalence (present in 32 individuals). However, due to the limited taxonomic resolution provided by our 16S DNA fragment, we are unable confirm whether the *Escherichia* reads originate from a pathogenic or commensal

Escherichia species, the latter of which are commonly found in the lower intestine of mammals (Tenaillon et al. 2010).

The percentage of pathogenic bacteria present in wolverines is low compared to bacteria which contribute to metabolism, which made up 45% of bacteria present. In other scavengers that are also potentially exposed to pathogenic bacteria associated with carrion, for example the Namibian black-backed jackal (*Canis mesomelas*), no abnormal levels of potentially pathogenic bacteria were found in the faecal microbiota (Menke et al. 2017). A similar picture is seen in New World vultures, which have evolved a notable tolerance to the bacterial pathogens of decaying meat, which is thought to result from the strong resilience of gastrointestinal bacteria in scavengers (Roggenbuck et al. 2014; Mendoza et al. 2018). The low level of pathogenic bacteria we detected in wolverines implies they are either not exposed to pathogens or the microbiota composition provides protection against pathogens associated with decomposing carcasses. It is important to note that wolverine caching behaviour may lead to a low level of pathogenic bacteria in the consumed carrion. Wolverines commonly cache their food in cold, structured microsites with low ambient temperatures and persistent spring snow cover, which is thought to limit competition from insects, bacteria and other scavengers (Inman et al. 2012). This behaviour is also seen in gray jays (*Perisoreus canadensis*), which also rely on cold temperatures (as well as the antimicrobial resins of coniferous trees) to preserve perishable cached food items such as insects, fungi and carrion (Waite and Strickland 2006; Strickland et al. 2011; Sutton et al. 2016). The behaviour of using cool microsites to preserve food items, known as the "refrigeration-zone hypothesis" may reduce the establishment of some pathogenic bacteria (Inman et al. 2012). Our results are consistent with this hypothesis, although confirmation of in-vivo methods would be required to support this point further.

The utilisation of cool caching sites to store prey items also serves the purpose of easing decomposition rate, which inhibits the loss of valuable prey biomass (Inman et al. 2012; Sutton et al. 2016). This may explain our finding that few bacteria which are typically prolific throughout the process of decomposition were detected within the gut microbiota of wolverines. Wohlfahrtiimonadaceae, which is the most dominant bacterial family of the saprophagous Black Soldier fly (*Hermetia illucens*) (Khamis et al. 2020) and carrion beetles (*Nicrophorus distinctus* and *Necrophila renatae*) (Setiawan et al. 2020), and is found in the thanatomicrobiome of the human mouth (Ashe 2019), was recovered only at a very low read count and prevalence within the wolverines. Similarly, the family

Corynebacteriaceae (which is prominent in the rectal microbiota of the bone eating scavenger species, the hyena (*Crocuta crocuta*) (Rojas et al. 2020)), and the psychrotolerant genus *Psychrobacter* (which is associated with food spoilage (Bjørkevoll et al. 2003)) were only detected at low read abundances and low prevalence. Furthermore, a low abundance of Pseudomonadaceae and Sphingobacteriaceae were recovered within our study, while the families Tissierellaceae and Caulobacteracea were entirely absent. The bacterial families Pseudomonadaceae, Clostridiaceae, Tissierellaceae, Caulobacteracea, and Sphingobacteriaceae are the most abundant bacterial families involved in the decomposition of human bone, listed here in order of descending abundance (Damann et al. 2015). The low read counts recovered for bacteria associated with decomposition may imply that the wolverine microbiota and/or stomach acid works efficiently as a barrier or filter against such bacteria. This may explain our finding that the pH tolerant bacterial order Lactobacillales was both highly abundant within the microbiota and highly prevalent amongst wolverines; this finding may also explain how wolverines are able to tolerate bone digestion. We did, however, detect a high abundance of Gammaproteobacteria relative to Alphaproteobacteria, which mirrors what is recovered from early stages of human bone decomposition—the relative abundance of Gammaproteobacteria typically decreases as decomposition progresses (Damann et al. 2015). A high Gammaproteobacteria to Alphaproteobacteria abundance may therefore reflect a wolverine's bone-eating diet—as may the dominant levels of Firmicutes detected, as partially skeletonised human remains maintain the highest proportion of Firmicutes compared to any other stage of human bone decomposition (Damann et al. 2015). Similarly, Clostridiaceae, which is highly abundant during human bone decomposition (Damann et al. 2015), was detected in high abundance within this study, ranking as the second most abundant bacterial family.

A high abundance of the family Peptostreptococcaceae, as detected within our study, is associated with moderate protein consumption and is considered to improve gut barrier function (Fan et al. 2017), which may reflect the scavenging diet of the wolverine. The biomass (and therefore protein) obtained from the consumption of cached prey items will almost always be inferior to that available after the immediate hunting of that item (Sutton et al. 2016). Typically, diet is the strongest driver in changes to gut microbial communities (Maslowski and Mackay 2011; Muegge et al. 2011; Schwab et al. 2011; David et al. 2013). We found significantly higher $\delta^{13}C$ in the harvest community of Kugluktuk. The higher $\delta^{13}C$ in the diet implies wolverines from Kugluktuk are perhaps scavenging on

more marine based items. However, this difference in dietary niche does not appear to be associated with changes to the gut microbiota diversity or abundance of the top five bacteria phyla detected within wolverines sampled within our study. We also found no association between the gut microbiota composition and wolverine age or sex. This could imply that a tightly specialised bacterial community is necessary to safely consume decomposing matter—which is required by all individuals regardless of geographic location, sex or age class.

Overall, our results suggest that the gut microbiota of wolverines may be an adaptation to a lifestyle of scavenging and caching behaviour, and hazardous food sources. The link between wolverine gut microbiota and their dietary niche is an important reflection considering that wolverines are known to be susceptible to the impacts of climate change and anthropogenic disruption (Aubry et al. 2007; Copeland et al. 2010), which influence their caching behaviour and prey availability. A deeper understanding of factors that influence wolverine intestinal health will feed into the development of future conservation strategies aiming to improve the persistence and resilience of this specialized Arctic carnivore.

5

Global change-driven use of onshore habitat impacts polar bear faecal microbiota

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Abstract

The gut microbiota plays a critical role in host health, yet remains poorly studied in wild species. Polar bears (*Ursus maritimus*), key indicators of Arctic ecosystem health and environmental change, are currently affected by rapid shifts in habitat that may alter gut homeostasis. Declining sea ice has led to a divide in the southern Beaufort Sea polar bear subpopulation such that an increasing proportion of individuals now inhabit onshore coastal regions during the open-water period ('onshore bears') while others continue to exhibit their typical behaviour of remaining on the ice ('offshore bears'). We propose that bears that have altered their habitat selection in response to climate change will exhibit a distinct gut microbiota diversity and composition, which may ultimately have important consequences for their health. Here, we perform the first assessment of abundance and diversity in the faecal microbiota of wild polar bears using 16S rRNA Illumina technology. We find that bacterial diversity is significantly higher in onshore bears compared to offshore bears. The most enriched OTU abundance in onshore bears belonged to the phylum Proteobacteria, while the most depleted OTU abundance within onshore bears was seen in the phylum Firmicutes. We conclude that climate-driven changes in polar bear land use are associated with distinct microbial communities. In doing so, we present the first case of global change mediated alterations in the gut microbiota of a free-roaming wild animal.

Introduction

As an apex predator with vulnerable conservation status (Regehr et al. 2016), the polar bear (*Ursus maritimus*) is widely acknowledged as a key indicator of Arctic ecosystem health (Amstrup et al. 2010), a model species for studying the effects of climatic and other anthropogenic stressors in the Arctic (Parmesan 2006; McKinney et al. 2011; Atwood et al. 2017), and a flagship for environmental change (Derocher et al. 2013). As one of the most ice dependent Arctic marine mammals (Laidre et al. 2008), polar bears require sea

ice for long-distance movements, mating and accessing prey (Regehr et al. 2010). One subpopulation of polar bear, the southern Beaufort Sea subpopulation, is exhibiting a distinct behavioural response to climate-driven changes in sea ice conditions. Historically, these polar bears remained year-round on the sea ice (hereafter referred to as 'offshore bears'), taking advantage of the biologically-productive continental shelf (Amstrup et al. 2008). Since the 2000s, however, substantial declines in the spatial and temporal availability of sea ice in summer and fall (Stroeve et al. 2014; Stern and Laidre 2016), extending well beyond the continental shelf, have driven a divide in polar bear behaviour whereby some continue to select the retreating ice habitat ('offshore bears') while others instead adopt a novel behaviour and move to coastal onshore habitat during the reduced ice period (‘onshore bears’)(Schliebe et al. 2008). The entire subpopulation uses the sea ice during the remainder of the year. Onshore bears have been associated with a range of dietary items that offshore bears are unable to access, notably ‘bone piles’, the remains of locally-harvested bowhead whales (*Balaena mysticetus*), along with the carcasses of fish, birds and caribou (*Rangifer tarandus*) (Herreman and Peacock 2013). Conversely, offshore bears primarily consume a traditional diet of ringed seal (*Pusa hispida*), bearded seal (*Erignathus barbatus*) and occasionally beluga whale (*Delphinapterus leucas*) (Herreman and Peacock 2013), which are inaccessible to onshore bears.

Changes in trophic interactions alter the exposure of polar bears to contaminants and novel parasites (McKinney et al. 2009; McKinney et al. 2010). For example, ringed seals (available only to offshore bears) are considered to occupy a high trophic position and so typically bioaccumulate higher levels of contaminants than species lower in the trophic chain such as the filter feeders (i.e. bowhead whales) and herbivores (i.e. caribou) (Dietz et al. 2000; Hoekstra et al. 2003; Bentzen et al. 2008), which are available only to onshore bears. In addition, bone piles, foraged on by onshore bears, are utilised as a food resource by other terrestrial species (Herreman and Peacock 2013; Miller et al. 2015) and lie within comparatively close range of human settlements, such as Kaktovik (70.13° N, 143.62° W) and Deadhorse (70.20° N, 148.46° W). Thus, onshore bears are potentially exposed to (and therefore at greater risk of infection from) novel parasites carried by terrestrial species, including humans and their domestic pets. For example, Atwood et al. (2017) found that southern Beaufort Sea polar bears exhibiting onshore behaviour have a greater risk of exposure to *Toxoplasma gondii* and lower exposure to certain contaminants than offshore bears. Thus, onshore bears are exposed to different biotic stressors compared to

offshore bears (Atwood et al. 2017; McKinney et al. 2017a), which have the potential to drive variation in the gut microbiota. In humans and mice, for example, helminth infection is associated with significant differences in the community composition of gut bacterial communities (Walk et al. 2010; Lee et al. 2014; Kreisinger et al. 2015), while contaminants such as herbicides and pesticides have been shown to inhibit the growth of a variety of beneficial gut bacteria (Shehata et al. 2013) and even cause dysbiosis (Joly et al. 2013).

The gut microbiota, a diverse community of bacteria that resides within the gastrointestinal tract, has a long co-evolutionary association with its host (Ley et al. 2008b), carrying out vital nutritional and physiological roles (Ley et al. 2008b; Maynard et al. 2012; Xing et al. 2013). In effect, the regular intestinal development and function of an individual is attributed to an array of specific bacterial groups or species, the composition and diversity of which are a function of complex interactions between host and environment (Round and Mazmanian 2009). Despite the importance of the gut microbiota to health, little is understood of the composition or community structure of the gut microbiota of wild fauna (Pascoe et al. 2017). In brown bears (*U. arctos*) however, we know a distinct gut microbiota profile is associated with active bears compared to those in hibernation phase – this specific community of bacteria is thought to play a role promoting adiposity while still maintaining normal gut metabolism (Sommer et al. 2016). A paucity of knowledge on wild microbiota is particularly concerning considering that in the face of rapid climate change tight host-gut microbiota associations could quickly become decoupled, negating millions of years of co-evolutionary adaptation (Ley et al. 2008b), and yet this too remains poorly understood.

A number of studies provide support for an association between host microbial communities and environmental fluctuations. Cold acclimated laboratory mice, for example, harbour a dramatically different gut microbiota composition to those raised at higher temperatures (Chevalier et al. 2015), while experimentally induced temperature increases of 2–3 °C cause a 34% loss of microbiota diversity in the common lizard (*Zootoca vivipara*)(Bestion et al. 2017). Outside a laboratory setting, variations in weather events have been linked to the increased occurrence of gastrointestinal illness in residents of Nunatsiavut, Canada (Harper et al. 2011). To the best of our knowledge, however, no study has demonstrated a climate change mediated alteration in the gut microbiota of free-roaming wildlife.

The gut microbiota has been examined once before in wild polar bears, specifically those from the Svalbard archipelago belonging to the Barents Sea subpopulation (Glad et al. 2010a). The authors found a low bacterial diversity, dissimilar to that reported in other Arctic carnivores (Glad et al. 2010b) and wild ursids (Xue et al. 2015; Sommer et al. 2016; Song et al. 2017), possibly attributed to the methodologies employed (having used 16S rRNA clone libraries as opposed to next generation sequencing techniques) and small sample size (Mardis 2008; Glad et al. 2010a). Here we use high-throughput sequencing techniques to conduct the first detailed investigation of the gut microbiota composition of a large sample (n = 112) of wild southern Beaufort Sea polar bears and to establish the diversity, abundance, and composition of gut bacteria associated with on- and offshore bears. In doing so, we are able to evaluate the effect of a climate driven change in habitat use on microbial composition. Reflecting methods widely used in other gut microbiota studies (Thomas et al. 2015), we use faeces as a proxy of gut microbiota, herein referred to as the faecal microbiota.

Methods

Polar bear capture and sampling

Polar bears were captured under the United States Geological Survey (USGS) Polar Bear Research Program (Marine Mammal Permit MA690038 to T.C.A.) in an area ranging approximately from Utqiagvik, Alaska (156°W) in the west to Demarcation Point (140°W) at the US-Canada border in the east, and extending from the shoreline to approximately 135 km north on sea ice (with the exception of one individual; Figure 16). In the spring and fall of 2008 and 2009, and the spring of 2010 and 2013, polar bears were encountered via helicopter and immobilized with a remote injection of zolazepam-tiletamine (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA, and Warner-Lambert Co., Groton, Connecticut, USA). A single faecal sample was collected directly from the rectum of each polar bear using a sterile latex glove and immediately transferred to a sterile Whirl-pak bag (Nasco, Fort Atkinson, Wisconsin, USA) for storage. In total, samples were taken from 112 individuals, including 89 adults and 23 subadults, (51 males and 61 females). All samples were stored at -20°C for the duration of the field season (approx. 5 weeks) before being stored at -80°C at the US Geological Survey, Alaska Science Center (Anchorage, Alaska, USA), and subsequently shipped on dry ice to the Fondazione Edmund Mach, Italy (CITES permit IT/IM/2015/MCE/01862 to S.W.).

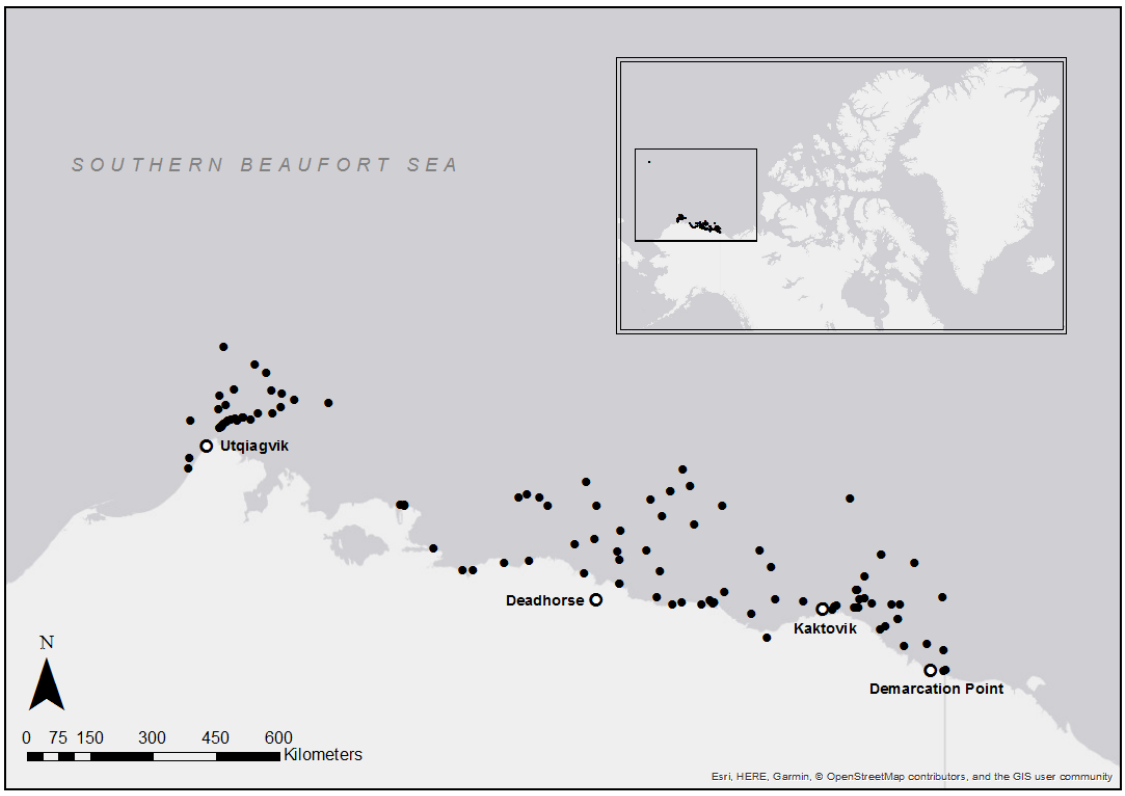

Figure 16. Map of study area showing the sampling locations of 112 southern Beaufort Sea polar bears along the north coast of Alaska. Inset map shows the location of the study area, highlighting that one sample originates from a more northerly location that the others

Age of subadults and adults was estimated by extracting and analysing the cementum annuli of a vestigial premolar tooth (Calvert and Ramsay 1998). In total, 85 of the 112 bears were known to be either onshore or offshore (onshore n = 46**;** offshore n = 39; Supplementary Table 3). Individuals were categorised as either 'onshore bears' or 'offshore bears' as described in (Atwood et al. 2017). Briefly, location data collected from satellite collars were used to identify adult females that used land (‘onshore’) or sea ice (‘offshore’) in summer and fall (Atwood et al. 2016). We classified both male and female individuals as onshore bears if they were detected (via genetic identification and cross-referencing with our database of known bears) at hair-snags erected in the fall around bowhead whale bone piles and from biopsy-darting during fall coastal surveys from 2010-2013. An individual was classified as onshore or offshore if spatial or genetic data suggested that the individual was onshore or offshore in summer and/or in the year of capture (for fall-captured bears) or immediately prior to capture (for spring-captured bears). Body condition for each polar bear was estimated using a ‘Body Condition Index’

metric (Cattet et al. 2002) and was classified as either above or below the mean body condition for our sample set. Year and season of capture was also recorded.

