Links between marine and gut bacterial communities and diet in thick-billed murres (*Uria lomvia*)

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A new consciousness is developing which sees the earth as a single organism and recognizes that an organism at war with itself is doomed. We are one planet.

– Carl Sagan

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Abstract

The use of diet generalizations for the study of an animal population facilitates sampling efforts and drawing conclusions from the collected data. However, using average values does not take into consideration the fact that a population is composed of individuals who may have different feeding behaviours. Individual prey specialization occurs in many animals and, particularly, in many seabirds. The study of this phenomenon is of importance for the understanding various aspects of the life history of these animals. This thesis contributes to the current knowledge of an Arctic seabird, the thick-billed murre (*Uria lomvia*) by investigating the interactions with bacteria that could determine the way these birds consume nutrients and accumulate mercury (Hg).

Seabirds are often used to monitor contaminant levels in the ocean because they integrate exposure signals over large areas and bring that signal back to a central location, their colonies, where they can be easily sampled. Individual prey specialization should be taken into consideration when using wildlife as monitors, given that diet plays an important role on the concentrations and type of contaminants that are accumulated by wildlife. We studied Hg accumulation patterns in an Arctic food web from Coats Island (Canada) comprised of invertebrates and fish that are common prey of the thick-billed murre. We characterized this food web using stable isotope signatures as proxy measurements of trophic level ($\delta^{15}N$), Hg methylation ($\delta^{34}S$), and carbon source ($\delta^{13}C$). $\delta^{34}S$ can be used as an indicator of the activity of sulfate-reducing bacteria, the main Hg methylators. $\delta^{34}S$ better explained Hg levels than the more widely used $\delta^{15}N$ when used individually and improved the correlation of $\delta^{15}N$ with Hg when the two ratios were combined.

As diet can affect the composition of the gut microbiome, it is expected that the bacteria inhabiting the intestines of prey specialists will vary with differential diets. Males and females also present variating feeding habits which could potentially change their gut bacterial communities.

We present the first description of the gut microbiome of the thick-billed murre. Diet categories were associated with variation in the gut microbiome. The bacteria that dominate the microbial community may be aiding their host to better metabolize the nutrients of their specialized diet. Differences in bacterial diversity were also found between males and females which can be explained by the variation in feeding times by sex that occurs at the studied colony.

Résumé

L'utilisation des généralisations de l'alimentation pour l'étude d'une population animale facilite les efforts d'échantillonnage et tirer des conclusions des données collectés. Cependant, en utilisant des valeurs moyennes ne tient pas compte du fait qu'une population est compris des individus qui peuvent avoir des comportements alimentaires différents. La spécialisation individuelle de proie se produit chez de nombreux animaux et, particulièrement, chez de nombreux oiseaux marins. L'étude de ce phénomène est importante pour la compréhension de divers aspects de l'histoire de la vie de ces animaux. Cette thèse contribue à la connaissance actuelle d'un oiseau marin arctique, le guillemot de Brünnich (*Uria lomvia*) en enquêtant les interactions avec les bactéries qui pourraient déterminer la façon dont ces oiseaux consomment des nutriments et accumulent du mercure (Hg).

Les oiseaux de mer sont souvent utilisés pour surveiller les niveaux des contaminants dans les océans parce qu'ils intègrent des signaux d'exposition de grandes zones et ils apportent le signal à un emplacement central, leurs colonies, où ils peuvent être échantillonnés facilement. La spécialisation individuelle de proie devrait être prise en considération en utilisant la faune comme des moniteurs, étant donné que l'alimentation joue un rôle important dans les concentrations et le type de contaminants qui sont accumulés par la faune. Nous avons étudié des modèles d'accumulation de Hg dans un réseau alimentaire arctique de l'île Coats (Canada) composé des invertébrés et du poissons qui sont des proies communes des guillemots. Nous avons caractérisé ce réseau alimentaire en utilisant des signatures à isotopes stables comme des mesures indirectes du niveau trophique ($\delta^{15}N$), de la méthylation de Hg ($\delta^{34}S$) et de la source du carbone ($\delta^{13}C$). $\delta^{34}S$ peut être utilisé comme un indicateur de l'activité des bactéries sulfato-réductrices, les principaux méthylateurs de Hg. $\delta^{34}S$ explique mieux les niveaux de Hg comparé à le plus utilisé $\delta^{15}N$ quand

ils sont utilisés individuellement et améliore la corrélation de $\delta^{15}N$ avec des niveaux de Hg quand les deux ratios sont combinés.

Comme le régime alimentaire peut affecter la composition du microbiome intestinal, il est prévu que les bactéries qui habitent les intestins des spécialistes de proie varient selon le régime. Les mâles et les femelles présentent aussi des habitudes alimentaires variables qui pourraient potentiellement changer leurs communautés bactériennes intestinales. Nous présentons la première description du microbiome intestinal du guillemot de Brünnich. Des catégories de régime étaient associés avec des changements dans le microbiome intestinal. Les bactéries qui dominent la communauté microbienne peuvent aider leur hôte à mieux métaboliser des nutriments dans leur régime spécialisé. Des différences dans la diversité bactérienne ont aussi été trouvées entre les mâles et les femelles et peuvent être expliquées par la variation des temps d'alimentation selon le sexe qui occurrent dans la colonie étudiée.

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Chapter 1. Introduction and Literature Review

1.1. Thick-billed murres: a generalist species made of individualists

Individual prey specialization (IPS), individuals of the same population using resources in their environment differently, is a widely distributed phenomenon occurring in a large number of taxa (Bolnick et al., 2003; Bolnick, Yang, Fordyce, Davis, & Svanbäck, 2002). The total variation in the types of prey of a population (the total niche width, TNW) can be divided into two components: the variation in the types of prey captured within an individual's diet (the within-individual component, WIC) and the variation in the prey between the diets of individuals that comprise the population (the between-individual component, BIC) so that TNW = WIC + BIC (Bolnick et al., 2003). IPS occurs when the BIC is larger than the WIC; individuals are specializing in small proportions of the TNW that vary from individual to individual (Fig. 1). The proportion of the within-individual component of the total niche width (WIC/TNW) has been used as a measurement for individual prey specialization. Because the two components add up to the TNW, when the BIC is large, the proportion between the WIC and the TNW (WIC/TNW) is small. IPS occurs with such a high frequency that it has been suggested that generalist populations may actually be composed of multiple specialist individuals (Bolnick et al., 2003; Provencher, Elliott, Gaston, & Braune, 2013). Although several explanations for the occurrence of this phenomenon have been proposed (Bolnick et al., 2003), in many cases, it is still not clear why IPS occurs.

Thick-billed murres (*Uria lomvia*) are seabirds found in a wide range of sites throughout the Arctic. Thick-billed murres are considered to be generalists, but they present various degrees and types of IPS within the same population (Woo, Elliott, Davidson, Gaston, & Davoren, 2008) and throughout the years (Elliott, Woo, & Gaston, 2009). Sex-specific feeding behaviours have

also been seen in various murre colonies, with males and females feeding at different times of the day while their mate stays at the colony taking care of their egg or chick (Elliott, Gaston, & Crump, 2010; Paredes, Jones, & Boness, 2006). This behaviour may lead to different types of prey being caught by each sex due to their abundance at different times of the day (Elliott et al., 2010).

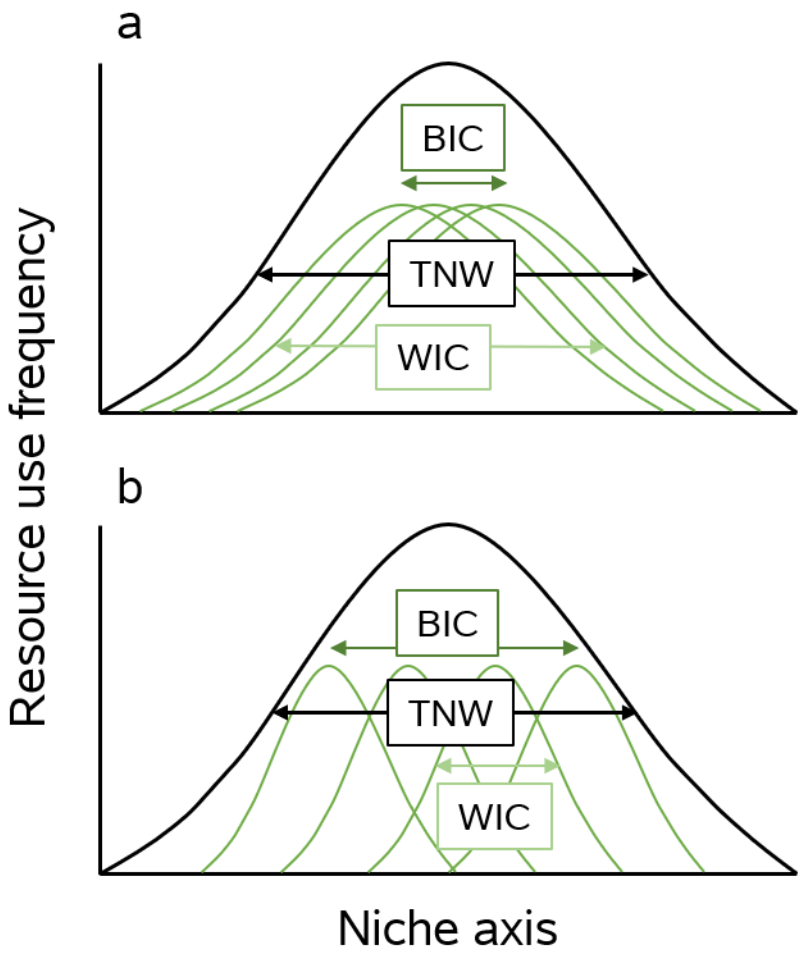

Figure 1.1. Diagram exemplifying the differences in niche use between generalist **(a)** and specialist **(b)** populations. The thick black curve represents the variation of resource use in a population (total niche width; TNW) and the thin green curves represent the niche use for a given individual. BIC: between-individual component of the niche width; WIC: within-individual component of the niche width. Modified from Bolnick et al., 2003.

1.2. Importance of sulfate-reducing bacteria in the mercury cycle

Mercury (Hg) is a naturally occurring element that is liberated from naturally occurring reservoirs into the atmosphere as elemental Hg (Hg^0) through geogenic (e.g. volcanoes, volatilization from soils, water sources, and plants) and anthropogenic processes (Amos, Jacob, Streets, & Sunderland, 2013; O'Driscoll, Rencz, Lean, & Beaney, 2005). Due to the high vapour pressure of Hg^0, it can remain in the atmosphere for prolonged periods of time (months to years; Driscoll, Mason, Chan, Jacob, & Pirrone, 2013; O'Driscoll et al., 2005). Hg^0 is then oxidized into ionic Hg (Hg^{2+}) which is removed from the atmosphere by dry or wet deposition (Driscoll et al., 2013). Hg speciation between its volatile and deposited forms can lead to processes such as global distillation during which Hg is transferred from equatorial or temperate regions into polar environments where it can be deposited due to atmospheric mercury depletion events (Ariya et al., 2004; Braune et al., 2015; O'Driscoll et al., 2005; Rigét et al., 2011; Skov et al., 2004). Because of this, Hg poses a special threat to polar regions (Fort, Robertson, Grémillet, Traisnel, & Bustamante, 2014; Kirk et al., 2012).

Worldwide Hg deposition has increased three-fold since preindustrial times (Driscoll et al., 2013; Selin, 2009). Currently, 1.9 to 4.0 Gg of Hg are released into the atmosphere every year from human primary sources (Driscoll et al., 2013; Selin, 2009) and it is estimated that 353 Gg of Hg are present in oceans worldwide (Amos et al., 2013). Between 2.08×10^{-1} and 3.25×10^{-1} Gg of Hg are deposited in the Arctic every year (Ariya et al., 2004; Skov et al., 2004), with an additional 1,656 ± 962 Gg of Hg present permafrost soils subject to being released into the environment due to increased thawing (Schuster et al., 2018). This makes the Arctic an ecosystem that is affected by Hg contamination, despite emissions in the region being low (Ariya et al., 2004; O'Driscoll et al., 2005; Skov et al., 2004).

Hg deposited in the environment can be bioaccumulated and later biomagnified in Arctic marine food webs (Atwell, Hobson, & Welch, 1998; Campbell et al., 2005). Methylmercury (MeHg), the neurotoxic organic form of Hg is predominantly assimilated and biomagnified by organisms (Campbell et al., 2005; Dietz et al., 2013; Liu et al., 2008). It is important to note that there may be different mechanisms depending on the level in the food web that is studied, such as the case of benthic detritivores which bioaccumulate but do not biomagnify Hg (Atwell et al., 1998). Individual vertebrates may also have unexplained variation in Hg concentrations irrespective of their trophic position. Such residual variation may be due to variation in diet from seasonal migrations and IPS for great skuas (Thompson, Hamer, & Furness, 1991) or foraging habitats with sulfate-reducing bacteria, the main producers of MeHg in many ecosystems (Elliott & Elliott, 2016; Góngora, Braune, & Elliott, 2018b).

Sulfate-reducing bacteria (and iron-reducing bacteria and methanogens, to a lesser extent) are the main drivers of the conversion of inorganic Hg into MeHg in many environments (Driscoll et al., 2013; Morel, Kraepiel, & Amyot, 1998; Selin, 2009). These bacteria, which inhabit anoxic aquatic environments, use sulfate as the final electron acceptor for respiration and can methylate Hg during the process, presumably by the involvement of the acetyl-coenzyme A pathway (Parks et al., 2013; Pollman & Axelrad, 2014; Selin, 2009). It is not clear why sulfate-reducing bacteria methylate Hg, but it may be a detoxification mechanism (Poulain & Barkay, 2013; Schaefer et al., 2011). The central role played by the sulfate-reducing bacteria suggests that the production of MeHg is not limited by the initial concentration of inorganic Hg but rather by sulfate concentrations and, thus, sulfate reduction rates. The addition of an inhibitor of sulfate reduction to anoxic sediments led to an almost complete reduction of MeHg production (Compeau & Bartha, 1985). Similarly, no methylation occurred in cultures of sulfate-reducing bacteria where no sulfate

was added (King, Kostka, Frischer, & Saunders, 2000). Conversely, increased concentrations of available sulfate in sediments and lakes results in an increase in MeHg production (Gilmour, Henry, & Mitchell, 1992).

