Gut bacterial composition and diversity of two geographically disparate polar bear (*Ursus maritimus*) subpopulations and the role of diet variation

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Natural Resource Sciences
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December 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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Abstract

The gut microbiome is an important component of host health and immunity, yet within and among wildlife populations, variation in gut microbial communities, and their main drivers, have received little attention to date. Notable drivers found in humans and lab animals are short- and long-term diets. Alterations in wildlife foraging behavior and food web dynamics can occur as a result of climate change-induced alterations to habitat, particularly in sensitive Arctic ecosystems experiencing extensive sea ice decline. As apex predators, polar bears (*Ursus maritimus*) serve as an important Arctic ecosystem indicator species. Different subpopulations of polar bears inhabit different ecoregions that are shaped by heterogeneous patterns of sea ice decline. This has led to regional variation in access to ice-associated seal species, their preferred prey, which could have implications for their respective gut microbiota. As such, this thesis aims: 1) to characterize the composition and diversity of the gut microbiota of two geographically disparate polar bear subpopulations—Southern Beaufort Sea (SB) and East Greenland (EG) subpopulations (Chapter 3) and 2) to develop a metagenomic diet assessment method to evaluate the role of feeding patterns in shaping gut microbial communities in polar bears (Chapter 4).

In Chapter 3, gut microbial communities were found to be distinct between SB and EG polar bears. A greater number of total (940 vs. 742) and unique (387 vs. 189) amplicon sequence variants (ASVs) were detected in SB compared to EG polar bears. Gut bacterial composition at bacterial class, genus, and ASV levels was also significantly different between the two subpopulations and among polar bear sex/age classes, possibly related to differences in foraging behavior. This hypothesis is further supported by findings for a subset of SB bears for which fatty acid (FA) signatures were available as chemical tracers of diet, showing that FA signatures were associated with both gut bacterial diversity and composition.

In Chapter 4, an indirect metagenomics-based approach to assessing polar bear diet from fecal samples was developed. It was validated relative to an established indirect method, quantitative fatty acid signature analysis (QFASA) from adipose biopsies, for a subset of SB polar bears. Ringed seal (*Pusa hispida*) was the predominant prey detected among SB and EG polar bears using both metagenomics-based and (for SB) QFASA diet assessment methods. Both methods detected bearded seal (*Erignathus barbatus*) as an important secondary prey item. The QFASA method quantified consumption of bowhead (*Balaena mysticetus*) and beluga whale (*Delphinapterus leucas*) among some SB polar bears, however DNA methods did not detect

cetacean prey. Short-term (DNA-based) diet was the main significant driver of variation in gut microbiome composition, but not diversity, for EG and SB polar bears.

The development of new and complementary diet assessment approaches provides a more complete picture of polar bear diet, and with this data it was possible to explicitly link diet as being an important driver of gut bacterial composition, information that is often lacking in studies on wild species of conservation concern. This research suggests that climate change induced habitat loss and subsequent alterations in food web dynamics may impact a critical aspect of polar bear health—the gut microbiome.

Résumé

Le microbiome intestinal est un aspect important de la santé et de l'immunité de l'hôte. Pourtant, au sein des populations d'animaux sauvages et entre elles, les variations des communautés microbiennes intestinales et leurs principaux facteurs ont reçu peu d'attention jusqu'à présent. L'un des facteurs les plus importants est le régime alimentaire à court et à long terme de l'espèce hôte. Des modifications du comportement de recherche de nourriture des individus et de la dynamique du réseau alimentaire peuvent survenir à la suite d'altérations de l'habitat induites par le changement climatique, en particulier dans les écosystèmes arctiques les plus sensibles qui subissent un déclin important de la glace de mer. En tant que prédateur suprême de l'Arctique, l'ours blanc (*Ursus maritimus*) est une importante espèce indicatrice des écosystèmes arctiques. Différentes sous-populations d'ours polaires habitent différentes écorégions qui sont façonnées par des modèles hétérogènes de déclin de la glace de mer. Ceci a conduit à une variation régionale dans l'accès aux espèces de phoques associées à la glace, leur proie préférée, ce qui pourrait avoir des implications sur leur microbiote intestinal respectif. Ainsi, cette thèse vise à 1) caractériser la composition et la diversité du microbiote intestinal de deux sous-populations d'ours polaires géographiquement disparates - les sous-populations du sud de la mer de Beaufort (SB) et de l'est du Groenland (EG) (Chapitre 3) et à 2) développer une méthode métagénomique d'évaluation du régime alimentaire, qui peut être utilisée pour évaluer le rôle des habitudes alimentaires dans la formation des communautés microbiennes intestinales des ours polaires (Chapitre 4).

Dans le chapitre 3, les communautés microbiennes intestinales se sont avérées distinctes entre les ours polaires SB et EG. Un plus grand nombre de variants de séquence d'amplicon (ASV) totaux (940 contre 742) et uniques (387 contre 189) a été détecté chez les ours polaires SB par rapport aux ours polaires EG. La composition bactérienne intestinale au niveau des classes bactériennes, des genres et des ASV était également très différente entre les deux sous-populations et entre les classes de sexe et d'âge des ours polaires, ce qui pourrait être lié à des différences dans le comportement de recherche de nourriture. Cette hypothèse est également soutenue par les résultats obtenus pour un sous-ensemble d'ours SB pour lesquels des signatures d'acides gras (AF) étaient disponibles comme traceurs chimiques du régime alimentaire, montrant que les signatures d'AF étaient associées à la fois à la diversité et à la composition des bactéries intestinales.

Dans le chapitre 4, une approche indirecte basée sur la métagénomique a été développée pour évaluer le régime alimentaire des ours polaires à partir d'échantillons fécaux. Elle a été validée par rapport à une méthode indirecte établie, l'analyse quantitative de la signature des acides gras (QFASA) à partir de biopsies adipeuses, pour un sous-ensemble d'ours polaires SB uniquement. Le phoque annelé (*Pusa hispida*) était la proie prédominante détectée chez les ours polaires SB et EG en utilisant les deux méthodes d'évaluation du régime alimentaire basées sur la métagénomique et la QFASA, et les deux méthodes ont détecté le phoque barbu (*Erignathus barbatus*) comme une proie secondaire importante. La méthode QFASA a permis de quantifier la consommation de baleine boréale (*Balaena mysticetus*) et de béluga (*Delphinapterus leucas*) chez certains ours polaires SB, mais les méthodes basées sur l'ADN n'ont pas détecté de proies cétacées. Le régime alimentaire à court terme (basé sur l'ADN) et les différences de sexe et d'âge des ours polaires se sont avérés être des facteurs importants de variation de la composition du microbiome intestinal, mais pas de la diversité, chez les ours polaires EG et SB.

Cette recherche nous permet de mieux comprendre comment la perte d'habitat induite par le changement climatique et les modifications subséquentes de la dynamique du réseau alimentaire peuvent avoir un impact sur un aspect essentiel de la santé des ours polaires, à savoir le microbiome intestinal. Le développement de nouvelles techniques complémentaires d'évaluation du régime alimentaire a permis de dresser un tableau plus complet de l'alimentation des ours polaires. Grâce à ces données, il a été possible d'établir un lien explicite entre le régime alimentaire et la composition des bactéries intestinales, une information qui fait souvent défaut dans les études sur les espèces sauvages dont la conservation est préoccupante.

Acknowledgements

Thank you to my supervisor Dr. Melissa McKinney, first for providing me with an opportunity to fly across the continent and join a new lab in a new country, and then, of course, for her support and guidance throughout the development and completion of this thesis. Her intense and detailed critiques have improved this work substantially. I have learned so much from her as a mentor and have been inspired by her research accomplishments over these last two and a half years. I would also like to thank my co-supervisor Lyle Whyte for allowing me to work in his laboratory and for introducing me to the Whyte Lab support crew. Especially Brady O'Connor for helping to get me settled in a busy and bustling lab.

I am grateful for both the academic and emotional support provided to me by several members of the McGill McKinney lab, past and present: Elena McCulloch, Rose Lacombe, Nasrin Golzadeh, Anaïs Remili, Nadia Facciola, Adam Pedersen, Haley Land-Miller, and in particular Kailee Hopkins who truly took care of me in the treacherous final few weeks of thesis crunch time—a blissful domestic partnership (or dependency?). Both forms of support are incredibly, if not equally, important aspects of the McKinney lab culture and I would not have made it to the finish line without such a supportive environment to conduct research in.

I was also fortunate to have support from several mentors turned co-authors of these thesis chapters, of which there are many. A special thank you to Sophie Watson, who provided help with experimental methods development and kind support in results interpretation, and kindly reminded me "there is no such thing as a stupid question." Thank you to Esteban Góngora who certainly earned his status as a co, co-supervisor and endured several rants related to my statistical analysis rough patches. Thank you to my committee members Kristin Laidre and Jessica Head for providing valuable feedback and validation of my degree progress at committee meetings. This was particularly valuable to me given the challenges of the COVID-19 pandemic and subsequent changes to the graduate student experience and work style.

Finally, my greatest thanks goes out to family and friends who picked up phone calls at the drop of a dime and did their best to enter my little research realm to try and understand and support me. Mama Jess, for the run chats and vent sessions (often occurring in below freezing temperatures) and a heck of a lot more. Mama Franz for pretty much everything—there are not enough words to describe how fortunate I have been to have you as my champion for success for all of the years of my life. My sister Emily and brother Matt for their endless advice and

affirmation, and just for being the coolest, most supportive siblings. My dad and Sandra for all their love and support, and for entertaining many fireside research chats about this project. If I have missed anyone I apologize and you have every right to call me out for it (but only if you read this entire thesis). Just know that even if I forgot to include you your support did not go unrecognized or unappreciated!

Contribution of Knowledge

This is a manuscript-based thesis following McGill guidelines and consists of two chapters prepared for publication. The candidate is the primary author on both data chapters, having performed the laboratory analyses, data analyses, results interpretation, and writing of the manuscripts.

Chapter 3 is co-authored by Melissa M. McKinney, Lyle G. Whyte, Todd C. Atwood, Kristin L. Laidre, Denis Roy, Sophie E. Watson, and Esteban Góngora. Melissa A. McKinney, Lyle G. Whyte, Todd C. Atwood, and Kristin L. Laidre designed the study and Todd C. Atwood and Kristin L. Laidre collected samples in the field. Megan Franz performed the laboratory work, analyzed the data, and wrote the initial draft of the manuscript. Lyle G. Whyte, Sophie Watson, and Esteban Góngora advised on lab protocols and interpretation of results. Denis Roy assisted with data analysis and visualization. All authors helped to critically review and edit the final version.

Chapter 4 is co-authored by Lyle G. Whyte, Todd C. Atwood, Damian Menning, Sarah A. Sonsthagen, Sandra L. Talbot, Kristin L. Laidre, Emmanuel Gonzalez, and Melissa A. McKinney. Melissa A. McKinney, Lyle G. Whyte, and Megan Franz designed the study and Todd C. Atwood and Kristin L. Laidre collected samples in the field. Sarah A. Sonsthagen, Sandra L. Talbot, and Damian Menning advised on aspects of experimental design, Megan Franz performed the laboratory work, analyzed the data, interpreted results, and wrote the initial draft of the manuscript. Emmanuel Gonzalez assisted with data processing and results interpretation. Melissa A. McKinney advised on interpretation of results. All authors helped to critically review and edit the manuscript.

Chapter 1: General Introduction

Climate change is a major driving force behind current ecosystem changes [1, 2], including shifts in species' abundances and distributions. Such species' redistributions can alter food web connections, which in turn, particularly when observed in apex predators, can often have cascading trophic level impacts [3, 4]. Dietary shifts may also impact predator health in a variety of ways, including by altering the composition of their gut microbiota. The gut microbiome serves key nutrient acquisition and immune defense roles for the host, and studies on experimental animals and humans indicate that gut microbial composition is strongly influenced by diet [5]. Nonetheless, the gut microbiome remains relatively understudied in wild populations, and moreover, its susceptibility to climate change-induced alterations in trophic ecology is virtually unknown [6].

The effects of global climate change are particularly profound in the Arctic, which is warming twice as fast as the global average [7]. Rapid loss of sea ice in the Arctic is having negative consequences for certain species, including the top predator polar bear (*Ursus maritimus*), with decreases in sea ice linked to declines in their body condition, vital rates, and population sizes for some subpopulations [8-11]. This is primarily because sea ice loss represents loss of crucial foraging habitat from which they hunt for their primary prey, ringed seals (*Pusa hispida*) [8, 9, 12, 13]. Yet, distinctive sea ice dynamics result in differing habitat and prey-type availability among polar bear subpopulations, for instance, those in East Greenland (EG) versus the Southern Beaufort Sea (SB) [9, 14]. The EG subpopulation inhabits one of the convergent ice ecoregions, meaning that sea ice from other regions converges in this area providing access to ice seal prey year-round [14]. While this has been suggested to potentially delay the impacts of sea ice loss compared to other polar bear subpopulations, sea ice decline in Greenland has coincided with certain sub-Arctic seal species — harp seals (*Phoca groenlandica*) and hooded seals (*Crystophora cristata*) — moving further northward for longer periods of time [15] into EG polar bear habitat [8]. For comparison, the SB polar bear subpopulation inhabits one of the divergent ice ecoregions, where a vast and growing open water area now develops between the shoreline and retreating ice edge during the summer and fall months [16]. Some bears in the SB population remain on the sea ice to continue hunting seals, albeit in a less productive region in the far north, while others shift to onshore habitat during the open water periods and consume

terrestrial-based food resources such as subsistence-harvested bowhead whale carcasses and shorebirds [17, 18]. Onshore and offshore SB polar bears have recently been shown to have significant differences in their gut microbial composition and diversity [10].

Many metabolic and immune system processes of higher-order organisms are carried out, in part, by the assemblage of microbes found within their gastrointestinal (GI) systems [19, 20]. Therefore, the gut microbiome can have a strong influence on individual health. While factors such as age, sex, local environment, host genetics, and host phylogeny are known to influence diversity of microbes in the gut, diet has consistently been identified as a driver of bacterial community composition [5, 21]. This relationship between diet and gut bacterial composition is well-established in human and experimental animal studies [5, 22, 23], it has only recently started to be explored in studies on wildlife [24, 25]. Multiple indirect methods of assessing the feeding patterns of wildlife have been developed and utilized, such as quantitative fatty acid signature analysis and stable isotopes, which have been applied to the study of polar bear diet [17, 26]. However, no method is currently available for polar bears that could provide dietary information from the same sample taken for microbiome analysis, i.e., a fecal swab. DNA metabarcoding of fecal samples was recently successfully applied to walrus (*Odobenus rosmarus*) [27], and may also provide critical insight into polar bear diets, if developed.

The primary aim of this thesis is to provide compare the gut microbial composition and diversity between the SB and EG polar bear subpopulations, and to develop a DNA metabarcoding approach, which can used to evaluate the role of diet in shaping gut microbial composition and diversity in these two subpopulations. The specific research objectives are as follows:

(1) To characterize the gut microbial community composition and diversity for EG and SB polar bear subpopulations and to preliminarily assess influence of diet on gut microbiota for a subset of SB polar bears;

(2) To develop a DNA metabarcoding approach to assess EG and SB polar bear diets from fecal samples, including a comparison to diet estimates obtained using quantitative fatty acid diet analysis for a subset of SB polar bears, and further to use the developed approach to investigate the role of diet differences in shaping the gut bacterial community of EG and SB polar bears.

This thesis has five chapters, including this general introduction and accompanying literature review. The literature review covers the functional role of the gut microbiome and factors known to influence gut bacterial composition and diversity, climate change as a driver of ecosystem-level change (particularly in sensitive Arctic ecosystems), key polar bear life history traits with an emphasis on historic diet and foraging behavior, and methods for assessing diets of free-ranging species. Chapter three focuses on characterizing the gut microbial community composition of EG and SB polar bears and describes the factors contributing to observed differences between the two subpopulations (Objective 1). Chapter four develops and validates a metabarcoding approach to assessing polar bear diet and compares results of this method to results from a previously established quantitative fatty acid signature analysis (QFASA) method of diet analysis, and further evaluates how variation in diet influences gut microbial community composition for the EG and SB polar bears (Objective 2). Chapter five includes a general discussion on key findings and conclusions from my MSc research with suggestions for future work.

Chapter 2: Literature Review

2.1 Diet as a driver of variation in gut bacterial composition and diversity

The gut microbiome—comprised of fungi, viruses, small eukaryotes, and predominantly bacteria—is an important component of organism health, and most notably the community of bacteria present in the gastrointestinal (GI) tract serve key nutrient uptake and immune regulation functions for their host [19, 28]. There are billions of bacteria present throughout the GI tract, meaning the collective genomic capacity of the bacteria found in the gut far exceeds that of the organisms [29]. As such, the functional capacities of gut bacteria are wide-ranging and cover metabolism of essential food macromolecules as well as other substances encountered by the host organism [29, 30]. Some gut bacteria have even been implicated as key metabolizers of synthetic, potentially harmful chemicals which suggests potential detoxification functions for the host species [31]. A large body of evidence also outlines the various immune defense roles of the gut microbiome as it can act as a barrier to pathogens encountered in the host's environment [32]. Given these important functions of the gut microbiome and its role in maintaining host health, it has been suggested that the adaptive potential of the gut microbiome is also likely an important aspect of host adaptation[29, 33].

The interplay of factors such as diet, host immune system, host phylogeny, early life exposure, and gut physiology can influence the presence or absence of particular bacterial species, as well as their abundances, within the gut [19, 34, 35]. Diet has been identified as a key long- and short-term driver of variation in gut bacterial composition and diversity [5, 21, 36-39]. In studies on the human gut microbiome and those using mouse models, it has been shown that many of the macronutrients the host organism consumes are modified or transformed by the bacteria present in the gut for use for energy and growth of the bacteria and host, as well as for downstream host metabolic processes [23]. While it is generally assumed that this is also the case for wild species [19, 28], it is more challenging to investigate diet impacts on the gut microbiome in wild populations. In the wild, other aspects related to diet such as individual foraging behavior and diet sources (i.e. the local environment and the diversity of coexisting species within an ecosystem) can also impact the bacteria found in the gut microbiome given the variety of gut bacterial colonization processes that exist [40]. One study on freshwater fish species found differences in gut bacterial diversity among individuals demonstrating generalist or specialist feeding patterns, with generalists having decreased gut bacterial diversity compared to

specialists, which was also associated with higher body condition [41]. The opposite pattern has also been found, however, and a predictable relationship between body condition and gut bacterial diversity has not been determined; nonetheless, there is a clear link between diet, gut bacterial diversity, and host health [41]. Diet macronutrient composition and diet sources have also been shown to introduce novel species to the gut, however whether or not successful inoculation and proliferation occurs depends on several other factors such as gut physiology, host immune system, and competition from preexisting bacterial species [40]. To fully understand the role of diet in relation to host gut microbiome composition, and ultimately how this relationship might impact host physiology and health for wild species, it may be important to improve diet analysis techniques to further explore associations between diet and gut bacterial composition and diversity in wildlife [42].

Considering the drastic global environmental changes occurring in the Anthropocene era, understanding the role of the gut microbiome for wildlife health, and the drivers of variation and change in the gut microbiome, should be of concern for wildlife research and conservation [42, 43]. Many species are threatened or vulnerable due to climate change-induced alterations of habitat and feeding habits, which can have downstream consequences on species health and survival [2, 44]. Gut microbiome research focused on wildlife is growing as we recognize the importance of this component of host health in relation to individual and species survival. However, it remains a challenge to measure and monitor baseline gut microbiome composition and diversity, predict how it might change over time, and to determine what the important drivers of gut microbiome composition and diversity are [43, 45]. Understanding individual-, species-, and population-level variation in the gut microbiome composition and diversity and what drives this variation could be valuable in its application to conservation of threatened species [42].

Diet has been well-documented in human and controlled lab studies as an important driver of gut microbiome composition and diversity, but diet-microbiome associations are only recently starting to be assessed in wildlife studies [5, 22, 23, 37, 38, 46]. A few recent studies have successfully identified relationships between diet and gut microbiome composition in wildlife. For example, Sugden et al. (2020) detected differences between the gut microbiota of urban and rural coyotes (*Canis latrans*), and directly tied this to differences in their diets by using a combination of stable isotope and stomach content data. Urban coyotes that fed on anthropogenic

food sources more frequently than rural coyotes were found to have higher gut bacterial alpha diversity, as well as altered gut bacterial composition, which reflected the more varied nature of the urban coyote diet and macronutrient differences [24]. Gongora et al. (2021) used stable isotope analysis to infer trophic position among thick-billed murres (*Uria lomvia*), and that trophic differences among sexes influenced their gut microbiome composition. Studies such as these showcase the ability to link broad-scale diet data to the gut microbiome of wild species. Yet, gaps remain as these types of diet analysis techniques might not cover appropriate dietary timescales for assessing diet:gut microbiome relationships, and often they cannot provide species-level resolution of prey. Moving forward, developing new dietary analysis techniques, or using multiple techniques in combination, could be useful for overcoming such barriers, helping to improve our understanding of the relationship between diet and gut microbiome diversity and composition and how it relates to host health and species persistence [42, 43, 47].

2.2 Climate change alters interspecific interactions

The variable and complex consequences of global climate change have been well-documented over the last several decades in the scientific literature. From case examples of shifts in local species ranges and distributions to follow their shifting habitat, to large-scale observed patterns of sea ice decline in the Arctic [44]. Increasing global average surface temperatures are directly linked to anthropogenic emissions of greenhouse gases [7]. Such increase in average global temperatures can lead to unpredictable weather patterns and shifts in microclimates, which can ultimately influence biome-level and ecosystem-level changes across the planet. Change is not an entirely novel concept when it comes to ecosystems and biodiversity of our planet, however the rapid rate of change occurring in the Anthropocene is unprecedented [48]. When faced with such drastic environmental change, a natural response is for organisms to adapt their behavior or redistribute to retain more preferable environmental conditions for survival [1, 49]. Examples of redistributions include terrestrial species shifting poleward as temperatures near the equator rise, or species shifting to higher elevation as lower elevation climates increase in temperature beyond what species have adapted to over time. Similarly, some marine species have also shifted poleward as water temperatures become less favorable, while others have been observed retreating to deeper, cooler waters to escape rising ocean temperatures [1, 50]. When these types

of distribution or range shifts occur, they can alter interspecific interactions or species-environment interactions and lead to overall disruption of ecosystem functioning [1, 2, 44].

One such example is potential habitat loss, which can affect key life history stages of organisms. Climate-driven habitat loss in some regions can lead to declines in food availability, leading to worsening body condition of individuals threatening both individual and population survival for some species [51]. Other risks of climate-driven environmental change include range expansion of pathogens and higher transmission rates due to simultaneous host range expansion [52]. Not only is new habitat opening up, which can increase competition and predation, but some areas are becoming more isolated which can interrupt interpopulation gene flow and lead to bottlenecks that reduce individual fitness and can impact population dynamics of a species [1].

The Arctic is uniquely sensitive to climate-driven environmental changes as it is experiencing annual temperature increases that are up to two times higher than the global average[7]. Arctic sea ice is an important component dictating global weather patterns, and it is being lost at an alarming rate [53, 54]. The loss of surface albedo, or radiative reflecting potential of the ice's white surface, further exacerbates warming forces by contributing to a positive feedback loop resulting in continued warming and further reductions in ice [55]. The interannual patterns of sea ice decline show minimum sea ice extent reductions of approximately 45,000km^2 per year (Post 2009), the consequences of which have been severe and numerous, particularly over the last few decades. Sea ice serves as substrate for microalgae and plankton growth, both of which contribute to nearly 50% of primary productivity in the Arctic. Sea ice is also critical habitat for most species found throughout the circumpolar Arctic which use sea ice for finding mates, making dens, raising young, and to forage for prey. Continued reductions in sea ice extent and duration will severely reduce habitat and food availability for many of these species [44].

2.3 Polar bears as an Arctic ecosystem indicator species

Polar bears are one such species listed as highly vulnerable on the IUCN red list due to reductions in sea ice habitat [56]. As an apex predator and keystone Arctic species, understanding polar bear population dynamics and ecology provides essential insight into the overall Arctic ecosystem health and functioning in this period of unprecedented sea ice decline [11, 15, 57].

Polar bears are highly specialized when it comes to their foraging ecology. They are, equipped with large, padded paws for enhanced swimming and sharp, hook-like canines for shearing tissue [58]. Most notably, they are able to rapidly convert lipid energy to fat storage due to their uniquely evolved metabolism, an adaptation that reflects thousands of years of diet specialization [59, 60]. While polar bears are also known to occasionally prey upon other species when seal prey is limited—including beluga whale (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), walrus (*Odobenus rosmarus*), bowhead whale (*Balaena mysticetus*), and terrestrial scavenge including berries, shorebirds, and washed-up carcasses [12, 13, 58, 61-64]—they are highly evolved to the calorie-rich high-fat diet of seal blubber [60]. Reduced access to seal prey has been linked to declines in polar bear body condition and population declines in certain subpopulations [11, 65-68].

Currently, there are 19 recognized subpopulations of polar bears distributed throughout the circumpolar Arctic, each distinguished by geographic barriers and characterized by the four different ice-ecoregion types within which they are found: polar basin divergent ice, polar basin convergent ice, Canadian archipelago ice, and seasonal ice [14, 56, 57, 65] (Fig 2.1). These ecoregions were defined based on their different spatial and temporal patterns of sea ice movement and circulation due to Arctic Ocean currents, as well as based on seasonal differences in sea ice melt and freezing. In divergent ice ecoregions there is dramatic formation and melting of annual sea ice, typically in the direction of the central polar basin and out via the Fram Strait (Fig. 2.1), while in convergent ice ecoregions sea ice converges and there is typically extensive multiyear ice. Historically, polar bears found in the polar basin and archipelago ecoregions remain on sea ice throughout the year [14]. Archipelago regions have both multiyear and annual sea ice that persists among the interisland channels, allowing polar bears to remain on sea ice. Five subpopulations occur in the seasonal ice ecoregion, where during the summer months, polar bears are forced entirely onshore and tend to have reduced access to food.

Southern Beaufort Sea (SB) polar bears are at potentially higher risk from declines in sea ice habitat than other subpopulations. The SB subpopulation is distributed along the northern shore of Alaska and the Yukon within the divergent ice ecoregion (Fig. 2.1), where patterns of summer sea ice melt are such that the ice diverges away from the highly productive continental shelf creating a vast open water space between the shoreline and the sea ice edge, which is energetically costly for polar bears to navigate between [69]. Thus, over the last several decades,

some polar bears in this subpopulation have been increasing their use of land-based habitat, and while there in the summer and fall months, are exploiting onshore food resources [12, 17, 70]. Polar bears that remain onshore during this time have access to subsistence-harvested bowhead whale carcasses, shorebirds, and other terrestrial foods [64], while bears that stay on the sea ice edge as it retreats, retain the possibility of accessing seals and other ice-associated prey. A large body of work on this on this subpopulation suggests many knock-on consequences from this change in behavior, from possible insufficient nutritional replacement to risks of increased exposure to novel pathogens and increased human-polar bear interactions [9, 12, 62, 63, 66, 70].

For comparison, the East Greenland (EG) polar bear subpopulation is found in the convergent ice ecoregion, wherein sea ice remains largely present year-round despite reductions (Fig. 2.1). This area receives additional sea ice from the polar basin, which flows southward along the East Greenland shoreline allowing polar bears relatively continuous access to sea ice as a hunting platform [14]. Although EG polar bears may continue to largely forage on high-fat seal species, they have shown increased consumption of sub-Arctic seal species over the past three decades [8]. These sub-Arctic seal species tend to be larger-bodied and higher in trophic position, and thus typically carry higher endocrine and immune disrupting contaminant loads [8]. Polar bears that show increased consumption of these seal species could be exposed to higher contaminant levels themselves by means of contaminant biomagnification processes. While the change in macronutrient content may be less with this type of dietary shift, risks such as these that are linked to diet changes over time will be important to continue monitoring over time.

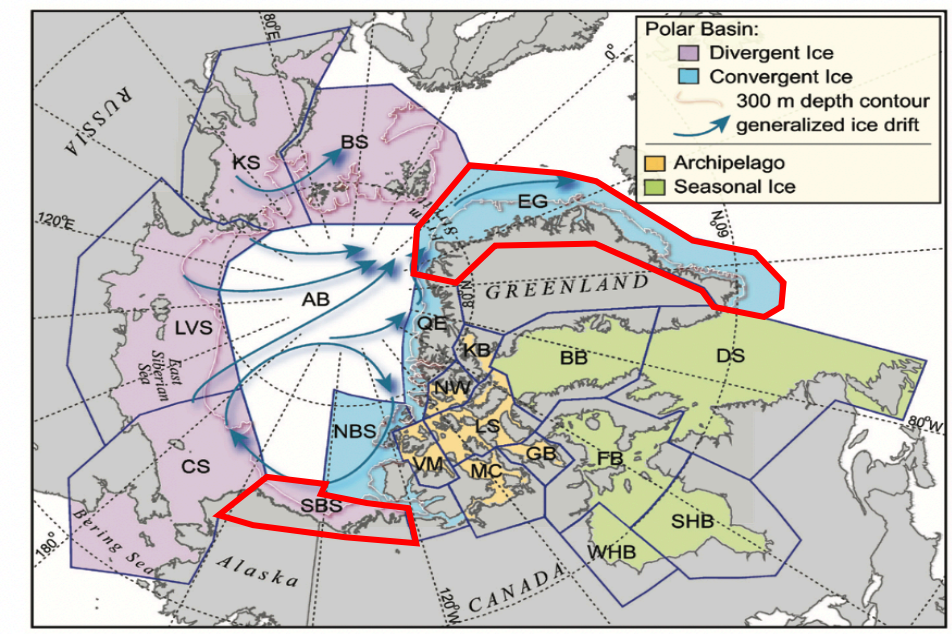

Figure 2.1 Map of the circumpolar Arctic showing the 19 polar bear subpopulations with sea ice ecoregion designations superimposed. Red borders indicate the Southern Beaufort Sea subpopulation (*noted here as SBS), which occurs in the divergent ice ecoregion (purple). and the East Greenland (EG) subpopulation, which occurs in the convergent ice ecoregion (blue). The remaining 17 subpopulations noted here are Chukchi Sea (CS), Laptev Sea (LVS), Kara Sea (KS), Barents Sea (BS), Queen Elizabeth (QE), Northern Beaufort Sea (NBS), southern Hudson Bay (SHB), western Hudson Bay (WHB), Foxe Basin (FB), Davis Strait (DS), and Baffin Bay (BB), Gulf of Boothia (GB), M'Clintock Channel (MC), Lancaster Sound(LS), Viscount-Melville Sound (VM), Norwegian Bay (NW) and Kane Basin (KB). Modified from Amstrup et al. 2008.

Dietary shifts such as those highlighted in the EG and SB subpopulations could lead to the alteration of gut homeostasis in polar bears, however, only a few studies in the last decade have sought to describe the gut microbiome of polar bears. Glad et al. (2010) examined bacterial diversity in feces from ten individual polar bears using 16S rRNA gene clone libraries. All clone libraries generated assigned back to the bacterial phylum Firmicutes, with a majority of these belonging to order Clostridiales and genus *Clostridium*. In general, Glad et al. (2010) concluded that these polar bears, which were sampled in Svalbard, Norway had lower gut bacterial diversity compared to other Arctic carnivore and Ursid species [71]. More recently, Watson et al. (2019) compared the gut microbiota of onshore and offshore polar bears from the SB subpopulation using 16S rRNA gene metabarcoding and next-generation sequencing methods (NGS).

‘Onshore’ describes the polar bears from the SB subpopulation that remain on land during the reduced ice season and thus having access to land-based food resources, such as subsistence-harvested bowhead whale carcasses, shorebirds, and other terrestrial foods. In contrast, ‘offshore’ polar bears remain on the sea ice as it retreats away from the shoreline and can continue to forage on ice-associated marine mammal prey. This study showed a greater number of operational taxonomic units (OTUs) and significantly higher alpha diversity for the gut microbiota of onshore compared to offshore bears. The NGS methodology employed in this study also enabled detection of a wider range of bacterial phyla and classes than the initial Glad et al. (2010) study. Overall, Watson et al (2019) showed that climate-induced alterations in habitat use are associated with shifts in the gut microbiota of polar bears. While their findings point to the possibility of these changes being driven by dietary differences between the two groups of SB polar bear, diet was not explicitly analyzed in this study [10].

2.4 Methods for assessing diet of wild species

Understanding the diets of individuals and species is essential for tracing food web dynamics and trophic interactions within an ecosystem and can be predictive of individual and species survival. Additionally, diet data can inform studies that examine the gut microbiome of wild species, connecting feeding patterns and changes to a known aspect of individual health (the microbiome), which can broaden our understanding of wildlife health and conservation threats [24, 25, 43]. Multiple methods of diet analysis for wild animal species have been developed, each with their respective benefits and limitations regarding the degree of dietary insight they provide and biases they have [47]. Earlier diet analysis approaches that are still used include feeding observations and assessment of stomach contents and hard parts (i.e. otoliths) analysis from stomach contents or feces [72]. It can be difficult to accurately identify certain prey items from stomach contents due to degradation of soft tissues. Further, it can often be challenging to observe species foraging in their natural environment. Both stomach content and observation-based diet assessments also only provide a snapshot into the most recent meal prior to sample collection [73]. To compliment these approaches and possibly avoid some of their limitations, bulk stable isotope (SI) assessment, fatty acid (FA) signature analysis, and DNA-based diet analysis approaches can be of use [74, 75].

FA signatures yield qualitative and quantitative insight into individual diet patterns and can help delineate ecological food web dynamics within a given ecosystem [47, 76, 77]. FAs are synthesized at the base of the food chain by primary producers and certain FAs are incorporated into fat storage tissues (e.g., adipose, blubber) of higher trophic level organisms in relatively unmodified or predictably modified proportions. As such, FA-based diet signatures typically reflect prey consumed in the previous few weeks to months, making FA diet estimates a useful metric indicating more long-term dietary habits [77]. In marine ecosystems there are approximately 30 or so "dietary" FAs that have been used to produce quantitative estimates of diet via quantitative fatty acid signature analysis (QFASA) [77]. This approach has been used to successfully estimate the proportions of different specified prey items consumed by a predator, however, can also be limited in its applications due to the requirement of developing a comprehensive prey library. Ideally, all possible prey species are represented in the prey library yet some could be missed as *a priori* knowledge of predator diet is required for analysis and library development. Further, prey species with similar FA signatures are sometimes poorly distinguished by the model, and the model also relies on predator-specific calibration coefficients that account for metabolic modifications of prey FAs as they are incorporated into the predator FA signature. Despite potential limitations, FA analysis and QFASA methods have been used numerous times in studies delineating polar bear diet, across many different subpopulations [8, 17, 61, 78, 79]

Recently-developed DNA-based diet analysis methods may be useful in improving our understanding of the diets of wild species [47]. DNA-based methods are a generally non-invasive as they require only a fecal sample or rectal sample of the study species and as next-generation sequencing (NGS) techniques become more affordable such an approach has the potential to provide vast amounts of high taxonomic resolution diet data [80]. There are many factors to consider when designing a DNA-based diet assessment approach, and in general there are two more frequently discussed and used DNA metabarcoding approaches, both of which involve polymerase chain reaction (PCR) amplification of a target gene region [80, 81]. The first is the use of group- or prey-specific primer sets that target only one or a few different prey species and produces data with high taxonomic specificity, and simultaneously minimizes co-amplification of host DNA that typically predominates in samples [73]. The second is the use of a more general primer set that captures a broad range of taxonomic diversity, however often at the cost

of reduced taxonomic resolution of potential prey species of interest. Further, this approach occasionally over-detects "rare" species that are ultimately biologically irrelevant in terms of their contribution to host diet [80]. For both of these metabarcoding approaches, choice in gene region target and primer set(s) is critical as both can strongly influence the range of prey species detectable and potentially introduce PCR bias in the form of preferential amplification of certain prey species relative to others. The use or development of a well-curated reference database also affects the final taxonomic resolution of prey species for both of these approaches and should be carefully considered in study design and methodology [80]. There are many choices to make along the way regarding DNA-based diet approaches, and the right ones are heavily dependent upon the study organism and specific research questions in mind [80, 82]. Another caveat to DNA-based methods of diet analysis relates to the current challenges in quantifying the proportions of different prey consumed using these approaches. In some cases, the use of quantitative PCR methods applied to experimentally controlled diet studies has allowed for accurate quantitative estimates of prey consumed [83-85], however, others have indicated that this may be a key limitation of the approach, especially when applied to studies of wild animals with complex diets, or in cases when another diet analysis approach cannot be concurrently conducted to validate the DNA-based findings [47, 80, 81]. Despite these challenges related to study design and execution, high-resolution DNA-based diet data could serve as an important biomonitoring tool with on-going testing and development.