Extraction of bacterial DNA

All faecal matter was collected from inside each sample glove using a sterile cotton swab (APTACA sterile transport swabs, Brescia, Italy). The swab was subsequently vortexed for 10 min in 1ml phosphate-buffered saline solution (PBS) and pelleted by centrifugation at 16 000 g for 12 min. Lysis buffer, 80 µl, (200 mM NaCl, 100 mM Tris, 20 mM EDTA, 20 mg/ml Lysozyme, pH 8.0); 5 mm stainless steel beads (Qiagen) were added to each sample before a three-minute homogenization step at 30Hz using a Mixer Mill MM200 (Retsch GmbH, Haan, Germany). Samples were then shaken at 37◦C for 40 minutes Grant-Bio PCMT Thermoshaker (500rpm). Microbial DNA was extracted using the QIAamp® DNA Mini Kits (QIAGEN©, Milan, Italy), following the manufacturer's Buccal Swab Spin Protocol for cotton swabs (QIAamp® DNA Mini and Blood Mini Handbook), but starting from step 2 (addition of Proteinase K).

16s rRNA gene amplification and sequencing

Using the bacteria-specific primer set 341F (5' CCTACGGGNGGCWGCAG 3') and 805Rmod (5' GACTACNVGGGTWTCTAATCC 3') (based on Klindworth *et al.* 2013 (Klindworth et al. 2013) with degenerate bases) with overhanging Illumina adapters, a ~460 base pair (bp) fragment of the 16S rRNA gene (variable region V3-V4)(Caporaso et al. 2012) was amplified using a GeneAmp PCR System 9700 (Thermo Fisher Scientific) and the following steps: 94°C for 5 minutes (one cycle), 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds (30 cycles), 72°C for 5 minutes (1 cycle). The PCR products were visualised on a 1.5% agarose gel and purified using Agencourt AMPure XP SPRI beads (Beckman Coulter, Brea, CA, USA) following manufacturer's instructions. Subsequently, Illumina® Nextera XT indices and sequencing adapters (Illumina®) were incorporated using seven cycles of PCR (16S Metagenomic Sequencing Library Preparation, Illumina®). The final libraries were quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific) by the Synergy2 microplate reader (Biotek), pooled in equimolar concentration before sequencing on an lllumina® MiSeq (2x300 bp reads) at the Next Generation Sequencing Platform, Fondazione Edmund Mach in collaboration with the Core Facility, CIBIO, University of Trento, Italy. All samples were sequenced in one Illumina MiSeq Standard Flow Cell targeting a depth of 20, 000 reads per sample.

Bioinformatic processing of 16s data

Reads were processed with MICCA v1.5.0 (Albanese et al. 2015). Briefly, paired-end reads were merged, and pairs diverging by more than 8 bp or overlapping by less than 100 bp were discarded. PCR amplification primers were trimmed (sequences not containing both PCR primer sequences were discarded). Finally, sequences were quality filtered at 0.5 % Expected Error (EE); those displaying greater than 0.5% EE were discarded along with those shorter than 400 bp or containing unknown base calls (N). Using the VSEARCH cluster_smallmem algorithm (Rognes et al. 2016), OTUs were created *de novo* by clustering sequences with 97% sequence identity, discarding chimeric sequences. Taxonomic assignments of representative sequences from each OTU were performed using the RDP Classifier v2.12 in conjunction with RDP 16S rRNA training set 15 (Wang et al. 2007). OTU sequences were aligned and phylogenetic analysis was performed using Nearest Alignment Space Termination (NAST) and a phylogeny reconstructed using FastTree (Price et al. 2009), both via MICCA (Albanese et al. 2015). The raw sequencing data can be found at the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) [Accession number: PRJNA542176].

Statistical analyses

Following initial processing, singletons were removed and all samples with fewer than 5000 reads were removed using the R package '*phyloseq*' (McMurdie and Holmes 2013), leaving a total of 511, 952 reads across 112 samples. The data were rarefied to an equal depth within 90% of the minimum observed sample size (specifically 4571 reads per sample). Generalized Linear Models (GLMs) with a Gamma error function were used to investigate whether metadata (onshore/offshore, age class, sex, body condition, year of capture and season of capture) were associated with alpha diversity of the faecal microbiota (Shannon, Inverse Simpson and Faith's Phylogenetic Diversity Indices). For Shannon and Faith's Phylogenetic Diversity measures, an identity link function was used, while a log link function was used when analysing an Inverse Simpson measure of diversity. All multivariate analyses on faecal microbiota structure according to host metadata (on-/offshore, age class, sex, body condition, year of capture and season) were assessed using PERMANOVA, based on Bray-Curtis dissimilarity and weighted UniFrac indices, using the '*adonis*' function in the R package '*vegan*' (Dixon 2003). An important assumption for PERMANOVA is homogenous dispersion of data among groups; for this

reason, the '*betadisper*' function in '*vegan*' was implemented to investigate the homogeneity of data. Data rows containing missing values (NAs) were removed from the dataset prior to conducting the PERMANOVA to ensure matrices were even between variables. To determine the differential abundance of OTUs between on- and offshore bears, sex and season were examined using the R package '*DESeq2*' (Love et al. 2014). To assess whether the microbiota profiles of polar bears is related to their geographic distribution, a GPS based pairwise distance matrix was constructed using the R package '*geosphere*' (Hijmans et al. 2017) and compared to a PCoA matrix (using both Bray-Curtis and weighted UniFrac) via a Mantel Test. All analyses were carried out using R statistical software package, version 3.2.0 (R Core Team 2018). Data was visualised using the R packages '*ggplot2*' (Wickham 2016) and '*metacoder*' (Foster et al. 2017).

Results

Faecal microbiota composition

The faecal microbiota of all 112 bears was composed of 1221 operational taxonomic units (OTUs) encompassing 25 bacterial phyla, with prevalence and abundance of specific phyla differing among individuals (Figure 17a). Across the population, the most abundant phyla (which composed 91% of the total reads and were present in all individuals) were Firmicutes (45%), Proteobacteria (25%) and Actinobacteria (21%), making up the core microbiota. All other phyla represented <9% of reads each (Figure 17a), and their prevalence among samples varied between 97% (Bacteroidetes) and 1% (Armatimonadetes, Deferribacteres, Lentisphaerae and Synergistetes). From the total number of reads obtained for the most dominant phylum (Firmicutes), 70% belonged to the class Clostridia, and 99% of those were from the order Clostridiales. The dominant orders for the remaining top bacterial phyla were Enterobacteriales (phyla: Proteobacteria) and Actinomycetales (phyla: Actinobacteria) (Figure 17b).

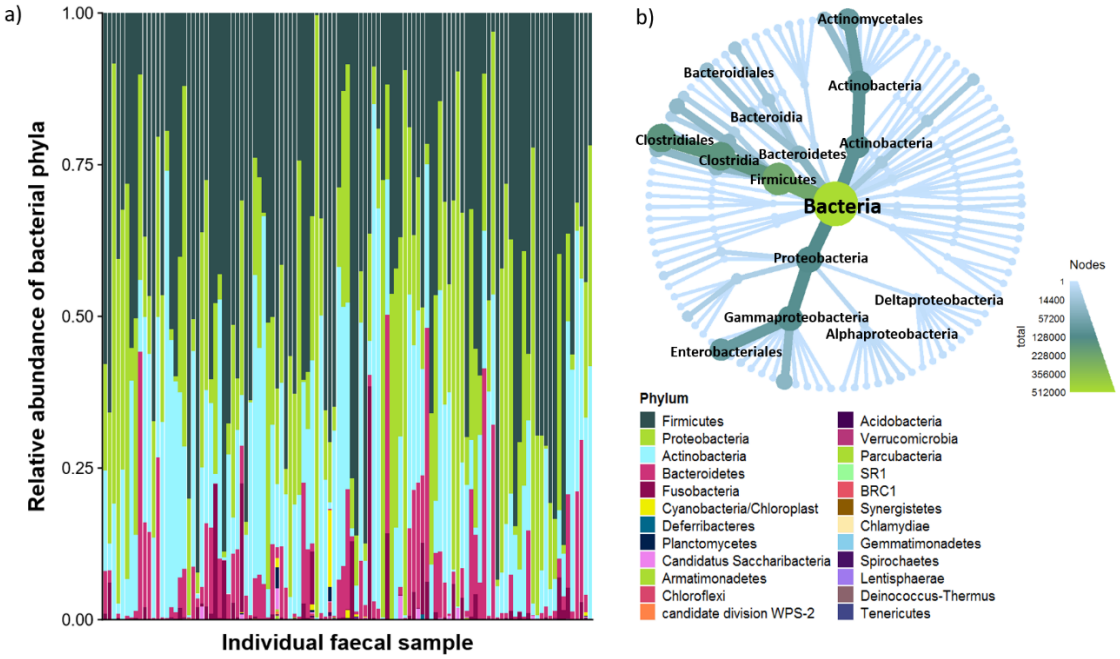

Figure 17a. Stacked bar chart of the relative abundance of 25 bacterial phyla in the faecal microbiota of 112 southern Beaufort Sea polar bears. Phyla in the legend are listed in order of decreasing abundance **b.** Inset is a metacoder heatmap plotted to order level: each node moving from the centre outwards represents a different taxonomic rank, whereby kingdom is the centre and nodes representing order appear on the outer edges. The map is weighted and coloured by read abundance

Onshore versus offshore microbiota

Using the subset of bears for which we had on- and offshore information (n = 85), we found alpha diversity was significantly higher in on- (n = 46) compared to offshore (n = 39) bears, for Shannon (adjusted R-squared = 0.06, $F_{1,83} = 6.32$, P = 0.014; Figure 18a and Supplementary Table 4) and Inverse Simpson (adjusted R-squared = 0.07, $F_{1,83} = 6.09$, P = 0.016; Figure 18b and Supplementary Table 4) indices but not for Faith's Phylogenetic Diversity index (Supplementary Table 5). Beta diversity did not differ between on- and offshore bears when using Bray-Curtis (Supplementary Figure 2) but differed significantly between on- and offshore bears when using a weighted UniFrac metric (adjusted R-squared = 0.03, $F_{1,80} = 2.53$, P = 0.029; Supplementary Figure 3). Data dispersion did not significantly differ between on- and offshore bears (P = 0.740).

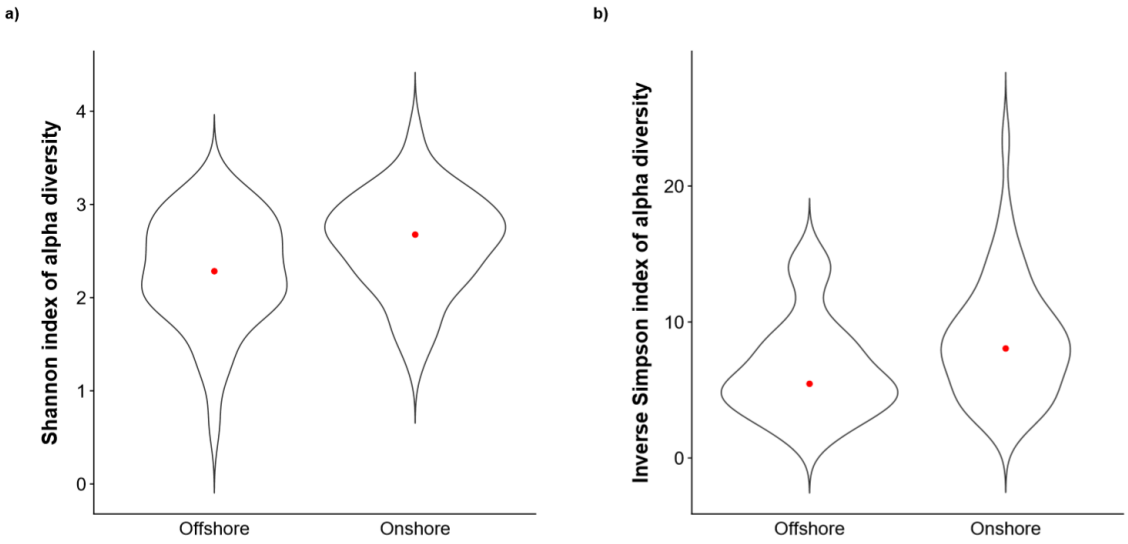

Figure 18. Violin plots of alpha diversity within the faecal microbiota of 85 southern Beaufort Sea polar bears for which 'onshore/offshore' land use is known (see text for definitions): **a.** Shannon diversity index **b.** Inverse Simpson diversity index. Violin plots combine a box plot with a density plot, and as such the width of each plot corresponds to the distribution of the data

The faecal microbiota of onshore bears consisted of 858 OTUs (19 bacterial phyla; 37 classes) compared to 635 OTUs (21 phyla; 35 classes) for offshore bears, of which 386 were shared between on- and offshore polar bears (Figure 19). Of the total number of OTUs found, 472 were unique to onshore bears, and a smaller number of OTUs (*n*= 249) were unique to offshore bears. Eleven OTUs (10 Firmicutes; 1 Proteobacteria) were significantly enriched and 6 OTUs (3 Bacteroidetes; 2 Firmicutes; 1 Proteobacteria) were significantly reduced in onshore bears (Figure 20; Supplementary Table 6). The majority (73%; n = 8) of OTUs that were enriched in onshore bears belonged to the order Clostridiales (Phylum: Firmicutes), although family level assignment varied across OTUs (Figure 20 and Supplementary Table 6). OTUs that were significantly decreased in on- compared to offshore bears varied in taxonomic assignment across taxonomic ranks (Supplementary Table 6). The most enriched OTU abundance in onshore bears belonged to the family Moraxellaceae (Phylum: Proteobacteria), with a 6.78 log2 fold change in abundance ($P < 0.001$), while the most depleted OTU abundance within onshore bears was seen in Clostridiaceae 1 (Phylum: Firmicutes) with a -8.04 log2 fold change in abundance ($P < 0.001$; Supplementary Table 6).