1.3. Seabirds: monitors of the oceans

Seabirds can cover large ranges across oceans but breed in more discrete areas. This is of particular interest for the study of contaminants because sampling a reduced number of birds in a breeding colony can provide representative information from large areas of the ocean (Elliott & Elliott, 2013; Furness & Camphuysen, 1997). Seabird eggs are one of the five wildlife groups used as sentinels for marine ecosystems by the Northern Contaminants Program (Scheuhammer et al., 2012) with thick-billed murres at Coats Island being monitored since 1993 and at Prince Leopold Island since 1975 (Braune, Gaston, Hobson, Gilchrist, & Mallory, 2014). Murre eggs have Hg levels lower than the lowest observed adverse effects level for reproductive impairment (Dietz et al., 2013; Scheuhammer et al., 2015) and are below the proposed indicative value for the protection against Hg-induced reproductive inhibition for 95% of the bird (non-marine) species (Scheuhammer et al., 2012; Shore, Pereira, Walker, & Thompson, 2011) as shown in Figure 2. Thus, although thick-billed murres appear not to be directly impacted by Hg at a population level, they remain a key monitoring species for Hg in the Canadian Arctic (Braune, 2007; Braune, Gaston, & Mallory, 2016).

Dietary analyses can be used to determine whether increasing Hg concentrations in animal tissue are due to an increase of available MeHg in the ocean or due to changes in the animal's feeding habits (Elliott & Elliott, 2016; Kidd, Hesslein, Fudge, & Hallard, 1995; McKinney et al., 2015; McKinney, Stirling, Lunn, Peacock, & Letcher, 2010). Stable isotope ratios have been

widely used as indicators of trophic level and feeding location when quantifying Hg in tissues (Atwell et al., 1998; Nisbet, Montoya, Burger, & Hatch, 2002; Overman & Parrish, 2001; Vo, Bank, Shine, & Edwards, 2011).

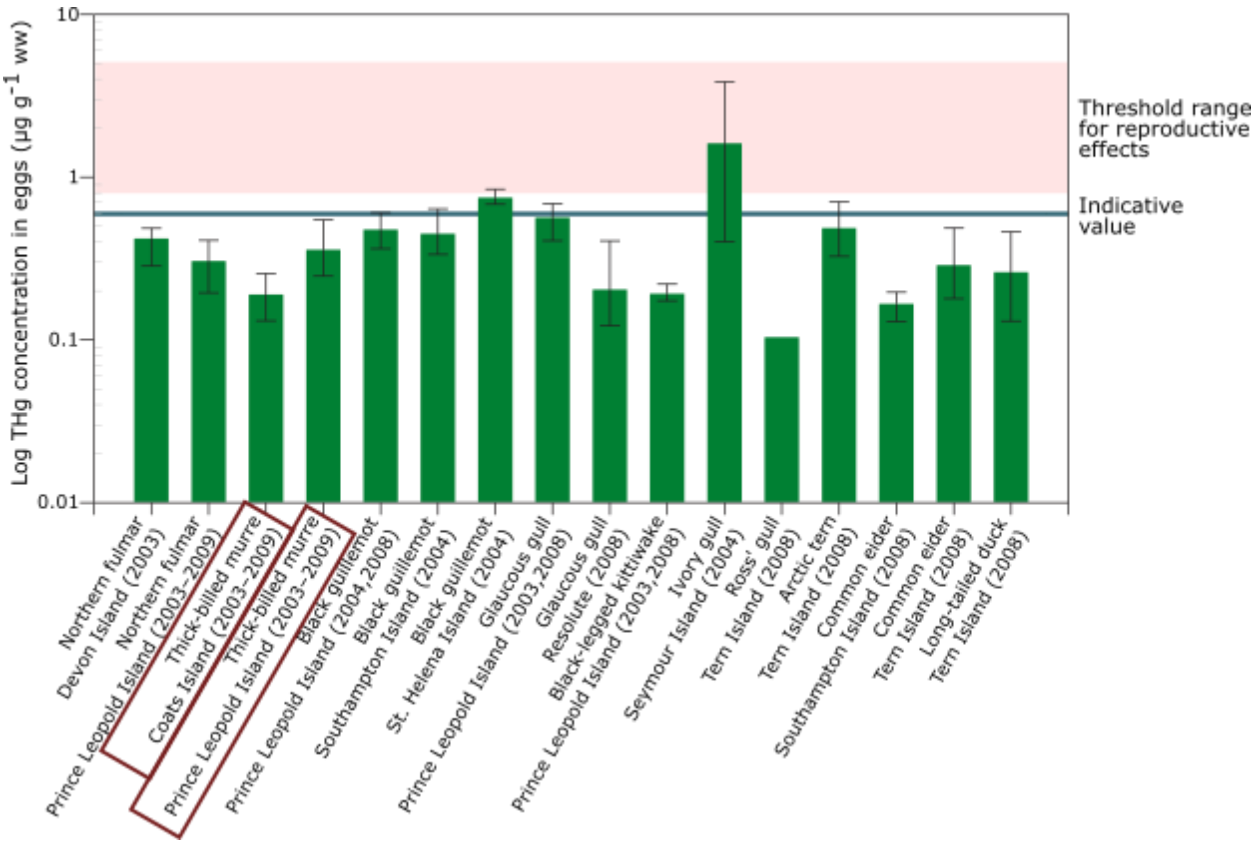

Figure 1.2. Mean total Hg (THg) concentrations in various Canadian Arctic seabird eggs (thick-billed murres are highlighted in red boxes). The indicative value refers to the Hg concentration for which 95% of the species are protected from Hg reproductive toxicity. Modified from Scheuhammer et al., 2012.

The $\delta^{15}N$ ratio (^{15}N to ^{14}N, expressed in relation to an international isotopic reference) is an index of the relative trophic position of an organism because ^{15}N content increases with trophic level (Carr et al., 2017; Hobson, Piatt, & Pitocchelli, 1994). As differences in trophic level can explain significant proportions of variation in Hg concentration, especially when temporal trends are being studied, correcting for the $\delta^{15}N$ ratio are often used to control for this confounding effect

(Bentzen et al., 2016; Kidd et al., 1995; McKinney et al., 2012; Vo et al., 2011). Another commonly used isotope signature is the ratio of ^{13}C to ^{14}C, $\delta^{13}C$ (as expressed relative to an international isotopic reference), which can describe changes in food sources associated with particular habitat types to a greater degree than trophic level. For example, benthic feeding organisms are enriched in ^{13}C compared to pelagic feeders (Carr et al., 2017; Hobson et al., 1994; Nisbet et al., 2002) and terrigenous organic carbon is associated with lower $\delta^{13}C$ values compared to marine carbon (Foster et al., 2012; Schell, Barnett, & Vinette, 1998). As sulfate-reducing bacteria respire, sulfur in the water column is converted from sulfate to sulfide causing the remaining sulfate to become enriched in the heavier sulfur isotope, ^{34}S (Krouse & Mayer, 2000; Peterson & Fry, 1987). This makes the ^{34}S to ^{32}S ratio as expressed relative to an international isotopic reference, $\delta^{34}S$, useful for the detection of sulfate reduction and, thus, mercury methylation via sulfate-reducing bacteria, so that variation in environmental MeHg levels can be accounted for (Elliott and Elliott 2016). $\delta^{34}S$ is also used as an indicator of diet source in a similar manner as $\delta^{13}C$ is used based on the fact that sulfate-reducing bacteria in different environments (e.g. lakes vs. rivers or seawater vs. saltwater) will have different rates of sulfate reduction which will result in varying $\delta^{34}S$ values for each different site (Carr et al., 2017; Fry & Chumchal, 2012; Hobson, 1999; Lavoie, Kyser, Friesen, & Campbell, 2015).

Thick-billed murre prey specialists appear to be foraging on higher trophic levels compared to prey generalists (Woo et al., 2008) which suggests that higher Hg levels may also correlate with a higher degree of specialization. A recent study showed that variation in Hg among both thick-billed murres and their prey was associated with variation in $\delta^{15}N$ (Braune et al., 2014a; Braune et al., 2014b). A decrease of $\delta^{15}N$ in murre eggs at Coats Island due to a dietary shift towards prey lower in $\delta^{15}N$ (a change of the main prey item from arctic cod to capelin caused by the decrease in

arctic cod populations with increasing seawater temperatures) masked an increase in Hg across time, and accounting for variation in $\delta^{15}N$ improved assessment of Hg trends over time (Braune et al., 2014b). Differential feeding from diverse habitats may also cause variation in the Hg concentrations uptaken. For instance, if Hg levels are higher in one habitat (i.e. benthos) than another habitat (i.e. pelagic waters), due to long range transport or other mechanisms, then an association between Hg and isotopic values might be expected. For example, among fish caught in the same river, those that were originally river residents had higher Hg levels than those that migrated from a lake; $\delta^{13}C$ and $\delta^{34}S$ could distinguish between the river residents and the lake migrants (Carr et al., 2017).

1.4. Gut bacteria: key players in diet and mercury accumulation

The role of bacteria in trophic interactions of free-ranging wildlife is increasingly being appreciated. Bacterial biofilms can be important food sources for western sandpipers (Kuwae et al., 2008). Bacteria can control levels of methylmercury in wild birds (Góngora et al., 2018b). The microbiome of wild animals can influence health and diet with production of compounds such as short-chained fatty acids (Waite & Taylor, 2015) and preventing the colonization of pathogens (Oakley et al., 2014). For example, variation in diet composition has been linked to changes in the gut microbiome in wild sticklebacks and perches, two species that present IPS (Bolnick et al., 2014; Bolnick et al., 2014). These studies also show that other types of interactions that alter the feeding behaviour (e.g. differential diets between sexes) can explain the variation in the microbiome (Bolnick et al., 2014; Lewis, Moore, & Wang, 2016). It has also been suggested that the gut microbiome may be crucial to the adaptation of the vertebrate host to ecological pressures, such as rapid environmental change or adverse conditions (Alberdi, Aizpurua, Bohmann, Zepeda-

Mendoza, & Gilbert, 2016; Ben-Yosef, Aharon, Jurkevitch, & Yuval, 2010; Lapanje, Zrimec, Drobne, & Rupnik, 2010; Lewis et al., 2016).

Bacterial communities change in structure and function throughout different sections of the avian gastrointestinal tract (Oakley et al., 2014; Sergeant et al., 2014; Waite & Taylor, 2014). In the crop, communities dominated by *Lactobacillus* primarily occur and they are associated with polysaccharide degradation and lactate fermentation (Oakley et al., 2014) although some cellulose degradation has been observed in hoatzins (Ophisthocomus hoazin; Waite & Taylor, 2014). Less diverse communities also dominated by *Lactobacillus* are present in the proventriculus and ventriculus due to the low pH (Oakley et al., 2014). The highest diversity of bacteria observed in the intestines, especially the ceca, fulfilling functions such as carbohydrate fermentation, production of vitamins and essential amino acids, and nitrogen waste compound recycling (Oakley et al., 2014; Sergeant et al., 2014; Waite & Taylor, 2014). Uric acid is the primary nitrogenous waste compound excreted by various animal groups such as birds, and some reptiles and insects (Karasawa, 1999; Kohl, 2012; Thong-On et al., 2012). Uric acid comes into contact with the gut microbial communities in birds due to the proximity of the urogenital and intestinal tracts in the cloaca (Kreisinger et al., 2017). Uric acid can travel from the cloaca into the ceca via the rectum due to retrograde peristalsis (Kohl, 2012; Pan & Yu, 2014; Vecherskii, Kuznetsova, & Stepan'kov, 2015; Vispo & Karasov, 1997). Once in the ceca, bacteria can degrade the transported uric acid into ammonia which can be re-absorbed and transformed by birds into nonessential amino acids, such as glutamine (Mortensen & Tindall, 1981a; Vispo & Karasov, 1997). Uric acid is normally fermented by anaerobic bacteria into ammonia, short-chained fatty acids such as acetic acid, and carbon dioxide (Kane, 1997; Mortensen & Tindall, 1981b; Vispo & Karasov, 1997). There are multiple examples of uricolytic bacteria present in the intestines of chickens, turkeys, guinea fowls,

pheasants, ducks, and even hummingbirds, which do not possess ceca (Kohl, 2012). Microbial uric acid recycling may enable the black grouse (*Lyrurus tetrix*) to reuse amounts of uric acid equal to their daily dietary nitrogen intake (Vecherskii et al., 2015). Uric acid recycling also occurs in eastern subterranean termites (*Reticulitermes flavipes*) where, despite the fact these termites have the ability to produce this compound, no uric acid is excreted but is rather taken up by gut bacteria and up to 30% of the total nitrogen in a termite colony can be recycled uric acid nitrogen (Kane, 1997).

Several studies have evidenced variation in the gut microbiome due to diet for multiple bird species using traditional microbiological approaches. Bacterial diversity in chickens shows differences in guanine-cytosine (G+C) content for different types of diet that are persistent throughout the years (Apajalahti, Kettunen, Bedford, & Holben, 2001). Chickens also had higher numbers of coliforms and faecal streptococci when foraging on whole wheat diets compared with conventional diets (Mead, Griffiths, Impey, & Coplestone, 1983). Captive raptors fed commercially prepared chicken presented differences in the isolated bacterial species and abundance when compared to raptors that were not fed chicken (Bangert, Ward, Stauber, Cho, & Widders, 1988). For penguins, crustacean-based diets, in opposition to fish-based diets, are related to a higher number of coliforms in various parts of the penguin gut (Soucek & Mushin, 1970). However, such variation was only seen between and not within penguin species. When comparing various bird species and families, a study showed that birds with insectivorous and granivorous diets tended to have lower numbers of *Campylobacter*. The authors also showed a tendency of variation in the prevalence of *Campylobacter* amongst bird guilds (Waldenström et al., 2002). Other behaviours not directly related to diet can affect the composition of gut microbiota. For

example, preening can cause environmental bacteria in feathers to enter the gut and end up in the cloaca where they can be sexually transmitted between individuals (Kulkarni & Heeb, 2007).

In recent years, research has been shifting from the detection of certain bacterial species or types of bacteria (e.g. pathogens) towards the study of total gut microbial communities for wild bird species (Waite & Taylor, 2015). Characterizing cloacal bacteria according to carbon use clustered the bacterial communities of various bird species from the order Passeriformes according to the species' diet (Maul, Gandhi, & Farris, 2005). The gut microbiome of two passerine species differed between the spring and fall migrations (Lewis et al., 2016) and between migrating and resident shorebirds (Risely, Waite, Ujvari, Hoye, & Klaassen, 2018), suggesting that environment, and possibly the highly changing diet at stopover sites, has a strong influence on the avian microbiome. Dewar *et al.* (2013) studied the gastrointestinal microbiota of four penguin species and observed that there is variation among individuals of the same species. Although a specific cause for this variation was not determined, it was suggested that diet could play an important role in the gut microbial composition of an individual. Results from a meta-analysis of the avian gut microbiota by Waite and Taylor (2014) illustrate that, even among phylogenetically and behaviourally diverse species, diet has an important effect on the gut microbiome composition.