Connecting text:

To answer the first research objective, in Chapter 3 we characterize the gut microbiomes for EG and SB polar bears using 16S rRNA gene metabarcoding techniques and compare inter- and intra-population variation in gut bacterial diversity and composition. Our goal was to determine the factors that could lead to differences observed in composition and diversity observed for EG and SB polar bears, and preliminarily explore the influence of diet on SB gut microbiota using fatty acid diet analysis approaches.

Chapter 3. Distinct gut microbiomes in two polar bear subpopulations inhabiting two sea ice ecoregions

Note on this chapter

This chapter corresponds exactly to the manuscript titled "Distinct gut microbiomes in two polar bear subpopulations inhabiting different sea ice ecoregions" which has been accepted for publication in Scientific Reports. The manuscript was written in collaboration with Lyle G. Whyte, Todd C. Atwood, Kristin L. Laidre, Denis Roy, Sophie E. Watson, Esteban Góngora, and Melissa A. McKinney. Melissa A. McKinney, Lyle G. Whyte, Todd C. Atwood, and Kristin L. Laidre designed the study and Todd C. Atwood and Kristin L. Laidre collected samples in the field. Megan Franz performed the laboratory work, analyzed the data, and wrote the initial draft of the manuscript. Lyle G. Whyte, Sophie Watson, and Esteban Góngora advised on lab protocols and interpretation of results. Denis Roy assisted with data analysis and visualization. All authors helped to critically review and edit the final version.

Distinct gut microbiomes in two polar bear subpopulations inhabiting two sea ice ecoregions

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3.1 Abstract

Gut microbiomes were analyzed by 16S rRNA gene metabarcoding for polar bears (*Ursus maritimus*) from the southern Beaufort Sea (SB), where sea ice loss has led to increased use of land-based food resources by bears, and from East Greenland (EG), where persistent sea ice has allowed hunting of ice-associate prey nearly year-round. SB polar bears showed a higher number of total (940 vs. 742) and unique (387 vs. 189) amplicon sequence variants (ASVs) and higher inter-individual variation compared to EG polar bears. Gut microbiome composition differed significantly between the two subpopulations and among sex/age classes, likely driven by diet variation and ontogenetic shifts in the gut microbiome. Dietary tracer analysis using fatty acid signatures for SB polar bears showed that diet explained more intrapopulation variation in gut microbiome composition and diversity than other tested variables, i.e., sex/age class, body condition, and capture year. Substantial differences in the SB gut microbiome relative to EG polar bears, and associations between SB gut microbiome and diet, suggest that the shifting foraging habits of SB polar bears tied to sea ice loss may be altering their gut microbiome, with potential consequences for nutrition and physiology.

3.2 Introduction

Many metabolic and immune system processes of higher-order organisms are carried out by an assemblage of microbes—predominantly bacteria—found within their gastrointestinal systems [19, 20]. Thus, the gut microbiome influences host nutrition, health, and resistance to enteric pathogenic diseases [19, 28, 86]. Although far less studied than those of human, laboratory, or domestic animals [6], the gut microbiomes of many wild animal species have recently been characterized [33]. Many of these species host Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Verrucomicrobia as the major bacterial phyla [87]. Yet, differences in the species composition within phyla among host species appears to be the norm [88]. In mammalian wildlife, variation in bacterial community composition among host species has been attributed to a combination of host phylogeny, habitat, and diet [20, 24, 25]; however, diet appears to be a predominant driver of interspecific variation in gut bacterial community composition and of intraspecific variation [5, 89, 90].

Recent research has argued that inter-individual variation can often provide insight into the adaptive potential of a species faced with environmental stressors [91, 92]. In general, inter-individual and inter-population variation among wild animal hosts in terms of their gut bacterial communities is understudied compared to work showing the role of biological and ecological drivers of intraspecific variation, (i.e. host sex, age, diet, and body condition) which has been shown for a number of wildlife species now [24, 25, 38, 93]. In wild bears, for example, four individual grizzly bears (*Ursus arctos*) from a population in Alberta, Canada, feeding in part on agricultural subsidies (cereals, domestic animals) showed significant differences in genus-level bacterial abundance compared to four grizzly bears from a population hunting wild prey (e.g., ungulates); both populations also showed wide variation among individuals and differences in gut bacteria compared to two captive grizzly bears [94]. In 16 individuals of *U. arctos* from Europe, changes in individual gut bacterial diversity and composition occurred among individuals between hibernation and active periods [95]. Thus, differences in the gut microbial community both within and among populations can shed light into the consequences of changes in habitat use—such as exposure to different environmental microbes, macrofauna, and climate factors that can influence microbial presence/abundance in a region—as well as differences in feeding habits within wild species.

Polar bears (*Ursus maritimus*) are distributed across the circumpolar Arctic in nineteen spatially segregated subpopulations (PBSG 2018). Similarity in habitats among some of these subpopulations has allowed for their classification into ecoregions, each of which has distinct sea ice characteristics that influence polar bear seasonal movements, foraging activities, and diets [13, 16, 96]. The 'convergent ecoregion' tends to receive supplemental sea ice formed within other regions and the Arctic Basin, providing polar bears, such as the East Greenland (EG) subpopulation, with near year-round access to sea ice and to the ice seals that comprise most of their diet [16, 97]. Within the divergent ecoregion, including polar bears in the Southern Beaufort Sea (SB) subpopulation, sea ice was present year-round before the 1980s [98] (Fig. 3.1). However, with climate change-mediated loss of sea ice over the last four decades, SB polar bears now spend longer periods of time onshore during the reduced ice season [9, 63]. This has led to increased access to onshore foods, including blubber, meat, and bones of bowhead whales leftover from local subsistence harvests ('bone piles'), as well as carcasses of fish, caribou, and birds left nearby [99].

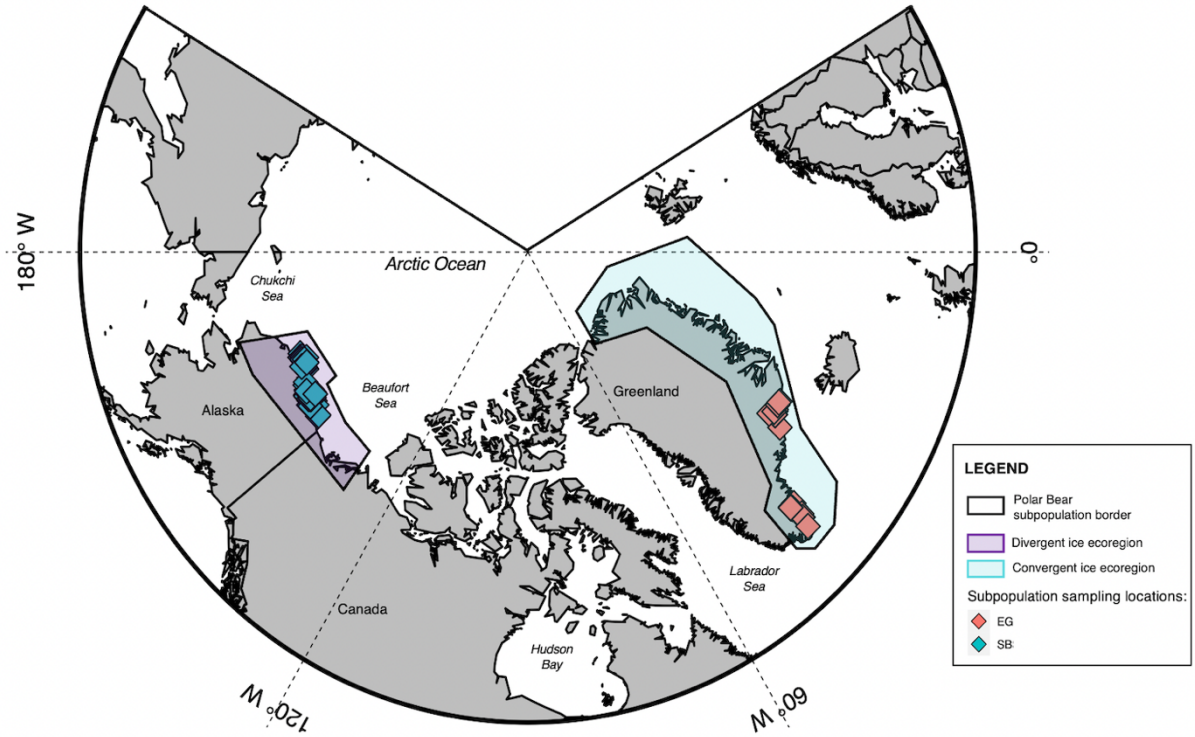


Figure 3.1 Map of sampling locations for the two polar bear subpopulations in this study. The East Greenland (EG) subpopulation is distributed along the east Greenland shoreline and occurs in a convergent ice ecoregion (blue), while the Southern Beaufort Sea (SB) polar bear subpopulation is distributed along the northern shore of Alaska and Canada and occurs in a divergent ice ecoregion (purple).

The distinct sea ice conditions that result in differing habitat use and feeding habits for EG and SB polar bears provides a unique opportunity to explore inter-population variation in the gut microbiota of a wild animal species and could provide insight into the ability of polar bears to cope with added environmental stressors introduced by climate change. Preliminary findings on the gut microbiota of a single polar bear subpopulation using 16S rRNA gene clone libraries detected just a single phylum, *Firmicutes*, suggesting low gut bacterial diversity relative to other mammalian species [71]. However, more recently, 16S rRNA metataxonomics using Illumina technology approaches found 25 bacterial phyla in the SB subpopulation and greater gut bacterial diversity for bears that spend part of the year onshore and that likely have a more diverse terrestrial-based diet relative to bears remaining offshore with likely narrower diets consisting largely of ice seals [10]. In this study, we use high-throughput 16S rRNA gene amplicon sequencing techniques to assess inter-population variation in gut microbial composition and diversity between EG and SB polar bears using samples collected during the same season (late-winter/early spring). We also explore how sex/age class, body mass (as an indicator of body condition), and (for SB bears) dietary patterns based on fatty acid (FA) signatures [8], are associated with inter-and (for SB bears) intra-population variation in gut microbial communities in two wild polar bear subpopulations. Given the evidence of dietary alterations occurring in the SB subpopulation and the distinct ice ecoregion differences that force some SB polar bears to spend greater amounts of time on land, we predict that SB gut microbiota will be more diverse and that we will see a higher degree of interindividual variation and a greater number of overall and unique bacterial species in the SB subpopulation compared to EG. We also expect that diet will be a significant driver of both gut bacterial diversity and composition in the subset of SB polar bears for which FA data was available.

3.3 Materials and Methods

Collection of polar bear fecal and adipose tissue

Fecal samples were collected from 34 EG polar bears in March-April of 2017 and from 59 SB polar bears in March-April of 2015, 2016, 2018, and 2019 (Fig. 3.1). Polar bears were immobilized from a helicopter and tissue samples were collected as part of long-term population assessments in each region. Biometric measurements were recorded, including sex and body mass. Ages were quantitatively estimated via growth layer groups from a vestigial premolar

tooth sampled on first capture[100]. Fecal samples were collected from the rectum of polar bears using sterile latex gloves placed in sterile whirlpak bags. Due to limitations imposed by the COVID-19 pandemic, only adipose tissue samples from SB polar bears could be shipped and analyzed for fatty acid-based assessment of diet. Adipose tissue biopsies were collected from 46 SB polar bears, representing a subset of the same SB individuals for which fecal samples were taken. Fecal and adipose samples were kept at -20 °C during the field season and then shipped on dry ice to McGill and stored at -80 °C prior to laboratory analysis. Samples were collected from SB polar bears as part of the U.S. Geological Survey (USGS) Polar Bear Research Program (U.S. Fish and Wildlife Service Permit# MA690038 to T.C.A) under capture protocols approved by the USGS Institutional Animal Care and Use Committee. Samples were collected from EG polar bears under case nr. 2017-5446, document 4710596 from the Department of Fisheries and Hunting, as part of a long-term monitoring program by the Greenland Institute of Natural Resources.

Fecal DNA extraction

Fecal samples were extracted in random order at McGill University according to the same procedures previously described for samples from 2009-2013 from the SB polar bear subpopulation [10]. Briefly, feces from the glove were swabbed with a sterile cotton-tipped applicator. Tips were transferred to a tube of 1 mL phosphate-buffered saline (PBS), vortexed, and spun down after removing the cotton tip to obtain a pellet. After adding a stainless-steel bead (Qiagen; Hilden, Germany) and lysis buffer (see Watson et al. 2019), samples were homogenized at 37 °C in a shaking water bath. The extraction protocol then continued at step 2 of the QIAamp Mini Kit Buccal Swab Spin Protocol (QIAamp DNA Mini and Blood Mini Handbook). Samples were spun down in a final volume of 100 µL elution buffer (Buffer AE) and 30 µL of each extract was aliquoted among two 96-well plates to facilitate downstream PCR reaction setup. For each batch of extractions, a separate sterile swab control was run alongside samples as a blank and stored with corresponding samples on the same 96-well plate. All DNA extracts were stored at -20 °C until further analysis.

16S rRNA gene amplification and sequencing

Gene amplification was performed as per previous analyses on SB polar bears [10] with minor modifications. In brief, a ~460 base pair (bp) region of the 16S rRNA gene was amplified using the universal bacterial primer set 341F (5′-CCTACGG GNGGCWGCAG-3′) and 805Rmod (5'-GACTACNVGGGTWTCTAATCC-3') with overhanging Illumina adaptors. PCR reaction wells contained 6.5 µL of Rnase free H_2O, 0.5 µL of 20 mg mL^{-1} BSA (bovine serum albumin), 1.5 µL of 10 µg $µL^{-1}$ of both 341F and 805Rmod primers, 12.5 µL of 2X Kapa Hifi Hot Start Ready Mix (Roche Diagnostics), and 2.5 µL template DNA with PCR cycling conditions as described [10]. Amplified DNA was purified using AMPure beads (0.8 bead to sample ratio; Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. Illumina® Nextera XT indices and sequencing adaptors (Illumina®, San Diego, CA) were annealed to PCR product in a subsequent 8-cycle PCR run as specified in the Illumina® 16S Library Preparation guide and purified again using AMPure beads (1.12 bead to sample ratio). Final indexed samples and negative controls were quantified using a Qubit fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and pooled at 4 nM to create the final library, which was then characterized and validated using the Agilent 2100 Bioanalyzer (Agilent Technologies) confirming uniform amplicon size (~600bp) before sequencing on a 2 x 250bp paired-end run with v2 chemistry on an Illumina® MiSeq platform at McGill University.

Fatty acid analysis

The 46 SB adipose tissue biopsies were processed for FA signatures to provide insight into feeding patterns according to methods previously used for SB polar bears from 2004-2016 [12, 101]. In short, lipids were extracted and then FAs were converted to fatty acid methyl esters (FAMEs) using the Hilditch reagent. FAMEs within each sample were then separated and analyzed on an Agilent (Santa Clara, CA, USA) 8860 gas chromatograph with flame ionization detector and quantified using OpenLab CDS Data Analysis software (V. 2.5) as mass percent of total FAME. FAs were abbreviated according to their carbon chain length (A), number of double bonds (B), and position of the first double bond counting from the methyl end of the carbon chain (X) as A:BnX.

FA signatures as dietary indicators

A principal components analysis (PCA) was conducted using selected FAs to reduce dimensionality of the diet data, and the significant PC axes were then used as explanatory variables in PERMANOVA and multiple linear regression models explaining variation in gut bacterial composition and diversity. Of the 70 marine-associated FAs that were detected and quantified, 30 FAs thought to be present in polar bear adipose tissue predominately due to dietary uptake and used in previous polar bear diet studies were initially selected [13, 77]. We did not include 20:1n11, as it has recently been suggested that this FA may not be informative in delineating polar bear feeding patterns [102]. We further only included the major dietary FAs, or those comprising on average > 1% of total FAME, to reduce the possible influence on FA proportions related to instrumental analytical variation [103]. The final set of nine FAs allowed us to meet the recommended 5:1 sample to variable ratio for conducting PCA analysis (Budge et al. 2006). Prior to PCA analysis, the FA proportions were log-ratio transformed as recommended to normalize the multivariate data (Aitchison 1986; Budge et al. 2006).

Microbial data analyses

Unless stated otherwise, all analyses were performed using R 4.0.3 (R Core Development Team 2020). Sequencing data was filtered, trimmed, de-replicated, and paired ends were merged using DADA2 [104]. The inferred amplicon sequence variants (ASVs) were taxonomically assigned using the SILVA reference database (version 132) as described in the DADA2 tutorial. Decontam [105] was used to identify and filter out any contaminant ASVs, (i.e., those detected in both sample PCR negative controls and in extraction kit blanks). MicrobiomeAnalystR [106] was then used to remove ASVs with less than 2 counts and zero variance and the resulting phyloseq object output was extracted and integrated into subsequent phyloseq (McMurdie, Holmes et al. 2013) and MicrobiomeAnalystR workflows [106, 107]. All samples produced > 10,000 reads and so none were eliminated. As recommended, data rarefaction was not performed [108].

MicrobiomeAnalyst was used to visually compare gut microbial composition between EG and SB polar bears at varying bacterial taxonomic levels. Shannon, Inverse Simpson, and Faith's phylogenetic alpha diversity indices were calculated separately for EG and SB polar bears at ASV-level and subsequently using MicrobiomeAnalyst Web version (as per Watson et al. 2019). To provide insight into the biological and ecological variables responsible for

differences in bacterial community composition (at bacterial phylum, class, genus, and ASV-level) within and among the EG and SB polar bears, permutational multivariate analysis of variance (PERMANOVA) tests were performed using the 'adonis' function in the vegan package in R (Oksanen et al. 2007). The Bray-Curtis distance method was used for all bacterial taxonomic levels to assess compositional patterns at multiple levels, and Weighted UniFrac distance was used at just the ASV-level to incorporate the influence of bacterial phylogeny in our community composition comparisons. Homogeneity of group dispersions (PERMDISP; Anderson 2006) for compared groups was checked prior to interpretation of PERMANOVA results. Subsequent analysis of composition with bias correction (ANCOMBC; Lin et al. 2020) tests were done to compare differential abundances of specific bacterial classes, genera, and ASVs contributing to compositional differences.

Both multiple linear regression models and PERMANOVAs were used to test for other ecological effects on alpha diversity indices as well as compositional differences (i.e., beta diversity differences). For both the PERMANOVAs and the linear models (LMs), the additional explanatory variables included subpopulation, sex/age class, body mass (as an indicator of body condition; [109]), and all biologically-relevant first-order interactions. The sex/age classes used were adult female (AF, n = 36), adult male (AM, n = 32), subadult (S, n = 15), and cub (C, n = 10). Year of capture was not included as an explanatory variable as EG bears were only captured in a single year and years did not overlap for the two subpopulations. When categorical explanatory variables were found to be significant in the PERMANOVAs or LMs, *post-hoc* univariate tests (ANOVAs) and ANCOMBC tests were performed to determine which means and bacterial classes, genera, and ASVs significantly differed between groups (e.g., sex/age classes).

Given that we only had FA signatures for SB polar bears, separate PERMANOVAs using the Bray-Curtis distance method (and *post-hoc* univariate tests, as appropriate) were also run to examine associations of bacterial composition with diet, using the significant PCs from the FA analysis (as described above), while also including sex/age class, body mass, and capture year (2016, 2017, and 2018). The sex/age classes used were adult female (AF, n = 16), adult male (AM, n = 24), and subadult (S, n = 6). Cubs are not included as adipose biopsies were not collected from this age class. Multiple linear regression models were run to test for ecological and dietary effects on gut bacterial alpha and beta diversity (represented by Bray Curtis and

Weighted UniFrac NMDS axes) indices for SB polar bears. Top models were selected using backwards model selection and Akaike information criterion (*AIC*) scores adjusted for smaller sample sizes (Burnham, Anderson and Huyvaert 2011 Behav. Ecol. SocioBiol). The backwards model selection process was conducted via stepwise dropping of terms in the model and AIC calculation. If dropping a term decreased the AIC it was removed and this process repeated until removal of variables did not result in lowering of the AIC score of the overall model.

3.4 Results

Gut bacterial diversity and composition of EG and SB polar bears

A total of 12,294,006 reads were obtained for both EG (n = 34) and SB (n = 59) samples combined, with an average of 81,960 reads per sample. Following DADA2 processing, 6,172 amplicon sequence variants (ASVs) were identified overall, which were then further reduced to 1,129 ASVs after removing ASVs with less than two counts and zero variance across all samples.

Although mean alpha diversity was qualitatively higher in SB than in EG polar bears for Shannon (SB: 2.74 +/- 0.06; EG: 2.65 +/- 0.07), Inverse Simpson (SB: 9.2 +/- 0.6; EG: 8.3 +/- 0.6), and Faith's Phylogenetic Diversity (SB: 13.3 +/- 0.4; EG: 12.9 +/- 0.5) (Supplementary Fig. S3.1), linear models showed no effect of subpopulation for any of these alpha diversity indices (Supplementary Table S3.1).

Differences in composition between EG and SB polar bears were assessed at multiple bacterial taxonomic levels—Phylum, Class, Genus, and ASV—and found to differ significantly at bacterial class (R^2 = 0.035, $F_{1,93}$ = 3.43, p = 0.008), genus (R^2 = 0.046, $F_{1,93}$ = 4.62, p < 0.001), and ASV-levels (R^2 = 0.052, $F_{1,93}$ = 5.20, p < 0.001) (Table 3.1). Of the seventeen detected phyla, five were predominant comprising ~97% of the total reads (Fig. 3.2A). Of the 24 detected classes detected, eight accounted for 99% of total reads in both polar bear subpopulations and varied in their proportional contributions among the two subpopulations (Fig. 3.2B). *Post-hoc* analysis of composition with bias correction (ANCOMBC) testing found that the abundances of two bacterial classes, Bacilli and Coriobacteria, differed significantly between EG and SB polar bears (Fig. 3.3, Supplementary Table S3.2).

Table 3.1 Summary of permutational analysis of variance (PERMANOVA) results* assessing differences in gut bacterial composition at bacterial phylum, class, genus, and ASV levels for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations using Bray-Curtis distance method.

Phylum-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	R^2	*Pr(>F)*
Sex/age class	3	0.270	0.090	1.370	0.044	0.210
Subpopulation	1	0.141	0.141	2.143	0.023	0.103
Body Condition	1	0.053	0.053	0.802	0.008	0.485
Subpopulation: Body Condition	1	0.087	0.087	1.320	0.014	0.268
Residuals	86	5.653	0.066	NA	0.911	NA
Total	92	6.203	NA	NA	1.000	NA
Class-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	R^2	*Pr(>F)*
Sex/age class	**3**	**1.058**	**0.353**	**2.511**	**0.076**	**0.004**
Subpopulation	**1**	**0.481**	**0.481**	**3.429**	**0.035**	**0.008**
Body Condition	1	0.089	0.089	0.635	0.006	0.665
Subpopulation: Body Condition	1	0.240	0.240	1.712	0.017	0.136
Residuals	86	12.073	0.140	NA	0.866	NA
Total	92	13.942	NA	NA	1.000	NA
Genus-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	R^2	*Pr(>F)*
Sex/age class	**3**	**2.077**	**0.692**	**2.658**	**0.079**	**< 0.001**
Subpopulation	**1**	**1.212**	**1.212**	**4.654**	**0.046**	**< 0.001**
Body Condition	1	0.151	0.151	0.579	0.006	0.881
Subpopulation: Body Condition	1	0.328	0.328	1.261	0.013	0.219
Residuals	86	22.400	0.260	NA	0.856	NA
Total	92	26.168	NA	NA	1.000	NA
ASV-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	R^2	*Pr(>F)*
Sex/age class	**3**	**2.172**	**0.724**	**2.731**	**0.081**	**< 0.001**
Subpopulation	**1**	**1.391**	**1.391**	**5.246**	**0.052**	**< 0.001**
Body Condition	1	0.157	0.157	0.592	0.006	0.852
Subpopulation: Body Condition	1	0.299	0.299	1.129	0.011	0.321
Residuals	86	22.798	0.265	NA	0.850	NA
Total	92	26.817	NA	NA	1.000	NA

*Significant terms are in bold.

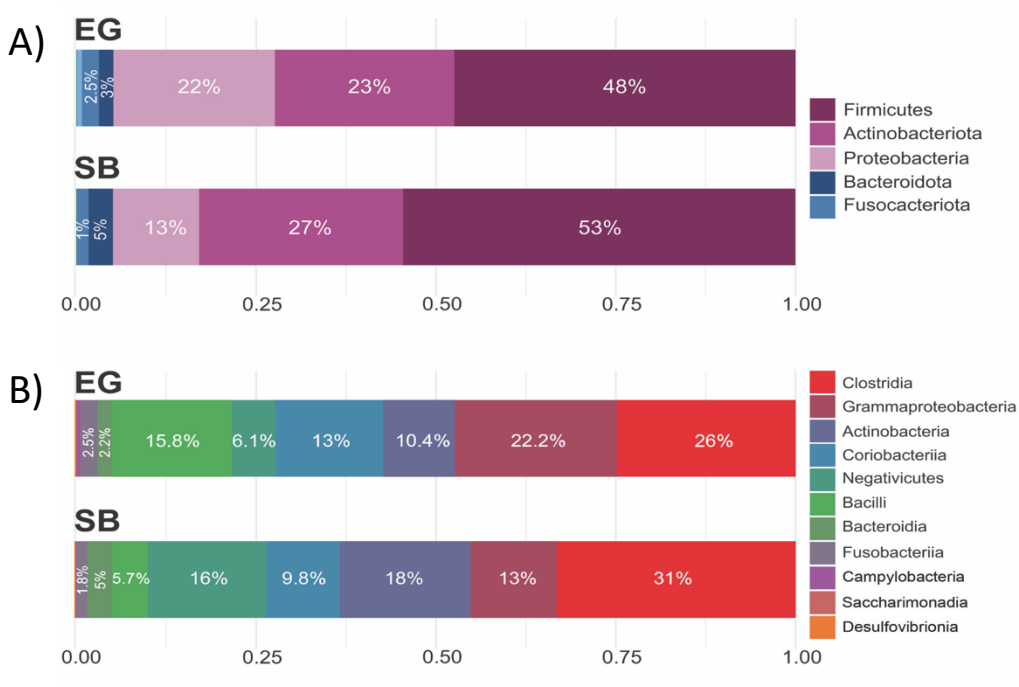


Figure 3.2 (A) Relative abundance bar plot showing the five most abundant bacterial phyla, averaged across all samples within each subpopulation (East Greenland [EG] and Southern Beaufort Sea [SB]) and (B) Relative abundance bar plot showing the eight most abundant bacterial classes, averaged across all samples within each subpopulation.

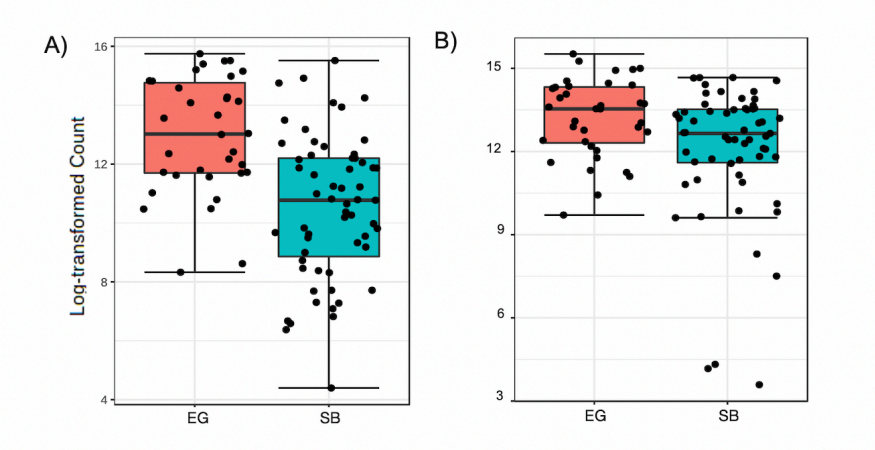


Figure 3.3 Boxplots of log-transformed counts for bacterial classes showing differential abundances of (A) Bacilli, significantly higher in East Greenland (EG) than Southern Beaufort Sea (SB) polar bears (Group means: EG: 13.0 ± 0.3; SB: 10.5 ± 0.3) (B) Coriobacteria, significantly higher in EG than in SB polar bears (Group means: EG: 13.1 ± 0.2; SB: 12.0 ± 0.3). Analysis of composition with bias correction (ANCOM-BC) test results summarized in Supplementary Table S3.2.

Of 203 total genera detected, 31 (the combined top 25 genera from each subpopulation) comprised ~90% of all reads for EG and SB bears and 12 were unique to EG while 51 were unique to SB. Despite observable inter-individual variation at the genus level for both EG and SB polar bears (Fig. 3.4, Table 3.1) *post-hoc* ANCOMBC analysis found that the abundances of seven of the top 31 most abundant genera still differed significantly between EG and SB bears (Fig. 3.5A, Supplementary Table S3.3). The remaining 13 differentially abundant genera contributed are listed in Supplementary Table S3.3.

A total of 742 ASVs were detected in EG polar bears and 940 ASVs were found for SB polar bears (Fig. 3.5B). Of the 553 shared ASVs, 48 differed significantly in their abundances between subpopulations (Supplementary Table S3.4). Significant differences in composition at the ASV level were found between subpopulations using both Bray-Curtis distances (PERMANOVA: $R^2 = 0.052$, $F_{1,93} = 5.20$, $p < 0.001$) (Fig. 3.6A) and the phylogenetic Weighted UniFrac Distances (PERMANOVA: $R^2 = 0.065$, $F_{1,93} = 6.49$, $p = 0.001$) (Fig. 3.6B). These results were not confounded by heterogeneity of subpopulation group dispersions (Bray-Curtis: PERMDISP: $F_{1,93} = 0.75$, $p = 0.39$; Weighted UniFrac: PERMDISP: $F_{1,93} = 0.61$, $p = 0.44$).

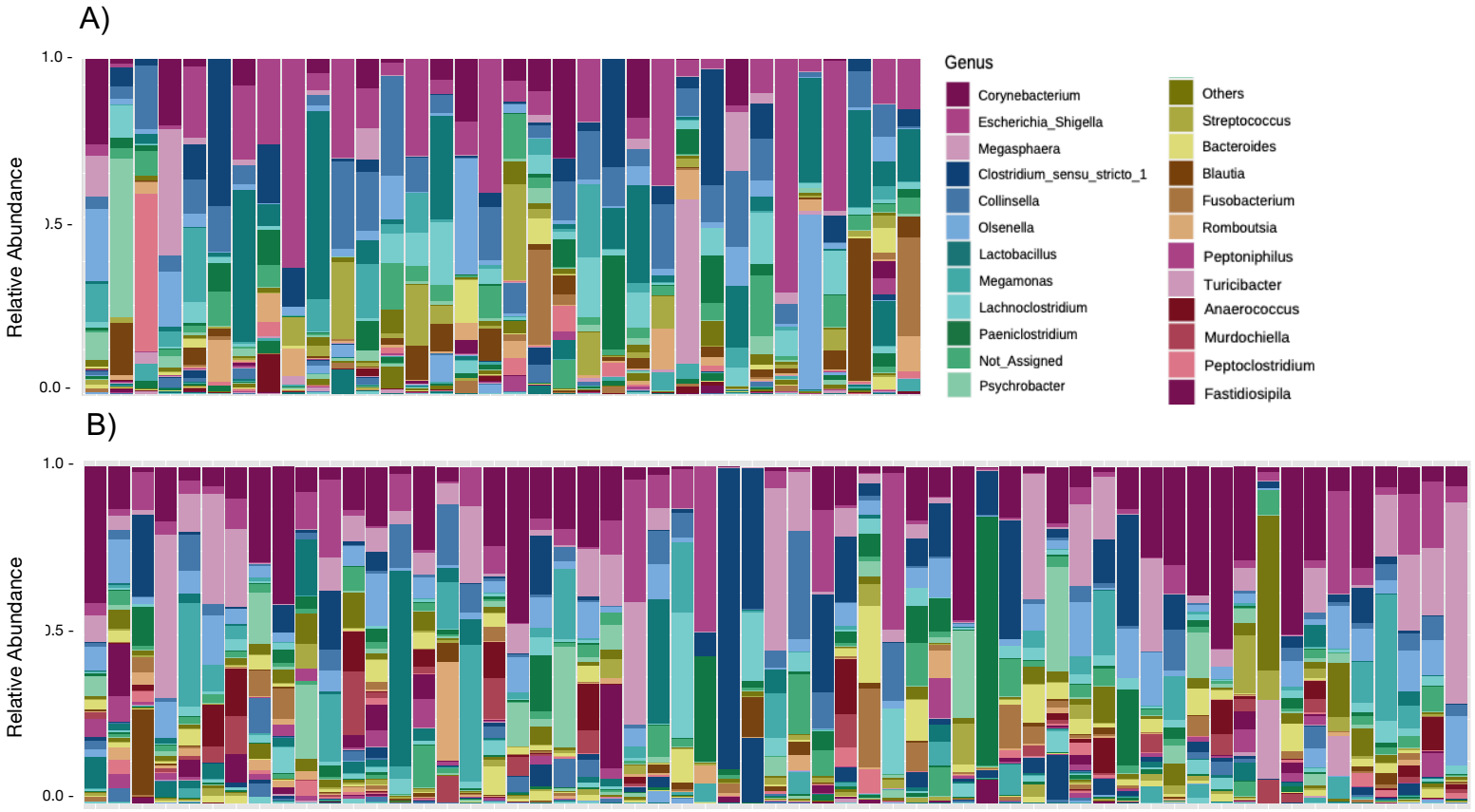


Figure 3.4 Relative abundance bar plots at genus level showing extent of interindividual variation among polar bears in the (A) East Greenland (EG) and (B) Southern Beaufort Sea (SB) subpopulations.