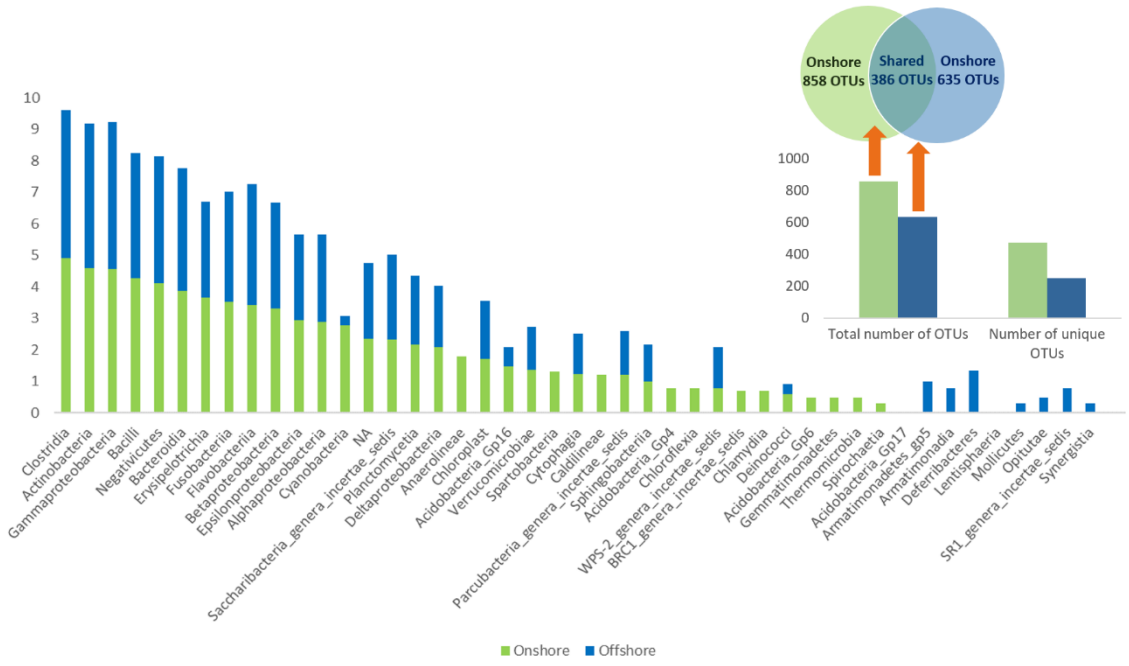

Figure 19. Log abundance of OTUs in the faecal microbiota of 'onshore' and 'offshore' bears, by bacterial Class. Inset shows shared number of OTUs by onshore (green) and offshore (blue) bears

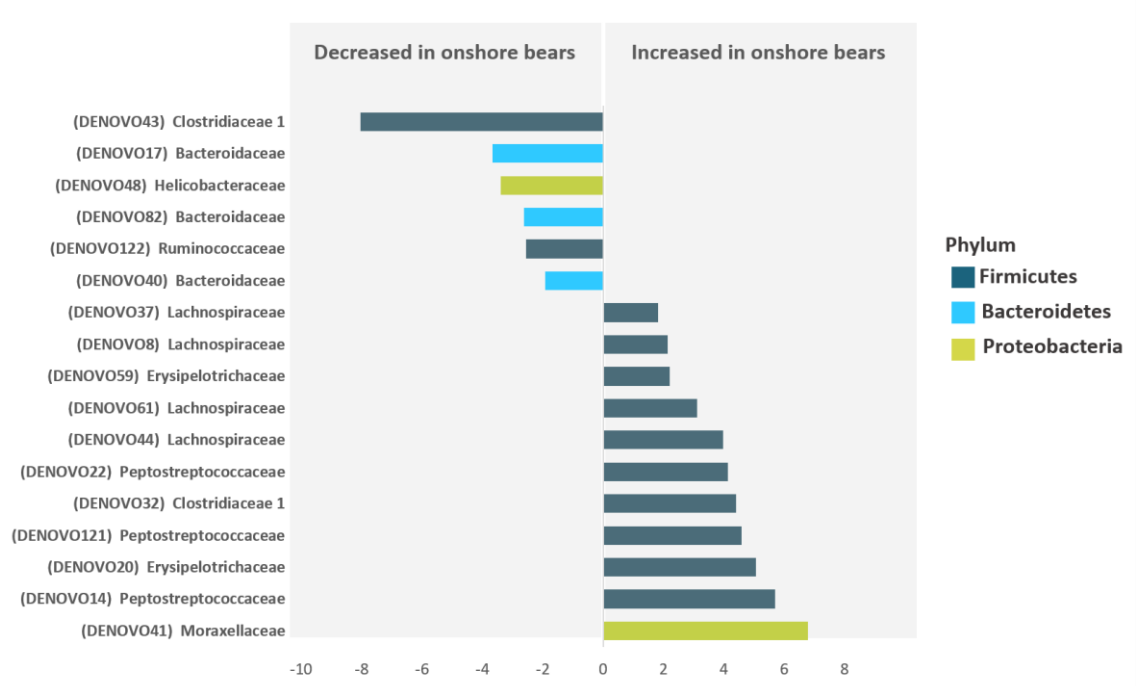

Figure 20. Differential OTU abundance of onshore compared to offshore bears from DESeq2 analysis, plotted with individual OTU number and associated family assignment

The gut microbiota composition of individuals was not associated with their geographic proximity to one another (P = 0.56 and P = 0.17; Mantel Test using Bray-Curtis and weighted Unifrac respectively).

Ecological factors and the microbiota

When using Faith's Phylogenetic Diversity Index, alpha diversity was significantly higher in females compared to males (adjusted R-squared = 0.30, $F_{2,109}$ = 25.18, P = 0.017), as well as in fall compared to spring captures (adjusted R-squared = 0.30, $F_{2,109}$ = 25.18, P < 0.001). However, alpha diversity did not differ with sex, season of capture, body condition, year or age class when using either a Shannon or Inverse Simpson index of diversity and no significant difference in alpha diversity was seen with body condition, year, or age class when using Faith's Phylogenetic Diversity. Beta diversity differed significantly with sex (Bray-Curtis; P = 0.001; weighted UniFrac P = 0.006) although data dispersion was seen to be significantly different between males and females (P = 0.018) and so the PERMANOVA should be interpreted with caution. Beta diversity also differed significantly with season when using Bray-Curtis (P = 0.005) but not weighted UniFrac (P = 0.184), where beta dispersion was P = 0.113. No differences in beta diversity were seen with year, age class or body condition when using either Bray-Curtis or a weighted UniFrac metric. When investigating the differential abundance of OTUs with sex, DESeq analysis showed that 66 OTUs were significantly different between males and females; 9 OTUs were significantly increased in males compared to females (the largest increase, of 5.40 log fold change, belonging to the family Clostridiales Incertae Sedis XI, phylum: Firmicutes) and 57 OTUs were significantly decreased (the largest decrease, of -10.04 log fold change, being seen in the family Flavobacteriaceae, phylum: Bacteroidetes). For season of capture, DESeq analysis revealed that 15 OTUs were significantly different between fall and spring captures; 2 OTUs were increased in spring compared to fall captures (the largest increase, of 3.01 log fold change, belonging to the family Veillonellaceae, phylum: Firmicutes) and 13 OTUs were significantly decreased (the largest decrease, of -7.50 log fold change, being seen in the family Peptostreptococcaceae, phylum: Firmicutes).

Discussion

Investigating factors which may influence the gut microbiota in a sentinel species experiencing rapid environmental change may improve our understanding of the role of the gut microbiota in wildlife health and conservation. Here we have shown that for the southern Beaufort Sea subpopulation of polar bears alpha diversity and bacterial composition are significantly different in the gut of onshore bears compared to those that

remain on the sea ice year-round. As such, our study shows for the first time, that global change driven alterations in habitat use are associated with changes in the gut microbial composition and diversity of a free-ranging species.

We detected 25 bacterial phyla, as opposed to just the one (Firmicutes) previously found by Glad et al. (2010a) in wild Barents Sea polar bears. This diversity closely mirrors that seen in other studies utilizing next generation sequencing methods to investigate the gut microbiota of ursids; for example, 24 bacterial phyla were detected in wild brown bears (Sommer et al. 2016). The most abundant phyla in polar bear faeces (Firmicutes, Proteobacteria and Actinobacteria), coincided with those of the core mammalian gut microbiota (Ley et al. 2008b), including that of Asiatic black bears (*Ursus thibetanus*) (Song et al. 2017). Our finding that Firmicutes constituted the majority of OTUs is noteworthy in that increased Firmicutes in genetically obese mice and humans suggests that this phylum plays an important role in promoting adiposity or energy resorption (Ley et al. 2006), although conflicting studies show no link between Firmicutes levels and obesity/high-fat intake (Fernandes et al. 2014). Interestingly, brown bears gaining weight for hibernation during summer months show simultaneously elevated levels of Firmicutes in the gut (Sommer et al. 2016), implying this phylum may also play a role in synthesising high energy inputs in large carnivores. More specifically, we show that 70% of reads assigned to the phylum Firmicutes belonged to the class Clostridia, and subsequently 99% were from the order Clostridiales – an outcome that coincides with the results of Glad et al. (2010), who showed all except one of the gene clones generated within their study were affiliated with the order Clostridiales. In a study using both wild type and laboratory mice, Hildebrandt et al. (2009) showed that levels of Clostridiales greatly increases after prolonged durations of time feeding on a high-fat diet.

Within this study we found that alpha diversity of bacterial OTUs was significantly higher in the faecal microbiota of onshore compared to offshore bears when using a Shannon or Inverse Simpson measure, but no association was found between alpha diversity and host metadata (age class, sex, body condition, year or season of capture) when using these indices. Much microbiota work focusing on humans has found sex and age influences microbiota dynamics (Mueller et al. 2006; Koenig et al. 2011; Dominianni et al. 2015). Although the majority of microbiota research has focused on humans, microbial studies of wild animals are increasing (Pascoe et al. 2017) and in some cases wild animals have been shown to follow similar trait-related stratification in microbiota. For example, the presence/absence of specific bacterial taxa were seen to correlate with specific age classes

within the gut microbiota of wild ring-tailed lemurs (*Lemur catta*) (Bennett et al. 2016). Similarly, sex-specific differences in bacterial diversity have been found in, for example, wild rufous mouse lemurs (*Microcebus rufus*), whereby females demonstrated higher bacterial diversity compared to their male counterparts (Aivelo et al. 2016). Further to this, season of capture has been seen to influence the gut microbiota composition. Sommer et al. (2016), for example, demonstrated that gut microbial composition of free-roaming brown bears is seasonally altered between summer and winter. This change in bacterial composition is thought to, in part, be influenced by extreme dietary shifts within brown bears between active and hibernation phase (Sommer et al. 2016). We also see this seasonal shift in gut microbial composition in other wild animal models such as wild wood mice (*Apodemus sylvaticus*) (Maurice et al. 2015), wild black howler monkey (*Alouatta pigra*) (Amato et al. 2015), and the giant panda (*Ailuropoda melanoleuca*) (Xue et al. 2015), probably also attributable to season-driven shifts in diet. None of these factors, however, were found to influence the gut microbiota composition of the polar bears sampled within this study when using a Shannon and Inverse Simpson index of diversity. However, when using Faith's Phylogenetic Diversity (i.e. a metric that characterises only the relatedness or distinctness of species and works under the assumption that different species make unequal contributions to diversity (Faith 1992)) we see a significant difference in diversity with sex and season only, whereby females had a higher bacterial diversity than males, and fall captures had a higher bacterial diversity than spring captures. Faith's phylogenetic diversity index does not incorporate the relative abundances of taxa within communities, but rather calculates phylogenetic diversity based on the presence or absence of species (Cadotte et al. 2010; Berg et al. 2016). Our results therefore imply that for sex and season, there was no difference in alpha diversity when considering the richness and evenness of species, but that there may be a number of species with deep and/or distinct branching that are making an unequal contribution to the diversity of those communities.

We posit that the differences in gut microbiota composition between on- and offshore bears is most likely driven by environmental factors, such as diet, contaminants and parasites which are known to differ between the two groups (Bentzen et al. 2007; Schliebe et al. 2008; Atwood et al. 2017; McKinney et al. 2017b) – although this hypothesis is yet to be tested. Diet, as one of the biggest drivers in gut microbial changes (Muegge et al. 2011; David et al. 2013; Carmody et al. 2015), likely plays the largest role in the observed differences in bacterial diversity. Historically, southern Beaufort Sea polar bears

remained offshore hunting ringed seal (*Pusa hispida*) and, to a lesser extent, bearded seal (*Erignathus barbatus*) (Stirling and Archibald 1977), primarily consuming high-calorie blubber with a specific, restricted nutritional input (Rode et al. 2015). In contrast, onshore bears have access to a more varied but less natural diet, including bowhead whale bone piles, which can consist of whale blubber, meat, and viscera, as well the carcasses of fish, birds and caribou (*Rangifer tarandus*) (Herreman and Peacock 2013; Atwood et al. 2016; McKinney et al. 2017c), a more varied food source in terms of both species and tissue types.

Not only do onshore bears consume a larger range of food items, but they also likely come into contact with more terrestrial species and their associated bacteria and pathogens. Whale bone piles are utilised by a range of other nearshore/terrestrial scavengers (Miller et al. 2015; Atwood et al. 2017) providing an inter-specific focal point for many species with which polar bears do not typically interact. Beach-cast bowhead whale remains frequently lie in close proximity to settlements and towns, increasing the potential for microbiota and pathogen spillover to polar bears from humans, and domestic animals. The high gut microbiota diversity seen in onshore bears may therefore be associated with this complex network of interspecific contacts. A secondary consequence of high inter-species contact could be a higher parasite load and/or diversity in polar bears, which is associated with high gut microbiota diversity in other species (Round and Mazmanian 2009; Broadhurst et al. 2012; Kreisinger et al. 2015).

Understanding the ways in which polar bears respond to climate-change mediated displacement from primary habitat is crucial in discerning their ability to cope with an increasingly changeable and uncertain environment (Atwood et al. 2016). Future management plans for polar bears could therefore benefit from a better understanding of the relationship between habitat availability, microbiota and health. Our results suggest that climate driven changes in land use by bears leads to changes in gut community composition, but further analyses are needed to determine whether these changes are linked to underlying causes such as diet, parasites and health. It has been suggested that researchers should incorporate health assessments into wildlife conservation practices (Deem et al. 2008; Patyk et al. 2015) and long term faecal microbiota monitoring could provide this framework.

6

Diet-driven mercury contamination is associated with polar bear gut microbiota

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Abstract

The gut microbiota may modulate the disposition and toxicity of environmental contaminants within a host but, conversely, contaminants may also impact gut bacteria. Yet, such contaminant-gut microbial connections, which could lead to alteration of host health, remain poorly known and are rarely studied in free-ranging wildlife. The polar bear (*Ursus maritimus*) is a long-lived, wide-ranging apex predator that feeds on a variety of high trophic position seal and cetacean species and, as such, is exposed to among the highest levels of biomagnifying contaminants of all Arctic species. Here, we investigate associations between a key Arctic contaminant, mercury, diet and the diversity and composition of the gut microbiota of southern Beaufort Sea polar bears, while accounting for host sex, age class and body condition. Bacterial diversity was negatively associated with seal consumption and mercury; a pattern seen for both a Shannon and Inverse Simpson measure of alpha diversity (adjusted R^2 = 0.35, $F_{1,18}$ = 8.00, P = 0.013 and adjusted R^2 = 0.26, $F_{1,18}$ = 6.04, P = 0.027, respectively), but no association was found with sex, age class or body condition of polar bears. The abundance of bacteria known to either be involved in mercury methylation or considered to be highly contaminant resistant were significantly lower in low mercury individuals; the largest of which were Lactobacillales, Bacillales and Aeromonadales. Conversely, we found significantly higher abundance of Bacteroidales, Selenomonadales and Coriobacteriales in low mercury individuals. These associations between diet-acquired mercury and microbiota illustrate the far-reaching implications of mercury accumulation to polar bears.

Background

The gut microbiota (the complex community of bacteria within the gut) plays a critical role in the regulation of physiological functions, and possibly also in metabolism and toxicity of environmental contaminants, in the host (Evariste et al. 2019). In particular, the microbiota is likely an important mediator for toxicity of heavy metals, with evidence of bacterial demethylation in the gut contributing to the elimination of mercury from a host (Breton et al. 2013; Claus et al. 2016). It has even been suggested that the gut microbiota matches, if not outweighs, the contribution of the host's liver to foreign compound metabolism (Scheline 1973). *In vivo* studies demonstrate that in laboratory mice and rats, a suppressed or depleted gut microbiota community is associated with lower faecal excretion of mercury and increased accumulation of mercury in host tissues (Nakamura et al. 1977; Rowland et al. 1980; Seko et al. 1981). Additionally, contaminants may negatively impact gut bacteria via effects on diversity and/or composition of bacterial communities. In the isopod *Porcellio scaber,* individuals from mercury polluted, compared to unpolluted, environments demonstrated a lower bacterial species richness in the gut, as well as elevated levels of bacteria from the genera *Pseudomonas*, *Listeria* and the phylum Bacteroidetes (Lapanje et al. 2010). In laboratory mice exposed to high mercury levels, the abundance of *Sporosarcina* sp., *Jeotgailcoccus* sp., and *Staphylococcus* sp. were significantly decreased in treatment groups relative to controls (Ruan et al. 2019). It is therefore possible that contaminant-induced alterations to the composition of gut bacteria may influence the toxicity of contaminants and, ultimately, impact host health (Ruan et al. 2019). This, alongside other well-documented physiological effects (Dietz et al. 2013; Desforges et al. 2016) makes host-contaminant interactions an important consideration, especially for top predators that bioaccumulate high levels of contaminants from their prey.

The polar bear (*Ursus maritimus*) is a long-lived, wide-ranging apex predator, which feed on a variety of high trophic position seal and cetacean species (Amstrup et al. 2003; McKinney et al. 2017c; Bourque et al. 2020). As such, of all the Arctic species, polar bears through their diet are exposed to among the highest levels of biomagnifying contaminants (Routti et al. 2019), including methylmercury (MeHg). After climate-driven loss of sea ice habitat, contaminant exposure is considered perhaps one of the most significant threats to polar bears (Routti et al. 2019) and has been linked to increasing incidences of alopecia within the southern Beaufort Sea subpopulation (Atwood et al. 2015; Bowen et al. 2015). Within the past century, concentrations of mercury have

increased substantially in the Arctic, with 94% of the mercury found in polar bear tissues estimated to be derived from anthropogenic sources, resulting from long-range transport to the Arctic (Dietz et al., 2006). Contaminants which are transported to the Arctic subsequently persist within the food chain (Hoekstra et al. 2003; Borgå et al. 2004).