Recent studies have observed that the contaminants a host comes into contact with can have an effect on their microbiome. Changes in the microbiome caused by these compounds have been associated with negative effects in the host's physiology and metabolism, in some cases leading to disease (Lu, Mahbub, & Fox, 2015). The microbiome of earthworms significantly changed when they were grown in soils treated with inorganic Hg and MeHg, and an increase in MeHg in the soils treated with inorganic Hg suggests that the bacteria inhabiting the bodies of earthworms were able to methylate the Hg in the soil (Rieder, Brunner, Daniel, Liu, & Frey, 2013).

Lu *et al.* (2014) found that arsenic causes significant variation in the composition of the mice gut microbiome which gives rise to the modification of the gut microbial metabolome. In the case of isopods, it has also been observed that the diversity of gut bacterial communities are affected by the ingestion of Hg in the diet (by chronic and acute exposure) of their isopod host (Lapanje, Rupnik, & Drobne, 2007; Lapanje et al., 2010). Additionally, there were larger number of Hg resistant bacteria in the guts of isopods from Hg polluted environments (Lapanje et al., 2010).

The presence of *merA*, a gene which encodes the mercuric reductase, one of the main proteins involved in the reduction of Hg^{2+} into Hg^{0}, was used as evidence to suggest that gut bacteria may help their host detoxify the Hg it consumes. In mice, gut bacteria may also stimulate their hosts ability to excrete Hg (Rowland, Robinson, & Doherty, 1984), which is believed to be caused by demethylation of the consumed MeHg (Rowland, 1988). Another study showed that Hg methylation could occur inside the intestines of various fish species (Rudd, Furutani, & Turner, 1980). Thus, studying gut bacteria may be useful to understand the cycling of Hg in Arctic food webs.

1.5. Research questions

Bacteria play key roles on various aspects of the life history of animals, but they are not traditionally taken into consideration by ecologists and ecotoxicologists. Understanding what is the relationship between bacteria and the feeding behaviour of an ecologically-relevant species, such as the thick-billed murre, could potentially allow a better understanding of this seabird. To the date, no studies have been made attempting to link the gut microbiome of thick-billed murres with IPS, or the presence of sulfate-reducing bacteria in thick-billed murre food webs with Hg

bioaccumulation. The three main questions (illustrated in Figure 3) that this thesis will aim to answer are:

(1) What are the Hg accumulation patterns of a food web used by thick-billed murres? (Chapter 1)

(2) Is there a significant influence of the activity of sulfate-reducing bacteria on Hg accumulation in the Canadian Arctic? (Chapter 1)

(3) Is there a difference in gut microbial diversity for the various types of prey specialists? (Chapter 2)

These questions lead us propose the following hypotheses along with their respective predictions:

- Hypothesis 1. Hg accumulation depends on multiple factors of an individual's diet such as trophic position and feeding habitat.

  Prediction: Increase in trophic position and selection of deep benthic feeding habitats, as assessed by $\delta^{15}N$ and $\delta^{34}S$ respectively, are key drivers of Hg bioaccumulation for the food web used by the thick-billed murre.
- Hypothesis 2. Hg bioaccumulation depends on contact with Hg methylation hotspots more than trophic interactions.

  Prediction: $\delta^{34}S$ will have a larger impact than $\delta^{15}N$ on Hg accumulation in Arctic food webs.
- Hypothesis 3: Individual prey specialization shapes the thick-billed murre gut microbiome.

  Prediction: Bacterial diversity varies with the type of IPS and individuals with different types of specializations will also have different gut microbiomes.

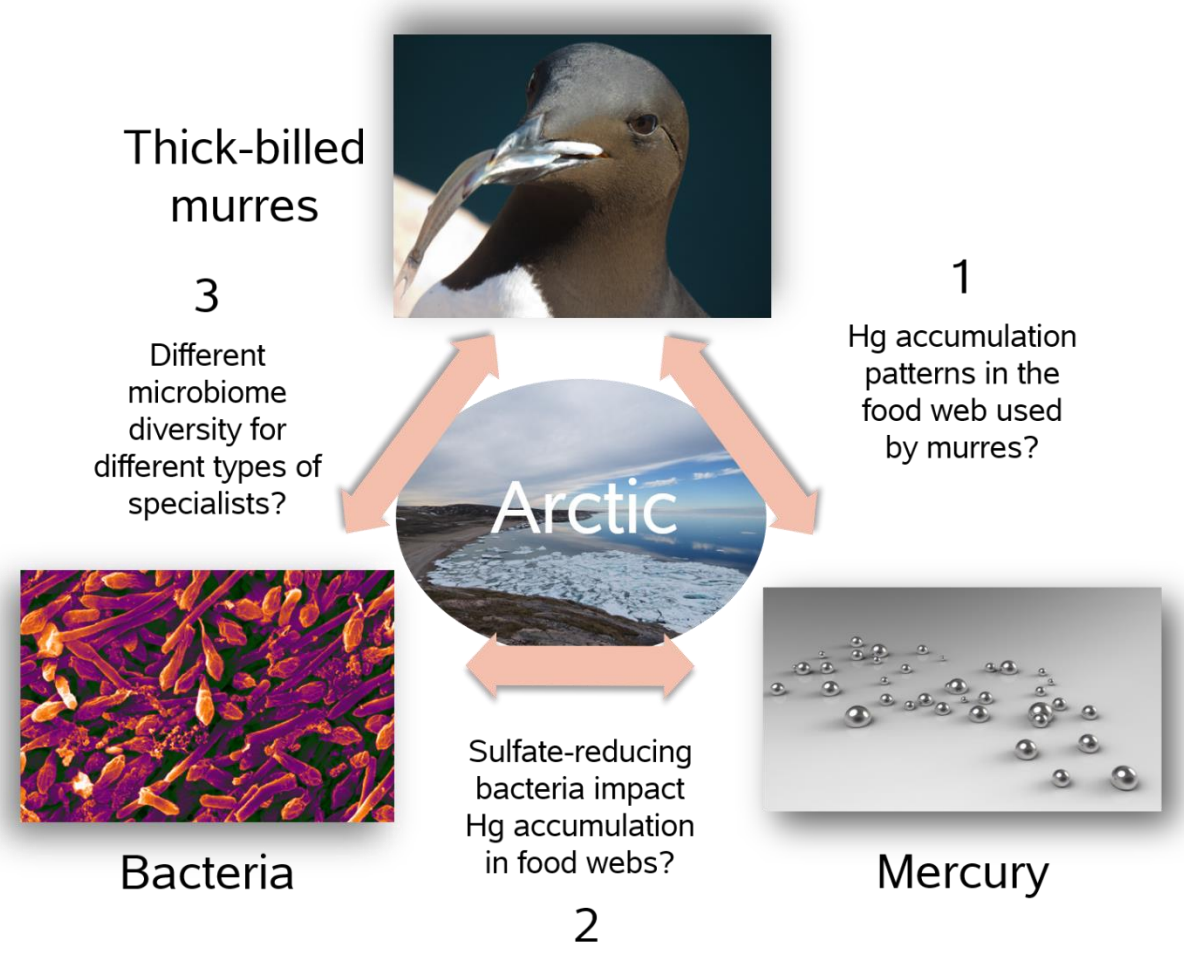

Figure 1. 3. Schematic illustrating the research questions the thesis aims to answer. Photo credit for the mercury image: ©g0b / Adobe Stock. All other images were taken and edited by Esteban Góngora.

Connecting text:

To answer the first and second research questions, in Chapter 2 we investigate Hg accumulation in a Coats Island trophic web using stable isotope analysis, with a special focus on the role that bacteria play in the accumulation of this heavy metal. Our objective was to determine the driving factors that caused Hg bioaccumulation through the various prey items that thick-billed murres consume.

Chapter 2. Nitrogen and sulfur isotopes predict variation in mercury levels in Arctic seabird prey

Note on this chapter

This chapter corresponds exactly to the paper titled “Nitrogen and sulfur isotopes predict variation in mercury levels in Arctic seabird prey” published in Marine Pollution Bulletin (Góngora et al., 2018b) which was made in collaboration with Birgit M. Braune from Environment and Climate Change Canada and Kyle H. Elliott from McGill University. Esteban Góngora performed the data analysis and wrote the manuscript. Samples were collected and processed as part of a previous study (Braune et al., 2014a). Birgit M. Braune and Kyle H. Elliott provided the data from a subsample of the results of the previous study (Braune et al., 2014a) and additional analyses performed on the samples for the present publication. Birgit M. Braune and Kyle H. Elliott also critically edited the manuscript.

2.1. Abstract

Mercury (Hg) biotransformation and biomagnification are processes that affect Hg burdens in wildlife. To interpret variation in Hg in seabird eggs, used as Hg bioindicators in the Arctic, it is important to understand how Hg biomagnifies through the food web. We evaluated the use of $\delta^{34}S$, along with other commonly used stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$), for the determination of possible sources of Hg in an Arctic food web (56 individuals of 15 species of fish and invertebrates). Hg correlated with $\delta^{34}S$ (R^2 = 0.72). When the combined effects of $\delta^{34}S$ and $\delta^{15}N$ were considered in mixed-effects models, both $\delta^{34}S$ and $\delta^{15}N$ together described Hg patterns in Arctic food webs better than either isotope alone. Our results demonstrate the usefulness of $\delta^{34}S$ to account for variation in Hg among marine animals and to study the possible underlying effects that MeHg production may have on Hg pathways in Arctic ecosystems.

2.2. Introduction

Human activity directly and indirectly produces multiple sources of pollutants that are affecting and modifying the environment (Persson et al., 2013; Rockström et al., 2009). For example, mercury (Hg) deposition has increased three-fold since preindustrial times (Driscoll et al., 2013; Selin, 2009). Currently, 1900-4000 t are released into the atmosphere every year from human primary sources (Driscoll et al., 2013; Selin, 2009). Hg poses a special threat to polar regions (Fort et al., 2014; Kirk et al., 2012). Hg speciation between its volatile and deposited forms can lead to processes such as global distillation and mercury depletion events during which Hg is transferred from equatorial or temperate regions into polar environments (Ariya et al., 2004; B. Braune et al., 2015; O'Driscoll et al., 2005; Rigét et al., 2011; Skov et al., 2004). Between 208 to 305 tons of Hg are deposited each year in the Arctic due to these types of processes despite local emissions in the region being low (Ariya et al., 2004; Skov et al., 2004). Mercury is most toxic in its organic form, methylmercury (MeHg), which is also easily assimilated and bioaccumulated by organisms (Dietz et al., 2013; Liu et al., 2008).

Animals, particularly seabirds, can be used to monitor contaminants in ecosystems because they can integrate signals over large foraging areas and return to a central site (colony) where they can be sampled relatively easily (Elliott & Elliott, 2013; Elliott & Elliott, 2016; Furness & Camphuysen, 1997). Dietary analyses can be used to determine whether increasing Hg concentrations in animal tissue are due to an increase of available MeHg in the ocean or due to changes in the animal's feeding habits (Elliott & Elliott, 2016; Kidd et al., 1995; McKinney et al., 2015, 2010). Stable isotope ratios have been widely used as indicators of trophic level and feeding location when quantifying Hg in tissues (Atwell et al., 1998; Nisbet et al., 2002; Overman & Parrish, 2001; Vo et al., 2011).

The $\delta^{15}N$ ratio (^{15}N to ^{14}N, expressed in relation to an international isotopic reference) is an index of the relative trophic position of an organism as ^{15}N content increases with trophic level (Carr et al., 2017; Hobson et al., 1994). As differences in dietary trophic level can explain significant proportions of Hg variation, especially when temporal trends are being studied, correcting for the $\delta^{15}N$ ratio can help to control for this confounding effect (Fig. 1: 'trophic position hypothesis'; Bentzen et al., 2016; Kidd et al., 1995; McKinney et al., 2012; Vo et al., 2011). Another commonly used isotope signature is the ratio of ^{13}C to ^{14}C, $\delta^{13}C$ (as expressed relative to an international isotopic reference), which can describe changes in food sources associated with habitat to a greater degree than trophic level. For example, benthic feeding organisms are enriched in ^{13}C compared to pelagic feeders (Carr et al., 2017; Hobson et al., 1994; Nisbet et al., 2002) and terrigenous organic carbon is associated with lower $\delta^{13}C$ values compared to marine carbon (Foster et al., 2012; Schell et al., 1998). Differential feeding from diverse habitats may cause variation in the Hg concentrations uptaken (Fig. 1: 'habitat variation hypothesis'). For instance, if Hg levels are higher in one habitat (i.e. benthos) than another habitat (i.e. pelagic waters), due to long range transport or other mechanisms, then an association between Hg and isotopic values might be expected. For example, fish that were originally river residents had higher Hg than other fish caught in the same rivers and that migrated from a lake; $\delta^{13}C$ and $\delta^{34}S$ (see below) could distinguish between the river residents and the lake migrants (Carr et al., 2017). These commonly used ratios can help to elucidate observed differences in Hg concentrations that are the result of the animal's feeding habits but fail to account for an important aspect of the mercury cycle that is independent of top predator's life history, the production of MeHg.

Sulfate-reducing bacteria (and iron-reducing or methanogenic bacteria, to a lesser extent) are the main drivers of the conversion of inorganic Hg into MeHg in many environments (Driscoll

et al., 2013; Morel et al., 1998; Selin, 2009). These bacteria, which inhabit anoxic aquatic environments, use sulfate as the final electron acceptor for respiration and can methylate Hg during the process, presumably by the involvement of the acetyl-coenzyme A pathway (Parks et al., 2013; Pollman & Axelrad, 2014; Selin, 2009). The central role played by the sulfate-reducing bacteria suggests that the production of MeHg is not limited by the initial concentration of inorganic Hg but rather by sulfate concentrations and, thus, sulfate reduction rates. The addition of an inhibitor of sulfate reduction to anoxic sediments led to an almost complete reduction of MeHg production (Compeau & Bartha, 1985). Similarly, no methylation occurred in cultures of sulfate-reducing bacteria where no sulfate was added (King et al., 2000). Increased concentration of available sulfate in sediments and lakes led to an increase in MeHg production (Gilmour et al., 1992). As these bacteria respire, sulfur in the water column is converted from sulfate to sulfide causing the remaining sulfate to become enriched in the heavier sulfur isotope, ^{34}S (Krouse & Mayer, 2000; Peterson & Fry, 1987). This makes the ^{34}S to ^{32}S ratio as expressed relative to an international isotopic reference, $\delta^{34}S$, useful for the detection of sulfate reduction and, thus, mercury methylation via sulfate-reducing bacteria, so that variation in environmental MeHg levels can be accounted for (Fig. 1: 'sulfate availability hypothesis'; Elliott and Elliott 2016). Conversely, if sulfate-reducing bacteria are limited by factors other than sulfate, we may expect a negative relationship between $\delta^{34}S$ and Hg (Carr et al., 2017; Fry & Chumchal, 2012). High levels of sulfide associated with low $\delta^{34}S$ values could be indicative of the active presence of sulfate-reducing bacteria (which, if limited by factors other than sulfate abundance, would convert most sulfate into sulfide) leading to higher methylation rates and thus, higher Hg bioaccumulation potential (Fig. 1: ‘sulfide abundance hypothesis’).