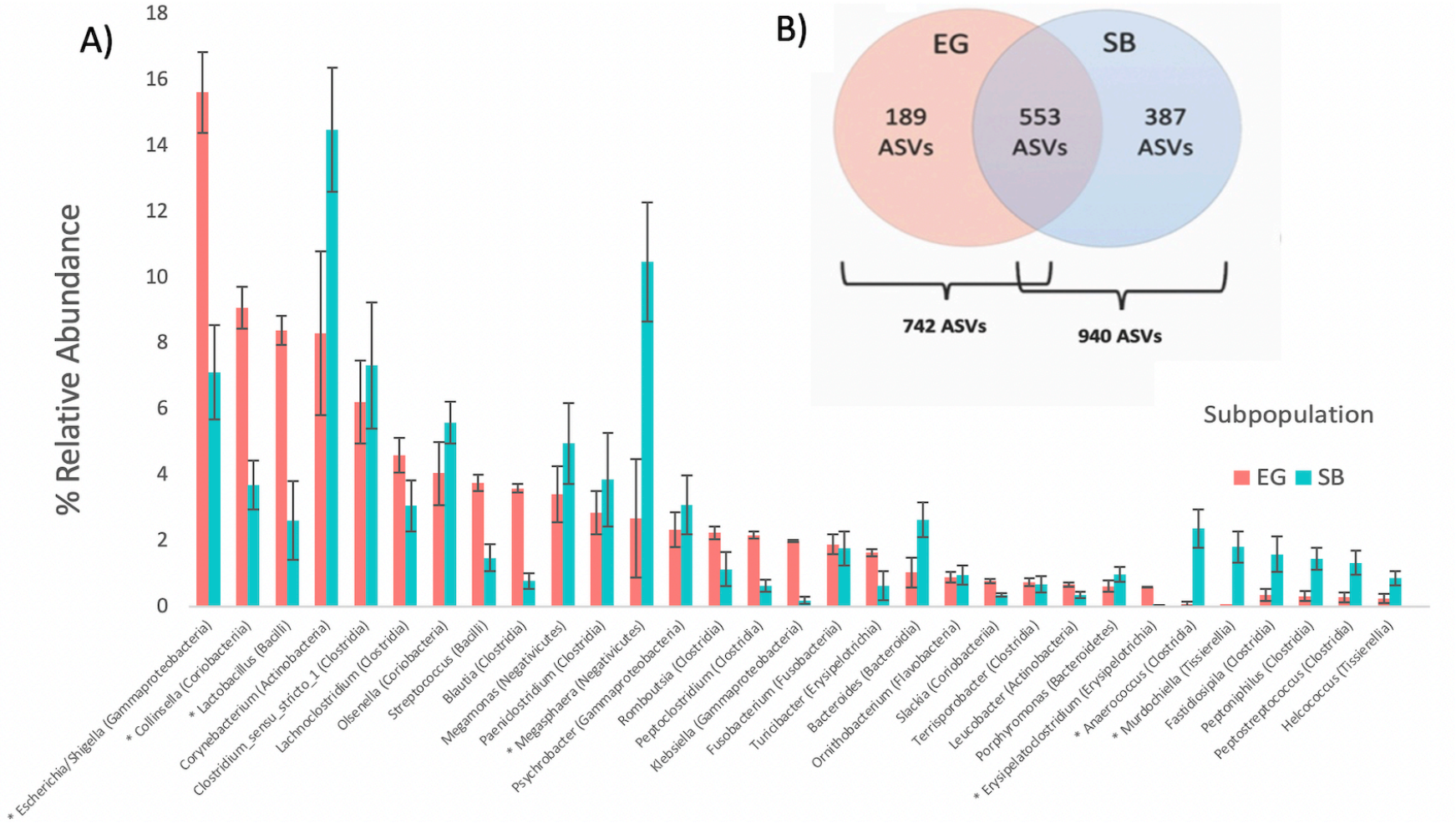


Figure 3.5 (A) Grouped bar plot showing the top 31 most abundant bacterial genera detected in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (associated bacterial class noted in parentheses). Relative abundances were averaged across all samples within each subpopulation (EG and SB). Asterisks (*) indicate genera with significantly different abundances between the two subpopulations (see Supplementary Table S3.3 for statistical results obtained using analysis of composition with bias

Neither sex/age class, body condition, nor any interaction terms significantly explained variation in Shannon and Inverse Simpson alpha diversity, although sex/age class was found to be a near-significant term in the linear model explaining variation Faiths Phylogenetic Diversity (Supplementary Table S3.1, Supplementary Fig. S3.2).

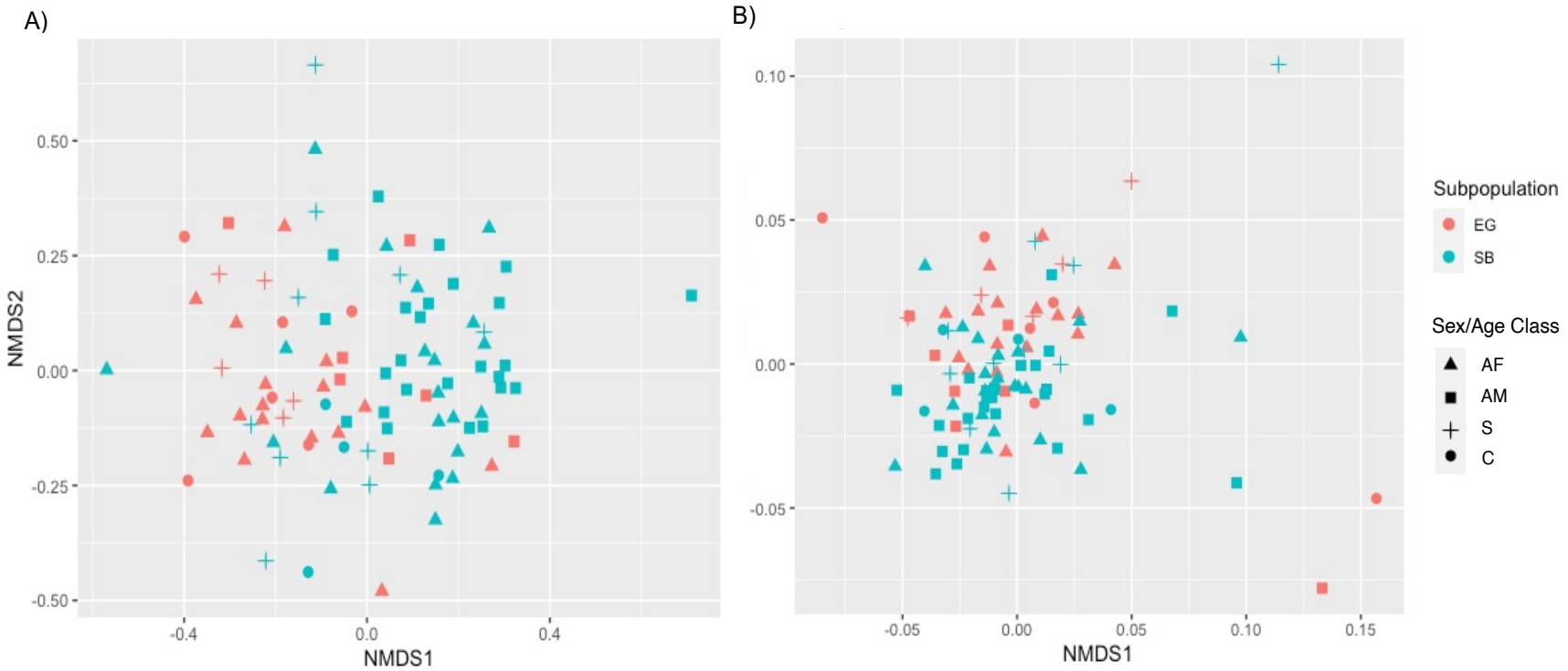


Figure 3.6 Non-metric multi-dimensional scaling (NMDS) plots showing gut bacterial communities for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations with color denoting subpopulation affiliation and shapes denoting Sex/Age Classes (adult females [AF], adult males [AM] and subadults [S] compared to cubs [C]) determined using (A) Bray-Curtis (Stress = 0.26; PERMANOVA: $R^2 = 0.06$, $p = 0.001$; PERMDISP: $p = 0.5$) and (B) weighted UniFrac distances (Stress = 0.16; PERMANOVA: $R^2 = 0.06$, $p = 0.001$; PERMDISP: $p = 0.5$) calculated at amplicon sequence variant (ASV)-level. Points represent individual fecal samples.

Significant differences in gut bacterial composition were found between polar bears of different sex/age classes (i.e. adult females [AF], adult males [AM], subadults [S], and cubs [C]) at the class (PERMANOVA: $R^2 = 0.076$, $F_{1,93} = 2.51$, $p = 0.004$), genus ($R^2 = 0.079$, $F_{1,93} = 2.66$, $p < 0.001$) and ASV-levels (Bray-Curtis distance: $R^2 = 0.081$, $F_{1,93} = 2.73$, $p < 0.001$; Weighted UniFrac distance: $R^2 = 0.079$, $F_{1,93} = 2.53$, $p = 0.002$) (Table 3.1, Supplementary Fig. S3.3). However, the assumption of homogeneity of multivariate group dispersions for the sex/age class groups was not met for either the Bray-Curtis or Weighted UniFrac indices at ASV-level (PERMDISP: $p = 0.005$ and $p = 0.041$, respectively), so results should be interpreted with some caution. *Post-hoc* ANCOMBC results showed that the abundances of three bacterial classes (Bacilli, Parcubacteria, and Saccharimonadia) (Supplementary Fig. S3.4), 21 bacterial genera and 65 ASVs differed significantly among the different sex/age class groups (Supplementary Tables S3.5, S3.6 and S3.7). There were no significant effects of body condition or any of the interaction terms at any taxonomic level (Table 3.1).

Influence of diet as a driver of gut bacterial diversity and composition in SB polar bears

For a subset of SB polar bears ($n = 46$), diet data was obtained using fatty acid (FA) signature analysis. The proportions of key dietary FAs were used in a principal components analysis (PCA) to reduce the number of variables from the fatty acids to just two principal components, which explained 83.3% of the total variation in polar bear FA signatures. Diet was represented in subsequent microbiome models by the individual's scores along FA_PC1 and FA_PC2 (Supplementary Fig. S3.5). PERMANOVAs and multiple linear regression models were run to assess how diet influences gut bacterial diversity and composition, respectively. FA_PC1 was a significant term in models explaining variation in Shannon and Inverse Simpson indices of alpha diversity for these bears and FA_PC2 was also a nearly-significant term in the model explaining differences in composition (Table 3.2, Supplementary Table S3.8). Athough diet did not explain variation in gut bacterial composition at class-level, significant effects of diet (FA_PC1 and FA_PC2) were found at bacterial genus-level and ASV-levels (Table 3.3).

Table 3.2 Summary of top models showing influence of diet (FA_PC1 and FA_PC2 axes) and other relevant metadata on variation in alpha diversity indices (Shannon, Inverse Simpson, Faith's phylogenetic distance) and beta diversity indices (Bray-Curtis and weighted UniFrac distances) for the subset of Southern Beaufort Sea (SB) polar bears for which diet data was available. There were no significant terms in models explaining variation using weighted UniFrac beta diversity axes.

Diversity index	Top Model	F	P	Mult R^2	Adj. R^2
Shannon	~ Sex/age class* + Body Condition + FA_PC1* +FA_PC2	2.73	0.033	0.25	0.16
Inverse Simpson	~ FA_PC1* + FA_PC2	3.51	0.039	0.14	0.10
Faiths Phylogenetic Diversity	~ Sex/age class* + FA_PC1	5.48	0.003	0.28	0.23
Bray Curtis (NMDS1)	~ Sex/age class *	5.58	0.007	0.21	0.17
Bray Curtis (NMDS2)	~ FA_PC2 . + Capture year	3.16	0.035	0.18	0.13
Weighted UniFrac (NMDS1)	~ 1 (NULL)	-	-	-	-
Weighted UniFrac (NMDS2)	~ 1 (NULL)	-	-	-	-

Similar to the analyses including both EG and SB subpopulations, sex/age class significantly explained variation in gut bacterial diversity and composition among SB polar bears (Table 3.2, Table 3.3, see Supplementary Text 3.1). Additionally, body condition was found to be a nearly-significant term in the Shannon alpha diversity model and capture year was found to be a significant term in the model for the Bray Curtis NMDS2 axis (Table 3.2, Supplementary Table S3.8). Capture year was also found to be a significant term in composition PERMANOVAs using Bray Curtis distance method (Table 3.3). There were no significant terms in models explaining variation in the Weighted UniFrac NMDS axes (Table 3.2, Supplementary Table S3.8).

Table 3.3 Summary of permutational analysis of variance (PERMANOVA) results* assessing differences in gut bacterial composition at bacterial class, genus, and amplicon sequence variant (ASV)-levels for the subset of Southern Beaufort Sea (SB) polar bears using Bray-Curtis distances.

Class-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	*R2*	*Pr(>F)*
Sex/age class	2	0.319	0.160	1.274	0.053	0.233
Body Condition	1	0.209	0.209	1.670	0.035	0.142
FA_PC1	1	0.126	0.126	1.005	0.021	0.411
FA_PC2	**1**	**0.262**	**0.262**	**2.092**	**0.043**	**0.085**
Capture year	**1**	**0.261**	**0.261**	**2.080**	**0.043**	**0.074**
Residuals	39	4.886	0.125		0.806	
Total	45	6.063			1	
Genus-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	*R2*	*Pr(>F)*
Sex/age class	**2**	**0.810**	**0.405**	**1.776**	**0.071**	**0.023**
Body Condition	1	0.209	0.208	0.914	0.018	0.527
FA_PC1	**1**	**0.504**	**0.504**	**2.208**	**0.044**	**0.024**
FA_PC2	**1**	**0.439**	**0.439**	**1.925**	**0.039**	**0.034**
Capture year	**1**	**0.483**	**0.483**	**2.118**	**0.043**	**0.019**
Residuals	39	8.895	0.228		0.784	
Total	45	11.339			1	
ASV-level	Analysis of Variance Table					
	Df	*SumsOfSqs*	*MeanSqs*	*F.Model*	*R2*	*Pr(>F)*
Sex/age class	**2**	**0.719**	**0.359**	**1.568**	**0.063**	**0.057**
Body Condition	1	0.205	0.205	0.894	0.018	0.519
FA_PC1	**1**	**0.557**	**0.557**	**2.431**	**0.049**	**0.009**
FA_PC2	**1**	**0.442**	**0.442**	**1.927**	**0.039**	**0.023**
Capture year	**1**	**0.485**	**0.485**	**2.116**	**0.043**	**0.024**
Residuals	39	8.937	0.229		0.788	
Total	45	11.344			1	

*Significant terms are in bold**.**

3.5 Discussion

Polar bears from the SB subpopulation showed significant differences in gut bacterial composition at multiple bacterial taxonomic levels compared to EG bears and an overall greater number of unique and total bacterial genera and ASVs. The particular bacterial classes and genera which were elevated in one subpopulation versus the other were consistent with a potentially altered and more varied gut microbiota in the more land-associated SB subpopulation relative to the more sea ice-based EG subpopulation. Relative to SB polar bears, those in the EG subpopulation had higher levels of bacteria from the class Bacilli, which has been suggested to

play an important role in restoring gut health and maintaining gut homeostasis [110] and from the class Coriobacteria which is a typical taxonomic group found in the human gut and known to play a role in gut microbiome health [111]. Although many of the most abundant bacterial genera were shared between the two subpopulations, some genera were significantly higher in EG bears compared to SB bears. Specifically, *Collinsella*, *Lactobacillus, Erysipelatoclostridium, and Escherichia-Shigella* were higher in EG, and some of these genera have important probiotic properties, at least based on human studies [112-115]. Bacteria from class Coriobacteria (e.g. *Collinsella*) have been suggested to aid with lipid metabolism in human studies [116] and with cholesterol metabolism in controlled studies on hamsters [117]. These differences could imply that EG bears have a healthier 'baseline' gut microbiome compared to SB bears, a reflection of their likely narrower dietary niche breadth and continued access to traditional lipid-rich prey species, however this is difficult to conclude given the lack of studies on functional roles of these bacteria in wildlife studies [6]. Alternatively, the differential bacterial classes and genera between SB and EG bears could simply reflect local regional adaptations based on differences food availability and other geographic and ecosystem variables such as exposure to sea ice vs. terrestrial habitat, exposure to different macro- and micro- fauna, etc.

Some bacterial classes and genera were elevated in SB polar bears compared to EG polar bears although not significantly so. Two genera *Megasphaera* and *Megamonas*, which contributed to ~15% of class Negativicutes reads within the SB subpopulation, were elevated in SB compared to EG polar bears (*Megasphaera* was significantly elevated) and may be important components of rumen microbiomes. Further, some Negativicutes species have metabolic properties related to the breakdown of polysaccharides and lactate into short chain fatty acids (SCFAs) which have been suggested to promote gut health [118-121]. This observation of elevated Negativicutes could potentially indicate increases in carbohydrates or starches in SB diets related to inputs from terrestrial foods, such as berries, which polar bears have been observed to eat while onshore [122, 123]. Bacteroidia were also elevated in SB relative to EG polar bears. Two genera of Bacteroidia comprised ~ 4% of reads for SB bears (compared to ~1.6% in EG bears): *Bacteroidetes* and *Porphyromonas*. *Bacteroidetes* have been described in human microbiome studies as having complex metabolic roles covering plant and polysaccharide degradation, protein metabolism, or just as a component of healthy adult gut microbiota [124]. Changes in abundance (i.e., increases or decreases) of *Bacteroidetes* have also been associated

with several GI tract diseases, such as obesity and irritable bowel syndrome in humans [125-128]. *Porphyromonas* species are asaccharolytic and are often associated with the oral microbiome and can occasionally become pathogenic [129-131]. Finally, a few genera that were significantly higher in SB compared to EG bears (i.e., *Megasphaera* and *Anaerococcus*) are typically part of the commensal microbiota. However, the latter has been linked to polymicrobial infections and can become pathogenic in humans or human-associated microbiomes [132-135]. While some of these bacterial genera that are more abundant in SB bears compared to EG bears have been previously linked to adverse health effects in human and controlled studies, they could also simply reflect a more varied and diverse diet for SB polar bears which would necessitate a shift in metabolic function of the gut microbiome. In general, the characteristics and functions of specific bacteria can vary depending on host species. As such, these bacteria might serve different functional roles in the polar bear gut microbiome compared to what has been shown in studies on the gut microbiomes of humans and other mammalian species. Further, higher or lower gut bacterial diversity and the presence or introduction of novel bacterial species could ultimately lead to the development of an adaptive gut microbiome, particularly when considering potential shifts toward protein and carbohydrate metabolism type functions of the bacterial species that are increased in the SB subpopulation. Alternatively, it could lead to gut dysbiosis and negative health consequences for an individual, population, or species[40]. While it is difficult to predict any long-term consequences that could result from these observed differences in bacterial composition and diversity between the SB and EG subpopulations, it will be important to continue to monitor such changes and investigate their health consequences.

Additional factors could be contributing to these compositional differences between subpopulations, such as host phylogeny, immune system effects, and environmental differences (biogeography, variety of cohabitating species present in the region, etc.) [19, 28, 35, 136, 137]. Although our dataset did not contain a sufficient number of capture years for both subpopulations to evaluate climate and ecological variation that could influence temporal trends in the gut microbiota, future work with additional years of collection data should assess this relationship. Nonetheless, differences in diet are likely important in explaining much of the differences in the gut microbiome between EG and SB bears, given the importance of diet in driving gut microbiome composition and separate studies pointing to dietary differences between these subpopulations [5, 20, 24, 25, 94]. In response to climate change, SB bears show increased

use of terrestrial habitat and terrestrially-based food resources in the late summer and fall months [9, 12, 62]. Reduced access to ice seal prey has been tied to declines in the SB polar bear population [66] and other studies speculate that alternative food resources will likely be nutritionally insufficient for polar bears[62, 63] which could have serious implications for long-term persistence of the species. Any changes in the gut microbiome could potentially impact immune functioning or impair nutrient uptake for polar bears in this region, further exacerbating these existing stressors faced by the SB subpopulation in a period of continued sea ice decline and habitat loss. Consumption of non-traditional prey species and tissue types also likely exposes them to novel pathogens (Watson et al, submitted) and gut microbiota, either via contact with other scavenging species or through changes in macronutrients of their prey [62, 70]. This hypothesis is supported by findings of higher gut bacterial diversity in onshore versus offshore polar bears in the SB subpopulation [10]. Although we did not have seasonal metadata distinguishing onshore vs. offshore SB bears as all fecal samples were collected in the spring before sea ice breakup, these differences in inter-individual foraging behavior likely contribute to increased rare/unique ASVs detected and overall larger variance in beta diversity within the SB subpopulation compared to the EG subpopulation. FA signatures from adipose samples collected in winter-spring of 1984-2011 suggests that EG polar bears mainly consume ice seals, and probably largely in the form of seal blubber. This is despite the proportion of Arctic seals such as ringed seals (*Pusa hispida)* declining, while the proportion of northward range-shifting sub-Arctic seals such as harp seals (*Phoca groenlandica)* and hooded seals (*Crystophora cristata*), increased [8]. Thus, while there is evidence that EG polar bears show decreased consumption of traditional ringed seal prey, the overall change in macronutrient composition between these prey types may be low (i.e., still predominantly blubber lipids) and could partially explain the detection of fewer bacterial genera and ASVs in EG compared to SB polar bears.

Further support for our hypothesis that diet is a driver of intra-species differences in the polar bear gut microbiome comes from the results that focused on the SB polar bears, for which we determined dietary patterns using FA signatures. For these polar bears, the FA-PC scores explained the largest amount of the variance in gut bacteria alpha diversity of all explanatory variables considered. These FA-PC scores also explained large amounts of variation in gut bacterial composition at most bacterial taxonomic levels, particularly at the ASV-level. Other studies on wildlife have identified diet as an important long and short term driver of gut bacterial

composition and diversity [24, 25], however the stable isotope diet analysis methods employed in previous wildlife work may not offer the resolution of dietary information relative to FAs [138]. Thus, our use of FA signatures to provide insight into variation in gut microbiome composition and diversity within and among wildlife populations shows considerable promise and suggest that future gut microbiome research could benefit from this approach. For example, quantitative fatty acid signature analysis (QFASA) and molecular-based diet analysis methods can provide species-level information on the diets of wild, free-ranging species which could enhance our understanding of how consumption of particular prey types influence the gut microbiome [17, 27, 61]

In both humans and animals, sex/age class has been shown to impact gut bacterial composition and diversity [22, 38, 95, 139]. Although the assumption of homogeneity of multivariate group dispersions for sex/age class groups was not met and we advise some caution of results interpretation of group comparisons, it is also possible that these differences in group dispersions could be related to important life history differences among polar bear sex/age classes. We found that bacteria from the class Negativicutes were higher in adult males compared to adult females, subadults, and cubs, while Saccharimonadia and Bacilli were higher in adult females and cubs compared to adult males and subadults. In addition to the probable health benefits of Bacilli discussed earlier, Bacilli may also be higher in females and cubs due to higher more *Lactobacillus* in the vaginal microbiome and in relation to milk production and lactation [35], and other studies have detected higher levels of *Lactobacillus* in females compared to males as well [36]. Some human and mouse model studies have also demonstrated strong interactions between sex-specific hormones and commensal gut bacteria, which could also be driving a portion of the sex/age class differences observed here [140-142]. In addition, considering the varied foraging behavior among polar bear sex/age classes, these compositional differences likely have underlying associations with dietary differences just as for subpopulation and the two factors may also interact [13, 143]. For example, adult male polar bears are much larger in body size and can more easily take down larger prey (bearded seal, beluga whale, etc.) when they are available, while adult females and subadults likely preferentially forage on smaller-bodied prey, such as ringed seal [8, 11]. Additionally, for the SB subpopulation in particular, it has also been shown that adult male polar bears use bowhead whale ‘bone piles’ more frequently than other sex/age classes [144], and consume higher amounts of bowhead

whales compared to adult females [12]. Cubs of the year generally have a different diet entirely, as they rely on high-fat milk from their mothers. Further, we found lower Faith's phylogenetic diversity in subadults compared to adult females which could be due to gut microbiomes of younger individuals being underdeveloped relative to adult microbiomes [38]. In general, diversity differences among sex/age classes likely reflect gut bacterial compositional differences that are tied to life history, physiological and diet differences among the sex/age classes.

We found no effect of body condition on variation in gut bacterial composition and diversity for SB and EG polar bears. It is possible that by choosing body mass as our indicator of body condition our results are confounded by other factors known to influence body mass of polar bears, such as sex and age class. However, to account for this, interaction terms were included in all models testing for associations between body condition and gut microbiota but none were found to be significant. While body condition has been identified as an important factor in some gut microbiome studies and potentially linked to diet [145], other studies have similarly found minimal or no importance of BMI on gut microbiome composition and diversity [39, 146]. Other biological and environmental factors could also contribute to differences in gut bacterial composition and diversity for EG and SB polar bears, including region-specific differences in contaminants, parasite types or loads, and differing interspecific interactions [10, 70, 147-149]. We were not able to account for these in our study, but such associations may be relevant to study in future work.

We found observable inter-individual variation within each subpopulation, which likely contributed to most (~85-90%) of the remaining unexplained variation in gut bacterial composition between the two subpopulations. However, it is important to note that other potential unmeasured biological factors, general stochasticity of the gut microbiome could also contribute to this unexplained variation [25, 150]. Despite a greater number of total and unique ASVs within the SB relative to EG subpopulation, the lack of significant subpopulation differences in any of the alpha diversity indices measured could also reflect that high inter-individual variation is typical. Other studies have also noted a lack of intra-species or inter-population differences in alpha diversity, while still detecting significant compositional differences between groups [90, 137]. Host phylogeny is another strong driver of gut bacterial composition and diversity and might, in part, explain the large overlap in bacterial species detected among EG and SB polar bears, the low separation between subpopulations along beta

diversity NMDS axes, and the large amount of unexplained variation in gut bacterial composition and diversity [19, 20] as it has been suggested that gut microbiota are vertically transmitted and coevolve with their host species [151]. Additional metrics we were unable to account for in our study but that could be useful to include in future studies include cortisol levels as an indicator of stress-levels[152, 153], female reproductive status[154], immune status of individuals by measuring cytokines[155, 156], assessment of individual contaminant loads[157-159], etc. Many studies on the gut microbiome have also found high proportions of unexplained variation which can reflect the convoluted nature of microbiome data [20, 150]. Given this typically is the case, and the fact that high inter-individual variation can sometimes mask generalized group differences, we can conclude there are relatively strong compositional differences in the gut bacteria for EG and SB polar bears.

Overall, this study showed differences in gut composition and diversity between two geographically distant polar bear subpopulations facing distinct sea ice conditions and prey availability. The SB subpopulation showed rarer and more unique ASVs and bacterial genera present compared to the EG subpopulation and indications of greater inter-individual diversity. These findings likely, in part, reflect the use of onshore foods for some members of the population during the reduced ice season [9, 18]. This interpretation is supported by the SB subset results indicating diet and intraspecific variation among polar bear sex/age classes are likely linked and are key drivers of alpha diversity and gut bacterial composition within the subpopulation. This study highlights the importance of considering both intra-species and inter-individual variation in gut bacterial composition, given the direct links between gut microbiota and host physiology, nutrition, and overall health [160, 161] and also acknowledges that because there are many parameters that influence the gut bacterial community it can be challenging to assess the influence of each in isolation, or to make direct conclusions when certain factors are unavailable for assessment. Polar bears are facing a myriad of anthropogenic stressors posing threats to their continued survival as a species. Moving forward, assessing the impacts of such stressors on the gut microbiome will likely be an important aspect of monitoring polar bear health.

3.6 Acknowledgements

Thanks to K. Simac, A. Pagano, E. Peacock, T. Donnelly, and G. Durner at the USGS Alaska Science Center for SB field sampling and sample coordination. Thanks to P. Hegelund and C. Isaksen for EG field work. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The SB work was funded by a U.S. Geological Survey grant (to M.A.M., #G19AC00335), the Canada Research Chairs Program (to M.A.M., 950−232183), and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants Program (to M.A.M., RGPIN-2019−05330). The EG work was funded by DANCEA. Thanks to additional support from a Graduate Excellence Award from the Department of Natural Resource Sciences at McGill University (to M.F.)

3.7 Supplementary Material

Supplementary Table S3.1. Summary of linear regression models showing that none of the explanatory variables explained a significant amount of the variation in Shannon and Inverse Simpson indices of alpha diversity. There was a tendency towards a significant effect of sex/age class in linear regression model for Faith's phylogenetic diversity (FPD), suggesting lower FPD in subadults compared to other sex/age classes. SB = Southern Beaufort Sea.

Shannon Diversity (Adj. $R^2 = 0.00$, $p = 0.67$)					
Full Model:	~ subpopulation + sex/age class + body condition + sex/age class*subpopulation + body condition*subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	2.52	0.33	7.72	<0.001
	Subpopulation (SB)	0.27	0.43	0.62	0.53
	Sex/age class (Adult male)	0.02	0.56	0.035	0.97
	Sex/age class (Cub)	0.10	0.27	0.39	0.70
	Sex/age class (Subadult)	-0.03	0.25	-0.12	0.91
	Body Condition	0.0005	0.002	0.29	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-0.09	0.63	-0.15	0.88
	Subpopulation (SB): Sex/age class (Cub)	-0.06	0.40	-0.15	0.88
	Subpopulation (SB): Sex/age class (Subadult)	-0.36	0.32	-1.12	0.27
	Subpopulation (SB): Body Condition	-0.0003	0.002	-0.15	0.88
Inverse Simpson Diversity (Adj. $R^2 = 0.00$, $p = 0.74$)					
Full Model:	~ subpopulation + sex/age class + body condition + sex/age class*subpopulation + body condition*subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	5.63	2.75	2.05	0.044
	Subpopulation (SB)	3.30	3.59	0.92	0.36
	Sex/age class (Adult male)	-1.97	4.72	-0.42	0.68
	Sex/age class (Cub)	0.85	2.24	0.38	0.71
	Sex/age class (Subadult)	-0.40	2.12	-0.19	0.85
	Body Condition	0.014	0.015	0.93	0.35

	Subpopulation (SB): Sex/age class (Adult male)	1.44	5.27	0.27	0.78
	Subpopulation (SB): Sex/age class (Cub)	-0.11	3.37	-0.032	0.97
	Subpopulation (SB): Sex/age class (Subadult)	-1.67	2.67	-0.63	0.53
	Subpopulation (SB): Body Condition	-0.011	0.018	-0.62	0.54
Faiths Phylogenetic Diversity (Adj. $R^2 = 0.06$, $p = 0.12$)					
Full Model:	~ subpopulation + sex/age class+ body condition + sex/age class*subpopulation + body condition*subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	13.64	1.80	7.58	4.4E-11
	Subpopulation (SB)	1.13	2.36	0.48	0.63
	Sex/age class (Adult male)	1.18	3.09	0.38	0.70
	Sex/age class (Cub)	-0.29	1.47	-0.19	0.85
	Sex/age class (Subadult)	**-2.49**	**1.39**	**-1.78**	**0.078**
	Body Condition	-0.0028	0.0096	-0.29	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-3.30	3.45	-0.96	0.34
	Subpopulation (SB): Sex/age class (Cub)	-1.32	2.21	-0.60	0.55
	Subpopulation (SB): Sex/age class (Subadult)	-0.42	1.75	-0.24	0.81
	Subpopulation (SB): Body Condition	0.0029	0.012	0.25	0.80

Supplementary Table S3.2. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis two classes of bacteria that differed in abundance between East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

	Class	Coef. (SB - EG)	*SE*	*Test statistic (W)*	*p-value*	*Adj. p-value*
1	Bacilli	-1.88	0.42	-4.49	<0.001	<0.001
2	Coriobacteriia	-0.95	0.32	-2.92	0.003	0.045

Supplementary Table S3.3. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis (for the 21 bacterial genera that differed in abundance between East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE. Asterisks (*) indicate differentially abundant genera that are also comprise a portion of the top ~90% of reads obtained for EG and SB polar bears combined.

	Genus	Coef. (SB vs. EG)	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*
1	**Anaerococcus*	3.16	0.55	5.71	<0.001	<0.001
2	**Murdochiella*	3.07	0.52	5.96	<0.001	<0.001
3	**Megasphaera*	2.70	0.72	3.76	<0.001	0.014
4	*Dialister*	1.89	0.50	3.81	<0.001	0.012
5	*Anaerobiospirillum*	1.68	0.43	3.94	<0.001	0.007
6	*Anoxybacillus*	1.50	0.38	3.93	<0.001	<0.001
7	*Hydrogenophilus*	1.29	0.33	3.94	<0.001	<0.001
8	*Parabacteroides*	0.60	0.28	2.15	<0.001	<0.001
9	*Anaerostipes*	0.46	0.26	1.79	<0.001	<0.001
10	*Cloacibacterium*	0.12	0.23	0.50	<0.001	<0.001
11	*Parvimonas*	-1.66	0.44	-3.77	<0.001	0.014
12	*Rothia*	-1.69	0.45	-3.78	<0.001	0.013
13	*TM7x*	-1.70	0.47	-3.64	<0.001	0.023
14	*Brachybacterium*	-1.78	0.42	-4.27	<0.001	0.002
15	*Leucobacter*	-1.80	0.52	-3.50	<0.001	0.037

16	**Escherichia_Shigella*	-1.87	0.43	-4.35	<0.001	0.001
17	**Collinsella*	-1.98	0.43	-4.59	<0.001	<0.001
18	*Turicibacter*	-2.29	0.62	-3.72	<0.001	0.017
19	*Enterococcus*	-2.42	0.57	-4.28	<0.001	0.002
20	**Erysipelatoclostridium*	-2.88	0.55	-5.28	<0.001	<0.001
21	**Lactobacillus*	-3.66	0.72	-5.07	<0.001	<0.001

Supplementary Table S3.4. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the 48 amplicon sequence variants (ASVs) that significantly differed in abundance between East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

	ASV	Class	Genus	Coefficient (SB vs. EG)	*SE.*	*Test statistic (W)*	*p-value*	*Adj. p-value*
1	ASV_49	Negativicutes	*Dialister*	3.54	0.41	8.59	<0.001	<0.001
2	ASV_20	Clostridia	*Anaerococcus*	2.76	0.57	4.81	<0.001	<0.001
3	ASV_26	Clostridia	*Murdochiella*	2.58	0.48	5.34	<0.001	<0.001
4	ASV_92	Clostridia	*Peptoniphilus*	2.06	0.37	5.57	<0.001	<0.001
5	ASV_46	Bacteroidia	*Porphyromonas*	2.00	0.44	4.51	<0.001	0.002
6	ASV_52	Negativicutes	*Megasphaera*	1.97	0.45	4.35	<0.001	<0.001
7	ASV_105	Clostridia	*Fastidiosipila*	1.78	0.37	4.87	<0.001	<0.001
8	ASV_72	Gammaproteobacteria	*Anaerobiospirillum*	1.73	0.39	4.46	<0.001	0.002
9	ASV_43	Clostridia	*Murdochiella*	1.45	0.43	3.39	<0.001	<0.001
10	ASV_136	Bacilli	*Anoxybacillus*	1.29	0.36	3.62	<0.001	<0.001
11	ASV_173	Gammaproteobacteria	*Hydrogenophilus*	1.22	0.31	3.90	<0.001	<0.001

12	ASV_120	Clostridia	*Helococcus*	1.21	0.37	3.31	<0.001	<0.001
13	ASV_121	Coriobacteriia	*Olsenella*	0.80	0.34	2.33	<0.001	<0.001
14	ASV_266	Campylobacteria	*Campylobacter*	0.75	0.29	2.57	<0.001	<0.001
15	ASV_159	Negativicutes	*Megasphaera*	0.73	0.34	2.12	<0.001	<0.001
16	ASV_363	Bacilli	*Anoxybacillus*	0.53	0.26	2.03	<0.001	<0.001
17	ASV_274	Clostridia	*NA*	0.50	0.27	1.83	<0.001	<0.001
18	ASV_252	Clostridia	*Peptostreptococcus*	0.47	0.30	1.53	<0.001	<0.001
19	ASV_277	Bacteroidia	*Bacteroides*	0.42	0.28	1.48	<0.001	<0.001
20	ASV_491	Clostridia	*Anaerostipes*	0.40	0.24	1.67	<0.001	<0.001
21	ASV_361	Bacteroidia	*Parabacteroides*	0.37	0.26	1.43	<0.001	<0.001
22	ASV_1799	Bacteroidia	*Bacteroides*	-0.70	0.18	-3.81	<0.001	0.029
23	ASV_978	Bacilli	*NA*	-0.99	0.25	-4.00	<0.001	0.014
24	ASV_1004	Bacilli	*Staphylococcus*	-1.09	0.27	-3.99	<0.001	0.015
25	ASV_525	Saccharimonadia	*TM7x*	-1.28	0.34	-3.78	<0.001	0.034
26	ASV_294	Bacilli	*Streptococcus*	-1.60	0.40	-3.96	<0.001	0.017
27	ASV_279	Clostridia	*Parvimonas*	-1.60	0.42	-3.81	<0.001	0.029
28	ASV_157	Actinobacteria	*Brachybacterium*	-1.84	0.42	-4.34	<0.001	0.003
29	ASV_312	Campylobacteria	*Campylobacter*	-1.85	0.41	-4.47	<0.001	0.002
30	ASV_356	Bacilli	*NA*	-1.91	0.37	-5.15	<0.001	<0.001
31	ASV_1	Gammaproteobacteria	*Escherichia/Shigella*	-1.91	0.41	-4.69	<0.001	<0.001
32	ASV_99	Bacilli	*Gemella*	-1.95	0.53	-3.71	<0.001	0.044
33	ASV_80	Gammaproteobacteria	*Klebsiella*	-1.98	0.52	-3.82	<0.001	0.028
34	ASV_160	Negativicutes	*Dialister*	-2.01	0.46	-4.36	<0.001	0.003
35	ASV_6	Coriobacteriia	*Collinsella*	-2.10	0.42	-4.94	<0.001	<0.001
36	ASV_115	Actinobacteria	*Actinomyces*	-2.16	0.51	-4.20	<0.001	0.006

37	ASV_24	Clostridia	*Lachnoclostridium*	-2.25	0.58	-3.92	<0.001	0.020
38	ASV_23	Gammaproteobacteria	*Klebsiella*	-2.35	0.62	-3.83	<0.001	0.028
39	ASV_39	Bacilli	*Turicibacter*	-2.36	0.60	-3.93	<0.001	0.018
40	ASV_60	Clostridia	*Clostridium_sensu_stricto_1*	-2.38	0.58	-4.09	<0.001	0.010
41	ASV_36	Clostridia	*Terrisporobacter*	-2.41	0.62	-3.91	<0.001	0.021
42	ASV_208	Actinobacteria	*Corynebacterium*	-2.47	0.47	-5.27	<0.001	<0.001
43	ASV_59	Bacilli	*Enterococcus*	-2.68	0.54	-4.99	<0.001	<0.001
44	ASV_16	Clostridia	*Blautia*	-2.82	0.68	-4.14	<0.001	0.008
45	ASV_62	Bacilli	*Erysipelatoclostridium*	-2.91	0.53	-5.55	<0.001	<0.001
46	ASV_9	Bacilli	*Streptococcus*	-2.94	0.77	-3.82	<0.001	0.029
47	ASV_14	Negativicutes	*Megamonas*	-2.97	0.71	-4.21	<0.001	0.006
48	ASV_8	Bacilli	*Lactobacillus*	-3.77	0.70	-5.39	<0.001	<0.001

Supplementary Table S3.5. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the three classes of bacteria that differed in abundance among sex/age classes (adult females [AF], adult males [AM] and subadults [S] compared to cubs [C]) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05).