For polar bears, substantial changes in the spatial and temporal extent of sea ice habitat since *c*. 2000 have led to subsequent shifts in prey availability and abundance (Ferguson et al. 2005; Johnston et al. 2005; Thiemann et al. 2008). Polar bears primarily consume a diet of seals; ringed seal (*Pusa hispida*) and bearded seal (*Erignathus barbatus*) (McKinney et al. 2017c; Bourque et al. 2020); species which occupy a high trophic position (Muir et al. 1988), and can potentially accumulate high levels of contaminants. Such diet-driven exposure to contaminants, such as mercury (McKinney et al. 2017a), may in turn may have a knock-on effect on gut microbiota diversity and composition. In itself, diet is considered an important determinant of changes to the gut microbiota (Ley et al. 2008a). Gut microbiota analysis of humans and 59 other mammalian species indicates that host diet has a strong influence on bacterial diversity and composition, which increases from carnivory to omnivory to herbivory (Ley et al. 2008a). In free-roaming brown bears (*Ursus arctos*), gut microbiota composition changes seasonally, which is thought to reflect extreme seasonal shifts in dietary intake (Sommer et al. 2016). Similarly, in Andean bears (*Tremarctos ornatus*), gut microbiota richness differs between captive and wild types, which is thought to reflect differences in the availability and diversity of food resources (Borbón-García et al. 2017).

Here, we investigate associations between dietary mercury, measured in hair samples, diet and the faecal microbiota diversity and composition of southern Beaufort Sea polar bears.

Methods

Polar bear capture and sampling

Polar bears were captured under the United States Geological Survey (USGS) Polar Bear Research Program (Marine Mammal Permit MA690038 to T.C.A.) in an area ranging approximately from Utqiagvik, Alaska (156°W) in the west to Demarcation Point (140°W) at the US-Canada border in the east (see Chapter 5 for sampling methods). A total of 91 bears were sampled; hair samples for mercury analysis were taken from 63 individuals, and adipose tissue samples for dietary analysis from 50 individuals with 22

individuals having both hair and adipose tissue sampled (See supplementary Table 7 for a breakdown of the samples and details of samples regime across individuals. Note, due to opportunistic sampling, not all individuals had both hair and adipose tissue samples collected). An adipose tissue sample was taken from each of 50 individuals using a 6mm biopsy punch, consisting of a full-layer fat core from skin to muscle. All adipose tissue samples were stored at -80°C prior to shipment to the McKinney lab for diet analysis. All samples were taken in years 2008, 2009, 2010 and 2013. A single faecal sample (for microbiota analysis) was collected directly from the rectum of polar bears (n = 91) using a sterile latex glove and immediately transferred to a sterile Whirlpak bag (Nasco, Fort Atkinson, Wisconsin, USA) for storage. All faecal samples were stored at −20 °C for the duration of the field season (~5 weeks) before being stored at −80°C at the US Geological Survey, Alaska Science Center (Anchorage, Alaska, USA), and subsequently shipped on dry ice to the Fondazione Edmund Mach, Italy (CITES permit IT/IM/2015/MCE/01862 to S.W.).

Extraction of bacterial DNA and 16S rRNA gene amplification and sequencing

All faecal matter was collected from inside each sample glove using a sterile cotton swab (APTACA sterile transport swabs, Brescia, Italy). The swab was subsequently vortexed for 10 min in 1 ml phosphate-buffered saline solution (PBS) and pelleted by centrifugation at 16,000 g for 12 min. Lysis buffer, 80 µl, (200 mM NaCl, 100 mM Tris, 20 mM EDTA, 20 mg/ml Lysozyme, pH 8.0); 5 mm stainless steel beads (Qiagen) were added to each sample before a three-minute homogenisation step at 30 Hz using a Mixer Mill MM200 (Retsch GmbH, Haan, Germany). Samples were shaken at 37 °C for 40 min Grant-Bio PCMT Thermoshaker (500 rpm). Microbial DNA was extracted using the QIAamp® DNA Mini Kits (QIAGEN©, Milan, Italy), following the manufacturer's Buccal Swab Spin Protocol for cotton swabs (QIAamp® DNA Mini and Blood Mini Handbook), but starting from step 2 (addition of Proteinase K). The primer set 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805Rmod (5′-GACTACNVGGGTWTCTAATCC-3′) (based on Klindworth et al. with degenerate bases) with overhanging Illumina adaptors, were used to target a ~460 base pair (bp) fragment of the 16S rRNA gene (variable region V3-V4). PCR products were purified and Illumina® Nextera XT indices and sequencing adaptors (Illumina®) were incorporated using seven cycles of PCR (16S Metagenomic Sequencing Library Preparation, Illumina®). Final quantified libraries were pooled in equimolar concentrations before sequencing on an lllumina® MiSeq (2 × 300 bp reads) at the Next

Generation Sequencing Platform, Fondazione Edmund Mach in collaboration with the Core Facility, CIBIO, University of Trento, Italy.

Bioinformatic analyses

Reads were processed with MICCA v1.5.0 (Albanese et al. 2015). Briefly, paired-end reads were merged, and pairs diverging by more than 8 bp or overlapping by less than 100 bp were discarded. PCR amplification primers were trimmed (sequences not containing both PCR primer sequences were discarded). Finally, sequences were quality filtered at 0.5 % Expected Error (EE); those displaying greater than 0.5% EE were discarded along with those shorter than 400 bp or containing unknown base calls (N). Using the VSEARCH cluster_smallmem algorithm (Rognes et al. 2016), OTUs were created *de novo* by clustering sequences with 97% sequence identity, discarding chimeric sequences. Taxonomic assignments of representative sequences from each OTU were performed using the RDP Classifier v2.12 in conjunction with RDP 16S rRNA training set 15 (Wang et al. 2007). OTU sequences were aligned and phylogenetic analysis was performed using Nearest Alignment Space Termination (NAST) and a phylogeny reconstructed using FastTree (Price et al. 2009), both via MICCA (Albanese et al. 2015). The raw sequencing data can be found at the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) [Accession number: PRJNA542176].

Mercury analysis

Samples were analysed for total mercury, and previously reported, as detailed in (McKinney et al. 2017a). In brief, surface contamination was removed from each hair sample by standard protocols (Dietz et al. 2006) before acid digestion. Mercury analysis was conducted by cold-vapour atomic absorption spectrometry, with total mercury concentrations (THg) reported as $\mu g\ g^{-1}$ dw. Blanks, sample duplicates and matrix spikes were included as quality-control procedures, with all blanks demonstrating levels below the detection limit (i.e. less than 0.3 $\mu g\ g^{-1}$, the limit based on an average sample size of ~0.001 g). This method was used to screen total mercury (THg) in µg/g dry weight in 63 individuals.

Fatty acid-based diet analysis

Quantitative fatty acid signature analysis (QFASA) was used to generate proportional diet estimates (% biomass) of bearded seal, beluga whale, bowhead whale and ringed seal for

50 individuals. Extraction and analysis of fatty acids from adipose tissue followed methods described by Iverson et al. (2004) and McKinney *et al.* (2014). In brief, to avoid potentially oxidized outer tissue, approximately 10−20 mg of inner adipose tissue was obtained from each polar bear. Chloroform containing 0.01% butylated hydroxytoluene (BHT) was added as an antioxidant. Samples were flushed with nitrogen, capped, and stored at 20°C until lipid extraction. Lipids were spiked with 5-a-cholestane as internal standard (100 lL of 20 mg/mL) and homogenized. Lipids were extracted twice using 8:4:3 chloroform/methanol/water containing BHT, at a volume/weight ratio of 20:1 solvent/sample. All solvent was evaporated from extracted lipids before being weighed by microbalance. To quantify internal standard recoveries, extracted fatty acids were derivatised to fatty acid methyl esters (FAMEs) and analysed using gas chromatography with flame ionization detection (GC-FID). Each FAME was calculated as the % of total dietary FAME.

Statistical analyses

Data were analysed using R, version 3.5.2 (R Core Team 2018). As Quantitative Fatty Acid Signature Analysis (QFASA) generates proportional diet estimates (% biomass) of a given species within individuals, there is a strong degree of collinearity across each of the diet variables. Low beluga and bowhead whale proportions were recorded within the polar bears used within this study, as such only total seal consumption (i.e. combined proportions of bearded and ringed seal) were included in downstream modelling and analyses. This follows the findings of Bourque et al. (2020) who found that seal consumption remains the predominant prey type in southern Beaufort Sea polar bears and also follows findings from a preliminary Principal Component Analysis (PCA) which we conducted to highlight the most important diet types in explaining the variation of our data. PCA revealed that two principal component variables (PC1 and PC2) explained 78.77% (cumulative proportion = 0.7877) of the variation across the four dietary variables. A polar bear with a high PC1 value has a high proportion of bearded seal in their diet (PC1 = 0.6427) while a polar bear with a high PC2 value has a high proportion of ringed seal in their diet (PC2 = 0.7991).

Generalized Linear Models (GLMs) were used to investigate associations between Shannon and Inverse Simpson measures of bacterial alpha diversity and the sex, age class, body condition, total mercury level, total proportion of seal consumption (i.e. combined proportion of bearded and ringed seal) and an interaction between mercury level and seal

consumption. A gamma family and identity link function was used to model a Shannon measure of alpha diversity, while an inverse gaussian family and log link function was used to model an Inverse Simpson measure of alpha diversity. Two data points were omitted from the model as their values fell more than three standard deviations from the mean. Model selection was based on fit of residual plots (Bolker et al. 2009) and backwards stepwise deletion of non-significant terms.

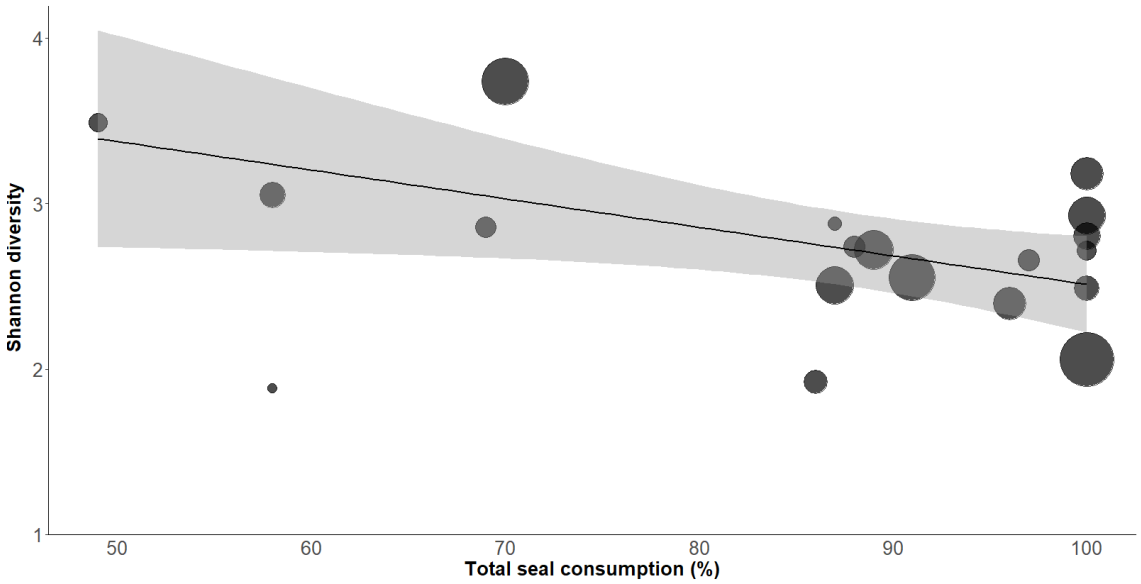
The revised human hair no-observed-effects-level (NOEL) threshold for THg is 6.0 μg g–1 dw (Grandjean and Budtz-Jørgensen 2007) which we use as a threshold, above which polar bear mercury levels are deemed unsafe (Dietz et al. 2011; Dietz et al. 2013). The differential abundance of OTUs for polar bears above versus below the 'safe threshold' was examined using the R package '*DESeq2*' (Love et al. 2014). To illustrate potential effects of mercury microbiota composition we constructed a heat tree, using the $\log_2$ ratio of median proportions for bacterial taxa associated with polar bears with mercury levels below and above threshold levels using the R package '*metacoder*' (Foster et al. 2017). The differential abundance of OTUs between polar bears with 'high' versus 'low' proportions of dietary bearded seal and ringed seal, were also examined using the R package '*DESeq2*' (Love et al. 2014). Dietary proportion was considered 'high' if the measurement detected was above or equal to the median across our samples, and considered 'low' if below the median.

Results

Associations between mercury, diet and gut microbiota diversity

Bacterial diversity had a negative association with seal consumption and mercury, a pattern seen for both a Shannon and Inverse Simpson measure of alpha diversity (adjusted R^2 = 0.35, $F_{1,18}$ = 8.00, P = 0.013 and adjusted R^2 = 0.26, $F_{1,18}$ = 6.04, P = 0.027, respectively; Figure 21a and b). No association was found between bacterial diversity and the sex, age class or body condition of polar bears.

a)



b)

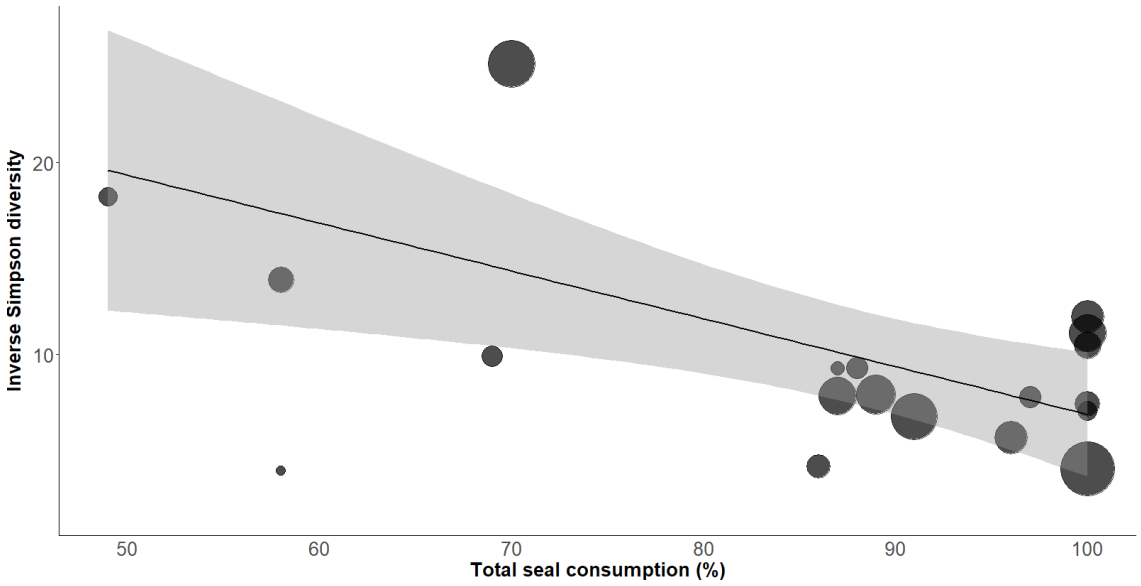


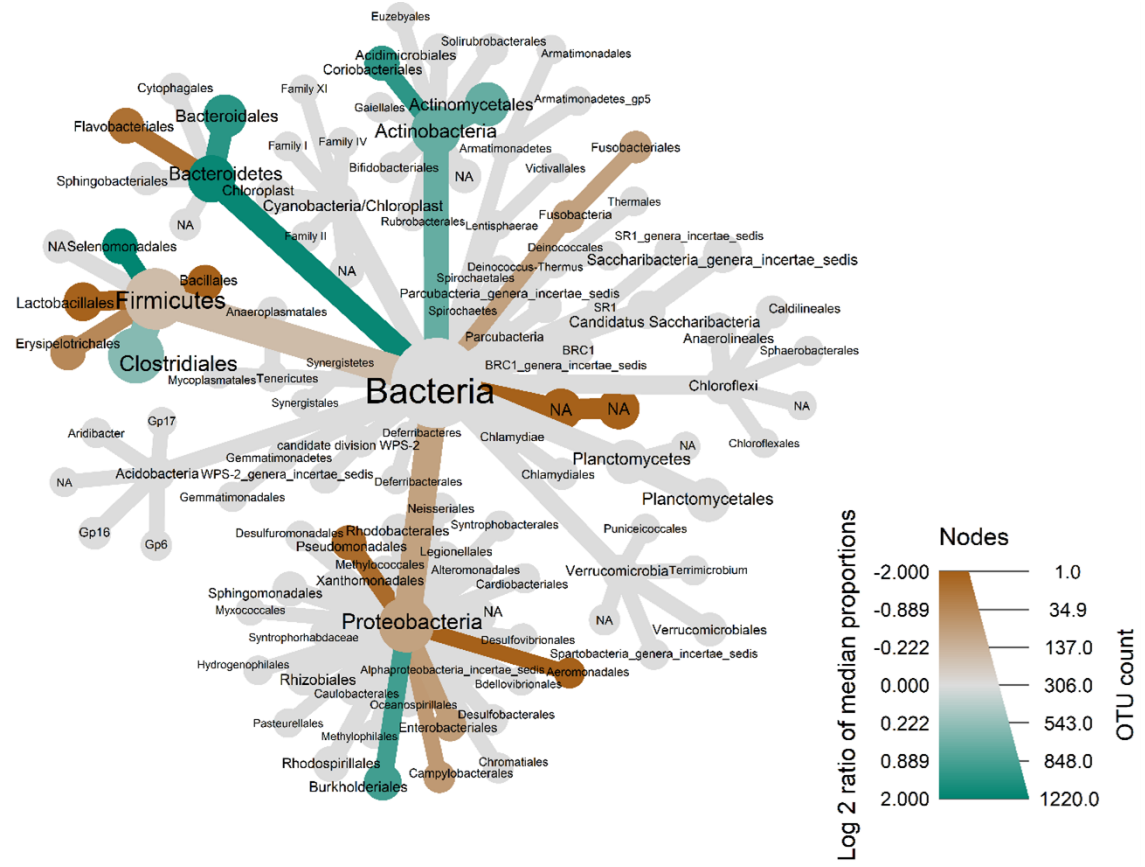
Figure 21. Associations between bacterial alpha diversity and percentage of total seal consumption in southern Beaufort Sea polar bears. Points are weighted by mercury level, where a larger point size reflects a higher mercury level detected within a given polar bear. Line of best fit takes in to account the weight of data points **a.** shows a Shannon measure of alpha diversity and **b.** shows an Inverse Simpson measure of alpha diversity

Associations between mercury and gut microbiota composition

Hair mercury concentrations in individual polar bears ranged widely from 0.9 to 22.94 THg μg/g, with a mean of 4.7 μg/g, (SD = 0.2), which is below mercury threshold of 6.0 μg/g (Grandjean and Budtz-Jørgensen 2007). Mercury levels ≥ 6.0 μg/g were detected in n = 16 (25%) individuals. Differences in specific bacterial taxa were detected in individuals falling above versus below threshold levels for mercury, with 12 phyla detected in individuals above the threshold, compared to 20 phyla for those below (for the latter of which 9 phyla were unique (Acidobacteria, Candidate division WPS-2, Chlamydiae, Chloroflexi, Deferribacteres, Gemmatimonadetes, Lentisphaerae, Planctomycetes, Tenericutes)).