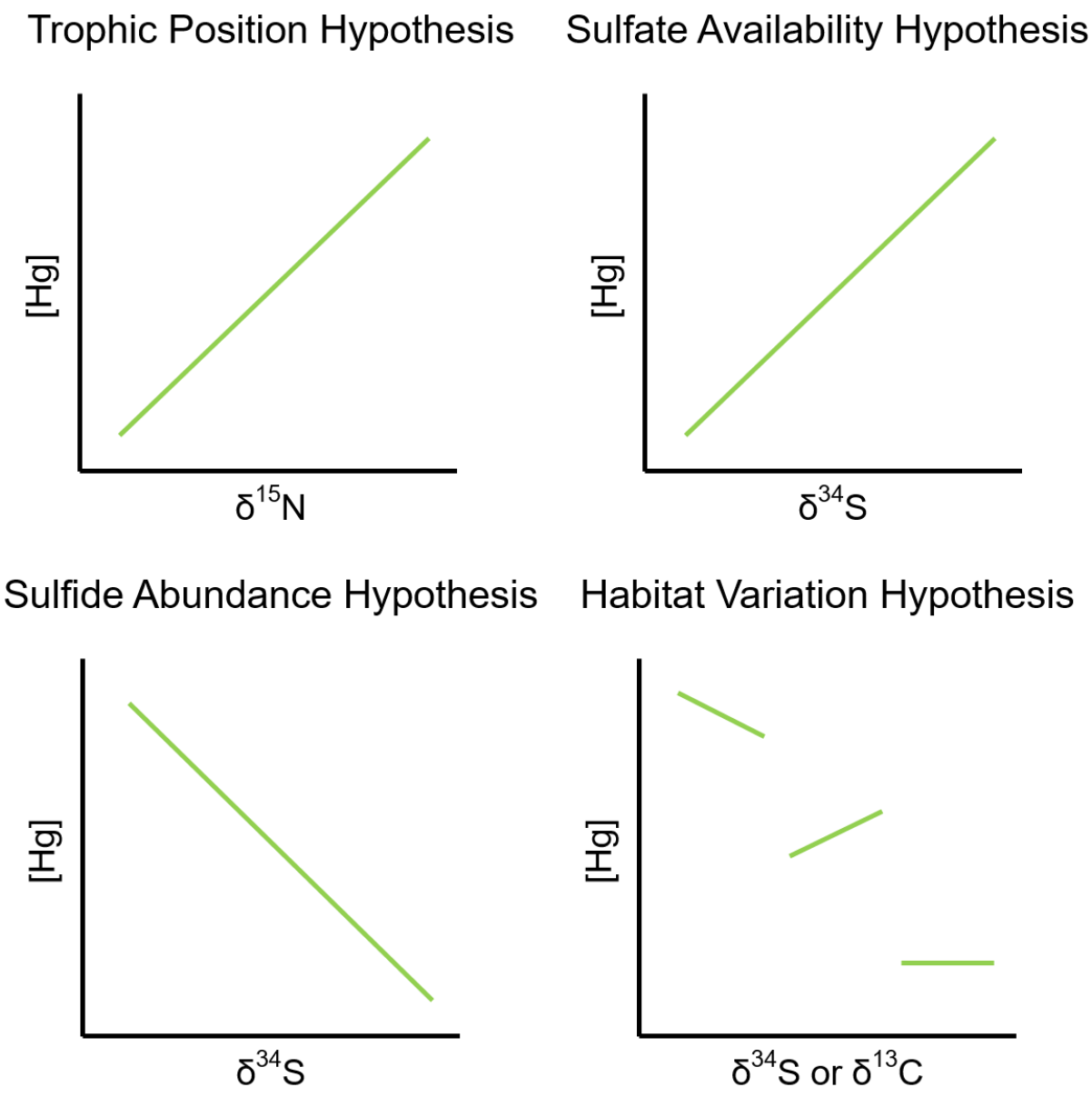

Figure 2.1. Hypotheses for relationship between Hg and isotopes tested within this paper: trophic position hypothesis, sulfate availability hypothesis, sulfide abundance hypothesis, and habitat variation hypothesis.

Thick-billed murres (*Uria lomvia*) are a key monitoring species for mercury in the Canadian Arctic (Birgit M. Braune, 2007; Birgit M. Braune et al., 2016). A recent study showed that variation in Hg among both thick-billed murres and their prey was associated with variation in $\delta^{15}N$ (Braune et al., 2014a; Braune et al., 2014b). A dietary shift towards prey lower in $\delta^{15}N$

masked an increase in Hg across time, and accounting for variation in $\delta^{15}N$ improved assessment of Hg trends over time (Braune et al., 2014b). Our study aims to compare the usefulness of $\delta^{34}S$, along with $\delta^{15}N$ and $\delta^{13}C$, for the determination of Hg bioaccumulation sources in Arctic food webs. To this end, we sampled individuals from 15 species of fish and invertebrates that are common prey of thick-billed murres breeding at Coats Island in northern Hudson Bay. We predicted that Hg levels would be associated with both sulfate availability ($\delta^{34}S$) and trophic position ($\delta^{15}N$).

2.3. Materials and Methods

Sample Collection and Preparation

Representative samples of small fish and invertebrates were collected opportunistically from the breeding ledges of thick-billed murres at Coats Island (62°98′N, 82°00′W) in northern Hudson Bay, Nunavut, Canada (see Braune et al. 2014a for details). Fifty-four individuals from 15 species were collected between 2007 and 2009. The samples were a subsample of those analyzed as part of Braune et al. (2014a), and Hg and $\delta^{15}N$ data used in this study were presented in that paper. Species sampled included Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), sand lance (*Ammodytes* spp.), Atlantic poacher (*Leptagonus decagonus*), Arctic shanny (*Stichaeus punctatus*), daubed shanny (*Leptoclinus maculatus*), banded gunnel (*Pholis fasciata*), fish doctor (*Gymnelus viridis*), fourline snake blenny (*Eumesogrammus praecisus*), sculpin (*Cottidae*), snailfish (*Liparis* sp.), sea butterfly (*Clione limacina*), squid (*Gonatus fabricii*), gammarid (*Gammaridae*), euphausiids (*Euphausiacea*), and jellyfish (*Medusozoa*). Fresh fish and invertebrates collected from the ledges were identified and measured as described by Elliott and Gaston (2008). Samples were washed, individually wrapped in foil, placed in plastic bags and

frozen at -20 °C in the field before being shipped to the National Wildlife Research Centre (NWRC), Ottawa, Ontario, where they were stored at −40 °C prior to chemical analysis. Fish were analyzed either individually or as composite samples (pools) comprised of 2–8 fish (see Braune et al. 2014a). Pooled samples were created by taking equal aliquots from each fish. In some cases, only sagittal sections were available after the other half of the fish was used for fatty acid analyses, but it was assumed that the sagittal sections were representative of the whole fish.

Mercury and Stable Isotope Analysis

For Hg quantification, samples were homogenized, freeze-dried, homogenized again, and weighed into nickel combustion boats. Total Hg was analyzed using an Advanced Mercury Analyzer (AMA-254) equipped with an ASS-254 autosampler for solid samples as described elsewhere (EPA Method 7473; see Braune et al. 2014a for details). Stable isotope analyses for $\delta^{34}S$, $\delta^{15}N$, and $\delta^{13}C$ were performed on the homogenate at the G. G. Hatch Stable Isotope Laboratory (Ottawa, ON; $\delta^{15}N$, $\delta^{13}C$, and all $\delta^{34}S$) or University of Winnipeg Stable Isotope Laboratory (Winnipeg, MB; only $\delta^{15}N$ and $\delta^{13}C$), with no significant inter-lab variability as reported by Braune et al. (2014b). Homogenates were freeze-dried and powdered. Lipids were removed using a 2:1 chloroform: methanol soak and rinse. Stable nitrogen and carbon isotope assays were performed on 1 mg subsamples of homogenized material loaded into tin cups. Samples were analyzed using an isotope cube elemental analyzer (Elementar, Germany) interfaced with a Delta Advantage continuous-flow isotope ratio mass spectrometer (Thermo, Germany) coupled with a ConFlo III (Thermo, Germany). A glutamic acid laboratory standard was included for every 10 unknown samples. Quality control was maintained by running sample duplicates. All measurements are reported in standard δ-notation in parts per thousand (‰) relative to the AIR international standard.

Replicate measurements of internal laboratory standards [C-55 (glutamic acid)] indicated measurement error of ±0.2‰, respectively. Stable sulfur isotope analyses were performed on 10 mg subsamples of the homogenized fish loaded into tin capsules (lipids were not extracted from homogenates as lipid extraction is known to alter $\delta^{34}S$ and lipids should not contain sulfur; Elliott et al. 2014). Samples were analyzed with an isotope cube elemental analyzer (Elementar, Germany) interfaced with a Finnigan DeltaPlus XP isotope ratio mass spectrometer (Thermo Germany) coupled with a ConFlo IV (Thermo Germany). All measurements are reported in parts per thousand (‰) relative to the VDCT international standard. Calibrated internal standards were used to normalize the data with a precision of ±0.4‰.

Statistical Analyses

Statistical analyses were conducted in R 3.3.2 using linear models and linear mixed-effect models with Hg concentrations (log-transformed) as the dependent variable and isotope ratio values as the independent variables. Linear and mixed-effect models with single isotopes (only $\delta^{15}N$, $\delta^{34}S$, and $\delta^{13}C$) and groupings of two and all three isotopes ($\delta^{15}N + \delta^{34}S$; $\delta^{15}N + \delta^{13}C$; $\delta^{34}S + \delta^{13}C$; and $\delta^{15}N + \delta^{34}S + \delta^{13}C$) were included and ranked using Akaike's Information Criterion, adjusted for small sample sizes (AICc; Burnham and Anderson, 2002). With the linear models, for species which more than one individual was collected, we used a single average data point, which was averaged across all individuals of a given species; data for species with a single collected individual were also included in these models. With the mixed-effect models, data were not pooled and species was included as a random effect. We report *P*-values for all top models. The top 6 AIC_c of the mixed-effect models with centralized predictors were used to develop a conditional model average (Grueber, Nakagawa, Laws, & Jamieson, 2011). Model averaging can rank and weigh these

models to obtain quantitative measures of relative support of each of the used model to a general, averaged model (Grueber et al., 2011).

2.4. Results

All three isotopes correlated weakly with each other (Fig. 2). $\delta^{13}C$ and $\delta^{15}N$ were positively correlated each other (t_{54} = 4.77, P < 0.00002). $\delta^{34}S$ was negatively correlated with $\delta^{13}C$ (t_{54} = -3.21, P < 0.003) and with $\delta^{15}N$ (t_{54} = -3.46, P < 0.002). Using an average species value of the raw $\delta^{15}N$ data (raw data available at: Góngora, Braune, & Elliott, 2018a), we observed that banded gunnels, poachers, fourline snake blennies, and Arctic shannies occupied the highest trophic position in the studied food web, and sea butterflies, gammarids, and jellyfish occupied the lowest. $\delta^{13}C$ and $\delta^{34}S$ (average value per species) imply that the species more associated with pelagic feeding habits were sea butterflies and jellyfish, and that the species with more benthic feeding habits included euphausiids, fourline snake blennies, daubed shannies, and poachers. However, the habitat assignation obtained from the values for those two isotopes were not always in accordance with each other; $\delta^{13}C$ characterized sand lance as pelagic feeders while $\delta^{34}S$ characterized them as benthopelagic feeders, for example. All data are archived at (Góngora et al., 2018a) and included in the Supplementary material.

For the data pooled by species (linear models), Hg variation was best explained by $\delta^{34}S$ (t_{15} = -6.20, P < 0.00002) in accordance with the AIC_c model selection (Table 1), as Hg decreased with increasing $\delta^{34}S$ (Fig. 3). AIC_c values and weights for the non-pooled data (mixed-effect models) did not show strong statistically significant evidence for the selection of a model (Table 1) but models including $\delta^{13}C$ were discarded as this regression coefficient was not statistically

significant. The averaged mixed-effect model showed that the $\delta^{15}N$ and $\delta^{34}S$ ratios were both important in explaining Hg variation. Hg concentrations increased as $\delta^{15}N$ values increased (averaged mixed-effect model, $z = 2.96$, $P_{|z|} < 0.004$) and decreased with increasing $\delta^{34}S$ (averaged mixed-effect model, $z = 2.40$, $P_{|z|} < 0.02$).

Table 2.1. Statistical output for models of Hg variation either pooled or not pooled by species.

	df	**ΔAIC_c**	**AICc Weight**
Linear Model (pooled by species)			
Null	2	18.604	9.09×10^{-05}
$\delta^{13}C$	3	12.952	0.00153395
$\delta^{15}N$	3	12.304	0.00212078
$\delta^{34}S$	3	0	0.99625437
Mixed-effect Model (not pooled by species)			
Null	3	7.4295	0.01774927
$\delta^{15}N$	4	2.4844	0.21036928
$\delta^{34}S$	4	5.6455	0.04330757
$\delta^{15}N + \delta^{34}S$	5	0	0.72857389

2.5. Discussion

The $\delta^{34}S$ ratio consistently explained variation in Hg levels, both across and within species. We observed a negative relationship between the $\delta^{34}S$ and Hg concentrations which is consistent with the sulfide abundance hypothesis (Fig. 1). $\delta^{15}N$ explained variation in Hg levels when all individual samples were included (mixed-effect models), in support of the trophic position hypothesis. Thus, across an Arctic food web from zooplankton to predatory fish, trophic position and sulfide abundance (an index of the abundance of sulfate-reducing bacteria and their activity) together predicted levels of Hg. We urge the inclusion of $\delta^{34}S$, alongside the commonly used $\delta^{15}N$, to predict levels of Hg in Arctic ecosystems.