	Class	Coef. AF-C	Coef. AM-C	Coef. S - C	SE AF-C	SE AM - C	SE S - C	W Statistic AF - C	W Statistic AM - C	W Statistic S - C	*p* - value AF-C	*p* - value AM - C	*p* - value S - C	FDR Adj. *p* - value AF - C	FDR Adj. *p* - value AM - C	FDR Adj. *p* - value S - C
1	Bacilli	-0.973	-2.32	-1.21	0.601	0.585	0.763	-1.62	-3.97	-1.58	0.105	0.00	0.113	1.00	0.001	1.00
2	Parcubacteria	0.059	0.379	0.378	0.448	0.473	0.491	0.131	0.802	0.771	0.00	0.00	0.00	0.00	0.00	0.00
3	Saccharimonadia	-0.424	-2.42	-0.915	0.845	0.797	0.918	-0.502	-3.03	-0.997	0.616	0.002	0.319	1.00	0.032	1.00

Supplementary Table S3.6. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the 21 bacterial genera that significantly differed in abundance sex/age classes (adult females [AF], adult males [AM] and subadults [S] compared to cubs [C]) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05)

Genus	Coef. AF-C	Coef. AM-C	Coef. S - C	SE AF-C	SE AM - C	SE S - C	W Statistic AF - C	W Statistic AM - C	W Statistic S - C	*p* - value AF-C	*p* - value AM - C	*p* - value S - C	FDR Adj. *p* - value AF - C	FDR Adj. *p* - value AM - C	FDR Adj. *p* - value S - C
Trueperella	1.600	3.596	-0.328	0.688	0.814	0.780	2.326	4.416	-0.421	0.020	0.000	0.674	1.000	0.001	1.000
Arthrobacter	0.428	0.004	0.365	0.524	0.510	0.685	0.816	0.007	0.533	0.000	0.000	0.000	0.000	0.000	0.000
Parabacteroides	0.178	1.515	0.459	0.312	0.459	0.459	0.571	3.302	1.002	0.000	0.000	0.000	0.000	0.000	0.000
Ignavigranum	-0.119	-1.254	-1.520	0.928	0.946	0.896	-0.128	-1.326	-1.696	0.000	0.000	0.000	0.000	0.000	0.000
Clostridium_sensu_stricto_7	0.538	1.924	0.944	0.456	0.539	0.586	1.181	3.571	1.611	0.000	0.000	0.000	0.000	0.000	0.000

Hathewaya	0.085	1.336	0.412	0.327	0.530	0.450	0.259	2.520	0.915	0.000	0.000	0.000	0.000	0.000	0.000
Tuzzerella	0.170	0.965	0.993	0.361	0.378	0.552	0.470	2.553	1.799	0.000	0.000	0.000	0.000	0.000	0.000
UBA1819	-0.170	1.335	0.400	0.291	0.445	0.410	-0.585	3.004	0.976	0.000	0.000	0.000	0.000	0.000	0.000
Peptostreptococcus	-0.796	2.542	-0.445	0.692	0.723	1.014	-1.150	3.516	-0.439	0.250	0.000	0.661	1.000	0.040	1.000
Anaerococcus	2.075	4.760	0.952	0.640	0.824	0.710	3.241	5.773	1.341	0.001	0.000	0.180	0.117	0.000	1.000
Ezakiella	-2.949	-3.921	-2.815	0.957	0.965	1.142	-3.080	-4.062	-2.464	0.002	0.000	0.014	0.200	0.005	1.000
Gallicola	-0.717	-0.830	-1.119	0.802	0.828	0.777	-0.893	-1.003	-1.440	0.000	0.000	0.000	0.000	0.000	0.000
Guggenheimella	0.173	0.109	0.480	0.404	0.429	0.559	0.428	0.254	0.859	0.000	0.000	0.000	0.000	0.000	0.000
Murdochiella	0.681	3.893	-0.208	0.794	0.952	0.792	0.858	4.090	-0.263	0.391	0.000	0.793	1.000	0.004	1.000
Soehngenia	0.075	-0.399	-0.189	0.488	0.512	0.591	0.154	-0.779	-0.320	0.000	0.000	0.000	0.000	0.000	0.000
W5053	0.287	-0.097	0.217	0.468	0.483	0.562	0.613	-0.202	0.387	0.000	0.000	0.000	0.000	0.000	0.000
Dialister	1.003	3.416	-0.052	0.734	0.776	0.890	1.366	4.405	-0.059	0.172	0.000	0.953	1.000	0.001	1.000
Methylorubrum	0.180	0.968	0.728	0.296	0.398	0.457	0.607	2.433	1.595	0.000	0.000	0.000	0.000	0.000	0.000
Enterobacter	0.108	1.049	0.157	0.334	0.444	0.305	0.324	2.365	0.513	0.000	0.000	0.000	0.000	0.000	0.000
Moraxella	-0.056	1.134	0.269	0.293	0.386	0.334	-0.191	2.940	0.805	0.000	0.000	0.000	0.000	0.000	0.000
Stenotrophomonas	-0.568	0.546	-0.368	0.463	0.584	0.466	-1.227	0.935	-0.790	0.000	0.000	0.000	0.000	0.000	0.000

Supplementary Table S3.7.Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the 78 amplicon sequence variants (ASVs) that significantly differed in abundance among polar bear sex/age classes (adult females [AF], adult males [AM] and subadults [S] compared to cubs [C]) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05)

ASV	Bacterial Class	Bacterial Genus	Coef. (AF - C)	Coef. (AM - C)	Coef. (S - C)	SE (AF - C)	SE (AM - C)	SE (S - C)	Test statistic [W] (AF - C)	Test statistic [W] (AM - C)	Test statistic [W] (S - C)	*p* -value (AF - C)	*p* -value (AM - C)	*p* -value (S - C)	FDR Adj. *p* -value (AF - C)	FDR Adj. *p* -value (AM - C)	FDR Adj. *p* -value (S - C)
ASV_15	Clostridia	*Peptostreptococcus*	0.102	3.085	0.189	0.850	0.830	1.135	0.120	3.718	0.166	0.905	0.000	0.868	1.000	0.049	1.000
ASV_20	Clostridia	*Anaerococcus*	1.875	4.529	0.736	0.555	0.693	0.500	3.379	6.540	1.473	0.000	0.000	0.000	0.000	0.000	0.000
ASV_22	Clostridia	*Fastidiosipila*	3.383	-0.108	1.140	0.697	0.545	0.906	4.854	-0.198	1.259	0.000	0.843	0.208	0.000	1.000	1.000
ASV_26	Clostridia	*Murdochiella*	0.815	3.382	0.148	0.572	0.721	0.588	1.427	4.689	0.251	0.154	0.000	0.801	1.000	0.001	1.000
ASV_29	Clostridia	*Ezakiella*	-2.941	-4.433	-2.846	0.981	0.937	1.157	-2.999	-4.733	-2.460	0.003	0.000	0.014	0.665	0.001	1.000
ASV_41	Actinobacteria	*Trueperella*	1.536	3.928	0.220	0.533	0.597	0.552	2.883	6.577	0.398	0.004	0.000	0.691	0.955	0.000	1.000
ASV_43	Clostridia	*Murdochiella*	0.093	1.575	-0.560	0.718	0.817	0.588	0.130	1.927	-0.952	0.000	0.000	0.000	0.000	0.000	0.000
ASV_73	Clostridia	*Clostridium_sensu_stricto_1*	0.545	1.087	1.423	0.435	0.492	0.719	1.253	2.210	1.977	0.000	0.000	0.000	0.000	0.000	0.000
ASV_87	Gammaproteobacteria	*Oligella*	1.164	-1.644	-1.174	0.873	0.804	0.821	1.333	-2.046	-1.430	0.000	0.000	0.000	0.000	0.000	0.000
ASV_91	Negativicutes	*Megamonas*	-3.276	-3.263	-3.689	1.094	1.113	1.048	-2.995	-2.932	-3.520	0.000	0.000	0.000	0.000	0.000	0.000
ASV_98	Bacteroidia	*Bacteroides*	-0.050	0.424	-0.336	0.526	0.536	0.392	-0.094	0.792	-0.859	0.000	0.000	0.000	0.000	0.000	0.000
ASV_107	Actinobacteria	*Corynebacterium*	-0.618	-2.392	-1.518	0.889	0.814	0.879	-0.694	-2.937	-1.727	0.000	0.000	0.000	0.000	0.000	0.000
ASV_108	Gammaproteobacteria	*Klebsiella*	0.062	-0.549	-0.807	0.800	0.764	0.707	0.077	-0.719	-1.141	0.000	0.000	0.000	0.000	0.000	0.000
ASV_110	Bacteroidia	*Bacteroides*	0.861	2.423	0.502	0.396	0.529	0.472	2.175	4.577	1.063	0.000	0.000	0.000	0.000	0.000	0.000
ASV_113	Bacteroidia	*NA*	0.361	-0.465	-0.457	0.554	0.458	0.429	0.651	-1.014	-1.065	0.000	0.000	0.000	0.000	0.000	0.000
ASV_118	Clostridia	*Clostridium_sensu_stricto_1*	0.462	0.496	2.623	0.425	0.380	0.895	1.088	1.305	2.931	0.000	0.000	0.000	0.000	0.000	0.000
ASV_120	Clostridia	*Helcococcus*	0.869	1.676	0.019	0.414	0.536	0.191	2.101	3.128	0.100	0.000	0.000	0.000	0.000	0.000	0.000
ASV_156	Clostridia	*Peptoclostridium*	0.109	1.154	0.306	0.268	0.455	0.302	0.406	2.535	1.012	0.000	0.000	0.000	0.000	0.000	0.000
ASV_159	Negativicutes	*Megasphaera*	-0.016	1.579	0.455	0.299	0.515	0.430	-0.055	3.068	1.058	0.000	0.000	0.000	0.000	0.000	0.000
ASV_164	Clostridia	*Clostridium_sensu_stricto_1*	0.242	0.502	1.200	0.370	0.349	0.627	0.655	1.441	1.915	0.000	0.000	0.000	0.000	0.000	0.000
ASV_168	Bacteroidia	*Bacteroides*	0.465	0.776	0.430	0.377	0.380	0.299	1.233	2.045	1.438	0.000	0.000	0.000	0.000	0.000	0.000
ASV_171	Actinobacteria	*Dietzia*	-0.403	-1.323	-1.341	0.749	0.702	0.672	-0.537	-1.886	-1.996	0.000	0.000	0.000	0.000	0.000	0.000
ASV_174	Clostridia	*Peptoclostridium*	0.113	1.123	0.337	0.273	0.449	0.328	0.415	2.504	1.029	0.000	0.000	0.000	0.000	0.000	0.000
ASV_177	Actinobacteria	*Corynebacterium*	-2.760	-3.082	-3.047	0.977	0.964	0.940	-2.824	-3.196	-3.241	0.000	0.000	0.000	0.000	0.000	0.000
ASV_179	Actinobacteria	*Corynebacterium*	0.521	-0.915	0.026	0.627	0.563	0.655	0.831	-1.626	0.040	0.000	0.000	0.000	0.000	0.000	0.000
ASV_186	Bacteroidia	*Bacteroides*	0.546	1.596	0.358	0.365	0.439	0.346	1.496	3.632	1.037	0.000	0.000	0.000	0.000	0.000	0.000
ASV_190	Clostridia	*Clostridium_sensu_stricto_7*	0.042	0.791	0.166	0.322	0.406	0.234	0.130	1.947	0.706	0.000	0.000	0.000	0.000	0.000	0.000
ASV_197	Clostridia	*Fastidiosipila*	0.593	-1.357	-0.513	0.722	0.666	0.800	0.820	-2.038	-0.641	0.000	0.000	0.000	0.000	0.000	0.000
ASV_202	Clostridia	*Hathewaya*	0.089	0.821	0.381	0.280	0.451	0.411	0.317	1.820	0.928	0.000	0.000	0.000	0.000	0.000	0.000
ASV_203	Bacilli	*Ignavigranum*	-0.199	-1.754	-1.669	0.894	0.863	0.840	-0.223	-2.033	-1.987	0.000	0.000	0.000	0.000	0.000	0.000
ASV_207	Actinobacteria	*Corynebacterium*	0.665	-0.552	0.389	0.583	0.487	0.581	1.141	-1.135	0.669	0.000	0.000	0.000	0.000	0.000	0.000
ASV_210	Bacilli	*Enterococcus*	1.142	0.089	0.755	0.401	0.283	0.494	2.849	0.314	1.528	0.000	0.000	0.000	0.000	0.000	0.000
ASV_214	Actinobacteria	*Corynebacterium*	0.461	-1.326	-0.991	0.684	0.622	0.644	0.673	-2.133	-1.540	0.000	0.000	0.000	0.000	0.000	0.000
ASV_216	Clostridia	*Clostridium_sensu_stricto_7*	0.149	0.446	0.660	0.335	0.344	0.539	0.446	1.295	1.225	0.000	0.000	0.000	0.000	0.000	0.000
ASV_217	Negativicutes	*Megamonas*	0.508	0.483	0.436	0.384	0.373	0.442	1.322	1.294	0.986	0.000	0.000	0.000	0.000	0.000	0.000
ASV_223	Clostridia	*Fastidiosipila*	0.742	-0.066	0.714	0.339	0.206	0.539	2.190	-0.321	1.324	0.000	0.000	0.000	0.000	0.000	0.000
ASV_224	Clostridia	*Lachnoclostridium*	0.180	1.403	0.456	0.288	0.440	0.430	0.627	3.187	1.058	0.000	0.000	0.000	0.000	0.000	0.000
ASV_235	Clostridia	*Gallicola*	-0.765	-1.316	-1.123	0.759	0.737	0.721	-1.008	-1.785	-1.557	0.000	0.000	0.000	0.000	0.000	0.000
ASV_252	Clostridia	*Peptostreptococcus*	-0.195	1.310	0.412	0.220	0.479	0.392	-0.886	2.732	1.052	0.000	0.000	0.000	0.000	0.000	0.000
ASV_269	Clostridia	*Helcococcus*	0.469	-0.422	-0.137	0.422	0.372	0.374	1.112	-1.134	-0.365	0.000	0.000	0.000	0.000	0.000	0.000
ASV_274	Clostridia	*NA*	0.217	1.085	0.019	0.304	0.398	0.191	0.713	2.723	0.100	0.000	0.000	0.000	0.000	0.000	0.000
ASV_277	Bacteroidia	*Bacteroides*	0.043	0.963	0.390	0.278	0.423	0.373	0.156	2.279	1.047	0.000	0.000	0.000	0.000	0.000	0.000
ASV_283	Actinobacteria	*Trueperella*	0.675	-0.380	-0.030	0.424	0.337	0.410	1.591	-1.127	-0.072	0.000	0.000	0.000	0.000	0.000	0.000
ASV_289	Clostridia	*Lachnoclostridium*	0.195	0.970	0.367	0.320	0.393	0.353	0.609	2.471	1.040	0.000	0.000	0.000	0.000	0.000	0.000
ASV_306	Gammaproteobacteria	*Acinetobacter*	0.548	0.122	0.376	0.315	0.267	0.337	1.740	0.457	1.115	0.000	0.000	0.000	0.000	0.000	0.000
ASV_323	Gammaproteobacteria	*Proteus*	0.663	0.966	0.019	0.348	0.390	0.191	1.905	2.480	0.100	0.000	0.000	0.000	0.000	0.000	0.000
ASV_327	Actinobacteria	*Flaviflexus*	-0.191	-0.805	-0.104	0.548	0.506	0.549	-0.348	-1.591	-0.189	0.000	0.000	0.000	0.000	0.000	0.000
ASV_341	Actinobacteria	*Arthrobacter*	0.287	-0.508	-0.284	0.475	0.420	0.421	0.605	-1.211	-0.675	0.000	0.000	0.000	0.000	0.000	0.000
ASV_353	Gammaproteobacteria	*Sutterella*	-0.206	0.404	-0.175	0.303	0.413	0.264	-0.679	0.980	-0.666	0.000	0.000	0.000	0.000	0.000	0.000
ASV_361	Bacteroidia	*Parabacteroides*	-0.040	0.945	0.429	0.248	0.375	0.407	-0.162	2.521	1.055	0.000	0.000	0.000	0.000	0.000	0.000

ASV_363	Bacilli	*Anoxybacillus*	0.524	0.666	0.313	0.348	0.322	0.337	1.506	2.068	0.930	0.000	0.000	0.000	0.000	0.000	0.000
ASV_393	Actinobacteria	*Corynebacterium*	-0.250	-1.385	-1.114	0.677	0.638	0.635	-0.370	-2.171	-1.754	0.000	0.000	0.000	0.000	0.000	0.000
ASV_395	Actinobacteria	*Leucobacter*	0.179	-0.744	-0.659	0.489	0.437	0.416	0.365	-1.704	-1.585	0.000	0.000	0.000	0.000	0.000	0.000
ASV_405	Clostridia	*W5053*	0.294	-0.609	0.187	0.457	0.418	0.546	0.644	-1.459	0.343	0.000	0.000	0.000	0.000	0.000	0.000
ASV_408	Clostridia	*Soehngenia*	0.083	-0.911	-0.219	0.474	0.447	0.574	0.175	-2.036	-0.382	0.000	0.000	0.000	0.000	0.000	0.000
ASV_444	Clostridia	*Guggenheimella*	0.181	-0.403	0.450	0.388	0.356	0.540	0.467	-1.131	0.832	0.000	0.000	0.000	0.000	0.000	0.000
ASV_453	Clostridia	*UBA1819*	-0.162	0.824	0.370	0.234	0.363	0.355	-0.696	2.268	1.041	0.000	0.000	0.000	0.000	0.000	0.000
ASV_474	Clostridia	*Fastidiosipila*	0.270	-0.315	0.278	0.443	0.390	0.486	0.610	-0.807	0.572	0.000	0.000	0.000	0.000	0.000	0.000
ASV_527	Gammaproteobacteria	*Halomonas*	0.170	0.443	0.092	0.270	0.311	0.200	0.629	1.424	0.462	0.000	0.000	0.000	0.000	0.000	0.000
ASV_553	Actinobacteria	*Corynebacterium*	0.394	0.441	0.179	0.304	0.274	0.263	1.298	1.611	0.682	0.000	0.000	0.000	0.000	0.000	0.000
ASV_647	Bacilli	*NA*	-0.714	-0.931	-0.327	0.573	0.552	0.598	-1.246	-1.686	-0.547	0.000	0.000	0.000	0.000	0.000	0.000
ASV_653	Gammaproteobacteria	*Moraxella*	-0.059	0.585	0.239	0.234	0.293	0.259	-0.252	1.998	0.922	0.000	0.000	0.000	0.000	0.000	0.000
ASV_755	Negativicutes	*Megasphaera*	-0.109	1.002	0.258	0.219	0.336	0.265	-0.501	2.984	0.973	0.000	0.000	0.000	0.000	0.000	0.000
ASV_915	Actinobacteria	*Actinomyces*	-0.009	-0.107	-0.299	0.298	0.278	0.219	-0.029	-0.385	-1.364	0.000	0.000	0.000	0.000	0.000	0.000
ASV_1004	Bacilli	*Staphylococcus*	-0.581	-0.766	-0.395	0.471	0.452	0.483	-1.233	-1.694	-0.819	0.000	0.000	0.000	0.000	0.000	0.000

Supplementary Table S3.8. Summary of top linear regression models found using backwards model selection and Akaike information criterion (AIC_c) scores. Models show significant terms (i.e., ecological factors) that explain variation in Shannon and Inverse Simpson alpha diversity, Faith's phylogenetic diversity, and Bray-Curtis and weighted UniFrac beta diversity indices for the subset of Southern Beaufort Sea (SB) polar bears for which fatty acid diet data was available. There were no significant terms in the models predicting variation in weighted UniFrac beta diversity NMDS axes.

Shannon Alpha Diversity (Adj. $R^2 = 0.16$, $p = 0.032$)					
Top Model:	~ Sex/age class* + Body Condition + FA_PC1* +FA_PC2				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	2.37	3.3E-01	7.25	8.5E-09
	Sex/age class (Adult male)	-0.90	0.38	-2.40	**0.021**
	Sex/age class (Subadult)	-0.34	0.21	-1.62	0.11
	Body Condition	0.003	0.0015	2.00	**0.052**
	FA_PC1	-0.086	0.037	-2.32	**0.026**
	FA_PC2	0.067	0.045	1.49	0.14
Inverse Simpson Alpha Diversity (Adj. $R^2 = 0.10$, $p = 0.039$)					
Top Model:	~ FA_PC1* + FA_PC2				

	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	0.86	0.011	78.80	<2e-16
	FA_PC1	-0.011	0.0048	-2.19	**0.034**
	FA_PC2	0.011	0.0074	1.49	0.14
Faiths Phylogenetic Diversity (Adj. R^2 = 0.23, p = 0.003)					
Top Model:	~ Sex/age class * + FA_PC1				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	15.66	0.66	23.78	<
	Sex/age class (Adult male)	-3.26	0.95	-3.44	**0.001**
	Sex/age class (Subadult)	-3.53	1.12	-3.15	**0.003**
	FA_PC1	-0.28	0.20	-1.43	0.16
Beta Diversity: Bray-Curtis NMDS 1 (Adj. R2 = 0.15, p = 0.011)					
Top Model:	~ Sex/age class * + FA_PC1				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	0.16	0.065	2.42	0.020
	Sex/age class (Adult male)	-0.22	0.084	-2.58	**0.013**
	Sex/age class (Subadult)	-0.34	0.12	-2.74	**0.009**
Beta Diversity: Bray-Curtis NMDS 2 (Adj. R2 = 0.14, p = 0.014)					
Top Model:	~ FA_PC2 **.** + Capture year*				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	124.0	58.35	2.13	0.039
	FA_PC2	-0.040	0.022	-1.84	**0.072**
	Capture year	-0.061	0.029	-2.13	**0.039**

Beta Diversity: Weighted UniFrac NMDS1 (Adj. R2 = -0.029, p = 0.58)					
Full Model:	~ Sex/age class + Condition + FA_PC1 + FA_PC2 + Capture year				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	1.8	0.10	0.18	0.86
	Sex/age class (Adult male)	1.1E-02	2.8E-02	0.41	0.69
	Sex/age class (Subadult)	2.9E-03	1.5E-02	0.19	0.85
	Body Condition	-1.9E-05	1.1E-04	-0.17	0.86
	FA_PC1	3.9E-03	3.0E-03	1.29	0.20
	FA_PC2	-4.2E-03	3.3E-03	-1.29	0.20
	Capture year	-9.2E-04	5.1E-03	-0.18	0.86
Beta Diversity: Weighted UniFrac NMDS2 (Adj. R2 = -0.069, p = 0.79)					
Full Model:	~ Sex/age class + Body Condition + FA_PC1 + FA_PC2 + Capture year				
	Coefficients	*Estimate*	*Std.Error*	*tvalue*	*Pr(>|t|)*
	(Intercept)	5.4E+00	6.99E+00	0.777	0.44
	Sex/age class (Adult male)	1.4E-04	1.9E-02	0.007	0.99
	Sex/age class (Subadult)	1.5E-02	1.0E-02	1.42	0.16
	Body Condition	9.3E-06	7.7E-05	0.12	0.90
	FA_PC1	3.3E-05	2.1E-03	0.016	0.99
	FA_PC2	1.2E-03	2.2E-03	0.54	0.60
	Capture year	-2.7E-03	3.5E-03	-0.78	0.44

Supplementary Table S3.9. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the 25 bacterial genera that significantly differed in abundance among polar bear sex/age classes sex/age classes (adult males [AM] and subadults [S] compared to adult females [AF], for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05)

	Genus	Coef. AM-AF	Coef. S - AF	SE AM - AF	SE S - AF	W Statistic AM - AF	W Statistic S - AF	*p* -value AM - AF	*p* -value S - AF	FDR Adj. *p* -value AM - AF	FDR Adj. *p* -value S - AF
1	*Brachybacterium*	-1.402	-0.984	0.589	0.603	-2.381	-1.631	0.000	0.000	0.000	0.000
2	*Helcobacillus*	-2.347	-2.146	0.651	0.642	-3.608	-3.344	0.000	0.000	0.000	0.000
3	*Arthrobacter*	-1.062	0.613	0.468	1.201	-2.270	0.510	0.000	0.000	0.000	0.000
4	*Atopobium*	0.666	1.704	0.360	0.972	1.851	1.753	0.000	0.000	0.000	0.000
5	*Proteiniphilum*	-0.371	0.013	0.410	0.379	-0.905	0.035	0.000	0.000	0.000	0.000
6	*Chryseobacterium*	-1.093	-0.592	0.574	0.589	-1.904	-1.005	0.000	0.000	0.000	0.000
7	*Bacillus*	-0.286	-0.069	0.462	0.410	-0.619	-0.168	0.000	0.000	0.000	0.000
8	*Ignavigranum*	-2.585	-1.976	0.533	0.561	-4.850	-3.523	0.000	0.000	0.000	0.000
9	*Vagococcus*	-1.673	-1.064	0.764	0.784	-2.191	-1.358	0.000	0.000	0.000	0.000
10	*Nosocomiicoccus*	-3.914	-2.793	0.638	0.919	-6.130	-3.040	0.000	0.002	0.000	0.241
11	*Hathewaya*	0.515	0.126	0.606	0.367	0.849	0.345	0.000	0.000	0.000	0.000
12	*Epulopiscium*	-0.949	0.203	0.568	0.754	-1.670	0.269	0.000	0.000	0.000	0.000
13	*Colidextribacter*	0.894	1.056	0.392	0.342	2.282	3.091	0.000	0.000	0.000	0.000
14	*UCG_005*	0.355	0.492	0.400	0.388	0.886	1.266	0.000	0.000	0.000	0.000
15	*Gallicola*	-1.005	-0.396	0.440	0.474	-2.282	-0.836	0.000	0.000	0.000	0.000
16	*Soehngenia*	-1.249	-0.640	0.387	0.424	-3.230	-1.508	0.000	0.000	0.000	0.000

17	*W5053*	-1.442	-0.833	0.405	0.441	-3.565	-1.891	0.000	0.000	0.000	0.000
18	*Phascolarctobacterium*	1.203	1.036	0.510	0.329	2.358	3.148	0.000	0.000	0.000	0.000
19	*Oligella*	-3.116	-2.025	0.646	0.765	-4.823	-2.649	0.000	0.008	0.000	0.799
20	*Burkholderia_Caballeronia_ Paraburkholderia*	0.391	0.473	0.362	0.326	1.077	1.450	0.000	0.000	0.000	0.000
21	*Ottowia*	-0.050	0.154	0.452	0.456	-0.111	0.337	0.000	0.000	0.000	0.000
22	*Schlegelella*	-0.174	0.173	0.313	0.347	-0.554	0.497	0.000	0.000	0.000	0.000
23	*Cronobacter*	-0.347	-0.001	0.515	0.517	-0.674	-0.002	0.000	0.000	0.000	0.000
24	*Edwardsiella*	0.444	-0.307	0.748	0.552	0.593	-0.556	0.000	0.000	0.000	0.000
25	*Stenotrophomonas*	0.249	0.317	0.414	0.344	0.602	0.920	0.000	0.000	0.000	0.000

Supplementary Table S3.10. Results of Analysis of Composition with bias correction (ANCOM-BC) analysis for the 99 amplicon sequence variants (ASVs) that significantly differed in abundance among polar bear sex/age classes (adult males [AM] and subadults [S] compared to adult females [AF], for East Greenland (EG) and Southern Beaufort Sea (SB) polar bear subpopulations (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05)

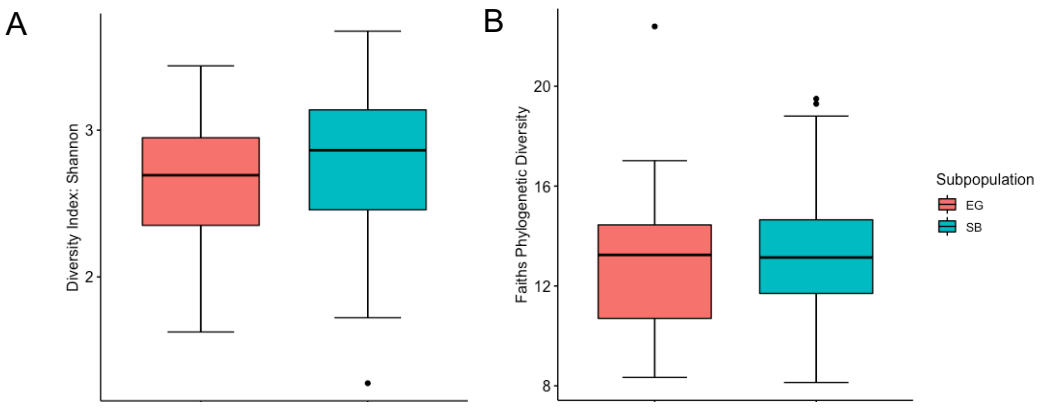
	ASV	Class	Genus	Coef. AM-AF	Coef. S - AF	SE AM - AF	SE S - AF	W Statistic AM - AF	W Statistic S - AF	*p* - value AM - AF	*p* - value S - AF	FDR Adj. *p* - value AM - AF	FDR Adj. *p* - value S - AF
1	ASV_22	Clostridia	*Fastidiosipila*	-4.34	-3.40	0.76	1.48	-5.70	-2.30	0.00	0.02	0.00	1.00
2	ASV_35	Clostridia	*Clostridium_sensu_stricto_1*	0.11	-1.29	0.88	0.65	0.12	-1.98	0.00	0.00	0.00	0.00
3	ASV_40	Gammaproteobacteria	*Edwardsiella*	0.30	-0.17	0.61	0.42	0.50	-0.39	0.00	0.00	0.00	0.00
4	ASV_42	Gammaproteobacteria	*Psychrobacter*	-3.29	-3.28	0.81	0.79	-4.06	-4.14	0.00	0.00	0.00	0.00
5	ASV_43	Clostridia	*Murdochiella*	0.79	-1.79	1.08	0.86	0.73	-2.09	0.00	0.00	0.00	0.00
6	ASV_60	Clostridia	*Clostridium_sensu_stricto_1*	-0.85	-1.45	0.69	0.60	-1.23	-2.43	0.00	0.00	0.00	0.00