For a number of taxa, the log fold ratios of median proportions differed between individuals above versus below the threshold for mercury (Figure 22). Median proportions of Firmicutes and Proteobacteria were lower, and Bacteroidetes and Actinobacteria higher, in individuals below the threshold for mercury (Figure 22a). At order level, the largest differences in median proportion was seen in lactobacillales, bacillales, aeromonadales and an unclassified taxonomic class (which were lower in individuals below the threshold for mercury) and bacteroidales, selenomonadales and coriobacteriales (which were higher in individuals below the threshold for mercury; Figure 22a). DESeq analysis revealed that 18 OTUs differed significantly between bears above versus below the mercury threshold. Specifically, the abundance of 16 OTUs was significantly higher in bears below the mercury threshold (the majority of which were assigned to families within the phylum Firmicutes), while the abundance of two OTUs were significantly reduced in bears below the mercury threshold (Figure 22b).

a)



b)

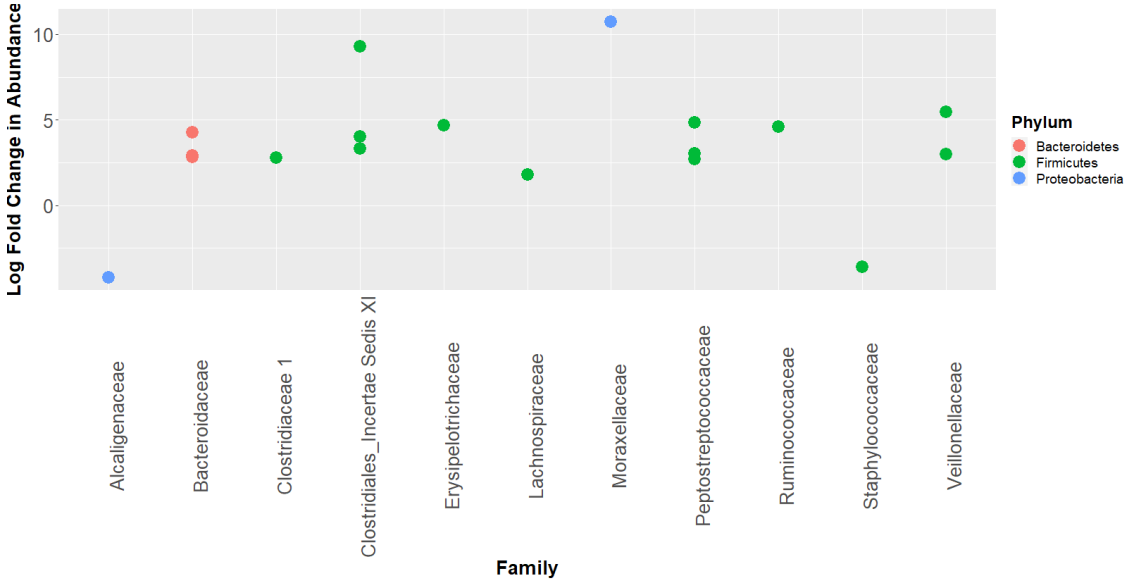


Figure 22a. Metacoder heat tree showing the $\log_2$ ratio of median proportions for bacterial taxa in polar bears demonstrating mercury levels below, compared to above, the 6.0 g g–1 dw no observed effect level (NOEL) threshold for humans. Nodes are weighted by OTU count and coloured on a gradient from largest decrease in median proportion (teal) to largest increase in median proportion (tan) **b.** Differential OTU abundance from DESeq2 analysis of polar bears demonstrating mercury levels below, compared to above, NOEL threshold for humans. Plotted at family level with associated phyla assignment

Associations between diet and gut microbiota composition

DESeq analysis revealed that a number of OTUs significantly differed in their relative abundance depending on the proportion of a given dietary item (Figure 23 and Table 3). Specifically, seven OTUs differed significantly between polar bears that had low proportions of bearded seal in their diet; six of which were significantly reduced in low bearded seal diet, and one of which was significantly increased. The largest difference in bacterial abundance was a 9.13 log fold decrease of DENOVO121 (Family; Peptostreptococcaceae, Genus; *Clostridium XI*) in the diet of individuals with a low proportion of bearded seal. A large decrease in the bacterial abundance of DENOVO78 (Family; Rhodobacteraceae, Genus; *Paracoccus*; 5.47 log fold decrease) was also seen in the diet of individuals with a low proportion of bearded seal. A similar decrease in bacterial abundance was seen in DENOVO14 (Family; Peptostreptococcaceae, Genus; *Romboutsia*; 5.03 log fold decrease) with a low proportion of bearded seal. The only OTU that significantly increased in abundance with a low proportion of bearded seal was DENOVO29 (Family; Veillonellaceae, Genus; *Dialister*; 3.06 log fold increase). Only one OTU significantly differed with proportion of ringed seal in the diet, where a 3.40 log fold change in the abundance of DENOVO5 (Family; Peptostreptococcaceae, Genus; *Clostridium XI*) was detected in individuals with low proportions of dietary ringed seal.

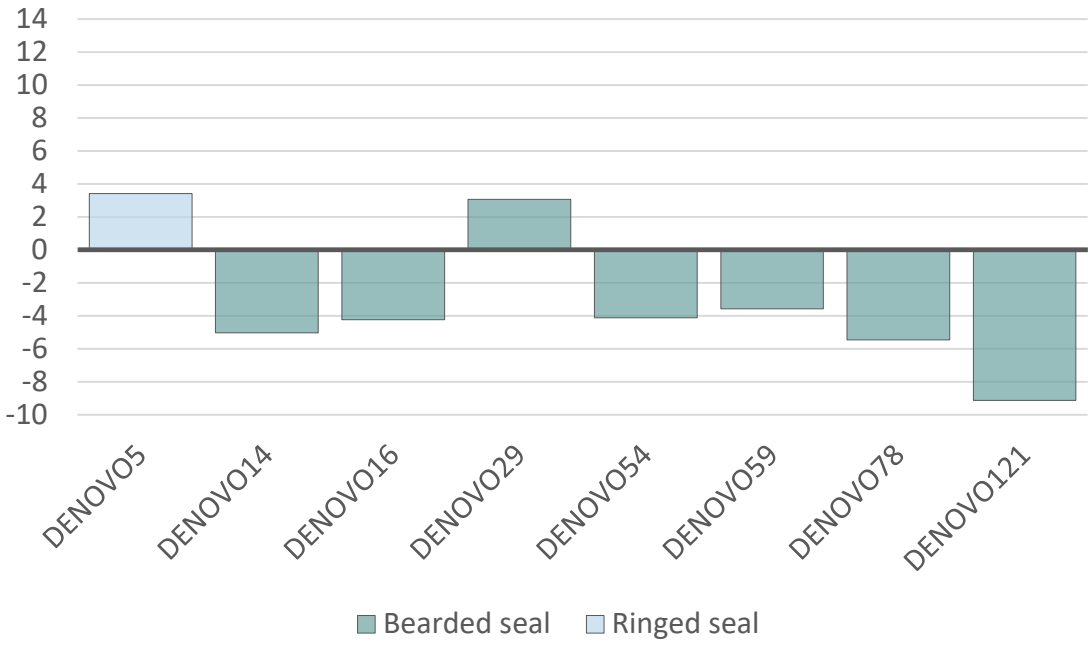


Figure 23. Log fold change in OTU abundance of polar bears demonstrating low levels of a given diet item (bearded seal or ringed seal) compared to those that demonstrated high levels. Dietary proportion was considered 'high' if the measurement detected was above or equal to the median across samples, and 'low' if below the median. Taxonomic information related to each OTU is given in Table 3

Table 3. List of OTUs that significantly differ in abundance within the gut microbiota of polar bears depending on diet type consumed (as shown in Figure 23). Taxonomic information for each OTU is listed to genus level

OTU	Phylum	Family	Genus
DENOVO5	Firmicutes	Peptostreptococcaceae	Clostridium XI
DENOVO14	Firmicutes	Peptostreptococcaceae	Romboutsia
DENOVO16	Bacteroidetes	Flavobacteriaceae	NA
DENOVO29	Firmicutes	Veillonellaceae	Dialister
DENOVO54	Actinobacteria	Actinomycetaceae	Flaviflexus
DENOVO59	Firmicutes	Erysipelotrichaceae	Clostridium XVIII
DENOVO78	Proteobacteria	Rhodobacteraceae	Paracoccus
DENOVO121	Firmicutes	Peptostreptococcaceae	Clostridium XI

Discussion

The gut microbiota may modulate the toxicity of environmental contaminants within a host but, conversely, contaminants may also impact gut bacteria communities. Here we have demonstrated that diet-driven mercury levels are associated with differences in the diversity and composition of polar bear (*Ursus maritimus*) gut microbiota. We found bacterial alpha diversity to be low when seal and mercury levels were high but no association was found with polar bear age class, sex or body condition. Additionally, we found that mercury accumulation and differing dietary items; ringed seal (*Pusa hispida*) and bearded seal (*Erignathus barbatus*) are associated with significant differences in the abundance of a number of bacterial taxa.

Our finding that bacterial diversity had a negative relationship with mercury contamination mirrors previous work in the isopod *Porcellio scaber*, whereby individuals from mercury contaminated, compared to uncontaminated, environments demonstrated a lower bacterial species richness in the gut (Lapanje et al. 2010). However, our findings contrast what is seen in laboratory mice and pregnant women, whereby no significant difference in bacterial diversity was associated with heavy metal exposure (Breton et al. 2013; Rothenberg et al. 2016; Wu et al. 2016). In zebrafish (*Danio rerio*) experimentally exposed to heavy metals, bacterial diversity instead significantly increases (Xia et al. 2018). It is possible that the depleted bacterial diversity we detect with mercury level within this study may demonstrate that a number of commensal gut bacteria within polar

bears are not mercury tolerant or, alternatively, that the polar bear gut microbiota responds negatively to contaminant-induced stress.

In addition to finding a lower bacterial diversity associated with mercury level, we also detected changes in the composition and abundance of bacteria. We found higher median proportions of Firmicutes and Proteobacteria, but lower median proportions of Bacteroidetes and Actinobacteria, in individuals above the safe threshold for mercury. This is similar to previous studies in laboratory mice that have been exposed to heavy metals (Wu et al. 2016). As seen in our study, the abundance of Firmicutes were higher, while Bacteroidetes abundance was reduced, in laboratory mice exposed to lead (Wu et al. 2016). However, Wu et al. (2016) found no differences in the abundance of Actinobacteria and Proteobacteria with heavy metal exposure, as we found in this study.

The ratio of Bacteroidetes:Firmicutes is important indicator of health, including determining bodyweight, with the ratio of Bacteroides:Firmicutes increasing as fat mass decreases (Ley et al. 2006; Turnbaugh et al. 2006). As such, changes to the Bacteroides:Firmicutes ratio influences the capacity of a host to harvest energy from the diet. At order level, one the largest differences in bacterial abundance between bears with high versus low mercury level was seen in lactobacillales (which was higher in individuals above the safe threshold for mercury). Some strains of lactobacilli are able to methylate mercury, perhaps explaining its higher abundance in polar bears with high mercury levels (Rowland et al. 1977). We also found the abundance of bacillales was greater in polar bears demonstrating high levels of mercury contamination. Members of the order bacillales are able to generate highly resistant dormant spores under conditions of nutrient depletion and can survive in the absence of nutrients, amongst other harsh environmental conditions, for long periods of time (Paredes-Sabja et al. 2011). It has also been demonstrated that spores from members of the order bacillales are resistant to a number of toxic chemicals (Setlow et al. 2017), again explaining their higher abundance in polar bears with high mercury levels. Further, members of lactobacillales and bacillales are considered efficient in preventing (or restoring gut health following) intestinal disorders, such as colitis and Irritable Bowel Syndrome (IBS) (see Ilinskaya et al. 2017). It is therefore possible that the higher abundance of lactobacillales and bacillales found within polar bears with higher mercury levels may reflect attempts to restore gut health from the stress of mercury contamination. Conversely, we found the abundance of bacteroidales was lower in polar bears demonstrating high mercury levels. Members of the order bacteroidales respond negatively to mercury level (Hiller-Bittrolff et al. 2018)

which is a concern considering that members of bacteroidales have been show to stimulate intraepithelial lymphocytes within the epithelial barrier, which promotes barrier integrity and protects the host from microbial invasion (Kuhn et al. 2018). Interestingly, we also found a lower abundance of selenomonadales in polar bears that have high levels of mercury, despite the fact that members of selenomonadales have been shown to be mercury resistant (Wei et al. 2017). Such mercury-dependent differences in microbiota composition are concerning, especially considering how the elevated mercury levels are acquired predominantly through consumption the polar bear's main prey choice, seal (Bourque et al. 2020).

As the largest, most carnivorous ursid species, polar bears have evolved to hunt lipid-rich marine mammals (i.e. ringed seals and bearded seals), which they rely on to satisfy the high energy consumption associated with living in the Arctic (Rode et al. 2015). However, the decreased bacterial diversity we found linked with greater dietary intake of seal (i.e. bearded seal and ringed seal) and associated increased mercury levels may indicate that the consumption of historical, typical polar bear prey items is becoming detrimental for polar bear gut health and resilience. A high bacterial diversity within the gut is typically associated with increased resilience and enhanced host health, and a low bacterial diversity (as we see within this study) the converse (Van Der Waaij et al. 1971; Girvan et al. 2005). Further, we found that the consumption of differing dietary items (i.e. ringed and bearded seal) was association with difference in gut microbiota composition and abundance. The largest difference in bacterial abundance was seen in the genus *Clostridium XI* (Family; Peptostreptococcaceae) which was significantly decreased in the diet of individuals with a low proportion of bearded seal. *Clostridium XI* is associated with high-fat diets and assimilating weight gain (Bishara et al. 2013; Cowan et al. 2014), which perhaps explains its diminished proportions in individuals demonstrating low dietary intake of bearded seal (i.e. a dietary item rich in blubber). Similarly, we detected a significantly decreased abundance of the genus *Paracoccus* (Family; Rhodobacteraceae) in polar bears that consume a low dietary intake of bearded seal. The family Rhodobacteraceae is among the most widely distributed bacterial lineages in marine habitats (Simon et al. 2017; Pohlner et al. 2019). The only OTU that significantly increased in abundance with a low proportion of bearded seal in polar bear diet was DENOVO29 (Family; Veillonellaceae, Genus; *Dialister*). The abundance of bacteria belonging to the family Veillonellaceae, including the genus *Dialister* is positively correlated with dietary fatty acid intake in laboratory mice, and are thought to play an

important role in energy metabolism (Castonguay-Paradis et al. 2020). Only one OTU significantly differed with proportion of ringed seal in the diet, DENOVO5 (Family; Peptostreptococcaceae, Genus; *Clostridium XI*), which was detected in a higher abundance in individuals with low proportions of ringed seal in the diet.

Diet, in itself, is considered a central driver in changes to the gut microbiota; gut microbiota analysis of humans and 59 other mammalian species indicates that host diet has a strong influence on bacterial diversity and composition (Ley et al. 2008a). Our findings that diet influences gut microbiota diversity and composition therefore reflects what is seen in other ursid species. In free-roaming brown bears (*Ursus arctos*), gut microbiota composition changes seasonally, which is thought to reflect extreme seasonal shifts in dietary intake (Sommer et al. 2016). Similarly, in Andean bears (*Tremarctos ornatus*), gut microbiota richness differs between captive and wild types, which is thought to reflect differences in the availability and diversity of food resources (Borbón-García et al. 2017).