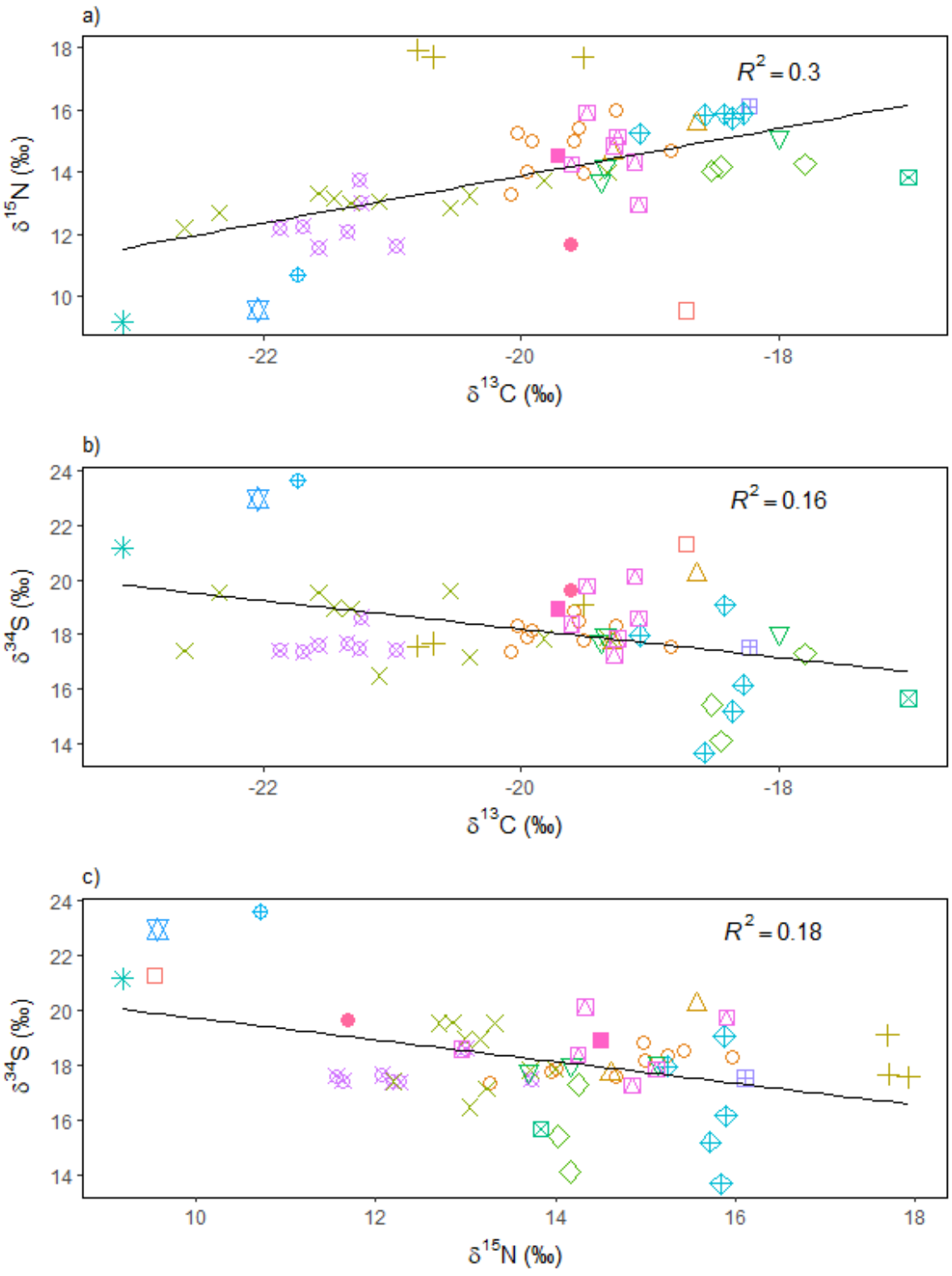

Figure 2.2. Relationships between a) $\delta^{13}C$ and $\delta^{15}N$, b) $\delta^{13}C$ and $\delta^{34}S$, and c) $\delta^{15}N$ and $\delta^{34}S$ for Arctic fish and invertebrates from Coats Island. Data from all (N = 56) individual samples was included and was not pooled by species. See Figure 3 for species legend.

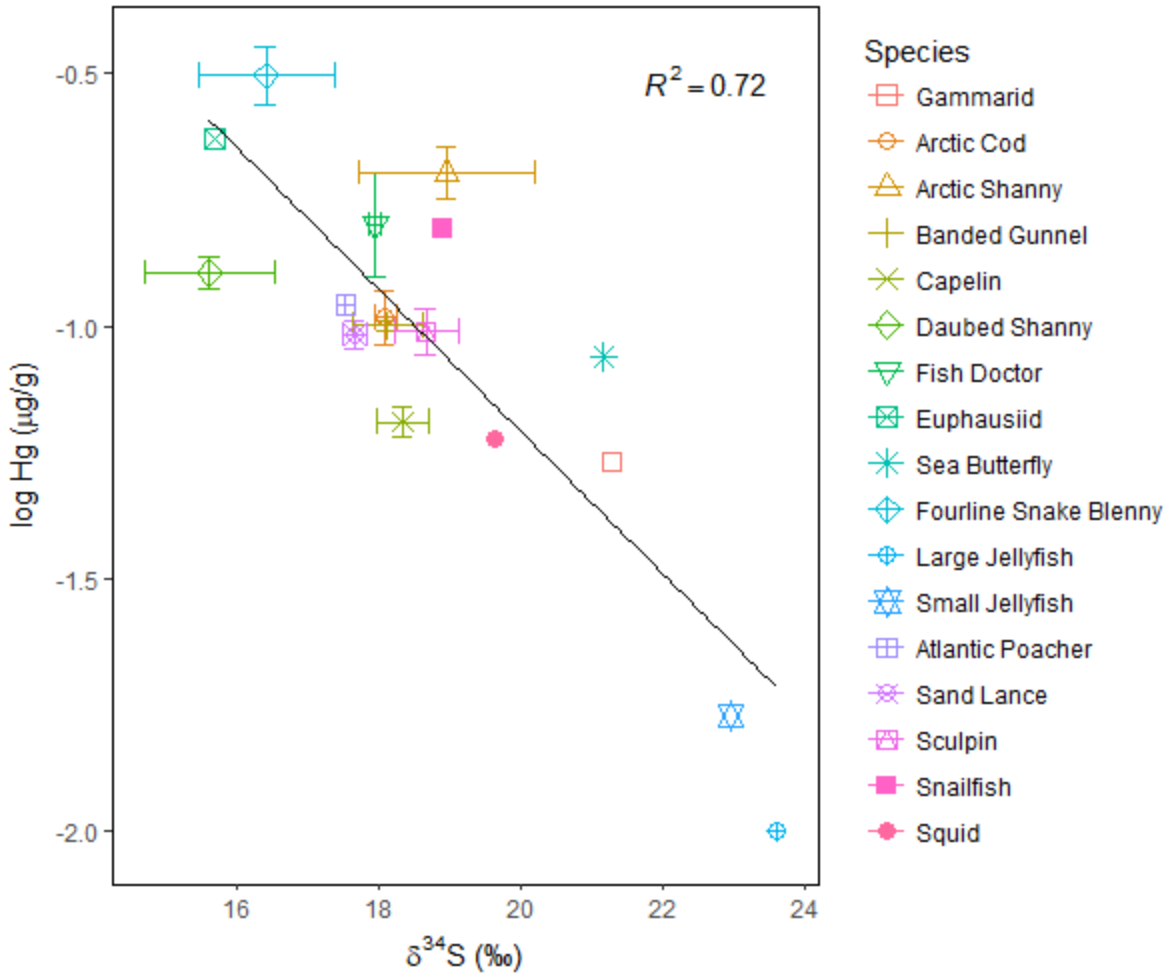

Figure 2.3. Log Hg decreased with $\delta^{34}S$ for samples averaged by species. Error bars represent SE for a given species; for species with no error bars, only one individual was collected for that species.

Significant relationships between Hg and $\delta^{34}S$ or $\delta^{15}N$ have also been observed for fish and invertebrates from estuarine and freshwater environments (Table 2). An explanation for the consistent relationships with $\delta^{34}S$ (8 out of 8 studies showing significant relationships, Table 2) compared with $\delta^{15}N$ (4 out of 8 studies showing consistent significant relationships for all sites/samples/species included; studies with variable results of positive and negative were excluded) is that previous studies of marine ecosystems were generally focused on a species or multiple species that are at relatively the same trophic level (Bentzen et al., 2016; Elliott & Elliott,

2016; Ramos et al., 2013). Thus, differential Hg accumulation is more likely caused by other factors (e.g. sulfate availability and sulfide abundance) when highly variable trophic levels are not present. Elliott and Elliott (2016) present an example of how $\delta^{34}S$ can explain patterns in Hg accumulation when $\delta^{15}N$ cannot; seabirds feeding further offshore, with the highest levels of $\delta^{34}S$, had the highest Hg concentrations compared to other seabirds feeding nearshore. Thus, species such as Leach's storm-petrel (*Oceanodroma leucorhoa*) had high levels of Hg despite having low trophic position (Table 2). In that study, both Hg and $\delta^{34}S$ declined over time in cormorants despite no variation in $\delta^{15}N$. If one only accounted for $\delta^{15}N$ and not $\delta^{34}S$, researchers would have concluded that Hg was decreasing when, in actuality, it was only diet that had changed. Another example of the future potential $\delta^{34}S$ as a complement of $\delta^{15}N$ in the study of Hg bioaccumulation is the unexpected result observed for halibut in the western Aleutian region in Bentzen et al. (2016); this region had the lowest $\delta^{15}N$ while having the highest Hg levels in the study. It is possible that a methylation hotspot could have been detected in this region if they would have also measured $\delta^{34}S$ for the same individuals (see footnote 2 in Table 2). Finally, in our own study, daubed shanny had higher Hg than banded gunnel (as correctly predicted by $\delta^{34}S$) despite being 1.5‰ lower in $\delta^{15}N$.

Table 2.2. Summary of published studies that use sulfur and nitrogen isotopes as explanatory variables for Hg concentrations.

Studied Organisms	**Location**	**Marine / Freshwater**	**Hg - $\delta^{15}N$ Correlation**	**Hg - $\delta^{34}S$ Correlation**	**Reference**
Fish and Invertebrates	Nunavut, Canada	Marine	Positive	Negative	This study
Seabirds	British Columbia, Canada	Marine	N/S	Positive	Elliott and Elliott, 2016

Fish	Northwest Territories, Canada	Freshwater	Positive	Negative	Carr et al., 2017
Halibut	Alaska, USA	Marine	Negative[1]	Not studied[2]	Bentzen et al., 2016
Cormorants and Terns	Ontario, Canada	Both	Negative to N/S[3]	Negative[3]	Lavoie et al., 2015
Yellow-legged gull	Eastern Iberian coast, Spain	Marine	N/S	Positive	Ramos et al., 2013
Fish	Ontario, Canada	Freshwater	N/S	Positive	Ethier et al., 2008
Fish and Invertebrates	Louisiana, USA	Estuary	Positive	Negative	Fry and Chumchal, 2012
Fish and Invertebrates	Missouri, USA	Freshwater	Negative to Positive	Negative to Positive	Schmitt et al., 2011
Fish and Invertebrates	Nova Scotia, Canada	Freshwater	Positive	Negative to Positive[4]	Clayden et al. 2013, 2017

[1]$\delta^{15}N$ for the western Aleutian region was the lowest from the study while having the highest total Hg concentrations; for the rest of the sites the correlation was positive. [2]Although this study doesn't analyze the $\delta^{34}S$ ratio, it does consider that a possible source of high Hg levels is marine Hg methylation, which could be evidenced if the $\delta^{34}S$ signature for the studied sites was measured. [3]At the ^{13}C-rich site, low $\delta^{15}N$ was associated with high total Hg values for cormorants while low $\delta^{34}S$ was associated with high total Hg for both cormorants and terns. [4]The relationship between $\delta^{34}S$ and Hg (total Hg and MeHg) was negative when data for all fish and invertebrates within a given lake were combined. Relationships for species considered individually were not significant, except for yellow perch, which was negative among all but one of the studied lakes.

The negative correlation between $\delta^{34}S$ and Hg observed in our study is consistent with the sulfide abundance hypothesis (high sulfide abundance evidencing high methylation and high Hg accumulation). Where bacteria are limited by sulfate availability, one might expect a positive

relationship between bacteria and sulfate as areas with high sulfate abundance can sustain high bacteria populations (Fig. 1). However, where bacteria are limited by other factors (such as oxygen concentrations, competition with other biota, sulfate recycling, temperature, presence of compounds complexing with Hg, or MeHg photodegradation; Marschall et al. 1993, Muyzer and Stams 2008, Point et al. 2011, Schaefer et al. 2011, O'Driscoll et al. 2011, Graham et al. 2012, Antler et al. 2013, St. Pierre et al. 2014, Schartup et al. 2015), one could expect that the abundance of sulfide might be an index of sulfate-reducing bacteria (more bacteria, more sulfate reduction, more sulfide), so that there is a negative relationship between bacteria, mercury, and $\delta^{34}S$. Both possibilities could explain the results presented in Table 2. As future studies continue to use $\delta^{34}S$, along with other commonly used stable isotope ratios, such as $\delta^{15}N$, recurring patterns will permit better understanding of $\delta^{34}S$ and its association with Hg. Increasing sampling efforts in the Arctic will help us to explain the role sulfur stable isotopes play in Hg accumulation in the Arctic marine ecosystems.

Previous studies used $\delta^{34}S$ primarily as a spatial indicator of the origin of food sources in parallel to how $\delta^{13}C$ is used and, thus, those studies used $\delta^{34}S$ to demonstrate differential Hg levels for samples originating from different habitats (i.e. lake vs. river or marine vs. freshwater vs. estuarine; Hobson 1999, Fry and Chumchal 2012, Lavoie et al. 2015, Carr et al. 2017). Indeed, the negative correlation could also be caused by spatial differences in the animals' feeding strategies (Fig. 1: 'habitat variation hypothesis'). Fry and Chumchal (2012) suggest the presence of Hg hotspots that may arise due to the interaction of sulfate-reducing bacteria with aquatic macrophytes and epiphytes. As most of the fish and invertebrates in our study are carnivorous, such an interaction was not considered. However, zooplankton feeding on particulate organic material and algae can play a central role in the introduction of MeHg into the food web (Chételat, Poulain,

Amyot, Cloutier, & Hintelmann, 2014; Pućko et al., 2014), and similar processes may be involved at our study site. Interactions at different levels of the ecosystem could cause the observed trends in our study. Foster et al. (2012) suggest that studying animals in low trophic position could provide information on what is occurring at higher trophic positions in terms of Hg trends. Foster et al. (2012) also emphasized that using $\delta^{15}N$ as a predictor of trophic level and trophic magnification factors is only meaningful if the specific interactions within a given food web can be identified. This proposal comes in opposition to using an entire data set without clearly identifying the species included and whether they co-occurred across food webs. Given that our sampling strategy depended on what the thick-billed murres brought back to their nests, it was not possible to characterize the entire food web in such detail but future studies should consider having greater sampling efforts that would allow for better descriptions of entire Arctic food webs. Some of the trends reported by Foster et al. (2012) might be clarified if $\delta^{34}S$ were also measured, and, indeed the lack of a correlation with $\delta^{15}N$ may be due to variation in sulfate-reducing bacteria at the base of their food web rather than variation in food web interactions.