7	ASV_76	Gammaproteobacteria	*Edwardsiella*	0.29	-0.21	0.64	0.49	0.46	-0.43	0.00	0.00	0.00	0.00
8	ASV_83	Bacilli	*Nosocomiicoccus*	-3.67	-2.79	0.64	0.89	-5.70	-3.11	0.00	0.00	0.00	0.50
9	ASV_87	Gammaproteobacteria	*Oligella*	-3.02	-2.65	0.63	0.65	-4.83	-4.08	0.00	0.00	0.00	0.00
10	ASV_91	Negativicutes	*Megamonas*	-0.10	-0.44	0.69	0.59	-0.14	-0.76	0.00	0.00	0.00	0.00
11	ASV_96	Actinobacteria	*Brevibacterium*	-3.04	-2.20	0.59	0.86	-5.15	-2.55	0.00	0.01	0.00	1.00
12	ASV_98	Bacteroidia	*Bacteroides*	0.34	-0.47	0.70	0.57	0.49	-0.83	0.00	0.00	0.00	0.00
13	ASV_100	Actinobacteria	*Helcobacillus*	-1.19	-1.04	0.58	0.59	-2.05	-1.78	0.00	0.00	0.00	0.00
14	ASV_103	Bacilli	*Vagococcus*	-1.76	-1.39	0.73	0.75	-2.40	-1.84	0.00	0.00	0.00	0.00
15	ASV_104	Actinobacteria	*Brevibacterium*	-2.30	-2.26	0.67	0.66	-3.46	-3.42	0.00	0.00	0.00	0.00
16	ASV_105	Clostridia	*Fastidiosipila*	-0.02	-2.51	0.87	0.69	-0.03	-3.64	0.00	0.00	0.00	0.00
17	ASV_107	Actinobacteria	*Corynebacterium*	-1.82	-1.45	0.64	0.67	-2.83	-2.18	0.00	0.00	0.00	0.00
18	ASV_108	Gammaproteobacteria	*Klebsiella*	-0.24	-0.35	0.54	0.48	-0.44	-0.73	0.00	0.00	0.00	0.00
19	ASV_113	Bacteroidia	*NA*	-1.11	-0.85	0.60	0.62	-1.85	-1.38	0.00	0.00	0.00	0.00
20	ASV_114	Actinobacteria	*Helcobacillus*	-2.08	-1.90	0.61	0.61	-3.43	-3.08	0.00	0.00	0.00	0.00
21	ASV_119	Actinobacteria	*Flaviflexus*	-0.36	-1.54	0.82	0.69	-0.45	-2.24	0.00	0.00	0.00	0.00
22	ASV_120	Clostridia	*Helcococcus*	-0.09	-1.97	0.91	0.71	-0.10	-2.77	0.00	0.00	0.00	0.00
23	ASV_123	Fusobacteriia	*Fusobacterium*	-0.71	-1.03	0.71	0.61	-1.00	-1.68	0.00	0.00	0.00	0.00
24	ASV_128	Fusobacteriia	*Fusobacterium*	-0.06	-0.43	0.58	0.46	-0.10	-0.93	0.00	0.00	0.00	0.00
25	ASV_139	Gammaproteobacteria	*Klebsiella*	0.34	-0.16	0.59	0.45	0.58	-0.36	0.00	0.00	0.00	0.00
26	ASV_142	Gammaproteobacteria	*Acinetobacter*	0.31	0.17	0.32	0.28	0.95	0.60	0.00	0.00	0.00	0.00
27	ASV_143	Actinobacteria	*Leucobacter*	-1.91	-1.81	0.66	0.67	-2.91	-2.72	0.00	0.00	0.00	0.00
28	ASV_148	Actinobacteria	*NA*	-2.18	-1.44	0.58	0.66	-3.79	-2.16	0.00	0.03	0.04	1.00
29	ASV_152	Clostridia	*Romboutsia*	0.27	-0.28	0.56	0.46	0.49	-0.61	0.00	0.00	0.00	0.00
30	ASV_157	Actinobacteria	*Brachybacterium*	-1.49	-1.31	0.58	0.59	-2.57	-2.21	0.00	0.00	0.00	0.00
31	ASV_170	Saccharimonadia	*NA*	-0.46	-0.19	0.35	0.37	-1.32	-0.52	0.00	0.00	0.00	0.00
32	ASV_179	Actinobacteria	*Corynebacterium*	-2.47	-1.72	0.52	0.69	-4.74	-2.47	0.00	0.00	0.00	0.00
33	ASV_184	Bacteroidia	*Bacteroides*	-0.85	-0.78	0.60	0.59	-1.40	-1.32	0.00	0.00	0.00	0.00
34	ASV_189	Clostridia	*Peptoniphilus*	-2.09	-1.17	0.53	0.81	-3.93	-1.43	0.00	0.15	0.02	1.00

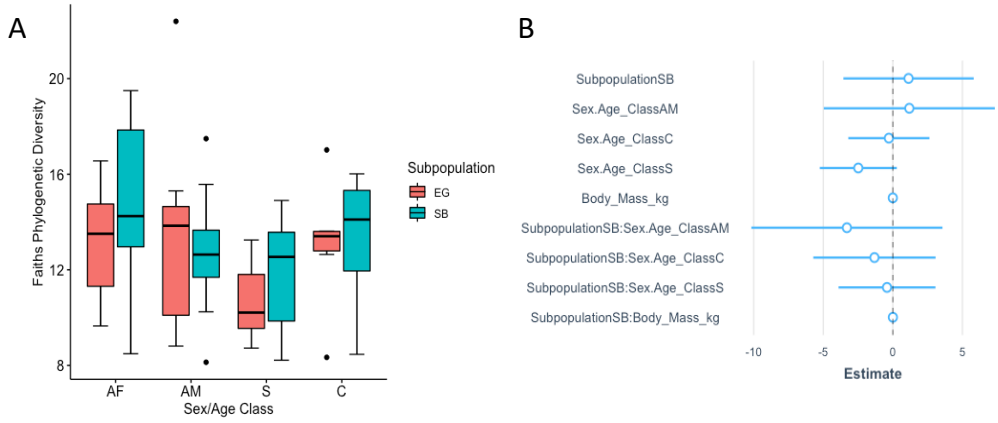
35	ASV_197	Clostridia	*Fastidiosipila*	-2.85	-1.63	0.52	0.94	-5.49	-1.73	0.00	0.00	0.00	0.00
36	ASV_199	Bacteroidia	*Bacteroides*	-1.25	-1.09	0.65	0.64	-1.91	-1.69	0.00	0.00	0.00	0.00
37	ASV_202	Clostridia	*Hathewaya*	0.43	-0.20	0.59	0.35	0.73	-0.56	0.00	0.00	0.00	0.00
38	ASV_203	Bacilli	*Ignavigranum*	-2.65	-2.28	0.51	0.54	-5.23	-4.25	0.00	0.00	0.00	0.00
39	ASV_207	Actinobacteria	*Corynebacterium*	-2.40	-1.69	0.55	0.65	-4.36	-2.60	0.00	0.00	0.00	0.00
40	ASV_209	Actinobacteria	*Corynebacterium*	0.71	0.04	0.57	0.40	1.25	0.09	0.00	0.00	0.00	0.00
41	ASV_214	Actinobacteria	*Corynebacterium*	-2.18	-1.81	0.56	0.58	-3.90	-3.09	0.00	0.00	0.00	0.00
42	ASV_223	Clostridia	*Fastidiosipila*	-1.27	-0.47	0.47	0.62	-2.72	-0.76	0.00	0.00	0.00	0.00
43	ASV_230	Clostridia	*Peptoclostridium*	0.72	0.03	0.53	0.39	1.35	0.07	0.00	0.00	0.00	0.00
44	ASV_235	Clostridia	*Gallicola*	-0.91	-0.55	0.41	0.44	-2.26	-1.24	0.00	0.00	0.00	0.00
45	ASV_237	Bacteroidia	*Porphyromonas*	0.21	-0.10	0.57	0.54	0.37	-0.18	0.00	0.00	0.00	0.00
46	ASV_240	Clostridia	*NA*	1.61	0.37	0.55	0.26	2.95	1.43	0.00	0.00	0.00	0.00
47	ASV_244	Bacilli	*Facklamia*	-0.74	-0.78	0.62	0.57	-1.19	-1.38	0.00	0.00	0.00	0.00
48	ASV_249	Bacteroidia	*Porphyromonas*	-1.53	-1.49	0.56	0.54	-2.72	-2.76	0.00	0.00	0.00	0.00
49	ASV_251	Clostridia	*Murdochiella*	0.67	0.09	0.56	0.39	1.20	0.24	0.00	0.00	0.00	0.00
50	ASV_253	Clostridia	*Clostridium_sensu_stricto_1*	-0.38	-0.32	0.52	0.49	-0.73	-0.65	0.00	0.00	0.00	0.00
51	ASV_262	Bacteroidia	*Bacteroides*	-1.03	-0.94	0.66	0.63	-1.56	-1.50	0.00	0.00	0.00	0.00
52	ASV_269	Clostridia	*Helcococcus*	-1.27	-0.90	0.41	0.44	-3.12	-2.04	0.00	0.00	0.00	0.00
53	ASV_274	Clostridia	*NA*	0.41	-0.74	0.66	0.55	0.62	-1.35	0.00	0.00	0.00	0.00
54	ASV_283	Actinobacteria	*Trueperella*	-1.80	-1.43	0.47	0.50	-3.83	-2.86	0.00	0.00	0.00	0.00
55	ASV_303	Clostridia	*Tuzzerella*	-0.19	-0.05	0.34	0.35	-0.54	-0.15	0.00	0.00	0.00	0.00
56	ASV_307	Clostridia	*Murdochiella*	-0.24	-0.61	0.67	0.58	-0.35	-1.05	0.00	0.00	0.00	0.00
57	ASV_318	Bacilli	*Streptococcus*	0.43	-0.15	0.51	0.42	0.83	-0.37	0.00	0.00	0.00	0.00
58	ASV_323	Gammaproteobacteria	*Proteus*	-0.44	-0.91	0.60	0.52	-0.73	-1.75	0.00	0.00	0.00	0.00
59	ASV_327	Actinobacteria	*Flaviflexus*	-1.19	-0.26	0.46	0.60	-2.59	-0.44	0.00	0.00	0.00	0.00
60	ASV_330	Clostridia	*Peptoclostridium*	0.72	0.02	0.54	0.40	1.32	0.04	0.00	0.00	0.00	0.00
61	ASV_341	Actinobacteria	*Arthrobacter*	-1.09	-0.72	0.44	0.47	-2.48	-1.53	0.00	0.00	0.00	0.00
62	ASV_343	Clostridia	*Anaerococcus*	0.20	-0.25	0.58	0.49	0.34	-0.52	0.00	0.00	0.00	0.00

63	ASV_345	Bacilli	*Nosocomiicoccus*	-1.45	-1.08	0.57	0.60	-2.52	-1.80	0.00	0.00	0.00	0.00
64	ASV_353	Gammaproteobacteria	*Sutterella*	0.14	-0.12	0.50	0.41	0.27	-0.29	0.00	0.00	0.00	0.00
65	ASV_369	Desulfovibrionia	*Bilophila*	0.96	1.20	0.38	0.67	2.55	1.80	0.00	0.00	0.00	0.00
66	ASV_393	Actinobacteria	*Corynebacterium*	-1.65	-1.28	0.49	0.52	-3.40	-2.48	0.00	0.00	0.00	0.00
67	ASV_395	Actinobacteria	*Leucobacter*	-1.02	-0.65	0.39	0.43	-2.64	-1.54	0.00	0.00	0.00	0.00
68	ASV_397	Gammaproteobacteria	*Psychrobacter*	-0.37	-0.28	0.43	0.40	-0.84	-0.70	0.00	0.00	0.00	0.00
69	ASV_401	Gammaproteobacteria	*Oligella*	-1.26	-0.89	0.45	0.48	-2.79	-1.84	0.00	0.00	0.00	0.00
70	ASV_405	Clostridia	*W5053*	-1.53	-1.16	0.39	0.43	-3.89	-2.69	0.00	0.00	0.00	0.00
71	ASV_408	Clostridia	*Soehngenia*	-1.33	-0.96	0.37	0.41	-3.62	-2.36	0.00	0.00	0.00	0.00
72	ASV_409	Clostridia	*Peptoclostridium*	0.07	-0.22	0.54	0.46	0.12	-0.48	0.00	0.00	0.00	0.00
73	ASV_410	Clostridia	*Helcococcus*	-1.10	-0.30	0.39	0.57	-2.83	-0.53	0.00	0.00	0.00	0.00
74	ASV_422	Gracilibacteria	*NA*	-0.88	-0.99	0.51	0.50	-1.73	-1.97	0.00	0.00	0.00	0.00
75	ASV_474	Clostridia	*Fastidiosipila*	-0.91	-0.16	0.40	0.46	-2.28	-0.35	0.00	0.00	0.00	0.00
76	ASV_502	Actinobacteria	*NA*	0.48	0.14	0.43	0.36	1.10	0.38	0.00	0.00	0.00	0.00
77	ASV_511	Clostridia	*Blautia*	-0.25	-0.26	0.42	0.39	-0.60	-0.67	0.00	0.00	0.00	0.00
78	ASV_516	Bacilli	*Staphylococcus*	0.27	-0.01	0.44	0.37	0.61	-0.02	0.00	0.00	0.00	0.00
79	ASV_538	Negativicutes	*Megasphaera*	1.10	1.17	0.43	0.64	2.54	1.83	0.00	0.00	0.00	0.00
80	ASV_547	Bacilli	*Streptococcus*	-0.40	-0.45	0.55	0.47	-0.73	-0.96	0.00	0.00	0.00	0.00
81	ASV_551	Coriobacteriia	*Atopobium*	0.52	0.78	0.33	0.45	1.60	1.75	0.00	0.00	0.00	0.00
82	ASV_563	Clostridia	*NA*	0.49	0.14	0.45	0.35	1.09	0.41	0.00	0.00	0.00	0.00
83	ASV_587	Actinobacteria	*Corynebacterium*	0.44	0.15	0.45	0.37	0.99	0.41	0.00	0.00	0.00	0.00
84	ASV_612	Clostridia	*Epulopiscium*	-1.03	-0.12	0.52	0.72	-1.99	-0.17	0.00	0.00	0.00	0.00
85	ASV_646	Gammaproteobacteria	*Schlegelella*	-0.26	-0.15	0.29	0.32	-0.88	-0.47	0.00	0.00	0.00	0.00
86	ASV_721	Bacteroidia	*Chryseobacterium*	-0.78	-0.41	0.34	0.38	-2.32	-1.08	0.00	0.00	0.00	0.00
87	ASV_758	Gammaproteobacteria	*Ottowia*	-0.13	-0.17	0.43	0.44	-0.31	-0.39	0.00	0.00	0.00	0.00
88	ASV_776	Actinobacteria	*Corynebacterium*	0.16	-0.34	0.41	0.37	0.39	-0.93	0.00	0.00	0.00	0.00
89	ASV_810	Alphaproteobacteria	*Methylobacterium-Methylorubrum*	0.35	0.26	0.35	0.29	1.01	0.91	0.00	0.00	0.00	0.00
90	ASV_833	Gammaproteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	0.31	0.15	0.32	0.29	0.95	0.51	0.00	0.00	0.00	0.00

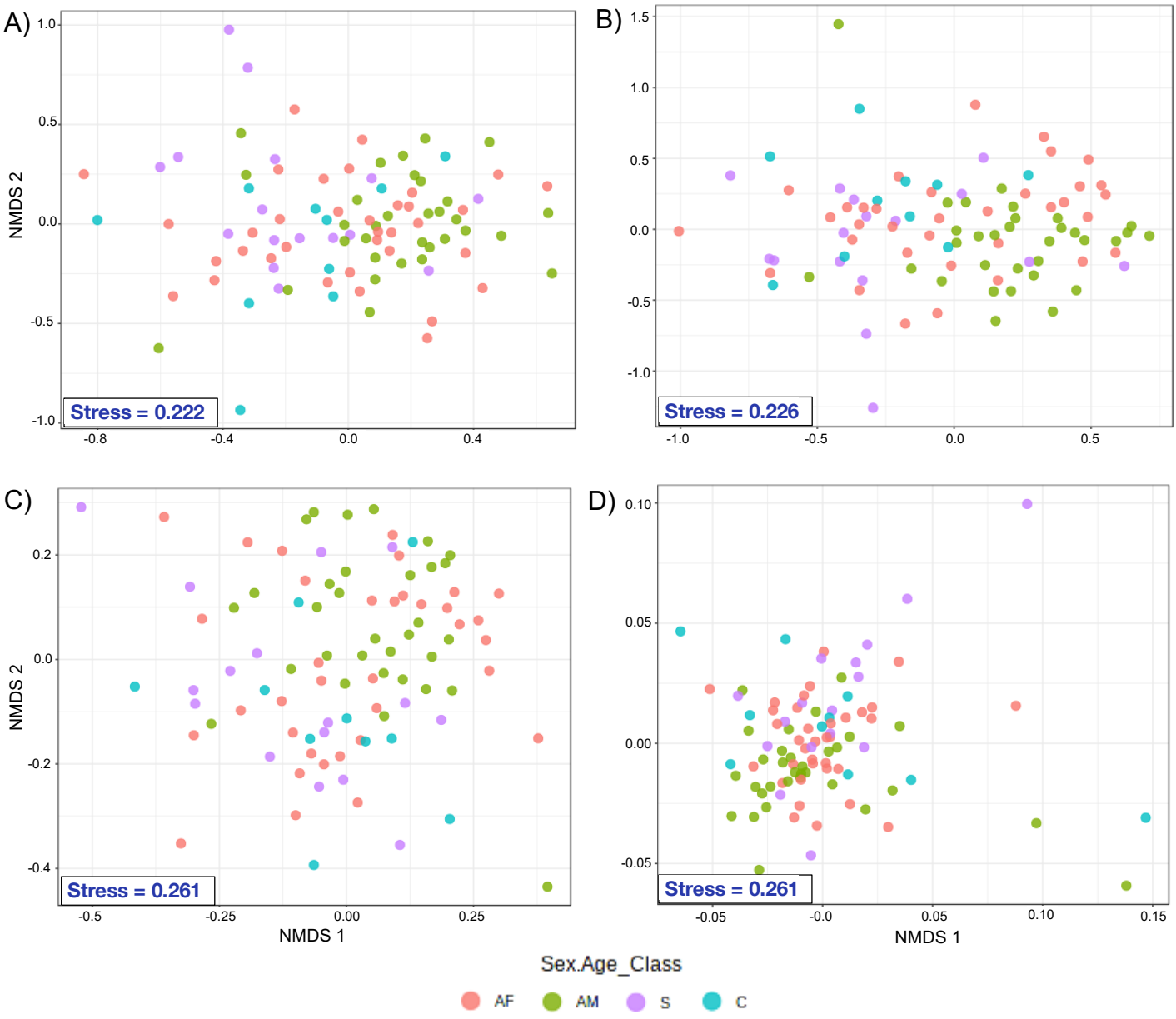
91	ASV_897	Negativicutes	*Megamonas*	0.25	0.15	0.35	0.32	0.71	0.46	0.00	0.00	0.00	0.00
92	ASV_915	Actinobacteria	*Actinomyces*	-0.27	-0.18	0.37	0.38	-0.73	-0.46	0.00	0.00	0.00	0.00
93	ASV_936	Gammaproteobacteria	*Enterobacter*	-0.34	-0.19	0.39	0.39	-0.86	-0.49	0.00	0.00	0.00	0.00
94	ASV_1027	Actinobacteria	*Dietzia*	0.50	0.73	0.31	0.41	1.63	1.78	0.00	0.00	0.00	0.00
95	ASV_1160	Clostridia	*NA*	0.64	0.92	0.29	0.42	2.18	2.17	0.00	0.00	0.00	0.00
96	ASV_1321	Clostridia	*Colidextribacter*	0.52	0.73	0.31	0.28	1.67	2.61	0.00	0.00	0.00	0.00
97	ASV_1541	Bacteroidia	*Bacteroides*	-0.01	0.04	0.36	0.35	-0.03	0.13	0.00	0.00	0.00	0.00
98	ASV_1850	Bacteroidia	*Parabacteroides*	-0.21	-0.09	0.33	0.35	-0.66	-0.27	0.00	0.00	0.00	0.00
99	ASV_1941	Negativicutes	*Megasphaera*	0.28	0.27	0.27	0.28	1.03	0.96	0.00	0.00	0.00	0.00



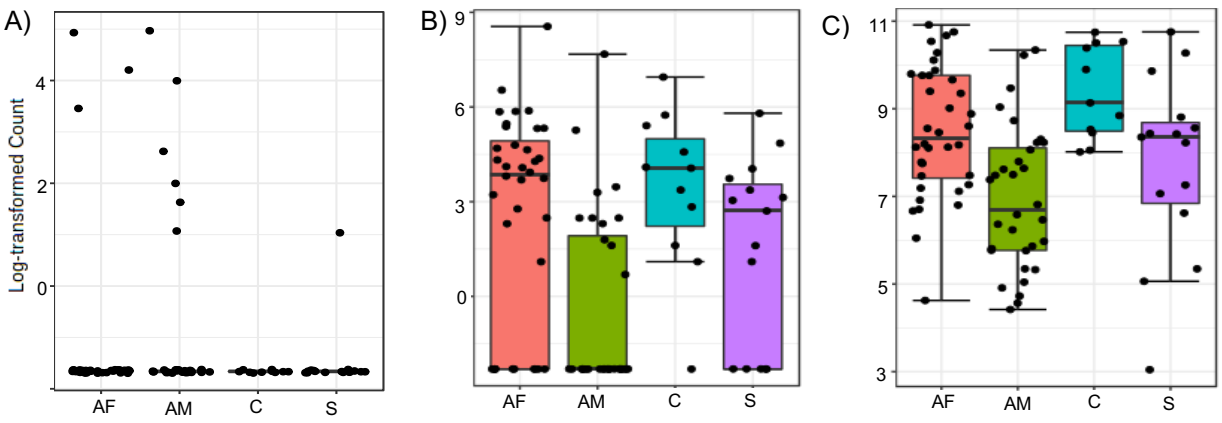
Supplementary Figure S3.1. Boxplots showing distribution of alpha diversity indices for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears for (A) Shannon alpha diversity indices (Group means: EG: 2.65 ± 0.07; SB: 2.74 ± 0.06), and (B) Faith's Phylogenetic Diversity indices (Group means: EG: 12.9 ± 0.5; SB: 13.3 ± 0.4).



Supplementary Figure S3.2. (A) Boxplots showing Faith's phylogenetic diversity (FPD) indices among sex/age classes (Adult females [AF], adult males [AM], subadults [S], and cubs [C]) for East Greenland (EG) (Group means: AF: 13.2 ± 0.5, AM: 13.5 ± 1.8, S: 10.7 ± 0.8, C: 13.1 ± 1.1) and Southern Beaufort Sea (SB) (Group means: AF: 14.8 ± 0.7, AM: 12.7 ± 0.4, S: 11.9 ± 0.7, C: 13.2 ± 1.7) polar bears. (B) Horizontal bar plots summarizing linear regression coefficients for FPD regression model. Bars indicate magnitudes of variable effects and 95% confidence intervals for terms included in the FPD linear regression model.

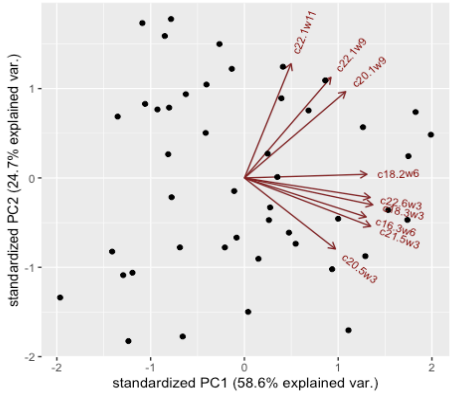


Supplementary Figure S3.3. Non-metric multi-dimensional scaling (NMDS) plots showing gut bacterial community composition differences among sex/age classes (Adult females [AF], adult males [AM], subadults [S], and cubs [C]) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears using Bray-Curtis distances at bacterial (A) class, (B) genus, and (C) amplicon sequence variant (ASV)-levels and using weighted UniFrac distance at (D) ASV-level.



Supplementary Figure S3.4. Boxplots showing differential abundances of log-transformed class counts indicating (A) higher Parcubacteria in adult females [AF] (Mean: 0.35 ± 0.2) , adult males [AM] (Mean: 0.5 ± 0.2) and subadults [S] (Mean: 0.07 ± 0.07) compared to cubs [C] (Not detected) and (B) higher Saccharimonadia in AF (Mean: 4.7 ± 0.6) and C (Mean: 5.0 ± 1.0) compared to AM (Mean: 1.5 ± 0.5) and S (3.2 ± 0.7), and (C) higher Bacilli in AF (Mean: 12.2 ± 0.4) and C (Mean: 13.4 ± 0.5) compared to AM (Mean: 10.0 ± 2.7) and S (Mean: 11.1 ± 0.4) sex/age classes for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. Analysis of composition with bias correction (ANCOM-BC) test results summarized in Supplementary Table S3.5.

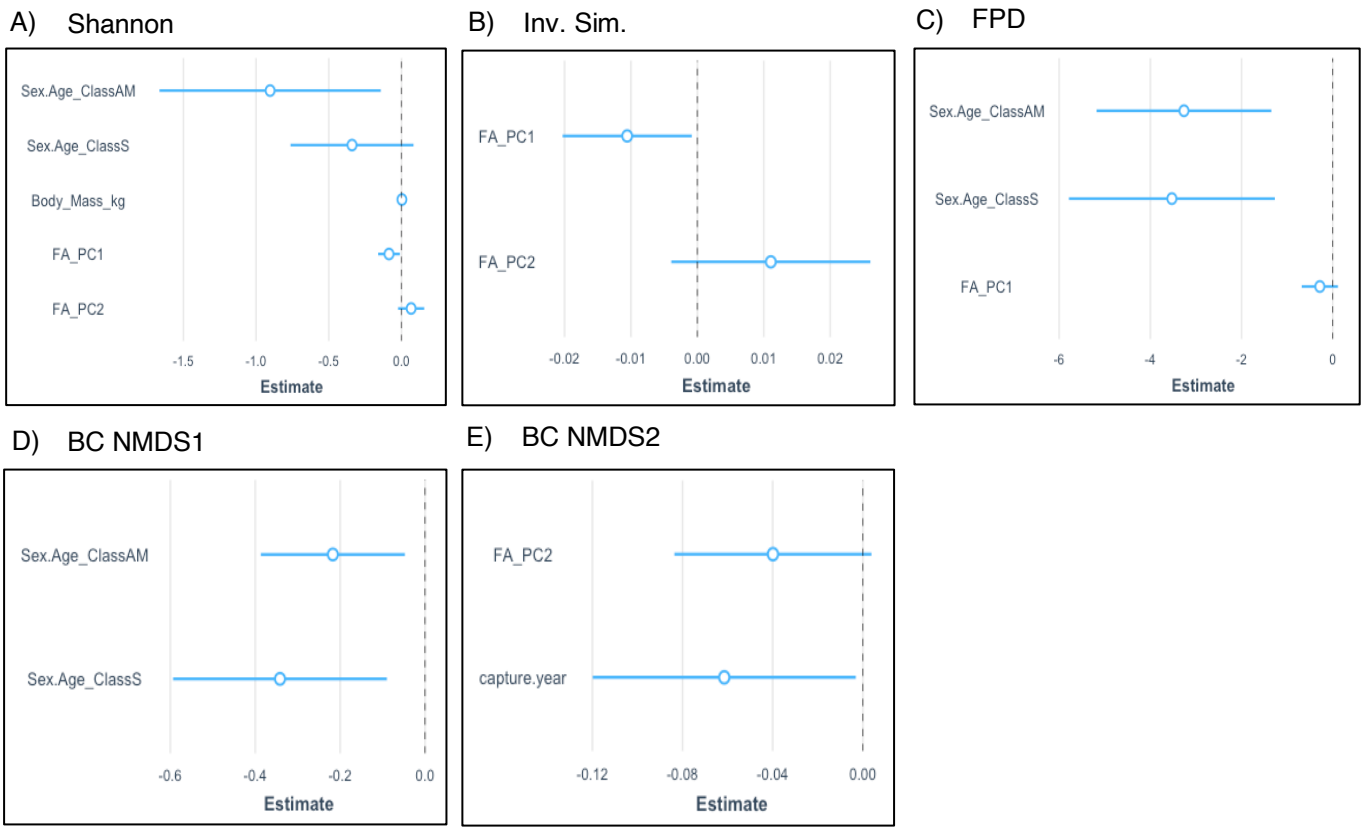
A)



B)

Fatty Acid	PC1 loading	PC2 loading
c16.3w6	0.373	-0.194
c18.2w6	0.376	0.0182
c18.3w3	0.394	-0.133
c20.1w9	0.311	0.428
c20.5w3	0.279	-0.352
c22.1w11	0.143	0.567
c22.1w9	0.265	0.499
c21.5w3	0.387	-0.24
c22.6w3	0.386	-0.097

Supplementary Figure S3.5. (A) Principal components analysis (PCA) showing loadings of the top nine fatty acids (FAs) which explained approximately 83.3% of the variation in diet among the 46 Southern Beaufort Sea (SB) samples for which diet data was available. (B) Table listing the top nine FAs and their associated PC1 and PC2 loading scores.



Supplementary Figure S3.6. Horizontal bar plots showing coefficient values and 95% confidence intervals for linear regression models incorporating diet as a driver of alpha and beta diversity within the subset of Southern Beaufort Sea (SB) polar bears for which diet data was available. (A) Shannon alpha diversity, (B) Inverse Simpson alpha diversity, (C) Faith's phylogenetic diversity (FPD), (D) first non-metric multidimensional scaling (NMDS) axis for Bray-Curtis distance (BC NDMDS1), second NMDS axis for Bray-Curtis distance (BC NMDS2). There were no significant terms in models explaining variation in weighted UniFrac NMDS coordinates for the subset of SB polar bears.

Supplementary Text S3.1.

Influence of sex/age class and body mass on gut bacterial composition and diversity for SB subset of polar bears

In addition to diet, other ecological drivers of variation in gut bacterial composition and diversity were identified. Sex/age class was identified as a significant term in models explaining variation in alpha diversity for Shannon and Faiths phylogenetic diversity (FPD) indices (Table 3.2,

Supplementary Table S3.8). Adult males and subadult bears appear to have lower Shannon Diversity and FPD compared to adult females (Supplementary Fig. S3.6A, S3.6C). Results of the PERMANOVAs found significant gut bacterial compositional differences among SB polar bear sex/age classes at the bacterial genus level and near-significant differences among sex/age classes at the ASV-level (Table 3.3). A total of 25 bacterial genera and 99 ASVs were differentially abundant among the different sex/age classes (Supplementary Tables S3.9 and S3.10). Adult males and subadult bears also appear to load lower on the Bray-Curtis NMDS1 axis compared to adult female bears (Supplementary Fig. S3.6D).

Connecting text:

To address the second research objective, in Chapter 4 we developed a novel DNA-based diet analysis approach for assessing diet of EG and SB polar bears using group-specific primer sets to detect pinniped (seal) and cetacean (whale) DNA in polar bear fecal samples. For a subset of SB polar bears, we compare results of DNA-based diet methods with quantitative fatty acid signature analysis (QFASA) methods of diet estimation. Finally, we test for associations between short-term (DNA-based) diet and variation in gut bacterial composition and diversity for EG and SB polar bears. Our objectives were to determine how the two diet methods compare in their ability to estimate polar bear diet, and to determine whether short-term diet is a significant driver of inter- and intra-population variation in gut bacterial diversity and composition for polar bears that we found in Chapter 3.

Chapter 4: Fecal DNA metabarcoding in polar bears (*Ursus maritimus*) from two different ecoregions provides credible short-term prey detections and explains variation in their gut microbiome

Note on this chapter

This chapter corresponds exactly to the manuscript titled "Fecal DNA metabarcoding in polar bears (*Ursus maritimus*) from two different ecoregions provides credible short-term prey detections and explains variation in their gut microbiome" which will be submitted to Marine Ecology Progress Series (MEPS). The manuscript was written in collaboration with Lyle G. Whyte, Todd C. Atwood, Damian Menning, Sarah A. Sonsthagen, Sandra L. Talbot, Kristin L. Laidre, Emmanuel Gonzalez, and Melissa A. McKinney. Melissa A. McKinney, Lyle G. Whyte, and Megan Franz designed the study and Todd C. Atwood and Kristin L. Laidre collected samples in the field. Sarah A. Sonsthagen, Sandra L. Talbot, and Damian Menning advised on experimental design, Megan Franz performed the laboratory work, analyzed the data, and wrote the initial draft of the manuscript. Emmanuel Gonzalez assisted with data processing. Melissa A. McKinney advised on interpretation of results. All authors helped to critically review and edit the manuscript.

Fecal DNA metabarcoding in polar bears (*Ursus maritimus*) from two different ecoregions provides credible short-term prey detections and explains variation in their gut microbiome

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4.1 Abstract

Polar bears (*Ursus maritimus*) occupy variable sea ice habitats across the circumpolar Arctic, which results in spatial variation in use of onshore habitats, food resources, and possibly in their gut microbiome. We used a metagenomic approach targeting pinniped and cetacean prey DNA to identify prey presence and preliminarily estimate prey relative abundance in fecal samples of East Greenland (EG) and Southern Beaufort Sea (SB) polar bears in the spring between 2015 to 2019. Ringed seal (*Pusa hispida*) was the predominant prey species present, identified in 100% of EG polar bears and 81% of SB polar bears. Bearded seal (*Erignathus barbatus*) DNA was found in 19% of SB polar bears. We compared prey presence and relative abundance to that estimated from quantitative fatty acid signature analysis (QFASA) for a subset of SB polar bears. Pinniped prey relative abundances from the DNA-based approach were consistent with the rank order found using QFASA but were not correlated with the QFASA proportional diet estimates (ringed seal: rho <0.15, $p > 0.54$). DNA-based prey detection ($R^2 = 0.03$), sex/age class ($R^2 = 0.07$) and subpopulation ($R^2 = 0.05$) significantly explained variation in gut bacterial composition of polar bears at multiple bacterial taxonomic levels. Polar bears with pinniped DNA identified in fecal samples showed higher abundance of bacteria from classes Clostridia and Bacilli, and reduced abundance of Negativicutes. Fecal DNA metabarcoding can be a useful approach for identifying recent prey fed on by polar bears, complimenting relatively longer-term estimates from QFASA, and can aid in interpreting individual variation in the polar bear gut microbiome.

4.2 Introduction

Understanding the feeding ecology of free-ranging wildlife provides critical insight into the structure and function of food webs, as well as into nutritional status of individuals which can be a predictor of reproductive success and survival [47, 162]. Feeding habits also influence the composition and diversity of the gut microbiome, a key aspect of health and immunity for wildlife host species [24, 25, 163]. While it can be challenging to assess wildlife diets, several methods exist, including direct observation, analysis of stomach contents, scats and hard parts, as well as chemical tracers like stable isotopes and fatty acid (FA) signatures [47]. Observational methods provide a glimpse into recent consumption but are biased against unobservable predation events and prey with soft tissues that degrade in the digestive tract [74, 164]. Stable isotope analysis of tissues can provide coarse resolution data on feeding habitats (e.g., $\delta^{13}C$ can differentiate benthic vs. pelagic feeding) and trophic position (e.g., $\delta^{15}N$ increases through food webs), but offers limited species-level resolution of prey items [47]. Quantitative fatty acid signature analysis (QFASA) can produce proportional estimates of prey consumed, yet may still be subject to false negatives or positives depending on the completeness and prey-distinguishability, respectively, of the reference library used [77]. To overcome limitations of applying single diet approaches, it has been recommended that multiple methods be used [47, 138, 165].

Improvements in molecular approaches to assessing the diets of wild species can provide species-level identification of prey, and possibly their relative abundances in the diet, and may help to overcome challenges in detecting rare occurrence or low-abundance prey species that may be missed by other methods [166]. Recently, Michaux et al. (2021) performed DNA-based prey identification on polar bear fecal samples using a universal mitochondrial cytochrome b (*Cytb*) gene region target to amplify vertebrate DNA in polar bear scat opportunistically collected from the M'Clintock Channel (MC) subpopulation of polar bears. Results were similar to what was reported for MC polar bears using QFASA [13], in that ringed seal detections predominated, but other lower abundance prey species were also identified. Nonetheless, there remain limitations to this approach, including over-representation of predator sequences, the inability to differentiate among some taxonomic groups, challenges in quantifying the amount of prey consumed, and possible environmental and human DNA contamination [61]. The

development of prey-specific or group-specific primers (DNA metabarcoding) that could reduce the amount of predator DNA amplified and improve species-level prey identifications, particularly when predator and prey are closely-related species with higher genetic similarity [167], such as is often the case for polar bears and their prey.

Certain fatty acids (FAs), which are synthesized at base levels of the food web, are incorporated from prey to predator tissues in proportions that are relatively unmodified or predictably modified, making them useful as dietary indicators [76]. QFASA allows for accurate proportional diet estimates of prey consumed by predators and is a more established quantitative approach for polar bears [8, 12, 77]. Despite some limitations [13, 78, 168], QFASA estimates may be useful in evaluating the utility of the DNA metabarcoding approach for identifying polar bear prey items. They may provide complementary information on the mechanisms by which climate-driven diet shifts may be impacting polar bear health, including in terms of changing contaminant exposures, infectious agents, and gut microbiota [12, 169].