Overall, within this study we have shown that diet driven mercury contamination has more intricate effects on host health than previously thought, with high seal consumption and associated high mercury concentrations impacting both diversity and composition of polar bear gut microbiota. Given that polar bears are apex predators and are vulnerable to multiple environmental stressors, they are considered a useful indicator species for the health of Arctic ecosystems. A deeper understanding of factors that influence polar bear intestinal health will feed into the development of future conservation strategies aiming to improve the persistence and resilience of this specialized high trophic feeding carnivore. We highlight the need to understand the interaction of multiple stressors, namely climate change, diet and contaminants, and their influence on the gut microbiota and health of Arctic wildlife.

7

General Discussion

Overview

Within the Arctic, there remains a paucity of data addressing two major influencers of host health, i) the composition of host gut microbiota and ii) parasitic infection. My thesis aimed to address these shortcomings using a whole-Arctic approach and two model apex species; the polar bear (*Ursus maritimus*) and the wolverine (*Gulo gulo*). A combination of data mining, high-throughput sequencing, and traditional parasite count approaches were used to i) establish the diversity and pervasiveness of parasites across Arctic species, and ii) to determine the gut bacterial communities and parasite diversity of polar bears and wolverines in association with contaminant profiles, changes in land use and diet.

A theme that resonates across all chapters within this thesis is creation of baseline gut biome data, which was previously lacking even for the most charismatic, ambassador species of the Arctic. Chapter 2 quantifies parasitism across the Arctic, and demonstrates the significant contribution to the Arctic parasitome of introduced host species (namely humans and their domestic pets). Humans and domestics dogs were shown to harbour a high frequency of parasites and were highly connected within the Arctic network. Domestic dogs have also been implicated as interspecific infection sources in other systems around the world, for example, they were considered the source of a canine distemper outbreak among lions (*Panthera leo*) in the Serengeti (Cleaveland et al. 2000) and phocine distemper virus (PDV) in harbour seals (*Phoca vitulina*) (Härkönen *et al.*, 2006). Our work therefore highlights the need for affordable, routine veterinary care for domestic dogs inhabiting remote northern communities, such as deworming and vaccination, as such resources are currently lacking (Salb et al. 2008). Our work also demonstrates that the most pervasive of each parasite type within the network (Influenza A, *Toxoplasma gondii*, *Trichinella nativa*, *Brucella* sp. and *Encephalitozoon cuniculi*) had zoonotic potential, which is a concern for the northern communities that rely upon a plethora of Arctic species for subsistence hunting. Our work indicates which parasite

species are zoonotic and which Arctic hosts are most likely to harbour them, therefore providing an inventory against which surveillance can be executed. However, the Arctic host-parasite sharing network in Chapter 2 served an additional purpose; by constructing a 'potential' and a 'realised' network of parasite sharing in the Arctic, we highlighted the substantial variation in sampling effort across taxa and geography. There was an overall paucity of parasite data across the Arctic as a whole, which we aimed to partially address in Chapter 3.

Chapter 3 aimed to contribute to the knowledge base of the wild gut biome of Artic hosts, namely in the wolverine (*Gulo gulo*). By combining molecular and traditional techniques, Chapter 3 demonstrated the broad diversity of parasites that Arctic dwelling wolverines can host. We described *Baylisascaris devosi* and *Taenia twitchelli* in Canadian wolverines for the first time since 1978, and extended the recorded geographic distribution of these parasites by ca 2,000 km to the east and into the tundra. Obtaining this information not only informs us as to the parasites of wolverines, but also sheds light on the ecology of the parasites themselves. For example, from our work it appears that the thermotolerance of *B. devosi* is even greater than previously thought (Sapp et al. 2017), although our results do imply that there is a northerly limit to their distribution. Our findings illustrate the value of combining molecular and traditional methods, encouraging additional work to improve the advancement of molecular screening methods for parasites. Currently, taxonomic resolution from metabarcoding methods is reliant on the availability and curation of data in reference databases (Bohmann et al. 2014; Taberlet et al. 2018), which is often lacking for wildlife parasites (Jex et al. 2010; Gogarten et al. 2020).

In addition to parasitic infection, there is a growing emphasis on the importance of gut microbiota communities to host health and infection resistance (Young 2012; Ducarmon et al. 2019). The work presented here used high throughput sequencing techniques to establish the gut microbiota of free-roaming wolverines and polar bears, for the first time (Chapters 4 and 5). Previously a study by Glad et al. (2010) used clone libraries to investigate the gut microbiota of polar bears from Svalbard but the technique lacks the resolution of modern sequencing technologies (Mardis 2008). In contrast to Glad et al. (2010), we demonstrated that the gut microbiota of polar bears is much more diverse than previously thought and closely mirrors the bacterial diversity detected in other ursids, such as brown bears (*Ursus arctos*) (Sommer et al. 2016) (Chapter 5). Not only does the research presented within this thesis provide a more accurate baseline against which to assess future environmental impacts, but also demonstrates the immediate effect that

some of the major threats to Arctic species populations are having right now. For example, this thesis has demonstrated that loss of sea ice habitat and heavy metal contamination are directly associated with differences in the intestinal microbiota of polar bears (Chapters 5 and 6). Changes to the gut microbiota composition influences the very internal physiology of the host (O'Hara and Shanahan 2006; Chow et al. 2010) and has the potential to uncouple host-microbiota interactions that developed under millions of years of evolutionary adaptation and selective pressure (Ley et al. 2008b). Many Arctic species are well adapted to extreme climates but poorly adapted to secondary stressors (Callaghan et al. 2004; Meltofte et al. 2013) and so understanding the effects of changing gut microbiota communities may play a central role in host success and survival.

In Chapter 4, we demonstrate that the gut microbiota of wolverines may be an adaptation to a lifestyle of scavenging and caching behaviour. The tight evolutionary coupling of hosts to their gastrointestinal bacteria may explain how scavengers are able to consume decomposed carrion and, in some cases, bone. Changes in gut bacterial communities of the wolverine may therefore impact their ability to consume and digest scavenged items. This is an important reflection considering that wolverines are known to be susceptible to the impacts of climate change and anthropogenic disruption (Aubry et al. 2007; Copeland et al. 2010), which influence their scavenging and caching behaviour. In the future, as climate change continues to alter cache site suitability and/or availability, and as such the rate of decomposition of cached items (Copeland et al. 2010; Inman et al. 2012), the gut microbiota may have to adapt in response to increased hazardous pathogen intake and lower biomass associated with cached prey items.

Overall, this thesis highlights that it is the seemingly concealed, more intricate implications of climatic and anthropogenic change that are perhaps the most important to highlight in conservation –to raise awareness of the far reaching effects that go beyond what can be observed with the naked eye. This thesis therefore, hopefully, challenges future researchers to discover knock-on effects that our changing world has on the whole biome of Arctic species. A plethora of research reveals the importance of the gut microbiota to human nutrition, health and disease. For example, metabolic products of gut bacteria are known to act as signalling molecules, inducing changes to host metabolism (Tremaroli and Bäckhed, 2012) and most complex carbohydrates and plant polysaccharides cannot be broken down by human enzymes but are instead digested by communities of gut bacteria (Hooper et al., 2002). Similarly, the gut microbiota is known to interact with the immune system, promoting immune cell maturation and the normal

development of immune functions (O'Hara and Shanahan 2006; Chow et al. 2010). In short, a large volume of research indicates the importance of the gut microbiota to humans, and within this thesis we have also demonstrated its importance in free-roaming wild species, which was previously largely overlooked (Pascoe et al. 2017).

Our understanding of the gut microbiota of wild species relies on a sampling effort which, for some Arctic species, is notoriously difficult to source. Both polar bears and wolverines are solitary species with large home ranges (Dawson et al. 2010; McCall et al. 2015), and both are important to northern indigenous communities (Banci 1994; Kendrick 2013). A previous deficiency of data may therefore be, in part, owed to the logistics of sampling in the Arctic and the expense of sampling remote and endangered species, which typically makes data difficult to obtain. The paucity of data surrounding these issues may mask the true extent to which climate change is impacting the health and long term survival of Arctic species.

Implications for future conservation

Our study demonstrates the first case of global change mediated alterations in the gut microbiota of a free-roaming wild animal; the polar bear (Chapter 5). As such, our research implies that the implications of climate change on host health are further reaching than previously thought. The work in this thesis highlights an additional consequence of climate changed induced sea ice loss on polar bears (Chapter 5), which is important considering the current predictions on the future of polar bears (Molnár et al. 2020). Coming onshore has consequential impacts, another reason polar bears may not survive if forced on land for the long term. Our work will form the baseline of future monitoring of the gut health of polar bears at the United States Geological Survey (USGS).

Implications for human health and northern communities

Output from Chapter 3 ("Parasites of an Arctic scavenger; the wolverine") will be used to inform the Nunavut government. Using this information, the Nunavut government will be able to assess which parasites may be of risk to wolverine trappers and generate guidance of best practice when processing harvested meat. Additionally, Chapter 2 ("Sharing isn't always caring: A host-parasite sharing network of the Arctic") highlights potential parasite risks to humans from the consumption of harvested Arctic species, such

as reindeer and polar bear. Chapter 2 therefore provides an inventory of potential parasites to be cautious of when harvesting and processing meat from Arctic species.

Conclusions

The research presented here has extended our current knowledge of gut macro- and microbiota within Arctic species and has provided a biomarker against which to assess the impacts of future stressors. A deeper understanding of factors that influence the intestinal health of polar bears and wolverines will feed into the development of future conservation strategies aiming to improve the persistence and resilience of these specialized Arctic species.

Additional Experiences

Conference Organiser, Wales – November 2017 and May 2018

- In November 2017 I co-organised and co-hosted the Wales Ecology and Evolution Network (WEEN), a conference run by postgraduate students of Wales, for postgraduate students of Wales. WEEN, which was based at the Center for Alternative Technology in North Wales, provided a platform for students across Welsh universities to present their research output/ ideas and to form future collaborations.
- In May 2018, I again co-organised and co-hosted a conference, this time a Cardiff University departmental conference called OnE Away Day. At OnE Away Day, all staff and postgraduate students from the Organisms and the Environment division come together to hear about current postgraduate research being conducted within the department and to attend a guest talk and workshop.

Safe and Effective Field Work Training, Svalbard – August 2019

- In August 2019 I participated in a British Antarctic Survey run training course in Svalbard. The course consisted of 3 days technical and logistical training at the British Antarctic Survey head offices in Cambridge followed by 2 weeks of field training (on land, on ice and over sea) conducted from the NERC Arctic Research Station in Ny-Ålesund. While in Ny-Ålesund I also obtained my Kings Bay rifle and polar bear training certificate.

ARCTIS 2020 UK-Russia Polar Network Field Course, Siberia, Russia – February 2020

- Arctic Interdisciplinary Studies (ARCTIS) 2020, is an interdisciplinary field course in the Russian Arctic organized by the Association of Polar Early Career Scientists in Russia (APECS Russia) and the UK Polar Network (UKPN) together with Kola Science Center of the Russian Academy of Sciences. The course aim was to facilitate bilateral and interdisciplinary cooperation (Atmosphere, Cryosphere, Terrestrial, Marine and Social & Humanitarian) of early career scientists from the United Kingdom and Russia in the Arctic natural and social studies. During the course, which aimed to provide an interactive platform to share ideas/knowledge and gain new experience, we developed collaborative science project concepts as a result of field training and meetings with local stakeholders (scientists, indigenous people, oil industry representatives and conservation groups).

Teaching and Outreach

- I supervised two Cardiff University Final Year Project Students (2017 and 2018) and the laboratory work for one Masters student at the McGill University, Canada (ongoing). Each year I have taught small mammal trapping on the week-long Ecology and Conservation Field Course (2017-2019), and on a number of occasions have lead some/part of the daily sessions. I have written a number of blogs about my research and experiences throughout my PhD, and have also presented my research at a number of national and international conferences. Additionally, in 2019 I was invited to create a research profile with 'Women in the Arctic and Antarctic', a group that promotes the work and voices of women researching, representing, and living in the North and the polar regions.

Supplementary Information

Supplementary Table 1. List of all 172 Arctic host species included within the Arctic host-parasite sharing network; including Latin name, common name and phylogenetic class to which each host belongs

Host Latin name	Host common name	Host class
Acanthis flammea	Redpoll	Aves
Actitis hypoleucos	Common Sandpiper	Aves
Actitis macularius	Spotted Sandpiper	Aves
Alca torda	Razorbill	Aves
Alces alces	Moose	Mammalia
Alces americanus	Moose	Mammalia
Alle alle	Little Auk	Aves
Anas acuta	Northern Pintail	Aves
Anas crecca	Common Teal	Aves
Anas crecca carolinensis	Green-winged Teal	Aves
Anas platyrhynchos	Mallard	Aves
Anser albifrons	Greater White-fronted Goose	Aves
Anser anser	Greylag Goose	Aves
Anser brachyrhynchus	Pink-footed Goose	Aves
Anser caerulescens	Snow Goose	Aves
Anser canagicus	Emperor Goose	Aves
Anser erythropus	Lesser White-fronted Goose	Aves
Anser fabalis serrirostris	Tundra Bean Goose	Aves
Anser rossii	Ross's Goose	Aves
Anthus pratensis	Meadow Pipit	Aves
Apodemus sylvaticus	Long-tailed Field Mouse	Mammalia
Aquila chrysaetos	Golden Eagle	Aves
Arenaria interpres	Ruddy Turnstone	Aves
Arenaria melanocephala	Black Turnstone	Aves
Arvicola amphibius	European Water Vole	Mammalia
Asio flammeus	Short-eared Owl	Aves
Aythya fuligula	Tufted Duck	Aves
Aythya marila	Greater Scaup	Aves
Balaena mysticetus	Bowhead Whale	Mammalia
Balaenoptera acutorostrata	Common Minke Whale	Mammalia
Balaenoptera borealis	Sei Whale	Mammalia
Balaenoptera musculus	Blue Whale	Mammalia
Balaenoptera physalus	Razorback, Finback	Mammalia
Branta bernicla	Brent Goose	Aves
Branta canadensis	Canada Goose	Aves
Branta hutchinsii	Cackling Goose	Aves
Branta leucopsis	Barnacle Goose	Aves
Branta ruficollis	Red-breasted Goose	Aves
Bubo scandiacus	Snowy Owl	Aves
Bucephala clangula	Common Goldeneye	Aves
Buteo lagopus	Buzzard	Aves
Calidris acuminata	Sharp-tailed Sandpiper	Aves

Calidris alba	Sanderling	Aves
Calidris alpina	Dunlin	Aves
Calidris bairdii	Baird's Sandpiper	Aves
Calidris ferruginea	Curlew Sandpiper	Aves
Calidris fuscicollis	White-rumped Sandpiper	Aves
Calidris mauri	Western Sandpiper	Aves
Calidris minuta	Little Stint	Aves
Calidris minutilla	Least Sandpiper	Aves
Calidris ptilocnemis	Rock Sandpiper	Aves
Calidris pugnax	Ruff	Aves
Calidris pusilla	Semipalmated Sandpiper	Aves
Calidris ruficollis	Red-necked Stint	Aves
Calidris temminckii	Calidris temminckii	Aves
Callorhinus ursinus	Northern Fur Seal	Mammalia
Canis latrans	Coyote	Mammalia
Canis lupus	Gray Wolf	Mammalia
Canis lupus familiaris	Dog	Mammalia
Castor canadensis	American Beaver	Mammalia
Catharus minimus	Grey-cheeked Thrush	Aves
Cepphus grylle	Black Guillemot	Aves
Charadrius hiaticula	Common Ringed Plover	Aves
Charadrius semipalmatus	Semipalmated Plover	Aves
Clangula hyemalis	Long-tailed duck	Aves
Corvus corax	Common Raven	Aves
Corvus cornix	Hooded Crow	Aves
Cygnus columbianus	Tundra Swan	Aves
Cygnus cygnus	Whooper Swan	Aves
Cystophora cristata	Hooded seal	Mammalia
Delphinapterus leucas	Beluga	Mammalia
Dicrostonyx groenlandicus	Neartic Collared Lemming	Mammalia
Dicrostonyx richardsoni	Richardson's Collared Lemming	Mammalia
Dicrostonyx torquatus	Arctic Lemming	Mammalia
Dicrostonyx vinogradovi	Wrangel Island Collared Lemming	Mammalia
Emberiza schoeniclus	Reed Bunting	Aves
Enhydra lutris	Sea otter	Mammalia
Erignathus barbatus	Bearded seal	Mammalia
Eschrichtius robustus	Gray Whale, Grey Whale	Mammalia
Eubalaena glacialis	North Atlantic Right Whale	Mammalia
Eubalaena japonica	North Pacific Right Whale	Mammalia
Eumetopias jubatus	Steller's Sea Lion	Mammalia
Falco columbarius	Merlin	Aves
Falco peregrinus	Peregrine Falcon	Aves
Falco rusticolus	Gyrfalcon	Aves
Felis catus	Domestic Cat	Mammalia
Fratercula arctica	Atlantic Puffin	Aves
Fulmarus glacialis	Northern Fulmar	Aves
Gavia immer	Common Loon	Aves
Gavia stellata	Red-throated Loon	Aves
Globicephala melas	Long-finned Pilot Whale	Mammalia