Other possible causes for the inverse correlation between Hg and $\delta^{34}S$ are high inputs of terrestrial carbon and organic matter originating from rivers and thaw ponds flowing into the Canadian Arctic Ocean, especially Hudson Bay, and the consequent external sources of Hg entering the food web (Braune et al., 2015; Braune et al., 2005; Foster et al., 2012; MacMillan, Girard, Chételat, Laurion, & Amyot, 2015). Dissolved organic matter (DOM) can increase Hg bioavailability by forming Hg nanoparticles that are taken up more easily by sulfate-reducing bacteria (Graham et al., 2012). Additionally, Hg methylation has been detected in methanogenic archaea that were isolated from northern peat lands, which suggests that MeHg could be imported from terrestrial environments through runoffs during thawing events in the summer months

(Gilmour et al., 2013). Although $\delta^{13}C$, widely-used to distinguish between terrestrial and marine carbon sources, was not a significant predictor of Hg concentration patterns in our dataset, sulfur isotopes may be used to distinguish Hg sources (Fig. 1: 'habitat variation hypothesis'). The negative association between $\delta^{34}S$ and Hg could have been caused by species with low $\delta^{34}S$ (more terrestrial input; Lott et al. 2003) feeding on prey more closely associated with high-Hg terrestrial runoffs. A marine-terrestrial cut-off, initially used to distinguish between high $\delta^{34}S$ marine raptors from other coastal birds with lower $\delta^{34}S$, was established at a value of 10‰ (Lott et al., 2003). Despite the large extent of the Hudson Bay watershed, we argue that terrestrial input was likely low in our system, given that our lowest $\delta^{34}S$ value was 13.7‰. Regardless, Hg bioaccumulation in freshwater and saltwater Arctic ecosystems, and the interaction between the two, is complex and warrants further study (Chételat et al., 2015; Chételat, Amyot, Cloutier, & Poulain, 2008).

Even though thick-billed murres are a highly-studied species in the Arctic, not much is known about the ecology of the prey they consume. Our stable isotope data can help elucidate some of the characteristics of these animals. As expected, zooplankton species such as gammarids and sea butterflies occupy a low trophic position. However, euphausiids (though we only studied one species) had a higher $\delta^{15}N$ than the rest of the studied zooplankton, even though euphausiids mostly feed on phytoplankton. Unlike fish, euphausiids have a relatively low MeHg:Hg ratio, and it is possible that the high level of Hg in euphausiids is not indicative of a high level of methylmercury. Fish occupied higher trophic positions, suggesting they feed on invertebrates (intermediate $\delta^{15}N$ values; i.e. capelin) or on other fish (high $\delta^{15}N$ values; i.e. sculpin). Nonetheless, if species foraged in different food webs, with different baselines or discrimination factors, then $\delta^{15}N$ may be an imperfect measure of trophic position (Foster et al., 2012). In general, $\delta^{13}C$ and $\delta^{34}S$ allowed us to describe the feeding habits of various fish and invertebrates. Capelin,

sea butterflies, and jellyfish are associated with pelagic feeding, while daubed shannies and fourline snake blennies are benthic feeders. However, for some other species, the resolution of the isotope ratios included in our study was not sufficient to clearly determine a feeding habit (i.e. sculpin, a benthic species, has intermediate values suggesting it is a benthopelagic feeder) or the isotopes evidenced different results, as occurred for sand lance. Whereas the $\delta^{13}C$ signal implied that sand lance fed primarily in pelagic waters, as they come to the surface at night to feed; $\delta^{34}S$, in contrast, implied that sand lance were benthic feeders.

2.6. Conclusions

Stable isotopes are a valuable tool for understanding multiple aspects of the ecology and toxicology of marine fauna which, otherwise, could not be studied. Using isotope ratios other than the more traditional $\delta^{15}N$ and $\delta^{13}C$ can expand our knowledge of multiple biological systems that cannot be completely explained. Given the ubiquity of Hg in polar ecosystems, we encourage the incorporation of $\delta^{34}S$ into Hg monitoring plans in the Arctic, along with more detailed studies of the links between $\delta^{34}S$, Hg, MeHg, sulfate and sulfate-reducing bacteria.

2.7. Acknowledgements

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and nitrogen stable isotopes were funded by the Northern Contaminants Program of Indigenous and Northern Affairs Canada (grant M-20). Sulfur isotope analysis was funded by the Canada Research Chair in Arctic Ecology. We thank Tony Gaston, Grant Gilchrist, Josiah Nakoolak, Jennifer Provencher, Paul Smith, and Kerry Woo for help collecting prey samples and organizing field seasons. Thanks also to Natural Resources Canada (Polar Continental Shelf Program) and Rick Armstrong at the Nunavut Research Institute for helping with logistics.

2.8. Conflicts of Interest

None

2.9. Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.07.075.

Connecting text:

To answer the third research question of whether there is a difference in gut microbial diversity for the various types of prey specialists, in Chapter 3 we describe the gut microbiome of the thick-billed murre and its association with murre diet and the stable isotope ratios used in Chapter 2 ($\delta^{34}S$, $\delta^{15}N$, and $\delta^{13}C$). Our objective was to determine how the gut microbiome changed with the diet of different individuals and whether the stable isotope ratios that help to describe Hg accumulation patterns in food webs are also associated with changes in microbial community composition.

Chapter 3. Individual prey specialization is associated with gut microbiome in seabirds

Note on this chapter

This chapter corresponds exactly to the manuscript titled “Individual prey specialization is associated with gut microbiome in seabirds” which will be submitted to FEMS Microbiology Ecology. The manuscript was made in collaboration with Lyle G. Whyte and Kyle H. Elliott from McGill University. Esteban Góngora performed the sample collection and processing, the data analysis and wrote the manuscript. Lyle G. Whyte and Kyle H. Elliott contributed with the overall experimental design and planning and helped to critically edit the manuscript.

3.1. Abstract

The role of the gut microbiome is increasingly being recognized by ecologists due to the importance of microorganisms in the life cycle of many animals. At the same time, there is growing awareness of the presence of individual prey specialization within populations of the same species. Variations in the gut microbiome could be the result of individual prey specialization. We evaluated the hypothesis that diet alters gut microbiome by testing the prediction that gut microbiome varies with sex, reproductive stage, and diet types. We present the first description of the fecal microbiome of the thick-billed murre (*Uria lomvia*) through a metabarcoding survey from samples collected in Coats Island (Canada) in 2017. We show the presence of large inter-individual variation in the composition of the gut microbiome occurring in murres. There were differences in unweighted UniFrac distances between males and females (PERMANOVA; P = 0.018) and incubating and chick-rearing birds (PERMANOVA; P = 0.006). The murre microbiome is dominated by bacteria belonging to the genus *Catellicoccus*, ubiquitous in the guts of many seabirds. We also present evidence of the possible role of bacteria in uric acid recycling which aids their host in compensating for nutritional deficiencies that may be encountered.

3.2. Introduction

Individual prey specialization (IPS) is a widely distributed phenomenon occurring in a large number of taxa (Bolnick et al., 2003, 2002) in which individuals of the same population use prey resources differentially. Thus, generalist populations may actually be composed of multiple specialist individuals (Provencher et al., 2013). Although IPS is well-documented and some mechanisms such as constraint in foraging performance, disruptive selection by sex, changes in morphology, variation in social status and mating strategy have been described, the causes of IPS remain poorly understood (Bolnick et al., 2003; Ceia & Ramos, 2015; Woo et al., 2008).

The role of bacteria in trophic interactions of top predators is increasingly being appreciated. For instance, bacterial biofilms can be important food sources for western sandpipers (Kuwae et al., 2008), bacteria can control levels of methylmercury in wild birds (Góngora et al., 2018b) and alterations in the microbiome of wild animals may be associated with variation in diet (Bolnick et al., 2014; Bolnick et al., 2014). Variation in diet composition was linked to changes in the gut microbiome in wild sticklebacks and perches, two fish species that present IPS (Bolnick et al., 2014; Bolnick et al., 2014). These studies also show that other types of interactions changing the feeding behaviour (e.g. differential diets between sexes) can explain variation in the microbiome (Bolnick et al., 2014; Lewis et al., 2016). The gut microbiome may also be crucial for the adaptation of the vertebrate host to ecological pressures, such rapid environmental change or adverse conditions (Alberdi et al., 2016; Ben-Yosef et al., 2010; Lapanje et al., 2010; Lewis et al., 2016).

Bacterial communities change in structure and function throughout different sections of the gastrointestinal tract with bacteria associated with polysaccharide degradation and lactate fermentation occurring primarily in the crop, with less diverse communities in the proventriculus

and ventriculus due to the low pH, where and with the highest diversity of bacteria observed in the intestines, especially the ceca, fulfilling functions such as carbohydrate fermentation, production of vitamins and essential amino acids, and nitrogen waste compound recycling (Oakley et al., 2014; Sergeant et al., 2014; Waite & Taylor, 2014). Uric acid is the primary nitrogenous waste compound excreted by various animal groups such as birds, and some reptiles and insects (Karasawa, 1999; Kohl, 2012; Thong-On et al., 2012). Uric acid comes into contact with the gut microbial communities in birds due to the proximity of the urogenital and intestinal tracts in the cloaca (Kreisinger et al., 2017). Uric acid can travel from the cloaca into the ceca via the rectum due to retrograde peristalsis (Kohl, 2012; Pan & Yu, 2014; Vecherskii et al., 2015; Vispo & Karasov, 1997). Once in the ceca, bacteria can degrade the transported uric acid into ammonia which can be re-absorbed and transformed by birds into nonessential amino acids, such as glutamine (Mortensen & Tindall, 1981a; Vispo & Karasov, 1997). Uric acid is normally fermented by anaerobic bacteria into ammonia, short-chained fatty acids such as acetic acid, and carbon dioxide (Kane, 1997; Mortensen & Tindall, 1981b; Vispo & Karasov, 1997). There are multiple examples of uricolytic bacteria present in the intestines of birds from the order Galliformes, ducks, and even hummingbirds, which do not possess ceca (Kohl, 2012). Microbial uric acid recycling may enable the black grouse (*Lyrurus tetrix*) to reuse amounts of uric acid equal to their daily dietary nitrogen intake (Vecherskii et al., 2015). Uric acid recycling also occurs in eastern subterranean termites (*Reticulitermes flavipes*) where, despite the fact these termites have the ability to produce this compound, no uric acid is excreted but is rather taken up by gut bacteria and up to 30% of the total nitrogen in a termite colony can be recycled uric acid nitrogen (Kane, 1997).

Most of the studies of the effects of diet on the avian gut microbiome are based on domestic or captive birds (chickens: Apajalahti *et al.* 2001; Apajalahti and Kettunen 2006; Oakley *et al.*

2014; raptors: Bangert *et al.* 1988) and have mainly focused on the detection of specific groups or species of bacteria (mostly pathogens; Soucek and Mushin 1970; Mead *et al.* 1983; Waldenström *et al.* 2002). Recently, total gut microbial communities for wild bird species have started to be described (Waite & Taylor, 2015). Maul, Gandhi and Farris (2005) characterized cloacal bacteria according to carbon use and clustered the bacterial communities of various passerine bird species according to the bird's diet. The gut microbiome of two passerine species differed between spring and fall migrations (Lewis et al., 2016) and between migrating and resident shorebirds (Risely et al., 2018), suggesting that the environment, and possibly diet, has a strong influence on the microbiome of wild birds. Dewar *et al.* (2013) reported individual variation in the gastrointestinal microbiota of four penguin species, which may have been associated with IPS. Results from the meta-analysis of the avian gut microbiota by Waite and Taylor (2014) illustrate that, even among phylogenetically and behaviourally diverse species, diet has an important effect on the gut microbiome composition.

Thick-billed murres (*Uria lomvia*; murres, from hereafter) are seabirds found in a wide range of sites throughout the Arctic. Murres are considered to be a generalist species but they present various degrees and types of IPS within the same population that is maintained within individuals across many years (Elliott et al., 2009; Woo et al., 2008). Sex-specific feeding behaviours are also present, with males and females feeding at different times of the day while their mate stays at the colony taking care of their egg or chick (Elliott et al., 2010; Paredes et al., 2006). This behaviour may lead to different types of prey being caught by each sex due to their abundance at different times of the day (Elliott et al., 2010). Females increase their trophic level when rearing chicks compared to when they are incubating their egg, although no significant

changes of trophic level could be observed for males between the two reproductive stages (Elliott et al., 2010).

To the date, no research has clearly linked variation in gut microbiome in wild birds with IPS. In this study we describe for the first time the gut microbiome of the thick-billed murre. We aimed to test the hypothesis that diet alters gut microbiome by testing the prediction that gut microbiome varies with sex, stage, and stable isotope values.

3.3. Materials and Methods

Sample collection and preparation

Thick-billed murres (N = 36) were sampled between July and August 2017 at a breeding colony at Coats Island (62°98′N, 82°00′W) located in northern Hudson Bay, Nunavut, Canada. Murres were captured using a neck pole. Fecal samples were aesceptically taken by inserting sterile PurFlock Ultra swabs (Puritan Diagnostics, Guilford, ME) into the cloaca (Fig. S1). The swab was swirled inside the cloaca to stimulate the release of feces. The recovered fecal matter was collected in sterile Falcon tubes, mixed with absolute ethanol (4:1 ethanol to feces ratio) and stored at -20°C until processed. This process was done at two different time points with 2 - 7 days in between each sampling event (Table S1). Ethanol was removed from the samples by centrifugation: polypropylene tubes containing the samples were centrifuged at 4500 RPM and 4°C for 5 min and the supernatant was discarded. The pellet was resuspended in 2 mL of sterile deionized water, centrifuged at 4500 RPM and 4°C for 5 min and the supernatant was discarded once more to assure any remaining ethanol was removed from the collected feces. Blood was taken from the brachial vein using heparinized syringes. Red blood cells (RBCs) were separated from plasma by

centrifugation, stored in gaseous nitrogen, and transported to the processing facility where they were stored at -80°C until processed.