Polar bears (*Ursus maritimus*) are currently identified as vulnerable on the IUCN red list due to reductions in the extent and seasonal duration of their sea ice habitat and consequently altered access to traditional ice seal prey and increased movement requirements [57, 65]. Changes in foraging behaviors have been observed in some subpopulations, such as those in the southern Beaufort Sea (SB) [8, 12, 17]. SB polar bears are distributed within the divergent ice ecoregion [14], wherein the seasonal formation of a large open water space between the continental shoreline and sea ice edge has led to increased use of land and land-based food resources by some SB polar bears during the summer and fall months [9, 12, 64, 70]. Polar bears of the East Greenland (EG) subpopulation occur within the convergent ice ecoregion, wherein the outflow of sea ice along the East Greenland shoreline currently continues to allow for some year-round access to ice seals, at least in the northern parts of the range [8, 170]. Dietary alterations of any kind have the potential to negatively impact polar bear population dynamics and individual health [70, 79]. For example, the gut bacterial composition of polar bears was recently shown to differ between SB polar bears using terrestrial habitats relative to those remaining on the sea ice during the reduced ice season [10], likely related to variation in feeding habits [171].

Here, we identify prey presence (i.e., 'prey detected' or 'not detected', as well as type of prey species detected) for EG and SB polar bears using newly-designed prey-specific primer sets. Due to the seasonal use of terrestrial habitats and food resources for SB but not EG polar bears, we hypothesize that prey detection frequencies and total number of prey species detected for each subpopulation differ between these two subpopulations. Second, we further assess the utility and possible limitations of this new prey-specific primer approach for estimating prey relative abundances by comparing these estimates to proportional diet estimates obtained via the QFASA method for a subset of SB polar bears for which we had data from both approaches. We predict that the approaches will yield different yet complementary estimates, due to different method-specific biases and expected differences in the dietary timeframe represented by each approach (i.e., DNA metabarcoding will provide short-term estimates, while QFASA will provide longer-term estimates). Third, we test associations between 'prey detected' vs. 'prey not detected' and gut microbiome diversity and composition for these two polar bear subpopulations, using existing bacterial 16S rRNA data from Franz et al. (2022).

4.3 Methods

Fecal and adipose tissue sample collection

Collection of EG and SB fecal samples and subsequent DNA extraction was previously described [171]. Briefly, a latex glove was used to obtain fecal residue from the rectum of 93 captured and anesthetized EG polar bears (n = 34) in 2017 and SB polar bears (n = 59) in 2015, 2016, 2018, and 2019. Sample collection occurred in the spring (March-April) for both subpopulations as part of routine annual population assessments and from all available sex/age classes (adult females [AF], adult males [AM], subadults [S], and cubs [C]—yearlings and dependent two-year-olds only, not cubs of the year). SB samples were collected as part of the U.S. Geological Survey (USGS) Polar Bear Research Program (U.S. Fish and Wildlife Service Permit# MA690038) under capture protocols approved by the USGS Institutional Animal Care and Use Committee. EG samples were collected by the Greenland Institute of Natural Resources as part of a long-term monitoring program under case nr. 2017-5446, document 4710596 from the Department of Fisheries and Hunting. Collection of adipose tissue from a rump biopsy for a subset (n = 46) of the same SB polar bears in 2016, 2018, and 2019 was also previously

described [171]. In brief, adipose biopsies were collected from adult female, adult male, and subadult sex/age classes only (no cubs), stored at -20 °C immediately following collection, and then stored at -80 °C until laboratory analysis.

DNA extraction

DNA extraction from fecal samples was performed using a modified protocol from the QIAamp Mini Kit Buccal Swab Spin Protocol (QIAamp DNA Mini and Blood Mini Handbook), as described previously [171]. Samples were eluted in a final volume of 100 µL elution buffer (buffer AE, Qiagen; Hilden, Germany) and used for prey-specific DNA amplification (see next section), as well as in subsequent 16S rRNA gene amplification analysis for gut bacteria [171].

Design and optimization of group-specific primer sets

Four prey-specific primer sets to amplify pinniped and cetacean DNA were developed using custom Python [172] and Biopython [173] scripts [174, 175]. Complementary DNA sequences of mitochondrial loci, cytochrome b (Cytb), and cytochrome oxidase 1 (CO1) were compiled from the NCBI GenBank nucleotide repository (http://www.ncbi.nlm.nih.gov) for all terrestrial and marine vertebrate species known to co-occur with or be a component of EG and SB polar bear diets and used for primer design and as a reference database. Sequences were aligned using MEGA7 [176], and four primer sets were designed: Cytb and CO1 pinniped primer sets and Cytb and CO1 cetacean primer sets (Supplementary Table S4.1). These primers captured all pinniped and cetacean species of interest for this study and amplify similar-sized DNA fragments.

The selected primer pairs were tested for successful amplification in cetacean and pinniped samples that were available in the lab from earlier projects (Supplementary Table S4.2), as well as polar bear to test for the extent to which host DNA might be amplified by these primer sets. Prey and polar bear DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). A series of temperature gradient PCRs were conducted to determine the optimal annealing temperature for each prey-specific primer set (Supplementary Table S4.1; Supplementary Figure S4.1). PCR reactions were performed in 25 µL volumes containing: 0.4 mg mL^{-1} BSA (bovine serum albumin), 0.6 µg $µL^{-1}$ of each primer, 1X Kapa Hifi Hot Start

Ready Mix (Roche Diagnostics), and 2.5 µL template prey DNA. Thermocycler conditions were 95 °C for 5 min, followed by 30 cycles of 98 °C for 30 sec, (prey-specific primer annealing temperature: 51-62 °C) for 30 sec, 72 °C for 40 sec, and concluded with 72 °C for 5 min. After successful amplification of the prey DNA and testing for amplification of polar bear DNA (Supplementary Figures S4.1, S4.2, and S4.3), additional PCRs were conducted on a subsample of randomly selected polar bear fecal DNA extracts to confirm successful amplification of prey DNA from desired samples using the PCR conditions described above (Supplementary Figure S4.4).

Prey DNA amplification, library preparation and sequencing

A 96-well plate containing the polar bear fecal extracts and four positive control prey DNA extracts (Cetacea positive controls: humpback whale (*Megaptera novaeangliae*) [HW005] and long-finned pilot whale (*Globicephala melas*) [PW 15]; pinniped positive controls: ringed seal (*Pusa hispida*) [RS 0001] and harp seal (*Pagophilus groenlandicus*) [H.Seal F]) were sent to Genome Quebec for metagenomic library preparation and sequencing. CS1 (5' – ACACTGACGACATGGTTCTACA – 3') and CS2 (5' – TACGGTAGCAGAGACTTGGTCT – 3') tags were added to primer sequences to each amplicon in addition to an adapter and unique barcodes. Amplification of prey DNA was performed separately for each of the four primer sets, with the appropriate corresponding humpback whale and pilot whale (cetacea) or ringed seal and harp seal (pinniped) positive controls. Due to issues amplifying the humpback whale (HW005) and pilot whale (PW 15) positive controls using the Cetacea_CO1_F4_R5 primer set with CS1 and CS2 tags added, this primer set was not used further in the study (Supplementary Figure S4.5).

PCR amplifications followed the above protocols for the remaining 3 primer sets (Supplementary Table S4.1) with two exceptions: BSA was not included and the template DNA was decreased from 2.5 µL to 1 µL. Verification of successful PCR amplification was visually confirmed on 2% agarose gel. Excess dNTPs and primers were removed with sparQ PureMag Beads (Quantabio) prior to the index PCR step. Index PCR reactions were in 20 µL volume and contained: 0.025 µL of FastStart High Fi 5U μL^{-1} (Roche Diagnostics), 1X Buffer (Roche

Diagnostics), 1.8 mM $MgCl_2$ (Roche Diagnostics), 1.0 µL of Dimethylsulfoxide (DMSO; Roche Diagnostics), 0.2 mM dnTP mix, 1.0 µL of PCR product per reaction well and 0.2 uM LNA™ modified custom primers (Exiqon). Thermocycler conditions were as follows: 95 °C for 10 min, 15 cycles of 95 °C for 15 sec, 60 °C for 30 sec, 72 °C for 60 sec, and concluded with 72 °C for 3 min. Verification of barcode incorporation for each sample was done on a 2% agarose gel. Amplicons were quantified using a Quant-iT™ PicoGreen dsDNA Assay Kit (Life Technologies). The library was generated by pooling the same quantity of each amplicon and with excess primers and dNTPs removed using sparQ PureMag Beads (Quantabio). The pooled libraries were quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). The average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Libraries (9 pM final concentration with 12% PhiX control) were sequenced on an Illumina MiSeq using a v3 600 cycle kit.

DNA-based prey detections

Sequence counts were processed and annotated using the ANCHOR pipeline and custom reference database for each primer set, separately, and output as exact sequence variants (ESVs) [177]. Only paired-end sequences with primer sequences present were selected, aligned, and dereplicated before selection of ESVs using a count threshold of 9 across all samples. Annotation queried two sequence repositories with strict BLASTn criteria (>99% identity and coverage): NCBI nt and either CO1 or Cytb custom made database. CO1 and Cytb databases were constructed from NCBI by downloading sequence IDs that were annotated as either (CO1 or Cytb). The CO1 and Cytb databases contained 3,369,178 and 577,858 sequences, respectively. Database versions were from August 2021. Note that all annotation was considered putative and subject to improvement as database errors are resolved and new species are characterized. Following taxonomic assignment and generation of ESV count data, 'Unknown' sequences (i.e. those that did not meet the 99% identity and coverage thresholds) were spot-checked against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure no potential species of interest went undetected in the final datasets. Unknown ESVs and ESVs that assigned to '*Ursus maritimus'* and '*Ursus arctos*' were removed from the datasets. Read counts of ESVs that

assigned to the same prey species were collapsed or merged into a single summed count for a given species for subsequent analyses and prior to merging datasets (done for the pinniped primer sets only). MicrobiomeAnalyst Web Version [106] was used to explore and compare primer performance in detecting prey DNA. Given the ANCHOR pipeline's strict filtering parameter settings, no additional filtering steps were applied to raw count data. However, the PCR blank for the Pinniped CO1 primer dataset contained 2 seal prey DNA counts, therefore a minimum count of 10 prey DNA sequence reads (5x the PCR blank) was applied to samples from that dataset. Pinniped prey DNA read counts were converted to relative abundances within each polar bear fecal sample in order to compare the pinniped primer performances (COI vs. Cytb). Pinniped Primer performance (COI vs. Cytb) was qualitatively assessed and reported as a comparison of the frequencies of detection of pinniped prey in the same set of polar bear fecal samples.

Fatty acid analysis and QFASA diet estimates

In this study, previously generated FA data were used [171] to estimate the diets of the SB polar bears using QFASA, as previously described for this subpopulation (McKinney et al 2017). In brief, adipose samples were lipid extracted, and the resulting FAs were converted to fatty acid methyl esters (FAMEs) and quantified by gas chromatography with flame ionization detection [171], producing proportions of each FA (mass % of total). A preexisting prey library was used consisting of bearded seal (*Erignathus barbatus*) (n = 20), beluga whale (*Delphinapterus leucas*) (n = 29), bowhead whale (*Balaena mysticetus*) (n = 64), and ringed seal (*Pusa hispida*) (n = 64) [12]. To account for the effects of metabolism and biosynthesis on predator FA signatures, calibration coefficients (CCs) from mink (*Mustela vison*) that had been fed a marine diet were used [77]. The model was run in R Studio [178] using the QFASA package [168] with a subset of the 30 dietary FAs (as per Iverson et al. 2004). The prey library used in the model was previously shown to identify prey with >90% accuracy [12]. To test the accuracy of predator diet estimates, a pseudo-predator diet was created and tested to see how well the QFASA estimate output matched a 'known' predator diet (25% bearded seal, 25% beluga whale, 25% narwhal, and 25% ringed seal) after 100 simulations (Supplementary Figure S4.6).

16S analysis

16S rRNA gene library preparation and sequencing were previously described for these samples, and sequence data are available (National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA): [Accession number: PRJNA773176] [171].

Data analyses

Differences in prey detection frequency (i.e., where prey DNA was detected or not) between subpopulations and among polar bear sex/age classes were assessed using Pearson's chi-square tests with an alpha-value cutoff of 0.05. This was done for all EG and SB samples combined, and if significant was followed with a chi-square post hoc test using the 'chisq.posthoc.test' function that uses the standardized residuals to calculate Bonferroni-adjusted p-values. R 4.0.3 (R Core Development Team 2020) was used to run this analysis as well as the remainder of statistical tests conducted throughout this study, unless stated otherwise.

To pseudo validate whether the. relative read abundances of prey detected from the metagenomic analysis are related to the proportional biomass of prey, we compared them to proportional diet estimates from the QFASA approach using a subset of SB samples for which seal prey DNA was detected (n = 20). The mean proportional estimates for each diet approach were visually compared among polar bear sex/age classes. In addition, pairwise Spearman's ranked correlation tests assessed correlations between the estimates produced by each method.

To examine the influence of diet on gut microbiota, we tested associations between prey presence and both microbiome diversity and composition by creating a new categorical variable: 'prey DNA detected' or 'no prey DNA detected'. This was done due to insufficient sample size of polar bears that had consumed each prey item in each subpopulation (i.e., bearded seal was only detected in SB polar bears). The Shannon, Inverse Simpson, and Faith's Phylogenetic alpha diversity indices were calculated at ASV-level for EG and SB polar bears as per Franz et al. (2022). Multiple linear regression models (LMs) were then used to examine relationships between feeding and alpha diversity indices. Other variables included were subpopulation, sex/age class (AF, n = 36; AM, n = 32; S, n = 15; C, n = 10), body mass as an indicator of body condition [179], and all relevant first-order interactions. Capture year was not included as an explanatory variable as the EG samples were collected in a single year, which did not overlap the

SB collection years. To test for variation in gut bacterial composition at multiple taxonomic levels (i.e., phylum, class, genus, and ASV), separate PERMANOVAs were run using the Bray-Curtis distance method using the same ecological variables and interaction terms as per the alpha diversity LMs. Homogeneity of group dispersions (PERMDISP; Anderson 2006) was assessed using the 'betadisper' function from the 'vegan' R package. For significant categorical variables, *post hoc* analyses of composition with bias correction tests (ANCOMBC; Lin et al. 2020) were done to determine which bacterial taxa were differentially abundant. For significant continuous variables, Spearman's ranked correlation tests determined to assess post hoc differences.

4.4 Results

Prey detection for EG and SB polar bears based on DNA metagenomicss

For both the pinniped Cytb and CO1 primer sets, ringed seal and bearded seal were the two prey species detected from the EG and SB polar bear fecal samples. After combining the pinniped Cytb and CO1datasets, pinniped prey DNA was detected in 53% of all fecal samples and represented the majority of prey detections for both EG (64.7%) and SB polar bears (46.6%). This prey detection frequency was not statistically different between the subpopulations (X^2 = 2.84, p = 0.09) (Table 4.1; Fig. 4.1). Cetacean DNA was not detected in any EG or SB fecal samples. Performance of the different prey-specific primer sets (i.e., how many samples each primer set detected prey DNA in, how effectively each primer set minimized predator DNA amplification, etc.) is reported in the Supplementary Information (Text S4.1, Table S4.3, and Figures S4.7, S4.8, S4.9, and S4.10).

Table 4.1. Percentages of seal prey detected among sex/age classes (adult females [AF], adult males [AM], subadults [S], and cubs [C]) of polar bears from the East Greenland (EG) and Southern Beaufort Sea (SB) subpopulations after combining prey DNA data from the pinniped cytochrome b and the pinniped cytochrome oxidase 1 primer sets.

Subpopulation	Initial Sample *n*	Samples *n* post-QC*	% samples prey detected	**Overall**** (EG: *n* = 34, SB: *n* = 58)			**AF** (EG: *n* = 16, SB: *n* = 20)			**AM** (EG: *n* = 7, SB: *n* = 24)			**S** (EG: *n* = 5, SB: *n* = 10)			**C** (EG: *n* = 6, SB: *n* = 4)		
				%RS	%BS	%NPD	%RS	%BS	%NPD	%RS	%BS	%NPD	%RS	%BS	%NPD	%RS	%BS	%NPD
EG	34	34	64.7	64.7	ND	35.3	62.5	6.3	37.5	28.6	ND	71.4	80.0	ND	20.0	100	ND	ND
SB	59	58	46.6	44.8	8.6	53.4	55.0	5.0	45.0	29.2	12.5	66.7	60.0	10.0	40.0	50.0	ND	50.0
Combined	93	92	53.3	52.2	6.5	46.7	58.3	5.6	41.7	29.0	9.7	67.7	66.7	6.67	33.3	80.0	ND	20.0

***** QC = 'quality check' and refers to samples that met sample filtering parameters.
** RS = ringed seal, BS = bearded seal, NPD = No prey DNA detected.
ND = not detected

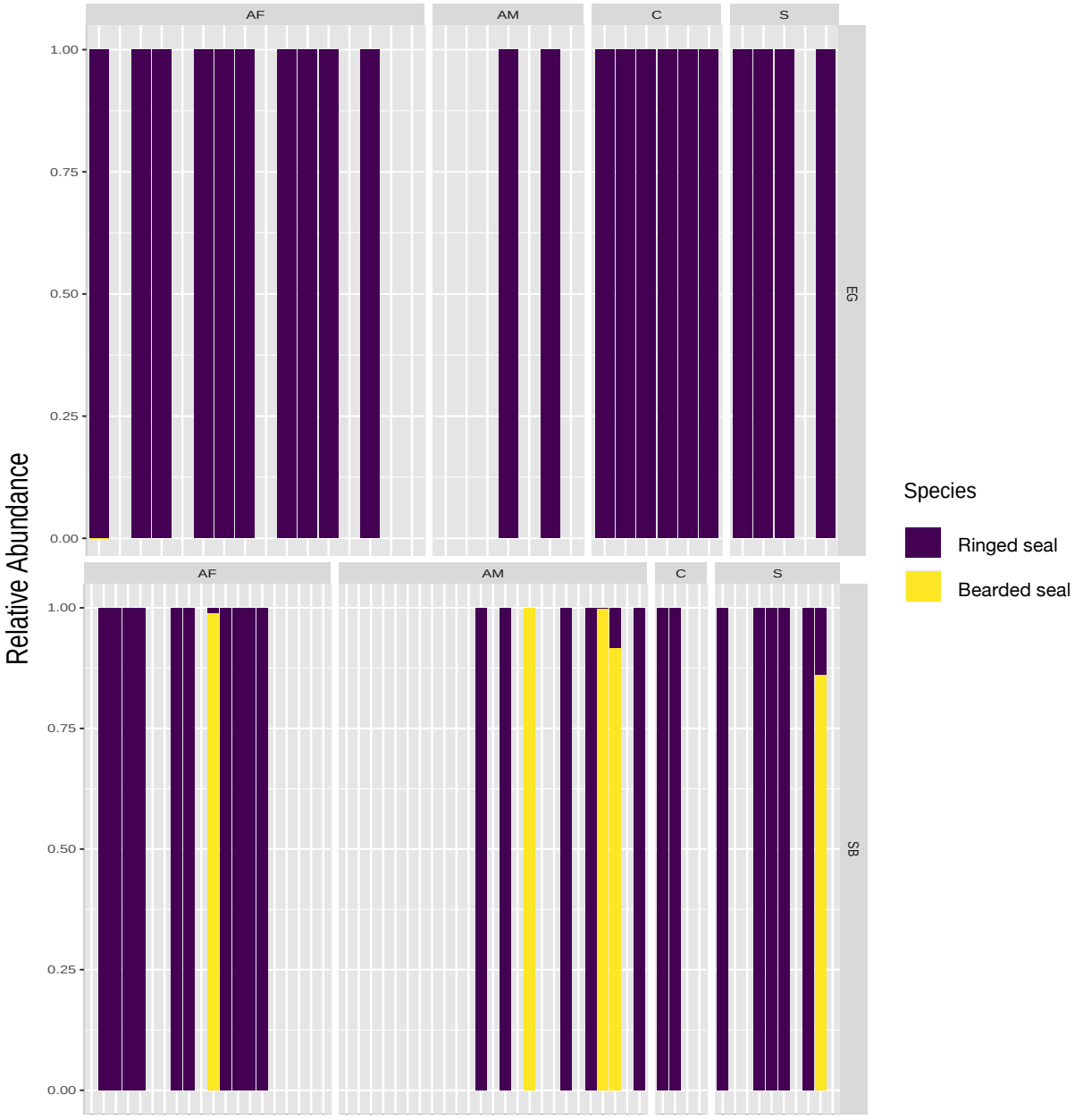


Figure 4.1. Bar plots showing the relative abundances of ringed seal and bearded seal prey using fecal DNA metabarcoding for East Greenland (EG; top panel) and Southern Beaufort Sea (SB; bottom panel) polar bears, grouped by sex/age class (adult female [AF], adult male [AM], cubs [C] and subadults [S]). Results were combined from the pinniped cytochrome b and pinniped CO1 datasets.

Ringed seal was found in 98% of all samples with prey detected (Fig. 1). Bearded seal DNA was detected in only 6.5% of samples, one from the EG subpopulation and five from the SB subpopulation (Fig. 4.1). For this one EG individual, bearded seal was only detected in trace

amounts, i.e., < 0.001% relative read abundance (RRA), while ringed seal was at > 99.99% RRA. Thus, the detection of bearded seal DNA in this individual may reflect contamination of the sample with environmental DNA or trace amounts of DNA from a previous meal. Given this, essentially all bearded seal detection was in the SB subpopulation. Bearded seal detection was nonetheless infrequent in SB polar bears as well, with DNA detected in only 8.6% of SB individuals (Table 4.1). For individuals for which both ringed and bearded seal were detected, the RRA of one of the prey species was consistently far higher (> 85%) than for the other prey species (Fig. 4.1).

For both subpopulations combined, patterns of prey DNA detection appeared to be similar among all sex/age classes ($X^2 = 7.26$, *p-adj.* = 0.07) (Table 4.1). Patterns of prey detection frequencies were also similar among sex/age classes within each of the subpopulations (EG: $X^2 = 7.5$, *p-adj.* = 0.06; SB: $X^2 = 2.80$, *p-adj.* = 0.42). Within the SB subpopulation, while bearded seal DNA detections were most frequent in adult males, no statistical analysis could be done to assess this pattern due to small sample sizes with bearded seal prey detected (AM: $n = 3$; AF: $n = 1$, S: $n = 1$) (Table 4.1; Fig. 4.1).

Comparison of prey DNA metabarcoding to QFASA

The presence of each prey species (proportion of polar bears where prey was detected) determined by the DNA-based and the QFASA-based approaches were compared for the subset of SB polar bears for which both sets of diet data were available (Table 4.2). For both techniques, ringed seal was the predominant prey species, detected in 95% of samples by DNA metagenomics and in 100% of samples using QFASA. Bearded seal was the second most detected prey by both approaches, but the detection rate of bearded seal among samples was much higher at 95% for QFASA versus 25% of samples by DNA metagenomics. No cetacean DNA was detected by DNA metagenomics; however, with QFASA, bowhead whale was estimated as a diet item in 85% of samples and beluga whale in 15% of samples.

Table 4.2. Percentage of polar bears for which a given prey item was detected in the diet using both prey DNA metagenomics and quantitative fatty acid signature analysis (QFASA) using a subset of Southern Beaufort Sea (SB) polar bears for which prey DNA was detected.

Prey type	% Detections (n = 20)	% AF (n = 9)	% AM (n = 7)	%S (n = 4)
Prey DNA metagenomics				
Ringed seal	95.0	100	85.7	100
Bearded seal	25.0	11.1	42.9	25.0
Beluga whale	ND*	ND	ND	ND
Bowhead whale	ND	ND	ND	ND
QFASA				
Ringed seal	100	100	100	100
Bearded seal	95.0	88.9	100	100
Beluga whale	15.0	33.3	0	0
Bowhead whale	85.0	77.8	100	75.0

* ND = not detected

To further compare the two methods, the relative abundance of prey DNA from metagenomics was compared to the proportional diet estimates from QFASA (Table 4.3). These estimates were not correlated for either proportional estimates of ringed seal consumption (Spearman's rho = 0.15, $p = 0.54$) or bearded seal consumption (rho = 0.10, $p = 0.68$). Despite lack of correlation in the individual-level proportional estimates obtained by these two approaches, the mean proportional estimates exhibited some consistencies in terms of rank order of the mean proportions of the main pinniped prey consumed. Ringed seal was estimated to be the predominant prey by both methods, with bearded seal second. Estimates using both methods were also similar in that they detected minimal or no contribution of cetacean species. Nonetheless, ringed seal estimates were higher for DNA metagenomics than for QFASA (76% vs 47%), while the reverse seemed to be the case for bearded seal and the cetacean species estimates. Broadly similar patterns of prey consumption among polar bear sex/age classes were found using both diet approaches; for both, adult females and subadults were estimated to consume more ringed seal than adult males, and adult males were estimated to consume more bearded seal than adult females and subadults (Fig. 4.2). Given the small sample sizes among polar bear sex/age classes after reducing to a sample size of $n = 20$ individuals, statistical analyses could not be done to assess these diet patterns.

Table 4.3. Comparison of the relative proportions (%) of each prey in the diet based on DNA metagenomics versus quantitative fatty acid signature analysis (QFASA) for a subset of Southern Beaufort Sea (SB) polar bears for which prey DNA was detected.

		Ringed seal (%)		**Bearded seal (%)**		**Beluga whale (%)**		**Bowhead whale(%)**	
SampleID	Sex/age Class	DNA-based	QFASA	DNA-based	QFASA	DNA-based	QFASA	DNA-based	QFASA
SB_5	AM	ND	33	100	60	-	0	-	7
SB_6	AM	0.2	44	99.8	35	-	0	-	21
SB_7	AM	8	27	92	17	-	0	-	56
SB_9	AM	100	38	ND	58	-	0	-	4
SB_40	AM	100	7.4	ND	73	-	0	-	19
SB_54	AM	100	18	ND	80	-	0	-	18
SB_58	AM	100	26	ND	23	-	0	-	51
SB_38	AF	1	42	99	50	-	0	-	8
SB_44	AF	100	0.2	ND	98	-	0	-	1
SB_45	AF	100	20	ND	73	-	0	-	8
SB_46	AF	100	89	ND	10	-	0	-	2
SB_14	AF	100	48	ND	1	-	8	-	42
SB_17	AF	100	77	ND	10	-	0	-	13
SB_20	AF	100	73	ND	0	-	27	-	0
SB_21	AF	100	69	ND	28	-	3	-	0
SB_48	AF	100	56	ND	42	-	0	-	1
SB_47	S	14	59	86	10	-	0	-	31
SB_39	S	100	83	ND	17	-	0	-	0
SB_22	S	100	81	ND	2	-	0	-	17
SB_43	S	100	55	ND	30	-	0	-	15
Mean proportion consumed		76.1 ± 9.5	47.3 ± 5.9	23.8 ± 9.5	35.8 ± 6.6	-	1.9 ± 1.4	-	15.7 ± 3.8

ND = not detected

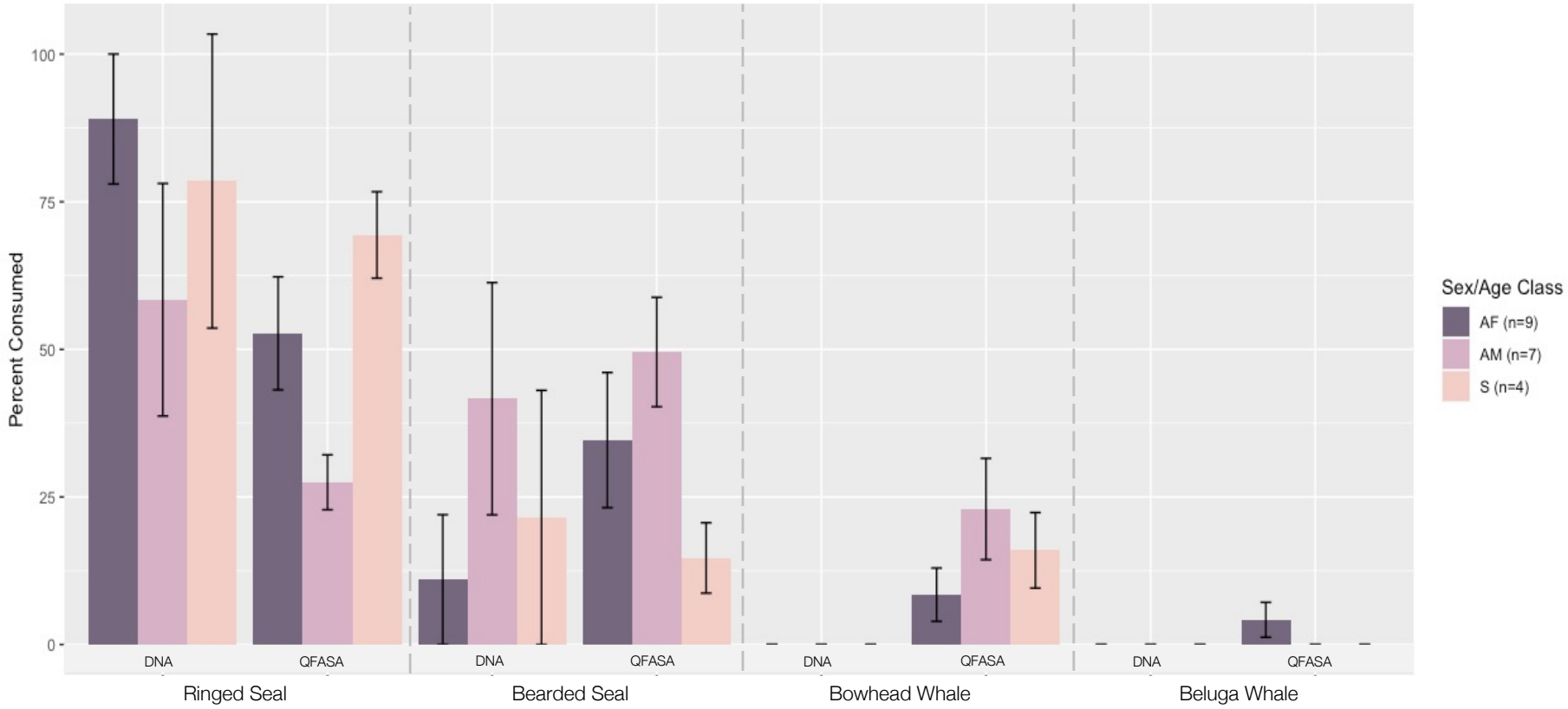


Figure 4.2. Comparison of diet estimates using DNA metabarcoding and quantitative fatty acid signature analysis (QFASA) for a subset of Southern Beaufort Sea polar bears for which prey DNA was detected (n = 20). Proportions of prey consumed are grouped by diet estimate approach (DNA- or QFASA-based) and further by sex/age class (Adult female [AF], adult male [AM], and subadult [S]). Error bars represent standard errors. Bowhead whale and beluga whale were not detected by DNA metabarcoding.

Influence of diet estimates from DNA metabarcoding on EG and SB gut microbiota

The DNA-based diet profiles did not significantly explain variation in Shannon, Inverse Simpson, and Faith's Phylogenetic alpha diversity indices (Supplementary Table S4.4). In contrast, gut bacterial composition was found to vary significantly with DNA-based diet profile category ('prey detected' versus 'prey not detected') at the bacterial class ($R^2 = 0.03$, $p = 0.01$), genus ($R^2 = 0.03$, $p = 0.003$), and ASV ($R^2 = 0.03$, $p < 0.001$) levels (Table 4.4). It should be noted that the homogeneity of group dispersion assumption was not met at the ASV-level for the Bray-Curtis distance, with 'prey detected' individuals having higher group dispersion (Distance to centroid: 0.59 ± 0.01) compared to 'prey not detected' individuals (Distance to centroid: 0.55 ± 0.01) (PERMDISP: F = 3.96, $p = 0.06$), therefore results from this model should be interpreted with some caution. Sex/age class and subpopulation were also significant in the models at bacterial class (Sex/age class: $R^2 = 0.06$, $p = 0.03$; Subpopulation: $R^2 = 0.03$, $p = 0.01$), genus (Sex/age class: $R^2 = 0.06$, $p = <0.001$; Subpopulation: $R^2 = 0.04$, $p = <0.001$), and ASV (Sex/age class: $R^2 = 0.06$, $p = 0.003$; Subpopulation: $R^2 = 0.05$, $p = 0.001$) levels (Table 4.4).

In general, bacteria within the classes Clostridia, Negativicutes, and Bacilli showed differential abundances between the DNA-based prey detection categories. At the class level, Clostridia were significantly higher (*Adj.* $p = 0.009$), and Negativicutes were nearly-significantly lower (*Adj.* $p = 0.12$), for 'prey detected' individuals than for those with 'prey not detected' (Fig. 4.3A; Supplementary Table S4.5). Two bacteria genera, Terrisporobacter (class: Clostridia) and Halomonas (class: Gammaproteobacteria), showed significantly different abundances according to DNA-based diet profile, and one showed a tendency towards differences, Clostridium_sensu_stricto_1 (class: Clostridia) (Figure 4.4B; Supplementary Table S4.6). Three ASVs were significantly differentially abundant or nearly-significantly so, and these ASVs assigned to the classes Clostridia and Bacilli (Supplementary Table S4.7).

Table 4.4. Permutational analysis of variance (PERMANOVA) results showing differences in gut bacterial composition (at bacterial phylum, class, genus, and ASV levels) related to DNA diet profile*, sex/age class and subpopulation for polar bears from East Greenland (EG) and the Southern Beaufort Sea (SB).