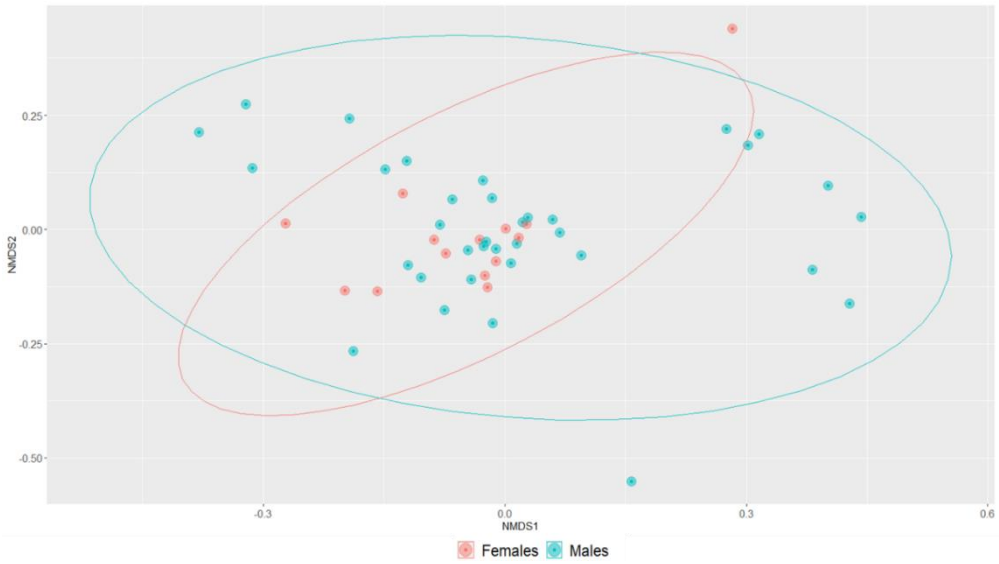
Grus canadensis	Sandhill Crane	Aves
Gulo gulo	Wolverine	Mammalia
Haematopus ostralegus	Eurasian Oystercatcher	Aves
Haliaeetus albicilla	White-tailed Sea-eagle	Aves
Halichoerus grypus	Gray seal	Mammalia
Histriophoca fasciata	Ribbon Seal	Mammalia
Homo sapiens	Human	Mammalia
Lagenorhynchus acutus	Atlantic White-sided Dolphin	Mammalia
Lagopus lagopus	Willow Grouse	Aves
Lagopus muta	Rock Ptarmigan	Aves
Larus argentatus	European Herring Gull	Aves
Larus canus	Mew Gull	Aves
Larus fuscus	Lesser Black-backed Gull	Aves
Larus hyperboreus	Glaucous Gull	Aves
Larus marinus	Great Black-backed Gull	Aves
Lemmus lemmus	Norway Lemming	Mammalia
Lemmus sibiricus	Siberian Brown Lemming	Mammalia
Lemmus trimucronatus	Nearctic Brown Lemming	Mammalia
Lepus americanus	Snowshoe Hare	Mammalia
Lepus timidus	Mountain Hare	Mammalia
Limosa limosa	Black-tailed Godwit	Aves
Lontra canadensis	North American River Otter	Mammalia
Lynx canadensis	Canada Lynx	Mammalia
Lynx lynx	Eurasian Lynx	Mammalia
Mareca americana	American Wigeon	Aves
Mareca penelope	Eurasian Wigeon	Aves
Melanitta perspicillata	Surf Scoter	Aves
Mergus serrator	Red-breasted Merganser	Aves
Microtus gregalis	Narrow-headed Vole	Mammalia
Microtus levis	East European Vole	Mammalia
Microtus miurus	Singing Vole	Mammalia
Microtus oeconomus	Tundra or Root Vole	Mammalia
Microtus pennsylvanicus	Meadow vole	Mammalia
Motacilla alba	White Wagtail	Aves
Mustela erminea	Stoat	Mammalia
Mustela nivalis	Least Weasel	Mammalia
Myodes rufocanus	Grey Red-backed Vole	Mammalia
Myodes rutilus	Northern Red-backed Vole	Mammalia
Neovison vison	American Mink	Mammalia
Ochotona collaris	Collared Pika	Mammalia
Ochotona hyperborea	Northern Pika	Mammalia
Odobenus rosmarus	Walrus	Mammalia
Ondatra zibethicus	Muskrat	Mammalia
Orcinus orca	Killer Whale	Mammalia
Ovibos moschatus	Muskox	Mammalia
Ovis dalli	Thinhorn Sheep	Mammalia
Phalacrocorax aristotelis	European Shag	Aves
Phalacrocorax carbo	Great Cormorant	Aves
Phalaropus lobatus	Red-necked Phalarope	Aves

Phoca groenlandica	Harp seal	Mammalia
Phoca largha	Spotted seal	Mammalia
Phoca vitulina	Harbor seal	Mammalia
Phocoena phocoena	Harbour Porpoise	Mammalia
Phylloscopus trochilus	Willow Warbler	Aves
Physeter macrocephalus	Sperm Whale	Mammalia
Pluvialis squatarola	Grey Plover	Aves
Pusa hispida	Ringed Seal	Mammalia
Rangifer tarandus	Reindeer	Mammalia
Rissa tridactyla	Black-legged Kittiwake	Aves
Sibirionetta formosa	Baikal Teal	Aves
Sicista betulina	Northern Birch Mouse	Mammalia
Somateria mollissima	Common Eider	Aves
Sorex araneus	Common Shrew	Mammalia
Sorex caecutiens	Eurasian Masked Shrew	Mammalia
Sorex cinereus	Cinereus Shrew	Mammalia
Sorex minutus	Eurasian Pygmy Shrew	Mammalia
Sorex roboratus	Flat-skulled Shrew	Mammalia
Sorex tundrensis	Tundra Shrew	Mammalia
Spatula clypeata	Northern Shoveler	Aves
Tringa solitaria	Solitary Sandpiper	Aves
Tringa totanus	Common Redshank	Aves
Turdus migratorius	American Robin	Aves
Uria aalge	Common Murre	Aves
Uria lomvia	Thick-billed Murre	Aves
Ursus americanus	American Black Bear	Mammalia
Ursus arctos	Brown Bear	Mammalia
Ursus maritimus	Polar Bear	Mammalia
Vulpes lagopus	Arctic Fox	Mammalia
Vulpes vulpes	Red Fox	Mammalia
Ziphius cavirostris	Cuvier's beaked whale	Mammalia
Zonotrichia leucophrys	White-crowned Sparrow	Aves

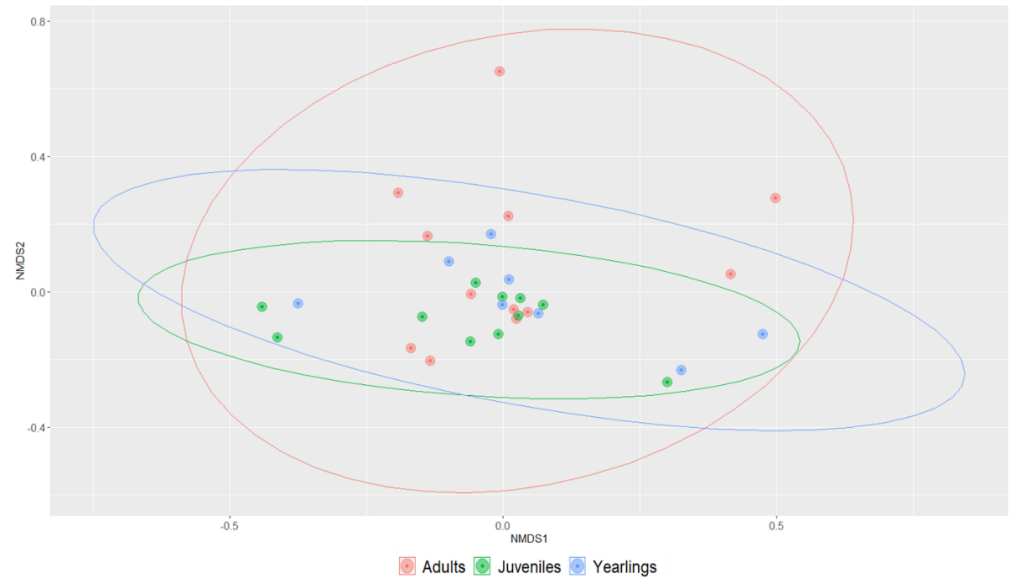
Supplementary Table 2. Infection prevalence for all six parasites detected in 54 wolverines when using 18S high-throughput sequencing. Due to discrepancies in taxonomic identification between SILVA and NCBI databases, parasites identifications are stated at the closest taxonomic rank that encompasses the identifications given in both databases. Prevalence is show against associated host and geographic metadata (where AR = Arviat, BL = Baker Lake, CB = Cambridge Bay, KU = Kugluktuk, and RB = Repulse Bay (Naujaat)). For age class, A = Adult, J = Juveniles, Y = yearling.

Parasite genera	ID	Location	Sex	Age class	Age (Years)	Length (cm)	Mass (Kg)
Order: Ascaridida	314	BL	M	Y	1	858	10.9
	1793	BL	F	J	0	742	8.2
	2924	CB	F	J	0	728	9.9
	3305	AR	F	Y	1	390	10.0
	4031	CB	M	Y	1	788	13.1
	664	BL	F	J	0	690	8.2
	716	AR	F	A	8	720	7.2
Family: Angiostrongylidae	2507	KU	F	J	0	789	8.5
Crenosoma sp.	3740	KU	F	A	7	757	10.3
Subclass: Eucestoda	35	AR	M	A	2	814	12.3
Sarcocystis sp.	612	BL	M	A	2	875	13.7
Trichinella sp.	23	AR	M	A	2	804	12.1

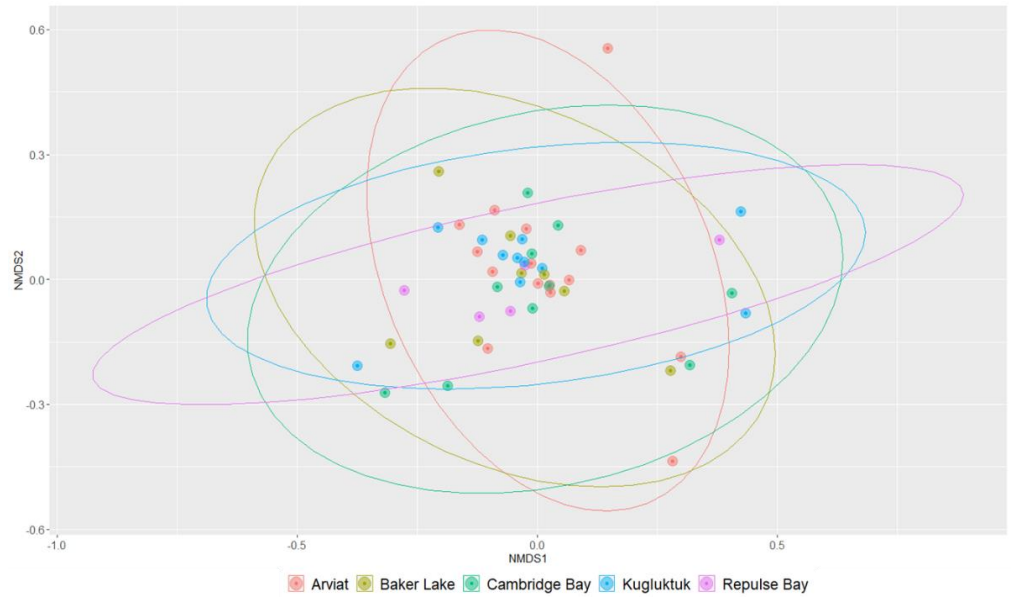
a)


0.25
0.00
-0.25
-0.50
NMDS2
-0.3
0.0
0.3
0.6
NMDS1
Females
Males

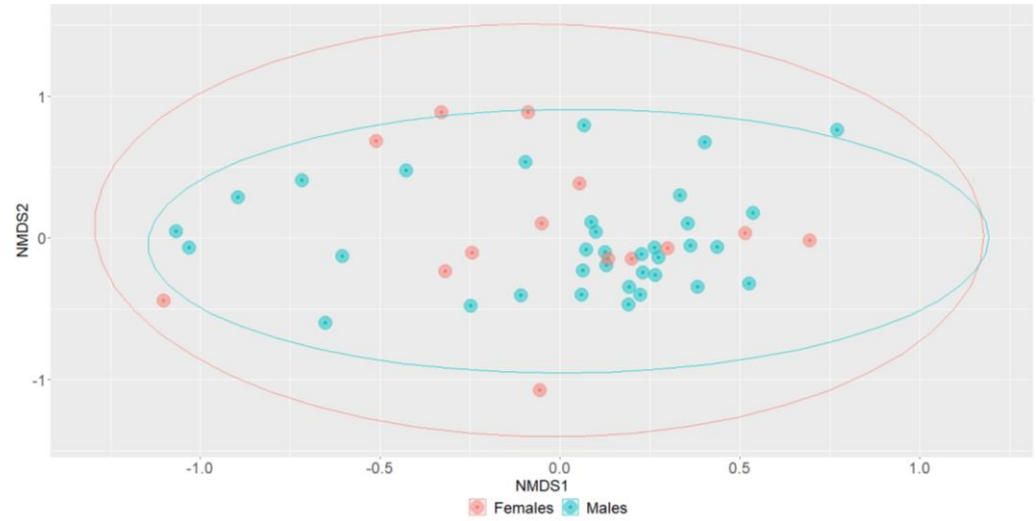
b)


0.8
0.4
0.0
-0.4
NMDS2
-0.5
0.0
0.5
NMDS1
Adults
Juveniles
Yearlings

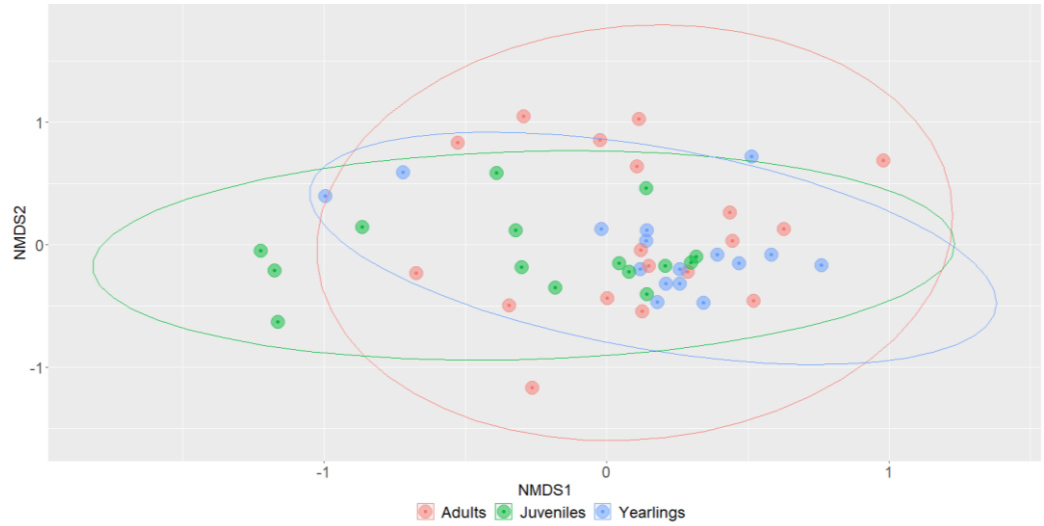
c)


0.6
0.3
0.0
-0.3
-0.6
NMDS2
-1.0
-0.5
0.0
0.5
NMDS1
Arviat
Baker Lake
Cambridge Bay
Kugluktuk
Repulse Bay

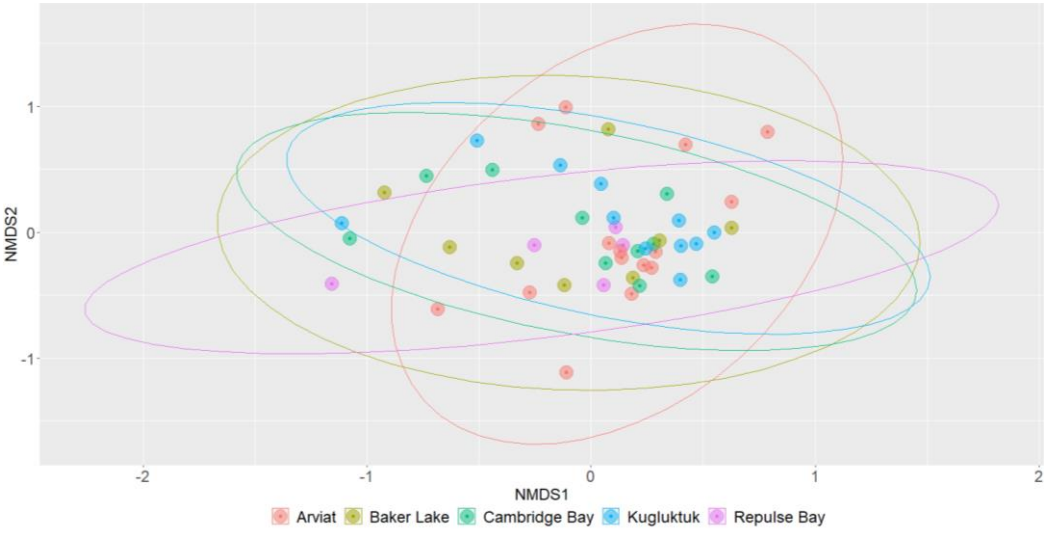
d)



e)



f)



Supplementary Figure 1. Beta diversity of wolverine microbiota using NMDS and unweighted unifrac measure for a) sex, b) age class and c) harvest community, versus using NMDS and Bray-curtis measure for d) sex, e) age class and f) harvest community

Supplementary Table 3. Metadata of on- and offshore bears used in the study, including body condition, sex, season of capture and age class.