DNA Extraction and Sequencing

Due to the high content of contaminants present in avian feces (e.g. uric acid) which might inhibit DNA extractions (Eriksson *et al.* 2017), we modified the protocols of commercially used DNA extraction and purification kits to improve the quality of the obtained nucleic acids. DNA was extracted from the resulting pellets of the fecal samples using the DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with minor modifications: samples were heated at 65°C for 10 min before bead beating, and, for the final elution step, nuclease-free water was heated to 70°C and used to elute the DNA. Extracted DNA was purified using Monarch PCR & DNA Cleanup Kit (New England Biolabs, Ipswitch, MA) according to the manufacturer's instructions with minor modifications: 20 µL of nuclease-free water heated to 70°C were used to elute the DNA.

The 16S rRNA gene was amplified using primers 515F and 926R (Parada, Needham, & Fuhrman, 2016) containing Illumina overhang adapter sequences. 25 µL PCR reactions containing 1X HotStarTaq Master Mix (Qiagen, Hilden, Germany), 0.6 µM of each primer, 0.4 mg mL^{-1} BSA (Sigma-Aldrich, St. Louis, MO), and 1 µL of DNA were performed under the following conditions: initial denaturation at 95°C for 15 min, followed by 25 to 35 cycles (depending on the DNA concentration of the sample) of 94°C for 1 min, 50°C for 45s, 72°C for 1 min, and a final extension step at 72°C for 10 min. Reactions were purified using Agencourt AMPure XP magnetic beads (0.8 bead-to-PCR volume ratio; Beckman Coulter, USA). Indexing was performed using the Nextera XT index kit (Illumina, San Diego, CA) following manufacturer's instructions with a

minor modification of 15 min at 95°C in the initial denaturation used to activate the polymerase (Qiagen HotStarTaq Master Mix). Indexed samples were purified with AMPure XP beads (1.12 bead-to-PCR volume ratio) and quantified using the Qubit fluorometer (Invitrogen, Thermo Fisher Scientific, USA). Samples (including negative controls of PCR reactions) were pooled in equimolar ratios of 4 nM and sequenced with a 2 × 250 bp run with v2 chemistry on a MiSeq platform (Illumina, San Diego, CA).

Sequencing data and statistical analyses

Raw sequencing data was quality filtered, merged, denoised, and dereplicated. Chimeras were removed and a preliminary taxonomic assignment was undertaken with the Silva database (Quast et al., 2012) version 132 release and using DADA2 (Callahan et al., 2016). Sequencing data was then analyzed using Qiime 2 (Bolyen *et al.* 2018; release 2018.8.0). Based on the preliminary classification, amplicon sequence variants (ASVs) predominantly present in the negative controls and also present in the fecal sample data were removed, samples with fewer than 1000 sequences and the negative controls were removed from further analysis. Taxonomic classification was performed with the q2-feature-classifier multinomial naive Bayes classifier (Bokulich et al., 2018a) using the SSU Ref NR 99% Silva database (Quast et al., 2012) version 132 release. ASVs that were not assigned a taxonomic classification, and mitochondrial and chloroplastic ASVs, were removed. Samples were rarefied to a sequencing depth of 9308.

Diversity analyses were performed with the q2-diversity plugin. Shannon diversity (Shannon & Weaver, 1949), Pielou's evenness (Pielou, 1966) and Faith's phylogenetic diversity (Faith's PD; Faith 1992) were calculated for the different diet types, between sexes, and between reproductive stages (incubating vs. chick-rearing). Kruskal-Wallis rank tests (Kruskal & Wallis,

1952) were used to observe statistical differences between said groups according to the three diversity metrics used. Bray-Curtis dissimilarities (Sørensen, 1948), and weighted and unweighted UniFrac distances (Lozupone, Hamady, Kelley, & Knight, 2007; Lozupone & Knight, 2005) were calculated to observe the community structure of the murre gut microbiome. After testing for the homogeneity of multivariate dispersions (PERMDISP; Anderson 2006), differences in community structure for the distance measurements among diets, between sexes and between reproductive stages were calculated using the permutational multivariate analysis of variance (PERMANOVA; Anderson 2001). Principal Coordinates Analysis (PCoA) plots and biplots (Legendre & Legendre, 2012) were created with Emperor (Vázquez-Baeza et al., 2017; Vázquez-Baeza, Pirrung, Gonzalez, & Knight, 2013) to visualize the differences obtained with PERMANOVA. BIOENV rank correlation (Clarke & Ainsworth, 1993) was used to determine if any of the isotopic signatures or the carbon, nitrogen or sulfur percentages quantified in murre blood, or a combination of these variables, explained the community composition. Based on the results of BIOENV, Mantel tests (Mantel, 1967) were used to infer the Spearman rank correlation (Spearman, 1904) between the community distance matrices and the selected variables.

Repeatability was evaluated for birds where two samples were obtained using the q2-longitudinal plugin (Bokulich et al., 2018b) to compare differences in terms of alpha diversity with the Wilcoxon signed-rank test (Wilcoxon, 1945). We also evaluated repeatability for the community composition between the two time points with R 3.5.1 by calculating the Pearson correlation (Pearson, 1895) for the weighted and unweighted UniFrac distances and the Spearman rank correlation for the Bray-Curtis dissimilarities after determining the normality of the distance metrics with the Shapiro-Wilk test (Shapiro & Wilk, 1965).

Stable isotope and elemental composition analysis

Stable isotope ratios have been used as indicators of trophic level and feeding location (Atwell et al., 1998; Nisbet et al., 2002; Overman & Parrish, 2001; Vo et al., 2011). Given that ^{15}N content increases with trophic level, the $\delta^{15}N$ ratio (^{15}N to ^{14}N, expressed in relation to an international isotopic reference) is an index of the relative trophic position of an organism (Carr et al., 2017; Hobson et al., 1994). $\delta^{13}C$, the ratio of ^{13}C to ^{14}C, (as expressed relative to an international isotopic reference), can describe changes in food sources associated with habitat to a greater degree than trophic level. Benthic feeding organisms are enriched in ^{13}C compared to pelagic feeders (Carr et al., 2017; Hobson et al., 1994; Nisbet et al., 2002). Because sulfate-reducing bacteria respire, sulfur in the water column is converted from sulfate to sulfide causing the remaining sulfate to become enriched in the heavier sulfur isotope, ^{34}S (Krouse & Mayer, 2000; Peterson & Fry, 1987). The ^{34}S to ^{32}S ratio as expressed relative to an international isotopic reference, $\delta^{34}S$, is useful for the detection of sulfate reduction and can be used to characterize food sources associated with anaerobic sediments, where sulfate reduction occurs (Elliott & Elliott, 2016; Góngora et al., 2018b).

Percentage of sulfur (%S) is positively associated with Hg accumulation as the S atm in amino acids containing sulfur, such as cysteine, are strong binding sites for methylmercury in tissue (Clayden et al., 2017; Lescord et al., 2018). Additionally, higher %S was associated with higher $\delta^{15}N$ values in 6 lake food webs from Cornwallis Island in the Canadian high Arctic which suggests that the amount of S-containing amino acids increases with trophic level (Lescord et al., 2018). The carbon to nitrogen (C:N) ratio is often used as a proxy measurement for body condition given that the relative lipid concentration in tissues given the high carbon and low nitrogen content of lipids (Dempson, Braithwaite, Doherty, & Power, 2010; Fagan, Koops, Arts, & Power, 2011;

Logan et al., 2008; Sweeting, Polunin, & Jennings, 2006). However, it must be noted that, the association between the C:N ratio and lipid content can be specific for each species and tissue type (Fagan et al., 2011; Sweeting et al., 2006).

RBCs were freeze-dried and powdered. Lipids were removed using a 2:1 chloroform: methanol soak and rinse. Stable isotope analyses for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ were performed by the G.G Hatch Stable Isotope Laboratory (Ottawa, ON) for 17 of the sampled birds that where also part of the Northern Contaminants Project of Aboriginal Affairs and Northern Development Canada. Stable nitrogen and carbon isotope assays were performed on 1 mg subsamples loaded into tin cups. Samples were analyzed using an isotope cube elemental analyzer (Elementar, Germany) interfaced with a Delta Advantage continuous-flow isotope ratio mass spectrometer (Thermo, Germany) coupled with a ConFlo III (Thermo, Germany). Replicate measurements of internal laboratory standards [C-55 (glutamic acid)] calibrated to international standards (IAEA-N1, IAEA-N2, USGS-40, and USGS-41 for $\delta^{15}N$ and IAEA-CH-6, NBS-22, USGS-40 and USGS-41 for δ C) for every 10 unknown samples indicated measurement error of ±0.2‰. Quality control was maintained by running sample duplicates for every 10 samples and assuring that the relative percent difference is less than 5%. All measurements are reported in standard δ-notation in parts per thousand (‰) relative to the AIR international standard. The percentages of carbon and nitrogen in the samples were also measured as part of this analysis. Stable sulfur isotope analyses were performed on 10 mg subsamples loaded into tin capsules. Samples were analyzed with an isotope cube elemental analyzer (Elementar, Germany) interfaced with a Finnigan DeltaPlus XP isotope ratio mass spectrometer (Thermo Germany) coupled with a ConFlo IV (Thermo Germany). All measurements are reported in parts per thousand (‰) relative to the VDCT international standard. Calibrated internal standards (S-6) were used to normalize the data with a precision of

±0.4‰. Quality control was maintained by running sample duplicates for every 10 samples and assuring that the relative percent difference is less than 5%. The percentage sulfur in the samples was also measured as part of this analysis.

Feces analysis

Murre feces could be categorized according to their color and consistency into one of three categories: red with fragments; yellow with a high oil content; and a diluted white paste (Fig. S1). Based on the known diet of birds from this murre colony, it is presumed that the red samples originated from a diet consisting mostly of arthropods and that the fragments could be parts of the exoskeletons that could not be digested (Croll, Gaston, Burger, & Konnoff, 1992). Yellow samples are assumed to belong to a diet consisting mostly on fish given the high content of fatty acids in Arctic fish (some yellow samples also had a green tint and were separated into a different category: green). We consider that white samples primarily consist of uric acid (Lonsdale & Sutor, 1971) excreted by birds that had not fed for an extended period. Some samples also presented combinations of white with yellow or red feces. These color categories will be used to describe the primary diet of the murres.

3.4. Results

After denoising and filtering, a total of 7 232 547 16S rRNA gene reads with an average of 76 942 ± 4960 S.E. reads per sample were obtained. We observed a large degree of inter-individual variation in the composition of the gut microbiome within the studied individuals (Fig. 1, Table S1). Firmicutes was the predominant phylum and ranged from over 12% to over 99% of the microbiome of the sampled murres with *Catellicoccus* being the dominant genus detected and

comprising 1.3% to 98.9% of an individual's gut microbiota (Fig. S2). Other major phyla in the murre gut microbiome include Fusobacteria, Proteobacteria, and Actinobacteria. In terms of taxonomy, we can see some general patterns with regards to the fecal color types (Fig. S3). Red samples were dominated by Firmicutes and, to a lesser extent, Fusobacteria. Yellow samples also had large proportions of Firmicutes and had a higher proportion of Fusobacteria, Proteobacteria, and Actinobacteria. White samples had, relatively, a smaller abundance of Firmicutes and had larger abundances of Proteobacteria and Actinobacteria. There is a strong influence of some ASVs on the composition of the murre gut microbiome (Fig. 2 and Fig. S4). Biplots representing the most important features determining the community composition and belonging to ASVs from the genus *Catellicoccus* were present in the PCoA plots of all three metrics. *Breznakia*, *Cetobacterium*, and *Campylobacter* ASV biplots were also present in two of the metrics.

Species diversity

There was a tendency for individuals with white samples to have more diverse and even species distribution in their gut microbiome (Table 1). This is evidenced by the observed differences in terms of Shannon diversity (Fig. 3a) and Pielou's evenness (Fig. 3b) among the main diet categories. Significant differences between sexes were observed for Faith's PD (Fig. 3c; Table 1). No significant differences were observed between reproductive stages.

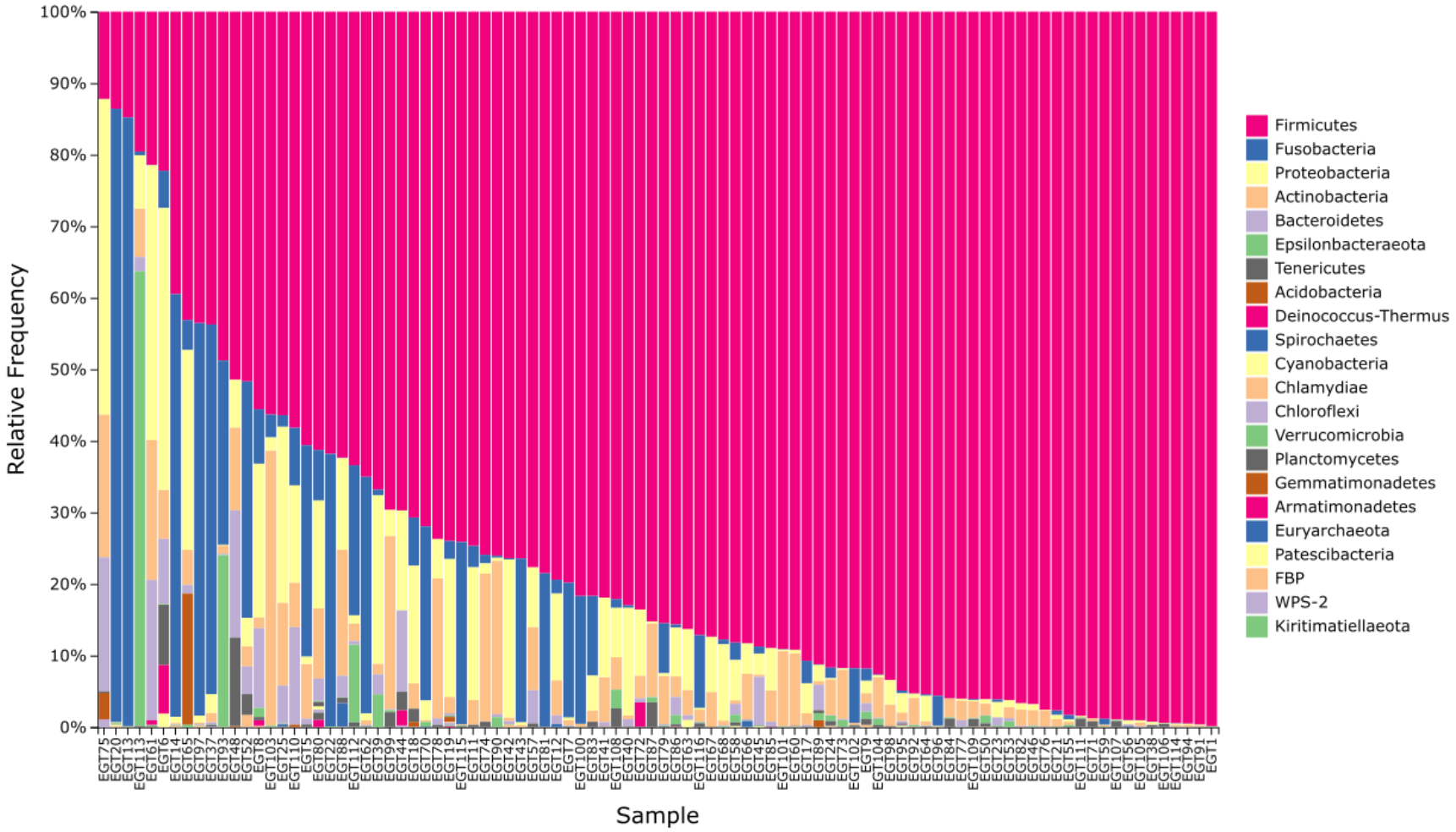

Figure 3. 1. Taxonomic classification at the Phylum level of the ASVs obtained from murre fecal samples showing a strong influence of bacteria belonging to the Phylum Firmicutes.