	Variable	# perm	DF (factor, total)	SS	MS	F.model	R^2	P	Sig. code
Phylum-level		1000							
	DNA diet profile		1, 92	0.11	0.11	1.67	0.02	0.18	
	Sex/Age Class		3, 92	0.24	0.08	1.24	0.04	0.28	
	Subpopulation		1, 92	0.15	0.15	2.29	0.02	0.09	
	Body Condition		1, 92	0.06	0.06	0.95	0.01	0.40	
	DNA diet profile *Sex/Age Class		3, 92	0.22	0.07	1.13	0.04	0.33	
	DNA diet profile *Subpopulation		1, 92	0.04	0.04	0.63	0.01	0.58	
	Subpopulation*Body Condition		1, 92	0.08	0.08	1.28	0.01	0.29	
	Residuals		81	5.29	0.07		0.85		
Class-level		1000							
	DNA diet profile		**1, 92**	**0.45**	**0.45**	**3.28**	**0.03**	**0.01**	**
	Sex/Age Class		**3, 92**	**0.81**	**0.27**	**1.95**	**0.06**	**0.03**	*
	Subpopulation		**1, 92**	**0.47**	**0.47**	**3.40**	**0.03**	**0.01**	**
	Body Condition		1, 92	0.10	0.10	0.71	0.01	0.60	
	DNA diet profile *Sex/Age Class		3, 92	0.65	0.22	1.57	0.05	0.09	
	DNA diet profile *Subpopulation		1, 92	0.10	0.10	0.70	0.01	0.61	
	Subpopulation*Body Condition		1, 92	0.20	0.20	1.46	0.01	0.21	
	Residuals		81	11.17	0.14		0.80		
Genus-level		1000							
	DNA diet profile		**1, 92**	**0.77**	**0.77**	**2.97**	**0.03**	**0.003**	**
	Sex/Age Class		**3, 92**	**1.67**	**0.56**	**2.15**	**0.06**	**<0.001**	***
	Subpopulation		**1, 92**	**1.14**	**1.14**	**4.40**	**0.04**	**<0.001**	***
	Body Condition		1, 92	0.15	0.15	0.59	0.01	0.87	
	DNA diet profile *Sex/Age Class		3, 92	1.01	0.34	1.30	0.04	0.12	
	DNA diet profile *Subpopulation		1, 92	0.17	0.17	0.65	0.01	0.81	
	Subpopulation*Body Condition		1, 92	0.28	0.28	1.07	0.01	0.36	
	Residuals		81	20.99	0.26		0.80		
ASV-level		1000							

	DNA diet profile		**1, 92**	**0.79**	**0.79**	**2.99**	**0.03**	**<0.001**	**
	Sex/Age Class		**3, 92**	**1.75**	**0.58**	**2.21**	**0.07**	**<0.001**	***
	Subpopulation		**1, 92**	**1.30**	**1.30**	**4.93**	**0.05**	**<0.001**	***
	Body Condition		1, 92	0.16	0.16	0.61	0.01	0.84	
	DNA diet profile*Sex/Age Class		3, 92	0.95	0.32	1.20	0.04	0.20	
	DNA diet profile*Subpopulation		1, 92	0.20	0.20	0.75	0.01	0.69	
	Subpopulation*Body Condition		1, 92	0.27	0.27	1.02	0.01	0.41	
	Residuals		81	21.39	0.26		0.80		

*Polar bear diet profile from DNA metagenomics, with two categorical diet variables: ‘prey DNA detected' and ‘prey DNA not detected’

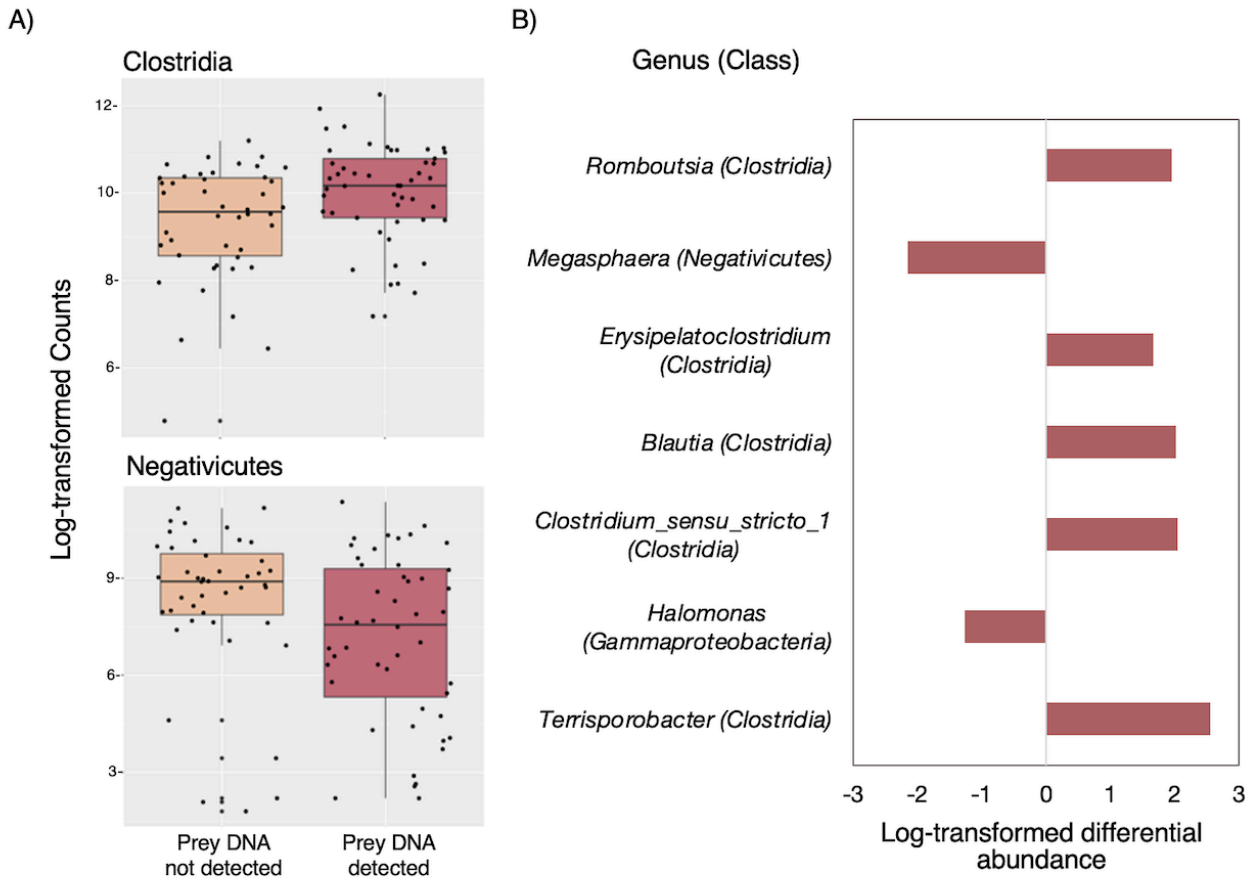


Figure 4.3. A) Boxplots showing log-transformed abundances of bacterial classes (A) and horizontal bar plot of bacterial genera (B) that were, or showed a tendency to be, differentially abundant (i.e., increased or decreased) in polar bears with 'prey DNA detected' vs. 'prey DNA not detected' DNA diet profiles. Statistical results are summarized in Supplementary Tables S4.5 and S4.6.

4.5 Discussion

We developed pinniped and cetacean group-specific prey primer sets to amplify prey DNA in polar bear fecal extracts. To our knowledge, this is only the second study to use DNA-based methods to assess polar bear diets [61], and it advances the approach by using group-specific primers instead of the universal *cytB* primer set. Use of targeted primer sets facilitated species-level resolution and reduced co-amplification of polar bear DNA. The estimates provided by the approach were also pseudo-validated by comparison to the estimates generated from the established QFASA method, with both methods underscoring the importance of ringed and bearded seals in polar bear diets. DNA metabarcoding results also showed promise for explaining variation in the gut microbiome of polar bears.

For most individual polar bears, when prey DNA was detected, it was largely just from a single species. This finding could be because polar bears are known to successfully capture prey somewhat infrequently, consuming perhaps the equivalent of around one adult ringed seal per week [180, 181]. Thus, the single prey species measured likely represents the detection of a single recent meal via prey DNA metagenomics. For a minority of polar bears, a second prey species was detected. The second prey always represented less than 15% RRA, suggesting perhaps several days had passed since this second prey species was consumed. This finding of just one or two prey species per polar bear diverges from the wider range of prey estimated using polar bear feces collected on the landscape and assessed by the universal *cytB* primer set [61]. The differences are likely related to differences in methodological approaches and study design/sample locations between the current study and Michaux et al. (2021) [61]. This previous study included a larger amount of starting material (scat), possibly greater chance of environmental contamination, prey misidentification, and seasonal variation due to opportunistic collection of fecal samples off the landscape, and also a more universal prey detection approach. An alternative explanation for low prey diversity seen in the DNA-based diet profiles is differential habitat preferences among pinniped species. For example, ringed and bearded seals have different sea ice habitat preferences, thus, with yearly variation in sea ice conditions in the Arctic one species might be more prevalent in a given region one year compared to the other [182, 183].

Ringed seal was the predominant prey species identified by DNA metagenomics for SB polar bears and was essentially the only prey item found for EG polar bears. This finding is

consistent with the well-documented tight predator-prey relationship between polar bears and ringed seals [180, 181, 184]. Polar bears were sampled in the spring, which is the most important feeding time for polar bears; during this period polar bears exhibit hyperphagia and, particularly for adult female polar bears and their cubs, it is adult female ringed seals and their newborn pups that are preyed on [15]. Previous QFASA studies have suggested EG polar bears increasingly feed on seasonally resident harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals with sea ice loss [8, 185]. However, no sub-Arctic seals were observed during the field work, which occurred from 19 March to 14 April 2017, consistent with the lack of detection of DNA of these sub-Arctic seals.

The DNA metagenomics method detected bearded seal as a minor prey species in the diets of SB but not EG polar bears, and no other seal species was detected as prey for either subpopulation. The Beaufort Sea coastal zone where the SB polar bears were sampled is characterized by shallow, productive waters, which represent the preferred habitat of bearded seal [15, 186]. Although polar bears prey on bearded seal less often than on ringed seal across the circumpolar Arctic, observation of prey kills has pointed to a higher reliance on bearded seals in the western Arctic, including the SB subpopulation, than in the eastern Arctic [181], consistent with bearded seal DNA detection in SB but not EG polar bears. A lack of detection of bearded seal and walrus prey DNA for EG polar bears is also consistent with previous diet estimates by QFASA of < 5% for bearded seal and no detection of walrus for the EG subpopulation [8], despite these two pinnipeds ranging within the habitat of the EG polar bear subpopulation [15]. Aside from ringed and bearded seals, no other pinniped species are thought to be accessible to SB polar bears [15, 67], which may explain a lack of detection of other pinniped prey DNA in this subpopulation.

No cetacean DNA was found in the spring-collected EG and SB polar bear fecal samples. Given evidence of positive control amplification, but lack of amplification in polar bear fecal samples, we conclude that cetaceans were likely not consumed by the EG or SB polar bears around the time period when they were sampled. The EG region supports narwhal and bowhead whale as potential cetacean prey [15]. However, bowhead whale are only accessible as beach-cast carcasses [62]. Previous QFASA estimates of narwhal consumption averaged < 1% for EG polar bears [8]. Beluga whale and bowhead whale are potential cetacean prey for polar bears within the SB region [15, 67]; however, there is limited evidence of springtime predation on

cetaceans for the SB subpopulation [187]. Some SB polar bears have been documented hunting and eating entrapped beluga whales in springtime [12, 187], but these events are considered to be infrequent, which may explain the lack of beluga DNA detected in SB polar bears by DNA metagenomics. In the summer and fall, some polar bears of the SB subpopulation shift to using onshore food resources as the sea ice extent declines [9, 18]. In particular, large numbers of SB polar bears access 'bone piles', which consist of remains of subsistence-harvested bowhead whales, as well as other species left in the area [9]. Since the DNA metagenomics approach probably only recovers recently consumed prey, if bowhead whale carcass consumption is largely in the fall, then it should be unlikely that bowhead DNA would be detected in fecal samples of spring sampled SB polar bears. For this reason, it would be useful to apply the DNA metabarcoding approach to analyze polar bear fecal samples collected in fall to provide greater insight into land-based feeding and seasonal differences in diet diversity.

DNA-based prey detections and relative abundances were compared to proportional diet estimates obtained using QFASA to assess the utility of the metagenomics approach and potential complementarity of both methods. DNA metagenomics presence/absence results were consistent with QFASA as well as other established methods (stable isotopes, observation, prey kills) [17, 181, 188] in identifying ringed seal as the predominant prey of the SB polar bears, followed by bearded seal. After converting prey DNA sequence count data to relative abundance data for comparison to QFASA proportional estimates, the pattern of higher bearded seal (or other larger prey) consumption by adult males and higher ringed seal consumption by adult females and subadults (although not statistically significant) was consistent with the QFASA estimates by sex/age class for SB polar bears (this study;[12]) and for other subpopulations [8, 13, 188]). However, DNA diet profiles from this study (both presence/absence and relative abundance results) suggested that cetacean species were not consumed, in contrast to the QFASA estimates and observations of SB polar bear feeding habits [12, 64, 187]. In addition, there was no correlation between the proportional diet estimates for these two approaches at the individual-level.

The divergence in estimates between the two methods is likely, in part, due to differences in the timescale represented by each method [61, 77]. Prey consumed more than 1-2 weeks prior to sample collection are unlikely to be detected by DNA-based approaches, due to prey DNA degradation by digestive enzymes and stomach bile [85, 189]. Nonetheless, in a controlled study

of captive polar bears fed a known diet, trace amounts of prey items were detected using a universal *cytB* primer set more than three weeks post-consumption [61]. Relative to DNA approaches, QFASA likely represents a longer feeding window, possibly in the order of multiple months for ursids. Controlled feeding trials on captive juvenile brown bears (*Ursus arctos*) found that QFASA estimates represented diet over the last one to three months [78]. The difference in time period represented by the two approaches may explain why, for instance, bowhead whale were indicated as prey by QFASA but not by DNA metagenomics, since SB polar bears are known to feed on bowhead carcasses onshore in the fall [9, 18], a time window unlikely to be captured by fecal DNA samples taken in the spring.

Possible explanations for differences in the proportional estimates from the two methods may be related to particularities of the methods themselves. It is currently impossible to convert DNA count data to reliable diet estimates given the multi-copy nature of the mtDNA target gene fragment in eukaryotes, and without conducting a controlled feeding study prior implementing newly-designed primers in studies on free-ranging wildlife [61, 81, 167, 190]. Certain primer sets can also show preferential amplification of certain prey species compared to others [81] and different tissues of the same prey species often have different mtDNA densities [191]. For our study, it is also possible that the probability of detection of cetacean species was diminished given that only one of the two cetacean primer sets was effective [81, 85]. In addition, both prey DNA and QFASA will only return prey they are set up to detect. Without specially designed primers or particular prey in the library for QFASA, respectively, certain prey species may not be identified, such as shorebirds or their eggs, which could be a minor component of the polar bears' diets [17]. Further, for QFASA, prey species that show similar FA composition can be difficult to differentiate [8, 77]. Despite these potential caveats, these two diet approaches may be complementary in representing diets in different seasons, and thus may contribute new insight into temporal variation in polar bear diets. Findings from the current study suggest that prey DNA results should be interpreted as 'short-term' presence/absence estimates, whereas QFASA better represents 'long-term' diet estimates.

The DNA diet profile term ('prey DNA detected' or 'prey DNA not detected') was significantly correlated with gut bacterial composition for EG and SB bears, but not of alpha diversity. Changes in composition but not alpha diversity of the gut microbiome have similarly

been found in studies examining the inherent dietary changes due to captivity on gut microbiota for multiple mammalian wildlife species [90, 137].

Although the interaction of DNA diet profile and polar bear sex/age class was not significant, both were independently significant. Morphological differences, energetic requirements, reproductive status, and spatial segregation vary among polar bear sex/age classes and thus lead to diet differences and differences in prey selection among them [12, 17, 70, 79]. As such, it is likely that the interplay of DNA diet profile differences and sex/age class differences overlap and explains some of the additional variation seen in polar bear gut bacterial composition.

For EG and SB polar bears, Clostridia and Bacilli abundances were higher in individuals where prey DNA was detected (ringed or bearded seal) compared to individuals where no prey DNA was detected. Clostridia is a bacterial class constituting a significant proportion of the polar bear core gut microbiome [10, 71], and more Clostridia with likely recent feeding supports its suggested functional role in lipid metabolism and fat deposition [10, 192]. Polar bears will often exclusively consume the lipid-rich blubber of marine mammal prey, leaving the rest of the carcass behind [193] and during the spring hyperphagic period must replenish fat stores lost over the less productive winter season [67]. Polar bears that had prey DNA detected likely had recently consumed a meal, and also showed higher levels of Bacilli compared to individuals where no prey DNA was detected. Bacilli are an important class of bacteria for maintaining gut health [110], so we can speculate that perhaps consumption of their traditional ice seal prey helps to maintain gut homeostasis in polar bears. Bacilli were previously found to be higher in EG and adult female polar bears compared to SB and adult male polar bears, respectively [171]. More EG polar bears and adult females tended to have had prey DNA detected compared to SB and adult male polar bears, suggesting that previously identified patterns of Bacilli with respect to subpopulation and sex/age class may in part be related to feeding.

Negativicutes were significantly higher in polar bears where no prey DNA was detected compared to those where prey DNA was detected, particularly those genera and ASVs assigning to *Megasphaera* species. More Negativicutes were previously found in SB polar bears than in EG polar bears [171]. Less is known about the class Negativicutes relative to Clostridia and Bacilli, yet, human gut microbiome studies suggest some genera found within the class Negativicutes serve as indicator species for host disease states following perturbation of the gut

flora [194]. Other research has suggested that certain Negativicutes are part of the commensal microbiota and can be involved with polysaccharide metabolism, producing short chain fatty acids that help maintain gut health [61, 118-120]. Deusch et al. (2014) found significant increases in *Megasphaera* in kittens fed a mixed proteinaceous/carbohydrate diet, while Franz et al. (2022) suggested that increased Negativicutes could reflect carbohydrate or starch input from onshore food resources or protein input from increased use of bowhead whale 'bone piles.'

Terrestrial foraging typically occurs in the summer and fall months for polar bears in the SB subpopulation, and the 'bone piles' are a particularly important food resource for adult male polar bears [144], which were found to have higher levels of Negativicutes compared to other sex/age classes [171]. It is possible that these more long-term diet habits of some of the onshore bears within the SB subpopulation contributed to this compositional pattern detected in individuals where no prey DNA was detected, as long-term diet is a known driver of gut bacterial composition [21]. Although certain gut bacteria have been shown to vary in function between host species, it is plausible that there are links between higher abundance of Negativicutes and use of land-based food resources in the SB subpopulation, as well adult male polar bear-specific foraging tendencies. We hypothesize that these processes could be leading to shifts in metabolic functionality of some bacteria in the gut microbiome of SB polar bears to accommodate seasonal reductions in ice-associated, lipid-rich prey availability. Studies on long-term changes in the polar bear gut microbiome and the functional role of particular bacterial groups within their wildlife hosts are needed.

With molecular diet analysis now available, this method will likely increase in use for studying the diets of free-ranging wildlife and, as we have shown here, can be helpful in interpreting gut bacterial community data. It is also useful that prey DNA metagenomics and gut microbiome data can be obtained from the same fecal DNA extract. The DNA-based diet profiles appeared to be credible, given the similar dietary patterns among sex/age classes as found with QFASA. The two techniques provided complimentary yet uncorrelated results, likely a consequence of the approaches representing different, minimally overlapping dietary time periods—DNA metabarcoding appeared to reflect more short-term diets while QFASA appeared to reflect more long-term diets. Thus, this study highlights the importance of using complementary diet assessment techniques to reveal spatio-temporal variation in dietary patterns for wild species [47, 61, 85, 195]. Further understanding of how temporal diet variation

influences the gut microbiome might come from incorporating these approaches simultaneously, providing important insight for polar bears currently as they undergo large-scale changes in foraging ecology because of climate change.

4.7 Supplementary Material

Supplementary Table S4.1. Cetacean and pinniped cytochrome oxidase 1 (CO1) and cytochrome b (cytb) prey-specific primer sequences and associated range of melting temperatures. F = forward direction , R = reverse direction.

Primer	Direction	Primer Sequence (5'-3')	Melting Temperature			Fragment size (bp)	Optimal annealing temperature (°C)
			Min. (°C)	Mean (°C)	Max. (°C)		
Pinniped_CO1_17	F	SGGRACYGGRTGAACCG	60.4	63.8	67.5	261	59
Pinniped_CO1_17	R	RRYATRGTRATRCCAGC	48.8	56.2	64.4		
*Cetacea_CO1_F4_R5	F	TAGCACATGCAGGAGC	60.7	60.5	60.8	269-273	62
*Cetacea_CO1_F4_R5	R	CCTCCDCCYGCMGGGTC	64	67.7	72.3		
Pinniped_Cytb1_2	F	YCAYCAGCACCCAAAGC	61	62.4	64.3	103-391	62
Pinniped_Cytb1_2	R	GCTTATATGCATGGGGC	-	58.7	-		
Cetacea_cytb_Forward_1	F	RYACAAATYYTAACAGG	48.8	52	56.3	266-269	51
Cetacea_cytb_Forward_1	R	ACRTARCCYACGAATGC	55.3	59	63.1		

*The Cetacea_CO1_F4_R5 primer set successfully amplified pure cetacean DNA extract in earlier lab trials, however following amplification issues after the addition of CS1 and CS2 tags (see main text) it was dropped from the current study

Supplementary Table S4.2. List of prey DNA extracts used for prey primer testing and optimization.

Sample origin	Sample ID	DNA Extract ID	Order	Genus	Species	Common name
East Greenland	Harp seal F	H.Seal F	Pinniped	*Pagophilus*	*groenlandicus*	Harp seal
East Greenland	Harp seal G	H.Seal G	Pinniped	*Pagophilus*	*groenlandicus*	Harp seal
SIMEP	ARRB-17-0001	RS 0001	Pinniped	*Pusa*	*hispida*	Ringed seal
SIMEP	ARRB-17-0004	RS 0004	Pinniped	*Pusa*	*hispida*	Ringed seal
SIMEP	ARIQ-DFO-2134	Walrus 1	Pinniped	*Odobenus*	*rosmarus*	Walrus
SIMEP	ARIQ-DFO-2126	Walrus 2	Pinniped	*Odobenus*	*rosmarus*	Walrus
East Greenland	GM-14	PW-14	Cetacea	*Globicephala*	*melas*	Pilot whale
East Greenland	GM-15	PW-15	Cetacea	*Globicephala*	*melas*	Pilot whale
East Greenland	Mn-17	H.Whale	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale
SIMEP	ARRB-XX-1403	Narwhal 1	Cetacea	*Monodon*	*monocerus*	Narwhal
SIMEP	ARRB-XX-1411	Narwhal 2	Cetacea	*Monodon*	*monocerus*	Narwhal
SIMEP	ARRB-XX-1415	Beluga 2	Cetacea	*Delphinapterus*	*leucas*	Beluga whale
SIMEP	ARRB-XX-1408	Beluga 2	Cetacea	*Delphinapterus*	*leucas*	Beluga whale
East Greenland	Ba-0007	Minke 1	Cetacea	*Balaenoptera*	*acutorostrata*	Minke whale
East Greenland	Ba-0008	Minke 2	Cetacea	*Balaenoptera*	*acutorostrata*	Minke whale
East Greenland	Orca 1 (Killer whale 1)	Orca 1	Cetacea	*Orcinus*	*orca*	Killer whale
East Greenland	2017_0003	Orca 2	Cetacea	*Orcinus*	*orca*	Killer whale
East Greenland	Mn_002	H. Whale 002	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale
East Greenland	Mn_005	H. Whale 005	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale

Supplementary Table S4.3. Summary of sequence reads obtained after sequence processing and quality check (QC) from the starting 93 polar bear fecal samples and percentages of samples for which ringed seal and bearded seal prey were detected using the Pinniped Cytochrome b [Pinniped Cytb] and Pinniped Cytochrome Oxidase 1 [Pinniped CO1]) primer sets. Individual and combined results are both listed.

Primer set	Starting # of Samples	# Samples after QC	Total # reads obtained	Average # Reads/Sample	Min count	Max count	% samples prey detected	% ringed seal detected	% bearded seal detected	%No prey detected
Pinniped Cytb	93	86	1,589,939	729± 350	0	116,521	26.7	25.6	2.33	73.3
Pinniped CO1	93	90	1,147,797	11,183± 3849	0	226,272	51.1	50	6.67	48.9
Combined Primer Sets	93	92	2,737,736	11,913± 4019	0	234,686	53.3	52.2	6.52	46.7

Supplementary Table S4.4. Summary of linear regression models showing that none of the explanatory variables explained a significant amount of the variation in Shannon and Inverse Simpson indices of alpha diversity for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. There was a near-significant effect of sex/age class in linear regression model for Faith's phylogenetic diversity (FPD) indicating lower FPD in subadults compared to other sex/age classes, however the overall model is not significant. Models incorporate DNA diet profiles (categorical diet variable: 'prey DNA detected' or 'prey DNA not detected').

Shannon Diversity (Adj. $R^2 = 0.00$, $p = 0.76$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	2.53	0.33	7.62	0.00
	DNA diet profile (Prey DNA detected)	-0.01	0.11	-0.11	0.92
	Sex/age class (Adult male)	0.01	0.57	0.02	0.99
	Sex/age class (Cub)	0.11	0.27	0.39	0.70
	Sex/age class (Subadult)	-0.03	0.25	-0.11	0.91
	Subpopulation (SB)	0.27	0.43	0.63	0.53
	Body Condition	0.00	0.00	0.30	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-0.08	0.64	-0.13	0.90
	Subpopulation (SB): Sex/age class (Cub)	-0.07	0.41	-0.16	0.87
	Subpopulation (SB): Sex/age class (Subadult)	-0.36	0.32	-1.11	0.27
	Subpopulation (SB): Body Condition	0.00	0.00	-0.16	0.87
Inverse Simpson Diversity (Adj. $R^2 = 0.00$, $p = 0.82$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	5.59	2.79	2.01	0.05
	DNA diet profile (Prey DNA detected)	0.10	0.93	0.11	0.91
	Sex/age class (Adult male)	-1.88	4.82	-0.39	0.70
	Sex/age class (Cub)	0.82	2.26	0.36	0.72
	Sex/age class (Subadult)	-0.41	2.14	-0.19	0.85

	Subpopulation (SB)	3.25	3.64	0.89	0.38
	Body Condition	0.01	0.01	0.91	0.37
	Subpopulation (SB): Sex/age class (Adult male)	1.35	5.37	0.25	0.80
	Subpopulation (SB): Sex/age class (Cub)	-0.06	3.42	-0.02	0.99
	Subpopulation (SB): Sex/age class (Subadult)	-1.66	2.69	-0.62	0.54
	Subpopulation (SB): Body Condition	-0.01	0.02	-0.59	0.56
Faiths Phylogenetic Diversity (Adj. $R^2 = 0.05$, $p = 0.18$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	13.65	1.83	7.47	0.00
	DNA diet profile (Prey DNA detected)	-0.01	0.61	-0.02	0.99
	Sex/age class (Adult male)	1.17	3.16	0.37	0.71
	Sex/age class (Cub)	-0.28	1.48	-0.19	0.85
	Sex/age class (Subadult)	-2.48	1.40	-1.77	0.08
	Subpopulation (SB)	1.13	2.39	0.48	0.64
	Body Condition	0.00	0.01	-0.29	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-3.30	3.52	-0.94	0.35
	Subpopulation (SB): Sex/age class (Cub)	-1.33	2.24	-0.59	0.56
	Subpopulation (SB): Sex/age class (Subadult)	-0.42	1.76	-0.24	0.81
	Subpopulation (SB): Body Condition	0.00	0.01	0.24	0.81

Supplementary Table S4.5. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial classes that differed or showed patterns of differential abundance based on DNA diet profile (i.e., increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

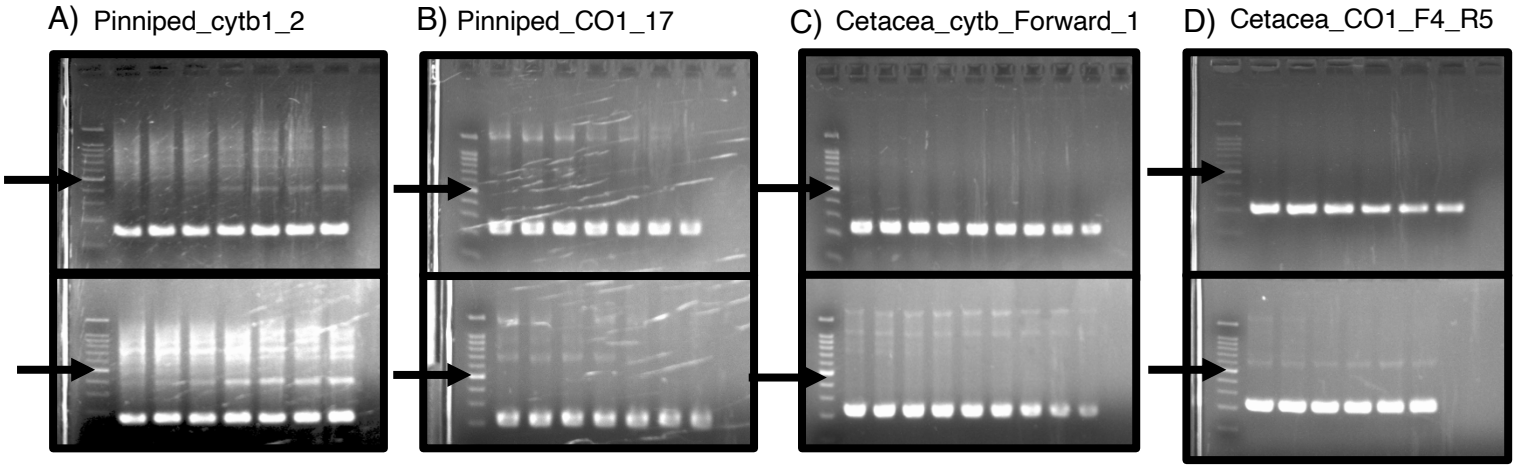
Class	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
Clostridia	**1.15**	**0.34**	**3.41**	**<0.001**	**0.009**	**TRUE**
Negativicutes	**-1.26**	**0.50**	**-2.52**	**0.01**	**0.12**	**FALSE**
Bacilli	0.90	0.44	2.07	0.04	0.41	FALSE

Supplementary Table S4.6. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial genera that differed or showed patterns of differential abundance based on DNA diet profile (i.e., increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

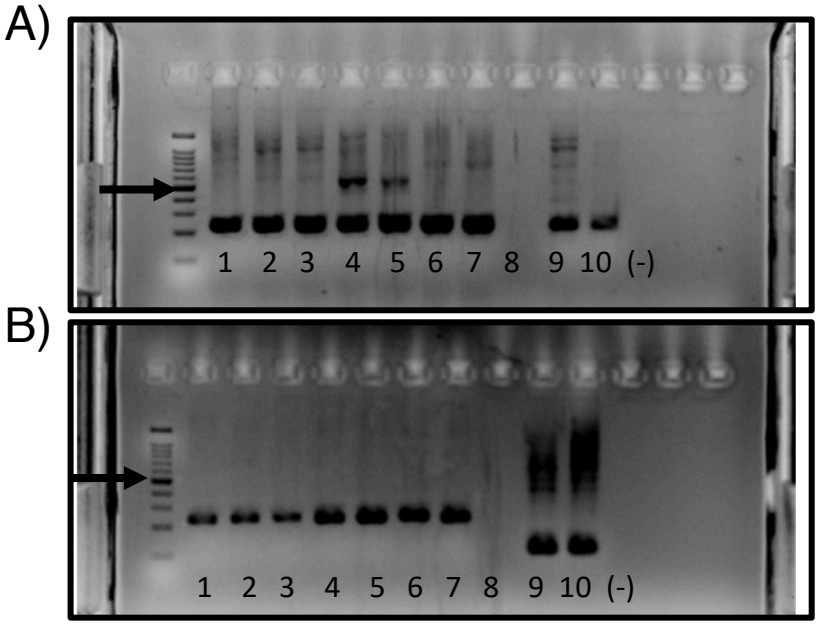
	Genus	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
1	***Terrisporobacter***	**2.55**	**0.60**	**4.27**	**0**	**0.002**	**TRUE**
2	***Halomonas***	**-1.26**	**0.35**	**-3.57**	**0**	**0.034**	**TRUE**
3	***Clostridium_sensu_stricto_1***	**2.04**	**0.61**	**3.34**	**0.001**	**0.08**	**FALSE**
4	*Blautia*	2.02	0.62	3.23	0.001	0.117	FALSE
5	*Erysipelatoclostridium*	1.66	0.52	3.23	0.001	0.118	FALSE
6	*Megasphaera*	-2.16	0.67	-3.21	0.001	0.123	FALSE
7	*Romboutsia*	1.95	0.63	3.07	0.002	0.199	FALSE

Supplementary Table S4.7. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial amplicon sequence variants (ASVs) that differed in abundance based on DNA diet profile (i.e., increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

	ASV	Class	Genus	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
1	**ASV_36**	**Clostridia**	***Terrisporobacter***	**2.04**	**0.58**	**3.54**	**< 0.001**	**0.01**	**TRUE**
2	**ASV_79**	**Clostridia**	***Paeniclostridium***	**1.69**	**0.45**	**3.73**	**< 0.001**	**0.05**	**TRUE**
3	**ASV_356**	**Bacilli**	***NA***	**1.19**	**0.33**	**3.62**	**< 0.001**	**0.07**	**FALSE**
4	ASV_3	Negativicutes	*Megasphaera*	-2.17	0.66	-3.31	< 0.001	0.23	FALSE
5	ASV_60	Clostridia	*Clostridium_sensu_stricto_1*	1.68	0.50	3.35	< 0.001	0.20	FALSE
6	ASV_62	Bacilli	*Erysipelatoclostridium*	1.58	0.49	3.20	< 0.001	0.34	FALSE
7	ASV_285	Clostridia	*Clostridium_sensu_stricto_1*	1.12	0.36	3.15	< 0.001	0.39	FALSE
8	ASV_321	Clostridia	*NA*	1.10	0.35	3.15	< 0.001	0.39	FALSE
9	ASV_220	Gammaproteobacteria	*Conchiformibius*	-1.03	0.35	-2.98	< 0.001	0.69	FALSE
10	ASV_150	Clostridia	*Clostridium_sensu_stricto_1*	1.14	0.40	2.89	< 0.001	0.91	FALSE



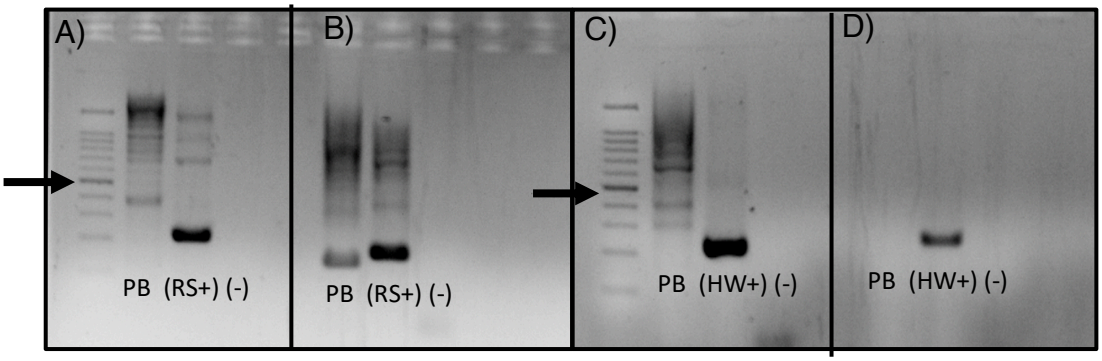
Supplementary Figure S4.1. Gel electrophoresis images showing results of separate temperature gradient polymerase chain reaction (PCR) reactions to find optimal primer annealing temperature for each polar bear prey DNA group primer set. **A)** Pinniped_cytb1_2: 51-57 °C gradient (Annealing temperatures, left to right: 51.1, 52.4, 53.7, 54.8, 55.8, 56.5, 56.9 °C). Top gel: harp seal (*Pagophilus groenlandicus*), bottom gel ringed seal (*Pusa hispida*); **B)** Pinniped_CO1_17: 55-60 °C gradient (Annealing temperatures, left to right: 55.0, 55.2, 55.8, 56.7, 57.8, 59.1, 60.4 °C). Top gel: harp seal (*Pagophilus groenlandicus*), bottom gel: ringed seal (*Pusa hispida*); **C)** Cetacea_cytb1_Forward_1: 47-55 °C gradient (Annealing temperatures, left to right: 47.1, 47.3, 47.9, 48.8, 49.9, 51.1, 52.4, 53.7, 54.8 °C). Top gel: Humpback whale (*Megaptera novaeagliae*), bottom gel: Long-finned pilot whale (*Globicephala melas)* **D)** Cetacea COI_F4_R5: 60-65 °C gradient (Annealing temperatures, left to right: 60.4, 61.7, 62.9, 63.9, 64.6, 64.9 °C). Top gel: Humpback whale (*Megaptera novaeagliae*), bottom gel: Long-finned pilot whale (*Globicephala melas).* 100 base pair (bp) ladder used in each image. Black arrow indicates 500bp fragment band.