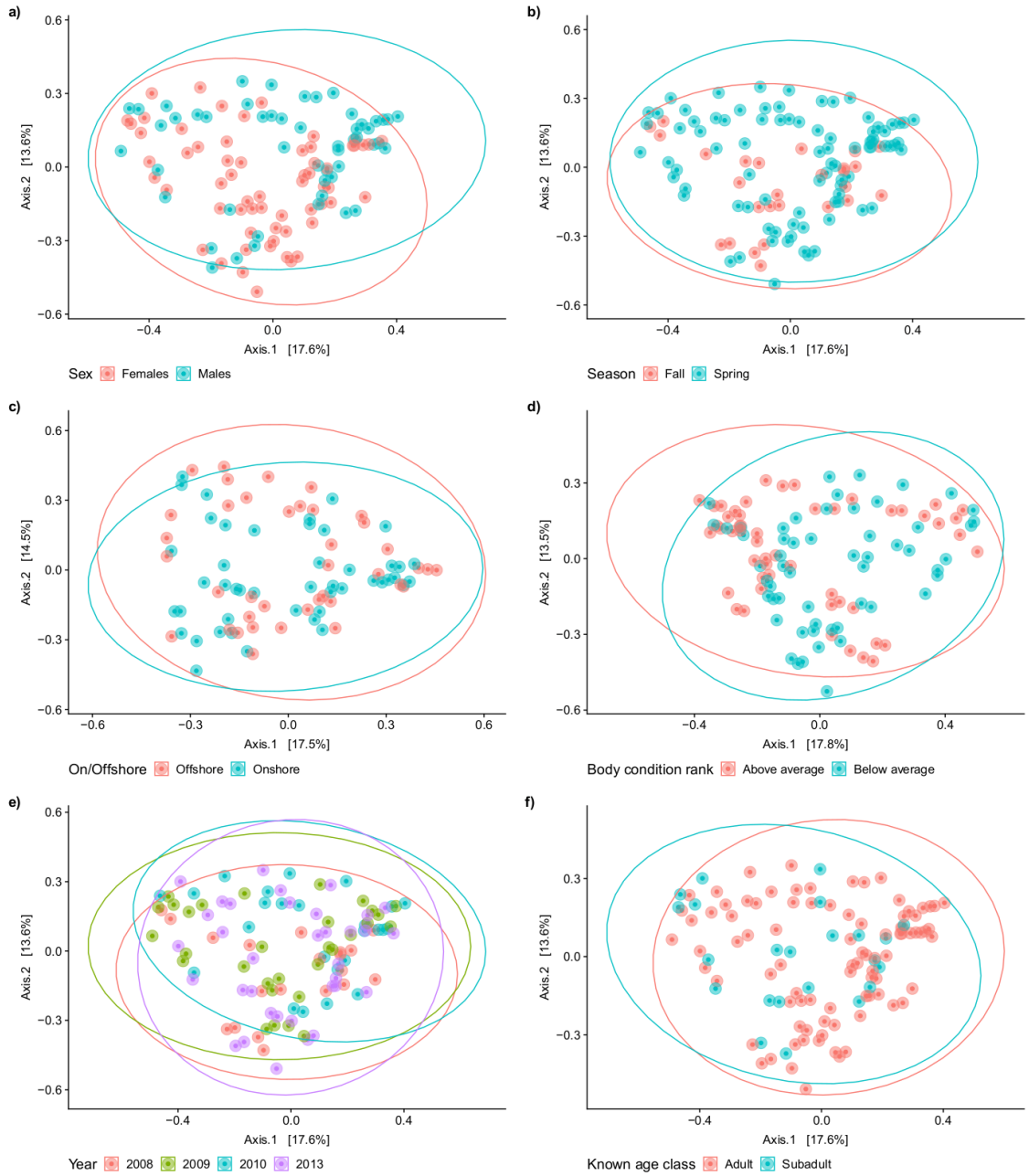
Metadata type	Onshore		Offshore	
Body condition*	**Above average**	**Below average**	**Above average**	**Below average**
	23	19	14	25
Sex	**Male**	**Female**	**Male**	**Female**
	18	28	22	17
Age class	**Adult**	**Subadult**	**Adult**	**Subadult**
	37	9	33	6
Season	**Spring**	**Fall**	**Spring**	**Fall**
	31	15	38	1

Supplementary Table 4. Results of the GLM showing that alpha diversity indices of polar bear faecal microbiota were not significantly different by sex, age class, body condition, year or season of capture, but only according to land use (on- vs offshore, P=0.02).

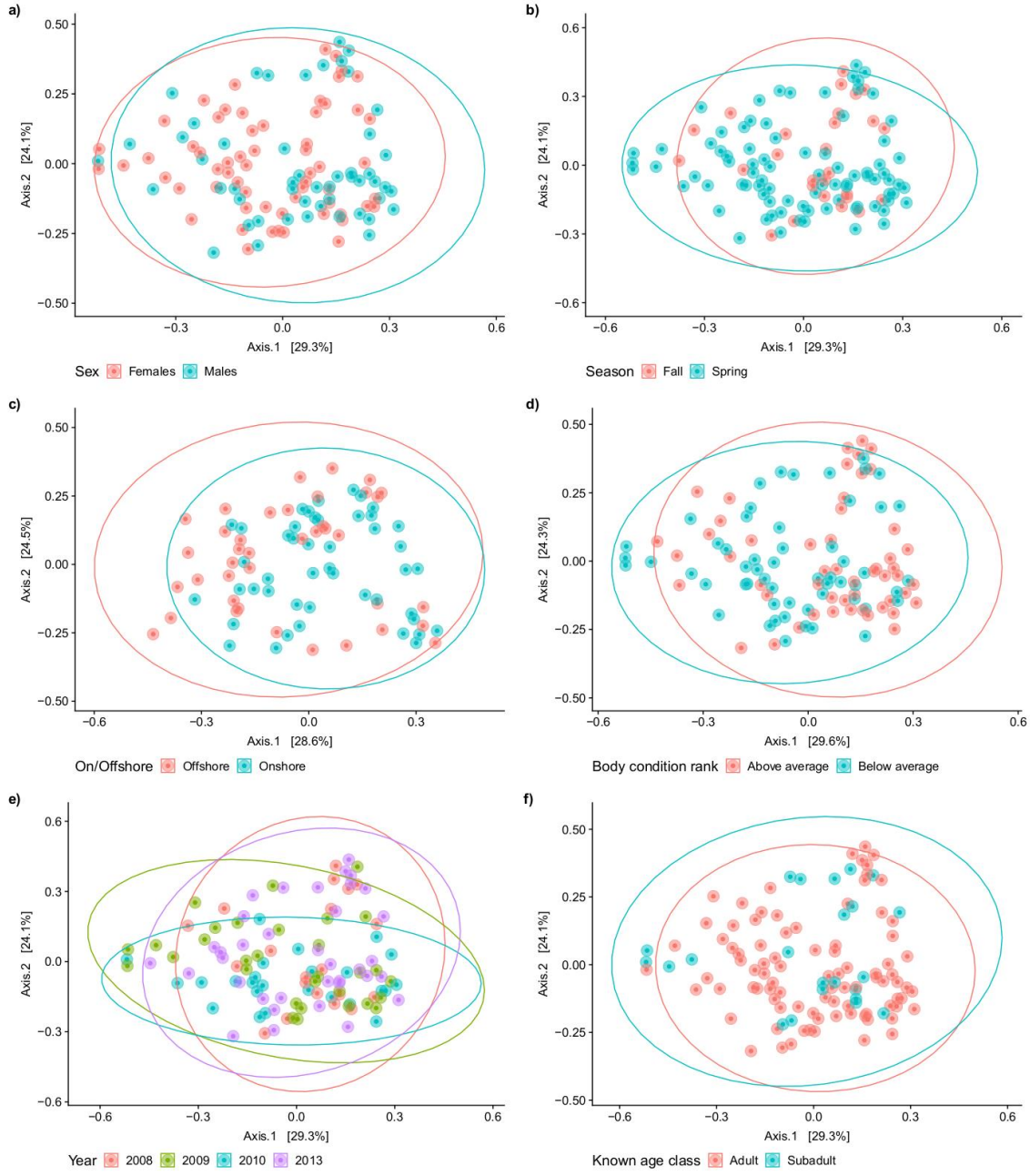
	Shannon index of diversity				Inverse Simpson index of diversity			
Variable within GLM	**Deviance**	**AIC**	**F-Value**	**P-Value**	**Deviance**	**AIC**	**F-Value**	**P-Value**
Year: Season	23.18	154.52	0	0.99	20.76	446.28	1.08	0.30
On/Offshore: Body Condition	23.18	152.53	0.02	0.9	21.76	445.45	1.01	0.32
Body Condition	23.21	150.64	0.09	0.76	21.12	443.75	0.26	0.61
On/Offshore: Year	23.51	151.99	0.18	0.68	20.26	448.25	0.48	0.49
Year	23.63	150.41	0.37	0.54	21.95	463.38	0.88	0.35
Sex: Age Class	23.81	149.07	0.6	0.44	20.45	447.01	0.63	0.43
Age Class	23.86	147.22	0.14	0.71	22.37	463.02	1.50	0.22
Sex: On/Offshore	24.07	146.00	0.73	0.4	22.94	463.2	2.08	0.15
On/Offshore: Season	24.39	145.09	1.04	0.31	20.12	449.66	0.26	0.60
Season	24.39	143.10	0.01	0.93	21.71	464.4	1.29	0.26
Sex	25.34	144.33	3.17	0.08	23.11	461.92	0.62	0.43
On/Offshore*	27.26	148.57	6.32	0.01	24.78	446.01	6.09	0.02

Supplementary Table 5. Results of the GLM showing that alpha diversity indices of polar bear faecal microbiota were not significantly different by land use (on- vs offshore), age class, body condition or year of capture, but only according to sex and season (P=0.017 and P<0.001, respectively).

	Faith's Phylogenetic Diversity			
Variable within GLM	**Deviance**	**AIC**	**F-Value**	**P-Value**
On/Offshore: Sex	2.76	906.4	0.34	0.56
Sex: Age Class	2.78	905.04	0.56	0.46
Age Class	2.78	903.09	0.03	0.86
On/Offshore: Year	2.8	901.64	0.48	0.49
Year	2.8	899.67	0.03	0.86
On/Offshore: Body Condition	2.89	900.43	2.46	0.12
On/Offshore	2.92	898.93	0.58	0.45
Body Condition	5.17	1222.4	0.08	0.78
Sex*	5.6	1270.8	5.83	0.02
Season*	7.39	1309.8	42.41	<0.001



Supplementary Figure 2. PCoA plots showing beta diversity for the variables **a.** sex; **b.** season of capture; **c.** land use (on- versus offshore); **d.** body condition; **e**. year of capture; **f.** age class, using the Bray-Curtis beta diversity index and 95% confidence interval (CI) ellipses.



Supplementary Figure 3. PCoA plots showing beta diversity for the variables **a.** sex; **b.** season of capture; **c.** land use (on versus offshore); **d.** body condition (rank); **e**. year of capture; **f.** age class, using the weighted UniFrac beta diversity index and 95% confidence interval (CI) ellipses.

Supplementary Table 6. Differential OTU abundance information from DESeq output, showing OTUs that were either significantly increased or significantly decreased in onshore compared to offshore bears.

	OTU	Phylum	Class	Order	Family	Log2 Fold Change	Adj P-value
Increased in onshore bears	DENOVO 41	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	6.78	<0.001
	DENOVO 14	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	5.69	<0.001
	DENOVO 20	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	5.07	<0.001
	DENOVO 121	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	4.58	0.01
	DENOVO 32	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	4.4	<0.001
	DENOVO 22	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	4.13	<0.001
	DENOVO 44	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	3.96	<0.001
	DENOVO 61	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	3.11	<0.001
	DENOVO 59	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	2.2	0.01
	DENOVO 8	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	2.15	<0.001
	DENOVO 37	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.81	<0.05
Decreased in onshore bears	DENOVO 40	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	-1.93	<0.05
	DENOVO 122	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	-2.55	<0.001
	DENOVO 82	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	-2.62	<0.05
	DENOVO 48	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	-3.39	0.01
	DENOVO 17	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	-3.66	<0.001
	DENOVO 43	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	-8.04	<0.001

Supplementary Table 7. Metadata associated with 91 southern Beaufort Sea polar bears for which faecal samples were collected for gut microbiota analysis. Individuals are separated by those for which i) mercury data was collected, ii) diet data was collected, and iii) both mercury and diet data was collected

Individuals with mercury data available only					
Polar bear	**Year**	**Age class**	**Sex**	**Mercury data available**	**Diet data available**
1	2008	Adult	Female	Yes	No
2	2009	Adult	Female	Yes	No
3	2009	Adult	Female	Yes	No
4	2013	Adult	Female	Yes	No
5	2013	Adult	Female	Yes	No
6	2013	Adult	Female	Yes	No
7	2013	Adult	Female	Yes	No
8	2013	Adult	Female	Yes	No
9	2013	Adult	Female	Yes	No
10	2013	Adult	Female	Yes	No
11	2013	Adult	Female	Yes	No
12	2013	Adult	Female	Yes	No
13	2013	Adult	Female	Yes	No
14	2013	Adult	Female	Yes	No
15	2013	Adult	Female	Yes	No
16	2013	Adult	Female	Yes	No
17	2013	Adult	Female	Yes	No
18	2013	Adult	Female	Yes	No
19	2013	Subadult	Female	Yes	No
20	2013	Subadult	Female	Yes	No
21	2013	Subadult	Female	Yes	No
22	2009	Adult	Male	Yes	No
23	2010	Adult	Male	Yes	No
24	2013	Adult	Male	Yes	No
25	2013	Adult	Male	Yes	No
26	2013	Adult	Male	Yes	No
27	2013	Adult	Male	Yes	No
28	2013	Adult	Male	Yes	No
29	2013	Adult	Male	Yes	No
30	2013	Adult	Male	Yes	No
31	2013	Adult	Male	Yes	No
32	2013	Adult	Male	Yes	No
33	2013	Adult	Male	Yes	No
34	2013	Adult	Male	Yes	No
35	2013	Adult	Male	Yes	No
36	2013	Adult	Male	Yes	No
37	2013	Adult	Male	Yes	No
38	2013	Subadult	Male	Yes	No
39	2013	Subadult	Male	Yes	No
40	2013	Subadult	Male	Yes	No
41	2013	Subadult	Male	Yes	No

Individuals with diet data available only					
Polar bear	**Year**	**Age class**	**Sex**	**Mercury data available**	**Diet data available**
42	2008	Adult	Female	No	Yes
43	2008	Adult	Female	No	Yes
44	2008	Adult	Female	No	Yes
45	2008	Adult	Female	No	Yes
46	2009	Adult	Female	No	Yes
47	2009	Adult	Female	No	Yes
48	2010	Adult	Female	No	Yes
49	2010	Adult	Female	No	Yes
50	2010	Adult	Female	No	Yes
51	2010	Adult	Female	No	Yes
52	2010	Adult	Female	No	Yes
53	2010	Adult	Female	No	Yes
54	2008	Subadult	Female	No	Yes
55	2008	Adult	Male	No	Yes
56	2009	Adult	Male	No	Yes
57	2009	Adult	Male	No	Yes
58	2009	Adult	Male	No	Yes
59	2009	Adult	Male	No	Yes
60	2009	Adult	Male	No	Yes
61	2010	Adult	Male	No	Yes
62	2010	Adult	Male	No	Yes
63	2010	Adult	Male	No	Yes
64	2010	Adult	Male	No	Yes
65	2008	Subadult	Male	No	Yes
66	2008	Subadult	Male	No	Yes
67	2009	Subadult	Male	No	Yes
68	2009	Subadult	Male	No	Yes
69	2009	Subadult	Male	No	Yes

Individuals with both mercury and diet data available					
Polar bear	**Year**	**Age class**	**Sex**	**Mercury data available**	**Diet data available**
70	2008	Adult	Female	Yes	Yes
71	2008	Adult	Female	Yes	Yes
72	2008	Adult	Female	Yes	Yes
73	2008	Adult	Female	Yes	Yes
74	2009	Adult	Female	Yes	Yes
75	2009	Adult	Female	Yes	Yes
76	2010	Adult	Female	Yes	Yes
77	2010	Adult	Female	Yes	Yes
78	2010	Adult	Female	Yes	Yes
79	2010	Adult	Female	Yes	Yes
80	2008	Subadult	Female	Yes	Yes
81	2009	Subadult	Female	Yes	Yes
82	2009	Adult	Male	Yes	Yes
83	2009	Adult	Male	Yes	Yes
84	2009	Adult	Male	Yes	Yes
85	2010	Adult	Male	Yes	Yes

86	2010	Adult	Male	Yes	Yes
87	2010	Adult	Male	Yes	Yes
88	2010	Adult	Male	Yes	Yes
89	2008	Subadult	Male	Yes	Yes
90	2010	Subadult	Male	Yes	Yes
91	2010	Subadult	Male	Yes	Yes

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