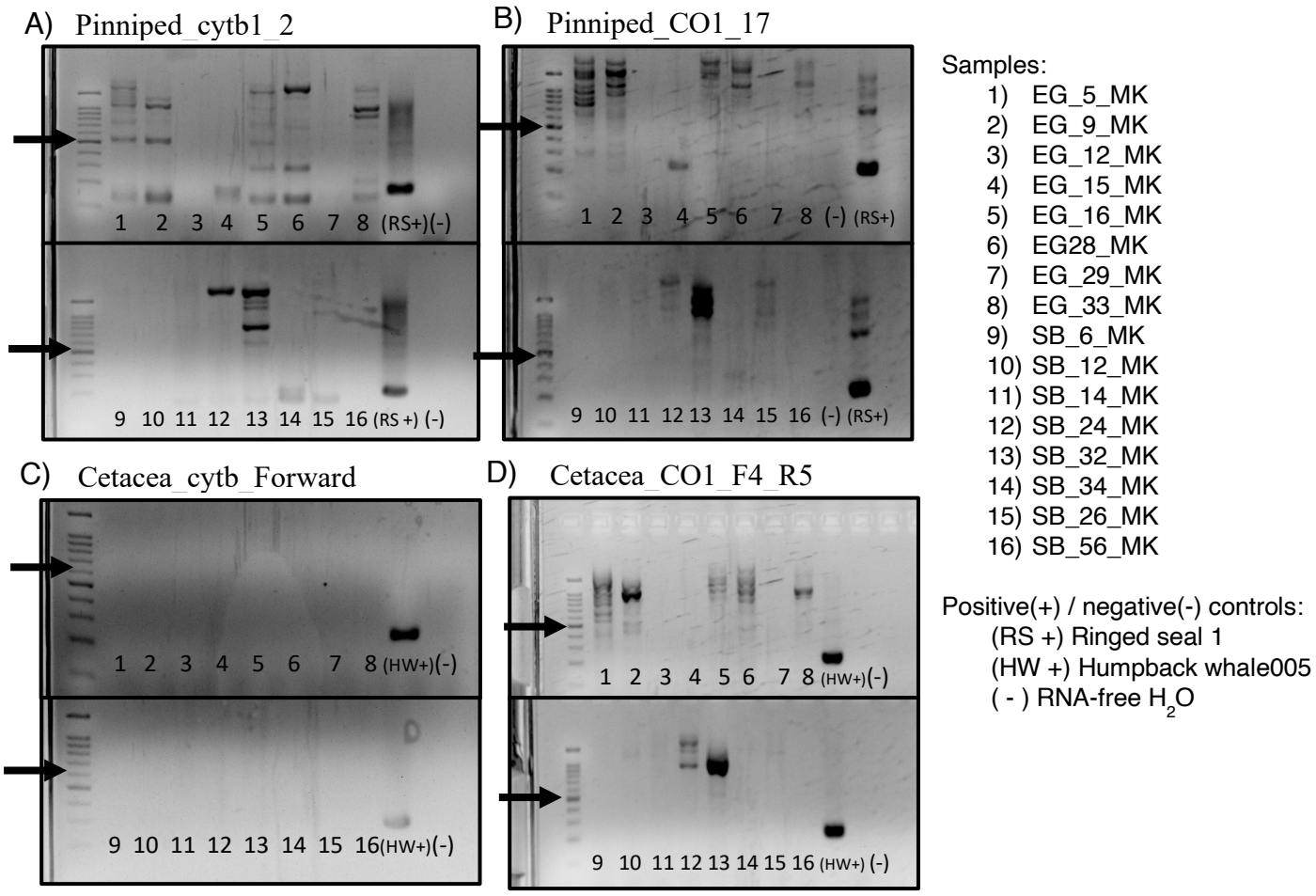
Samples:

1) Beluga 1
2) Narwhal 1
3) Narwhal 2
4) Minke 1
5) Minke 2
6) Orca 1
7) Orca 2
8) (blank/empty)
9) Walrus 1
10) Walrus 2
11) Negative control (blank)

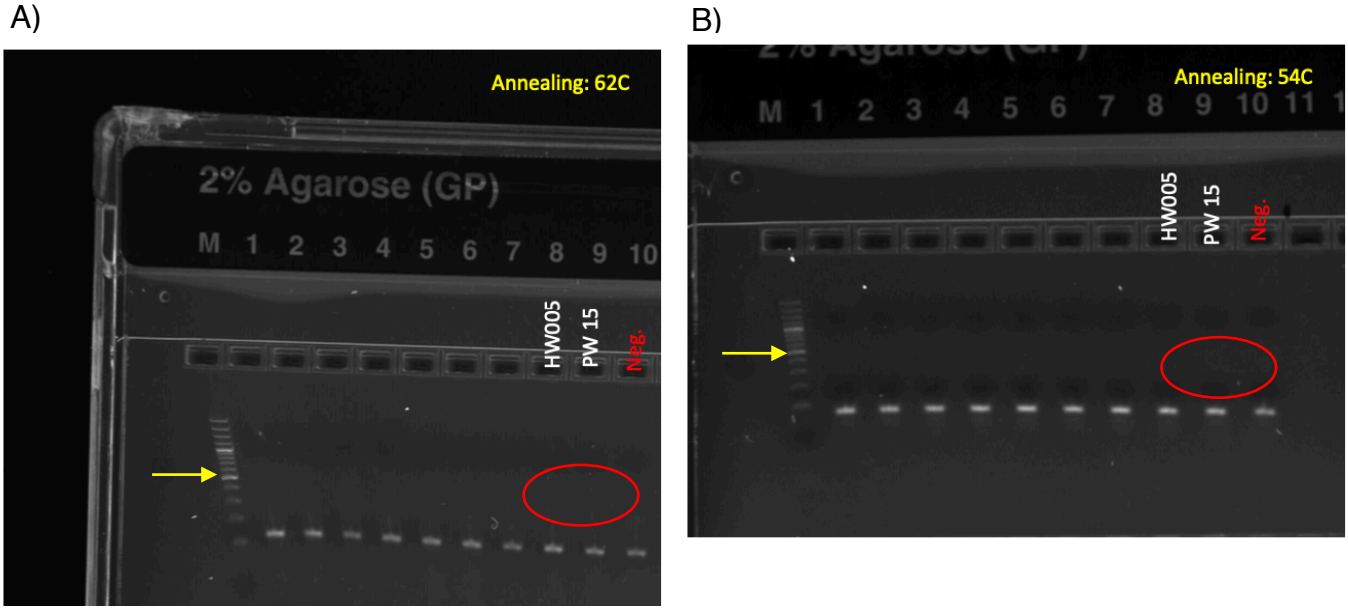
Supplementary Figure S4.2. Gel electrophoresis images showing successful amplification of remaining available prey DNA extracts using the optimal annealing temperatures determined previously for the newly developed prey-specific primer sets . A) Samples 1-7 were amplified using the cetacea_cytb_Forward_1 primer set and samples 9-10 were amplified using the pinniped_cytb1_2 primer set B) Samples 1-7 were amplified using the cetacea_CO1_F4_R5 primer set and samples 9-10 were amplified using the pinniped_CO1_17 primer set. A 100 base pair (bp) ladder was used in both images and black arrows indicate the 500bp fragment size. All PCR reactions were run using the shared optimal annealing temperature of 54 °C. Well 11 contained an Rnase-free water negative control sample.



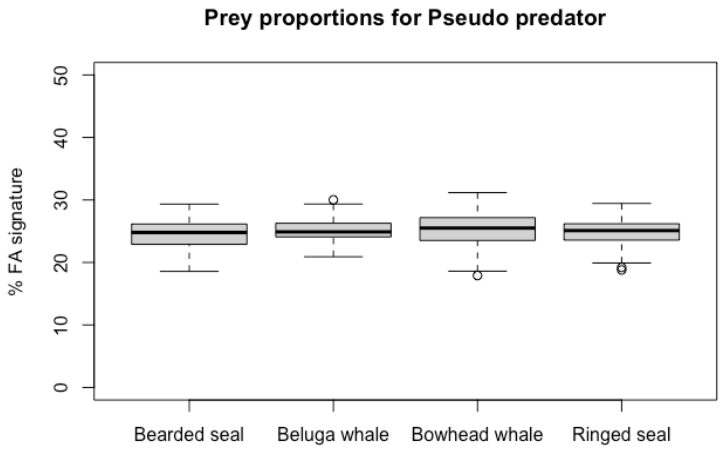
Supplementary Figure S4.3. Gel electrophoresis images showing polymerase chain reaction (PCR) test results for polar bear DNA extract (PB) using the **A)** pinniped_CO1_17 primer set **B)** pinniped_cytb1_2 primer set **C)** cetacea_CO1_F4_R5 primer set and **D)** cetacea_cytb_Forward_1 primer set. For both pinniped primer sets the ringed seal 1 positive control (RS+) was used and for both cetacea primer sets the humpback whale 005 positive control (HW+) was used. All negative controls (-) were Rnase-free water PCR blanks. A 100 base pair (bp) ladder was used in all images and black arrows indicate the 500bp fragment size band. All PCR reactions were run using a shared optimal annealing temperature of 54 °C.



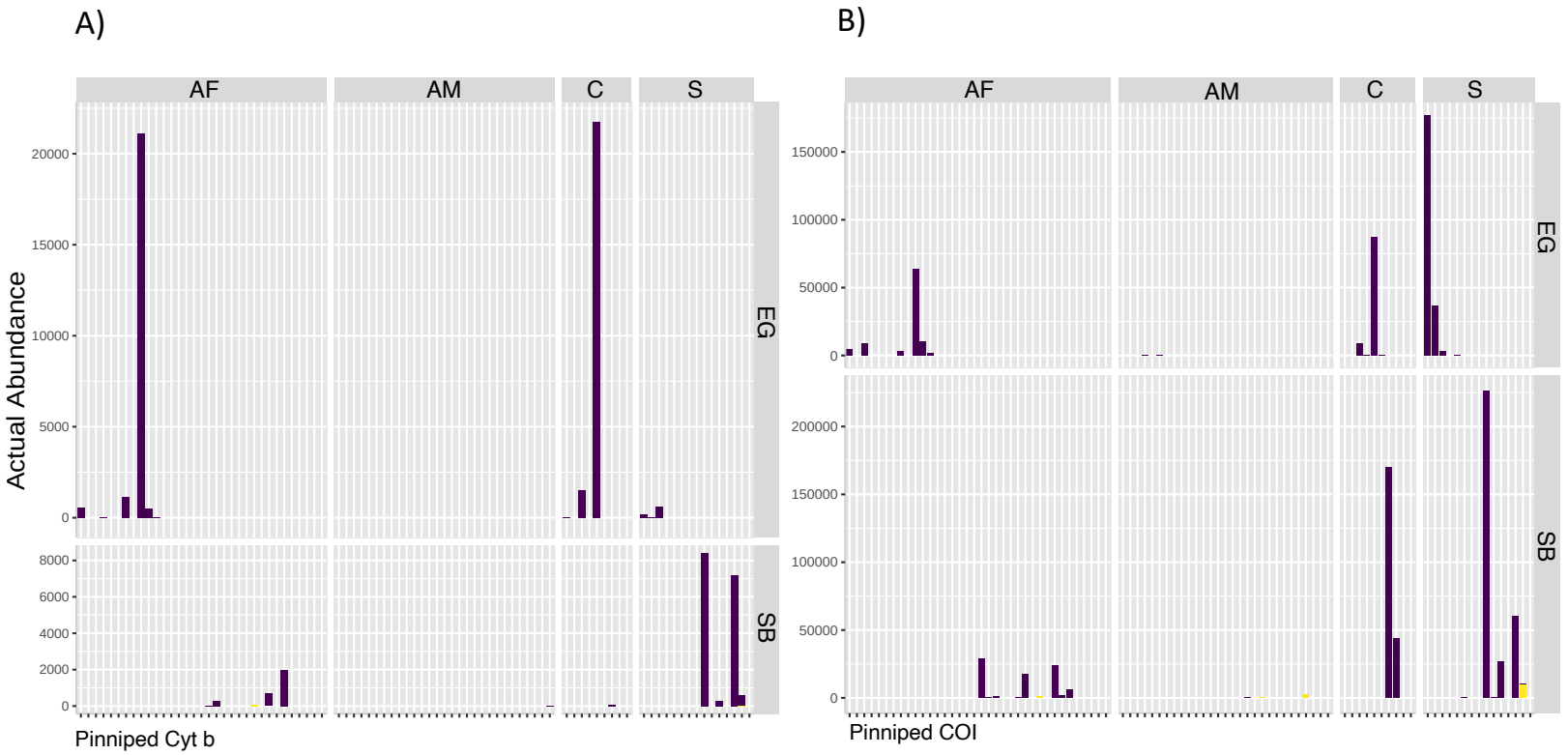
Supplementary Figure S4.4. Gel electrophoresis images showing polymerase chain reaction (PCR) test results for sixteen randomly selected East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal extract samples using the **A)** pinniped_cytb1_2 primer set **B)** pinniped_CO1_17 primer set C) cetacea_cytb_Forward_1 primer set and **D)** cetacea_CO1_F4_R5 primer set. A 100 base pair (bp) ladder was used in all images and black arrows indicate the 500bp fragment size band. All PCR reactions were run using a shared optimal annealing temperature of 54 °C.



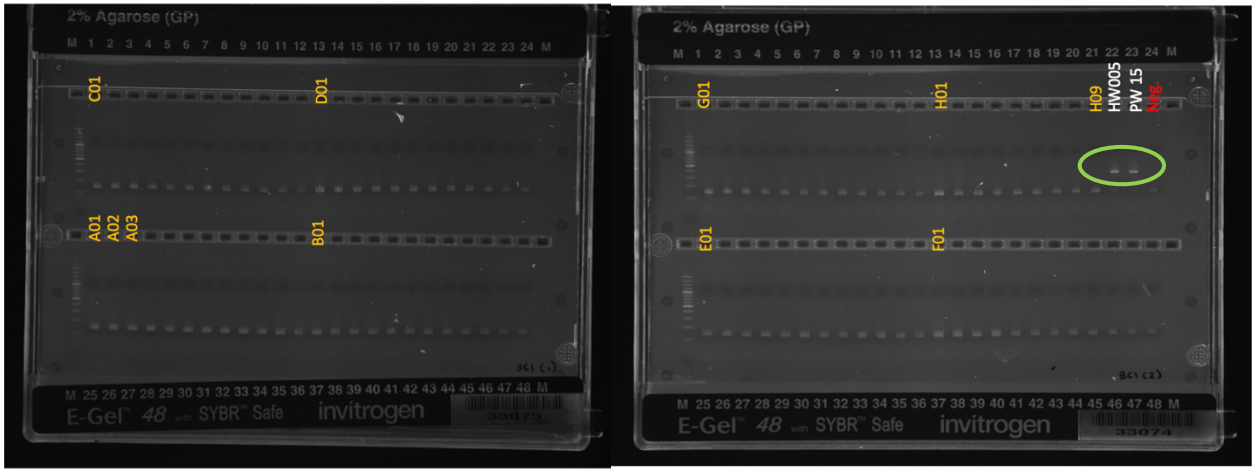
Supplementary Figure S4.5. Gel electrophoresis images showing results of two test polymerase chain reaction (PCR) experiments run at a A) 62 °C annealing temperature and a B) 54 °C annealing temperature on a few randomly selected polar bear fecal extracts and using humpback whale (HW005) and pilot whale (PW 15) PCR positive controls. Absence of positive control bands at the expected ~260 base pair (bp) fragment size (red circles) indicates failed PCR amplification. A 100bp ladder was used in both images. Bands that appear at the bottom of each gel image indicate primer-dimer formation. Yellow arrows indicate 500bp fragment size.



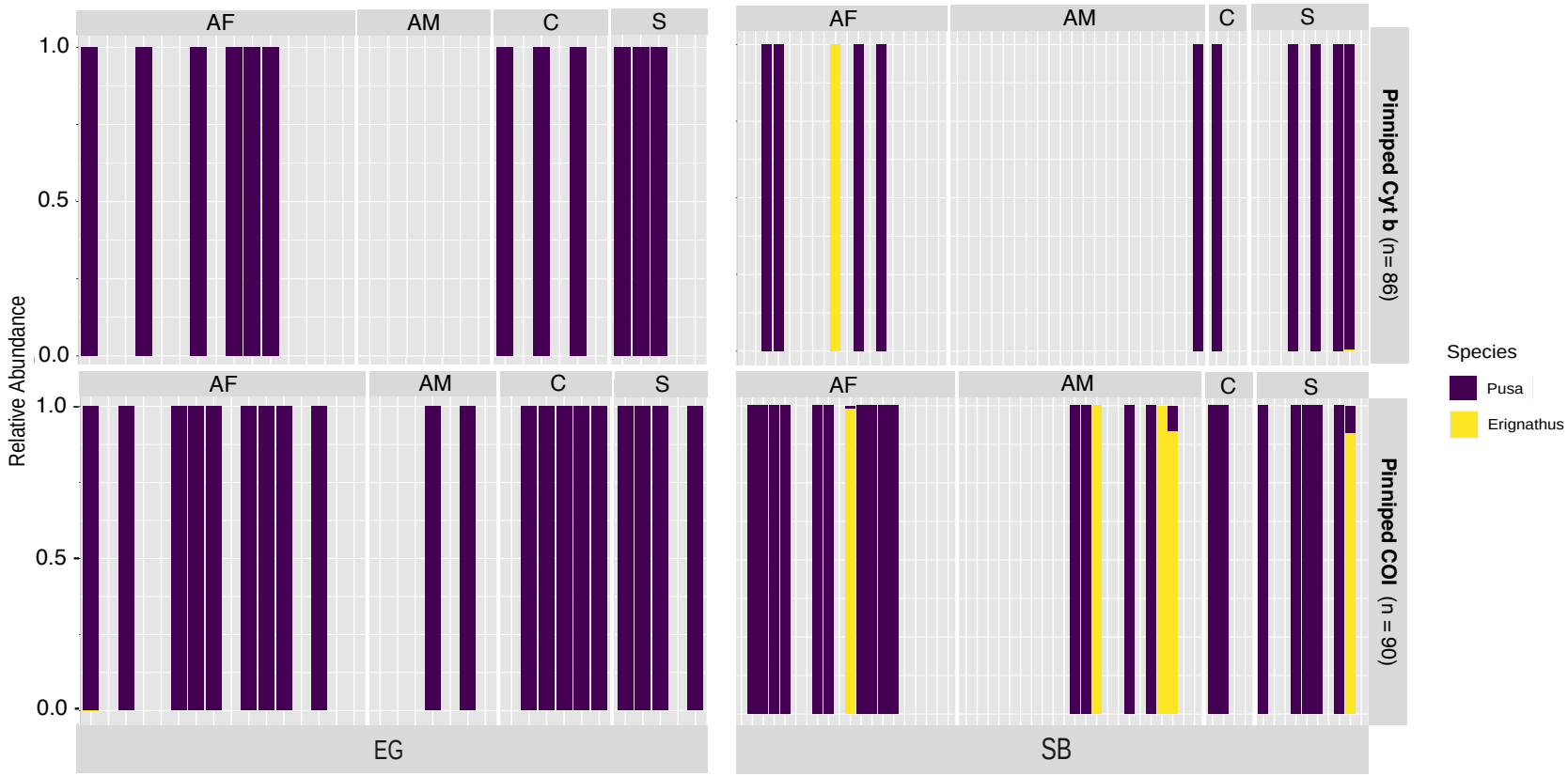
Supplementary Figure S4.6. Boxplots showing results of quantitative fatty acid signature analysis (QFASA) pseudo predator analysis. Psuedo-predator diet prey proportions were set at 25% Bearded seal, 25% Beluga whale, 25% bowhead whale, and 25% ringed seal prior to running the simulation and were estimated with high accuracy.



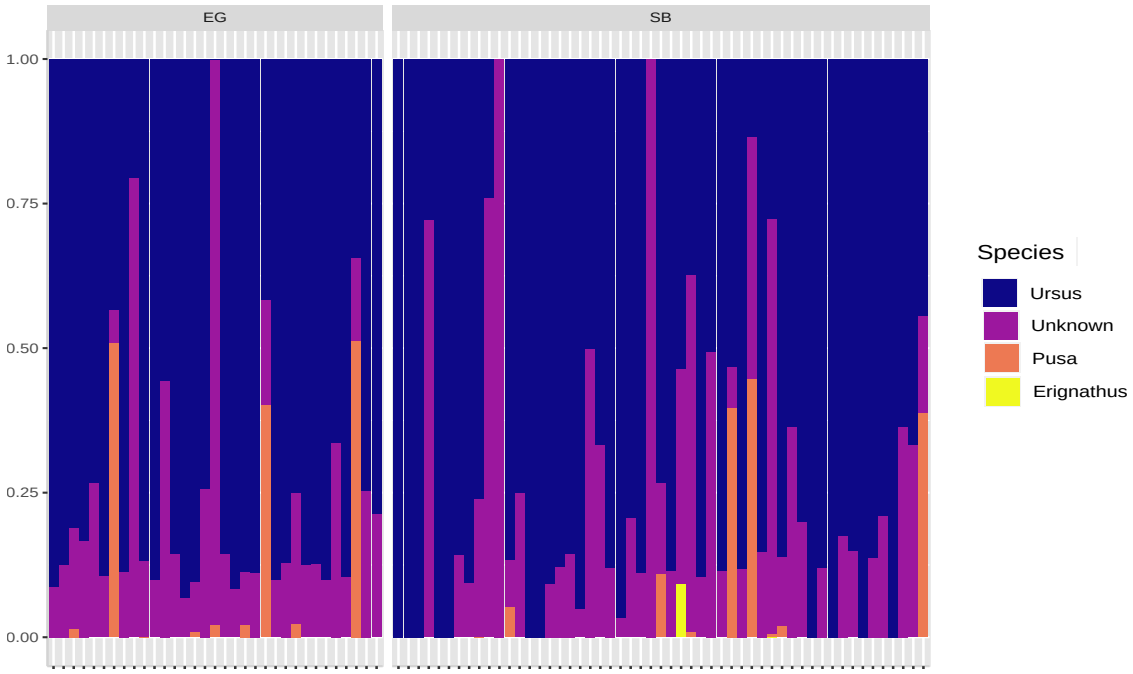
Supplementary Figure S4.7. Grouped bar plot showing the actual abundances (i.e. sample sequence coverage) of ringed seal (Pusa) and bearded seal (Erignathus) prey detected in individual polar bears and among polar bear sex/age classes (Adult female [AF], adult male [AM], cubs [C] and subadults [S] in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using A) the Pinniped_Cytb1_2b (Pinniped Cyt b) primer set, B) the Pinniped_CO1_17 (Pinniped CO1) primer set, and C) the Cetacea_cytb_Forward_1 (Cetacea Cyt b) primer set.



Supplementary Figure S4.8. Gel electrophoresis image showing successful amplification of humpback whale 005 (HW005) and pilot whale 15 (PW 15) positive controls (green circle) using the Cetacea_Cytb_F1 primer set. Image also shows minimal or nonexistent amplification of cetacean DNA in the 93 polar bear fecal extracts (i.e., lack of bands at ~260 base pair (bp) fragment size for the remaining gel wells pictured). A 100bp ladder was used in both images.



Supplementary Figure S4.9. Grouped bar plot showing the relative abundances of ringed seal (Pusa) and bearded seal (Erignathus) prey detected among different polar bear sex/age classes (Adult female [AF], adult male [AM], cubs [C] and subadults [S] in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using A) the Pinniped_Cytb1_2b (Pinniped Cytb) primer set and B) the Pinniped_CO1_17 (Pinniped CO1) primer set.



Supplementary Figure S4.10. Grouped relative abundance bar plot showing the proportions of polar bear and brown bear (grouped under 'Ursus'), unknown exact sequence variants (Unknown), ringed seal (Pusa) and bearded seal (Erignathus) sequences detected in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using the Pinniped_Cytb1_2b (Pinniped Cyt b) primer set. Polar bear DNA was found in a majority of samples (83/86, or 96.5%). Each vertical bar represents an individual polar bear.

Supplementary Text S4.1.

Performance of prey-specific primer sets

A total of 10,559,615 combined raw reads were obtained from the multiplexed sequencing run containing Pinniped_Cytb1_2b (pinniped Cytb), Pinniped_CO1_17 (pinniped CO1), and Cetacea_cytb_Forward_1 (cetacea Cytb) amplicons. Following sequence quality filtering and taxonomic assignment for each primer set using the reference database, 1,589,393 reads were obtained for pinniped Cytb, 1,147,797 reads for pinniped COI, and 3,000 reads for cetacea Cytb. Sequencing coverage for the pinniped primer sets was highly variable and ranged from 0 – 116,521 reads for Pinniped Cytb and 0 – 226,272 reads for pinniped COI (Supplementary Table S4.3; Supplementary Fig. S4.7).

No usable prey DNA was obtained using the cetacea Cytb primer set. After quality filtering there were 1 – 1163 reads per sample and sequence data was obtained for just 38 of the original 93 fecal samples. Five ESVs were identified, but none assigned to the cetacean species in the reference database. After searching the obtained ESV sequences in the NCBI database they were identified as being *Clostridium perfringens*, *Malassezia pachydermatis*, *Pusa hispida*, and *Streptococcus pasteurianus*, reflecting non-target cetacea Cytb primer binding and low levels of amplification of non-target DNA within the fecal sample DNA extracts, or possible PCR contamination. Although not sequenced, the humpback whale (HW005) and pilot whale (PW-15) positive controls (Supplementary Table S4.2) successfully amplified during library preparation using this primer set (Supplementary Fig. S4.8). We therefore concluded that no cetacean DNA present in the polar bear fecal samples.

For the pinniped Cytb primer set, 86 of the original 93 polar bear fecal extract samples yielded usable sequence data following filtering steps (Supplementary Fig. S4.9). 309 exact sequence variants (ESVs) were obtained, and 23 were identified to species-level using the curated reference database, corresponding to four species: *Ursus maritimus* (polar bear), *Ursus arctos* (brown bear), *Pusa hispida* (ringed seal) and *Erignathus barbatus* (bearded seal). When spot-checked against the NCBI blastn database, the remaining 286 unknown ESVs predominantly assigned to the same four species but were labeled as 'Unknowns' in the analysis as they did not meet the 99% coverage threshold used for taxonomic assignment. After removing 'Unknown' ESVs from the analysis and grouping sequence counts from ESVs that assigned to the same species, a total of 1,214,487 reads remained with an average of 14,122 +/- 2,684 reads

per sample (Range: 0 – 106,701 reads). *Ursid* DNA predominated and was found in ~97% of samples (Supplementary Fig. S4.10)

For the pinniped CO1 primer set, 90 of the original 93 polar bear fecal extract samples yielded usable sequence data following filtering steps (Supplementary Fig. S4.9). 45 ESVs were obtained, and six ESVs assigned to species-level using the reference database: five sequence variants assigned to ringed seal and one sequence variant assigned to bearded seal. The remaining 39 'Unknown' ESVs were spot-checked using the NCBI blastn database and a majority were found to assign back to either polar bear or bacterial species. The 'Unknown' ESVs were removed prior to downstream analysis and the counts from the six remaining ESVs were grouped according to seal prey species they assigned to. Following this, 1,028,970 reads remained with an average of 11,307 +/- 3,888 reads per sample (range: 0 – 226,272 reads)

The Cetacea_CO1_F4_R5 primer set did not amplify our humpback whale and pilot whale cetacea positive controls samples after the addition of the CS1 and CS2 tags. After checking this primer set with the added CS1/CS2 tags with the OligoAnalyzer 3.1 by Integrated DNA Technologies (http://www.idtdna.com/calc/analyzer), it appeared that the addition of these tags increased the guanine/cytosine (G/C) content of the primer sequence, thus increasing its melting temperature and preventing successful primer annealing and elongation of target cetacea CO1 mtDNA in complex fecal DNA extract mixture. This cetacea CO1 primer set should undergo re-development and preliminary testing with sequencing tags added to troubleshoot this issue.

The cetacea Cytb primer set successfully amplified cetacean prey DNA extracts (i.e., humpback whale and pilot whale positive controls), however no useable sequence data was obtained from the sequencing run as none of the ESVs identified with this primer set assigned to cetacean sequences of interest included in the custom reference database. This could be due to the primer set performing poorly in a complex metagenomic mixture [61], but this was not verifiable in the initial testing without a positive control metagenomic extract sample.

The pinniped CO1 and pinniped Cytb primer sets produced high-quality, usable data for DNA-based diet analysis of EG and SB polar bears; however, the pinniped CO1 primer set appeared to perform better than the pinniped Cytb primer set. The CO1 detected pinniped DNA in a more polar bear fecal samples and with a higher average number of sequence reads/sample than the Cytb. CO1 also showed minimal to no host (i.e., polar bear/Ursid) DNA amplification,

whereas large amounts of host DNA amplified with the Cytb primer set (Supplementary Figures S4.9 and S4.10, Supplementary Table S4.3). There could be greater sequence similarity at the Cytochrome b gene region between Ursid and Pinniped species which led to high amplification of host DNA that competed with and interfered with prey DNA amplification in the PCR reactions [27, 82, 85]. Thus, the CO1 gene region may be a better choice of primer in a study such as this where the prey species of interest are closely-related to the predator or host species and these gene regions are likely very similar [61]. As a result, there could be greater sequence similarity at the Cytochrome b gene region between Ursid and Pinniped species which resulted in the high amplification of host DNA that could have competed with and interfered with prey DNA amplification in the PCR reactions [27, 82, 85]. Despite these differences in primer performance, both the CO1 and Cytb pinniped primer sets detected the same two pinniped species from the same individual polar bears on a relatively consistent basis. Using both primer sets in combination ultimately enabled increased sample coverage and as such we recommend complementary use of these two group-specific primer sets to ensure maximum opportunity for prey DNA detection in polar bear fecal samples.

Chapter 5: General Discussion

This thesis assessed the influence of diet as a driver of inter- and intra- population variation in gut microbiome composition and diversity for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears—two geographically disparate polar bear subpopulations suggested to be experiencing varied access to traditional ice-associated seal prey. In chapter 3, I characterized the gut microbiota of EG and SB polar bears using 16S rRNA gene metabarcoding techniques and preliminarily assessed the influence of diet and other ecological factors on gut bacterial diversity and composition using fatty acid (FA) dietary tracers. In chapter 4, I further examined the influence of DNA diet profiles on the gut microbiota of EG and SB polar bears by developing novel group-specific prey primer sets for DNA-based diet analysis. I also assessed the complementary nature of DNA-based and QFASA diet estimates in Chapter 4.

5.1 Inter- and intra- population variation of EG and SB gut microbiota

The results of chapter 3 indicate that the gut microbial composition of EG and SB polar bears were distinct and that intra-population differences among polar bear sex/age classes was a key driver of some of the variation observed. A greater number of overall and unique bacterial species (ASVs) were detected in SB polar bears compared to EG polar bears and there were several bacterial classes, genera, and ASVs that were differentially abundant between the two subpopulations. Although we found no significant differences, there were patterns of increased and more variable alpha diversity in SB compared to EG polar bears. Beta diversity analyses indicated a greater degree of interindividual variation among SB polar bears compared to EG polar bears. These results are in line with previous work indicating there are differences in the gut microbiota of onshore and offshore SB polar bears, with onshore bears showing higher diversity and a greater number of unique bacteria detected compared to offshore bears that remain on the sea ice edge as it retreats [10]. Together, these results preliminarily support the notion that interpopulation variation between highly segregated populations of the same species exists and can be detected in the gut microbiome signature.

Our results indicate that intrapopulation differences among polar bear sex/age classes and sex/age class-related diet differences explains a considerable amount of the variation in gut bacterial composition and diversity for EG and SB polar bears. Subadults were found to have significantly decreased Faith's phylogenetic diversity, likely related to age-specific differences in

the development and maturity of the gut microflora but could also reflect other life history differences [196]. There were several bacterial classes, genera, and ASVs that were differentially abundant among EG and SB polar bear sex/age classes. Although not measured for both subpopulations in our study, differences in polar bear life history traits—sex-specific hormones, habitat use, foraging behavior—are important drivers of variation in gut bacterial diversity and composition and could have potentially accounted for remaining unexplained variation for the interpopulation differences [25, 35, 154, 197]. Our results showing diet and sex/age class were significant drivers of gut bacterial diversity composition for the subset of SB polar bears support what is known about varied foraging behavior among polar bear sex/age classes [8, 17], and suggests that inter-population diet differences between subpopulations and sex/age classes could be contributing to the distinct subpopulation differences detected. Thus, in chapter 3 of this thesis we explored the role of diet in explaining interpopulation differences in gut bacterial diversity and composition for EG and SB polar bears.

5.2 Short-term diet drives variation in polar bear gut bacterial composition

In chapter 3 we successfully developed pinniped and cetacean group-specific prey primer sets for DNA-based diet analysis of EG and SB polar bears. For EG and SB fecal samples collected in early spring (March/April) we detected two seal prey species (Ringed seal and bearded seal) and no cetacean prey species. Our results indicate there are dietary differences between EG and SB polar bears, with seal prey (specifically, ringed seal) being detected more frequently in EG polar bears compared to SB polar bears, and with SB polar bears having a more varied diet compared to EG polar bears as bearded seal was detected only in a few individuals from the SB subpopulation. These findings suggest that in early springtime (March/April) EG polar bears might have increased access to preferred ringed seal prey compared to SB polar bears, results that are concurrent with other studies on the SB subpopulation that show temporal increases in fasting among SB polar bears [198, 199]. We found no correlation between DNA-based diet estimates and QFASA diet estimates for the same individual polar bears, and instead concluded that DNA-based estimates reflect more short-term diet while QFASA estimates reflect more long-term diet for polar bears. We found that short-term diet did not explain variation in gut bacterial diversity for EG and SB polar bears but was significantly correlated with variation in gut bacterial composition. Increases in Clostridia were associated with seal prey DNA detection,

a bacterial group known to play a role in lipid metabolism and fat deposition [192]. There were also increases in Bacilli—a bacterial group associated with gut health and homeostasis [110]—and decreases in Negativicutes—a less-studied group potentially associated with more terrestrial-based feeding (i.e. higher starch inputs)—associated with prey DNA detection. These bacterial groups are also known to vary among adult female and adult male polar bears which supports links between gut bacterial composition and differences in sex/age class foraging behavior and diet.

Conclusions

This thesis found that the gut microbiota of EG and SB polar bears are compositionally distinct, and that there are strong intra-population drivers shaping these differences—particularly varied diet among polar bear sex/age classes. Both short- and long-term diet have been shown to be important drivers of gut bacterial composition in mammal and human studies [197, 200], however in chapter 4 of this thesis we explicitly quantify this relationship by incorporating measures of short-term diet in models predicting gut bacterial diversity and composition for a vulnerable, keystone Arctic predator. These results demonstrate promising applications of this method in studies on the gut microbiota of other at-risk wild species and populations experiencing habitat alterations and subsequent shifts in forage availability.

For now, it appears that some of the shifts in bacterial abundances observed between EG and SB bears—and within the subset of SB bears—are likely a result of dietary shifts and differences in prey availability between EG and SB bears, and sex/age class-specific differences in foraging behavior that translate to subsequent shifts in the metabolic functions of their associated gut flora. This thesis reports only the second attempt at characterizing the gut bacterial composition and diversity of free-roaming polar bears using high-resolution, next-generation sequencing methods. Additional work needs to be done to improve our understanding of 'baseline' gut bacterial composition for polar bears, from which we can begin to interpret patterns of deviance from the norm or signs of dysbiosis that could impact host health [38, 43].

It is evident that incorporating host diet data in the study of gut microbiomes of wild species adds to our ability to explain variation in gut bacterial composition and existing patterns of differentially abundant bacteria. Such information lends to our conclusions about potential functional plasticity of the gut microbiome, a likely important component of polar bear adaptability as they face further habitat loss in light of climate change. Results from this thesis also highlight the broadly applicable importance of considering both inter- and intra-population variation when considering the effects of climate change, habitat alterations, shifts in forage availability, and the impact that such shifts could have on an important aspect of host health—the gut microbiome.

References

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