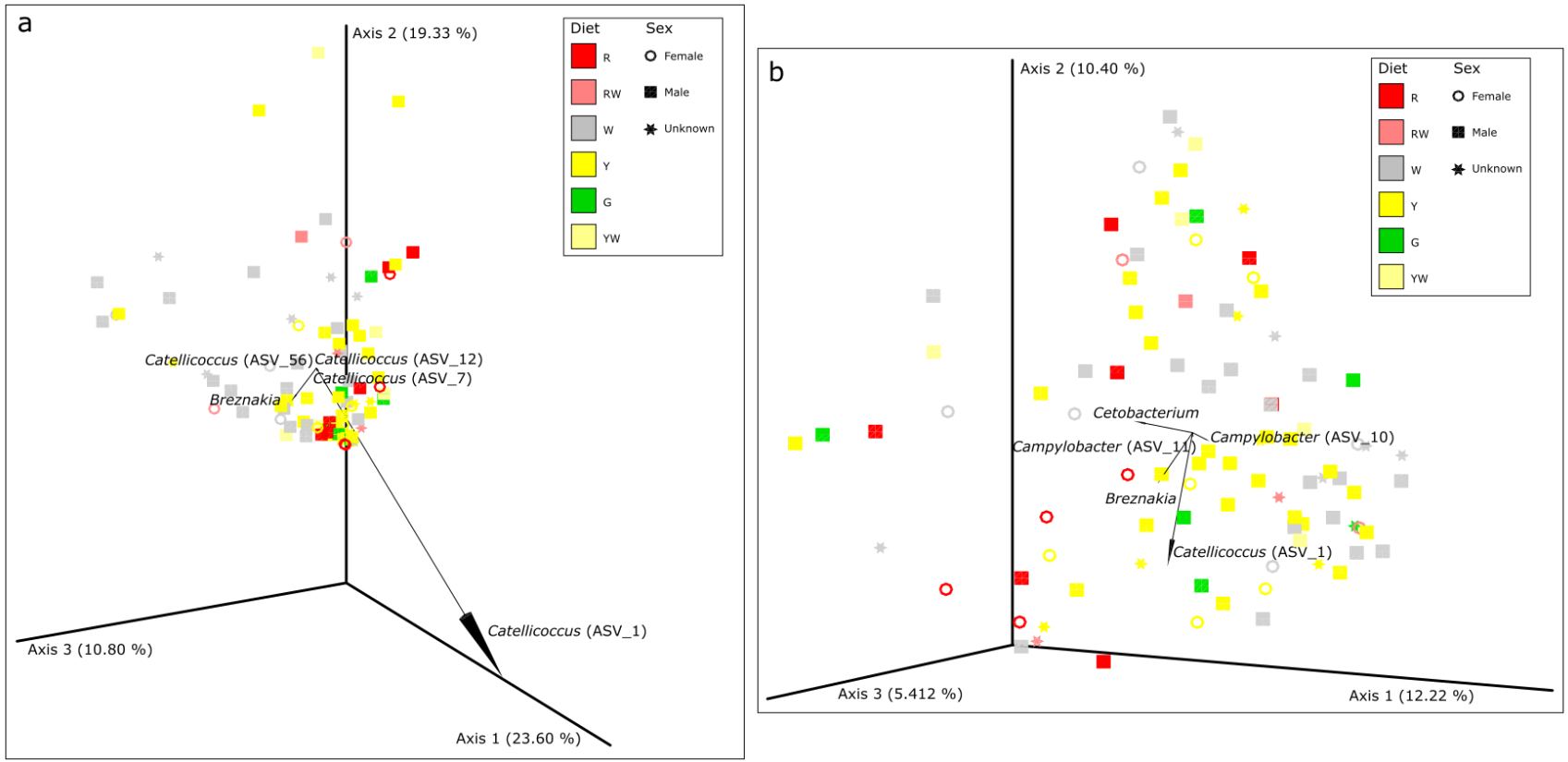

Figure 3.2. Principal Coordinates Analysis (PCoA) plots for the gut community composition using **(a)** weighted and **(b)** unweighted UniFrac metrics. R = Red; RW = Red and white; W = White; Y = Yellow; G = Green; YW = Yellow and white. Biplots show the taxonomy of the ASVs with the top 5 effects on the community composition for each metric with position of the arrow indicating the direction of the effect.

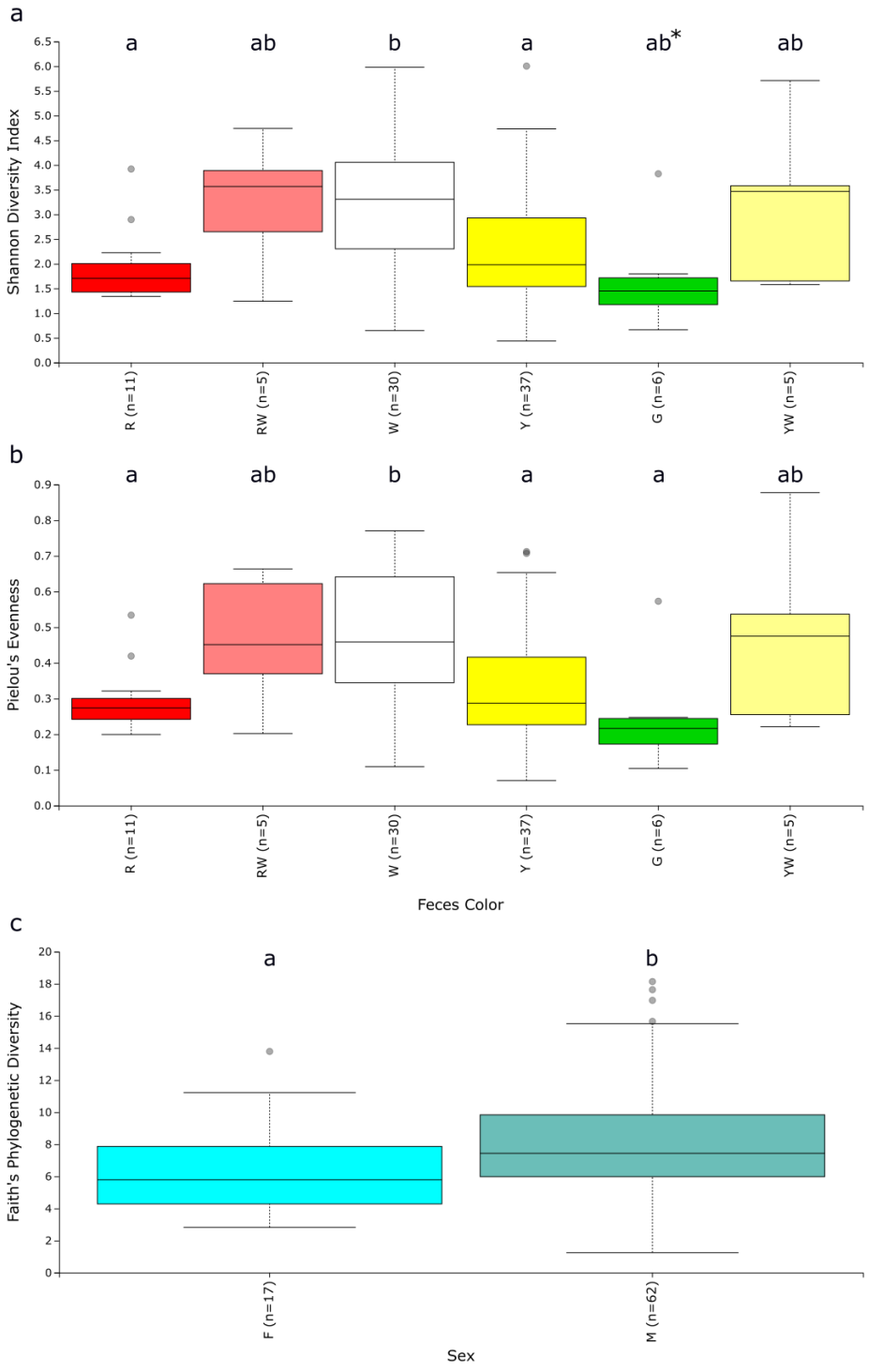

Figure 3. 3. Alpha diversity metrics for the gut microbiome evidencing differences between diets for (a) Shannon Diversity Index and (b) Pielou's Evenness and for differences between sexes for (c) Faith's Phylogenetic Diversity (Faith's PD). Letters above boxplots represent statistically significant differences between groups. For **(a)** the asterisk (*) represents a marginally significant difference (P = 0.054) between white and green samples. R = Red; RW = Red and white; W = White; Y = Yellow; G = Green; YW = Yellow and white; F = Female; M = Male.

Table 3.1. Diversity indices and statistical analyses among diets and between sexes. MS = Marginally significant.

	Feces Color	Mean	S.D.	Comparison	*H*	*Q*
Shannon Diversity	Red	1.95	0.8	Red - White	9.36	0.029
	White	3.27	1.35	White - Yellow	8.34	0.029
	Yellow	2.32	1.32	White - Green (MS)	6.49	0.054
	Green	1.71	1.10			
Pielou's Evenness	Red	0.30	0.10	Comparison	*H*	*Q*
	White	0.47	0.17	Red - White	9.01	0.020
	Yellow	0.34	0.18	White - Yellow	9.23	0.020
	Green	0.25	0.16	White - Green	7.15	0.037
	Sex	Mean	S.D.	Comparison	*H*	*P*
Faith's PD	Female	6.40	2.93	Female - Male	4.71	0.030
	Male	8.09	3.56			

Association between microbiome and diet, sex and reproductive stage

Murres appeared to have similar microbial communities amongst the different diet categories and between sexes. A significant effect of diet was observed for the studied birds (PERMANOVA: pseudo-F = 1.91, P = 0.007) caused by differences between red and white (Pairwise PERMANOVA: pseudo-F = 4.65, Q = 0.038) and white and yellow (Pairwise PERMANOVA: pseudo-F = 2.62, Q = 0.038) samples for weighted UniFrac distances (Fig. 2a). A significant effect of diet was also observed for unweighted UniFrac distances (PERMANOVA: pseudo-F = 1.31, P = 0.017) due to differences between the red and white (Pairwise PERMANOVA: pseudo-F = 2.60, Q = 0.015) and red and yellow (Pairwise PERMANOVA: pseudo-F = 2.16, Q = 0.045) predicted diets. For unweighted UniFrac distances, a significant effect of sex was also observed (PERMANOVA: pseudo-F = 1.62, P = 0.018; Fig. 2b). There was also a significant effect of reproductive stage for unweighted UniFrac distances (PERMANOVA: pseudo-F = 1.87, P = 0.006; Fig. S5) but, the multivariate dispersions are not homogeneous between incubating and

chick-rearing birds (PERMDISP: $F = 5.54$, $P = 0.019$;). No significant effects of diet, sex or stage were observed for the Bray-Curtis dissimilarities (Fig. S4).

Association of microbiome with stable isotope signatures and elemental composition

Using the stable isotope data in BIOENV we determined the variable or variable combinations that best explain the microbiome community composition. For weighted UniFrac, a combination of $\delta^{13}C$ and $\delta^{34}S$ had the highest explanatory power ($\rho_w = 0.12$). A combination of %S and C:N ratio were the variables with the highest correlation for unweighted UniFrac ($\rho_w = 0.23$). A combination of δ13C, δ15N, δ34S, %S, and C:N ratio were negatively correlated with the Bray-Curtis dissimilarities ($\rho_w = -0.24$). However, after testing the significance of these correlations via a two-sided Mantel tests, none of the correlations between these variables and the different metrics were statistically significant.

Repeatability analyses

No statistical differences were observed between the two sample points in terms of alpha diversity (Fig. S6). For the community composition, there was no significant correlations between samples taken from the same birds at the two different sample points (Fig. S7a, c, and e). Given that some birds had different sample colors at the first and second time points (Table S1), we re-calculated the correlations using the birds that had the same color for both samples, but no significant correlations were found either (Fig. S7b, d, and f).

3.5. Discussion

The gut microbiome of the thick-billed murre is consistent with other avian microbiomes which are mainly comprised of the phyla Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria (Waite & Taylor, 2015) although there was only a small proportion of Bacteroidetes in our samples (Fig. 1, Table S1). The murre gut microbiome was dominated by bacteria belonging to the genus *Catellicoccus*. This genus was first described with isolates from harbour porpoise (*Phocoena phocoena*) and grey seal (*Halichoerius grypus*) carcasses (Lawson, Collins, Falsen, & Foster, 2006) and, since then, have been found to be ubiquitous in the gut microbiome of various avian species. *Catellicoccus marimammalium* is used as a marker for detection of gull fecal contamination (Koskey, Fisher, Traudt, Newton, & McLellan, 2014; J. Lu, Santo Domingo, Lamendella, Edge, & Hill, 2008; Ryu et al., 2012; Sinigalliano et al., 2013). Bacteria from the genus *Catellicoccus* have also been found in the guts of zebra finches (*Taeniopygia guttata*; Benskin *et al.* 2010), barn swallows (*Hirundo rustica*; Kreisinger *et al.* 2017), red knots and ruddy turnstones (*Calidris canutus* and *Arenaria interpres*, respectively; Grond *et al.* 2014), black-tailed godwits, black-winged stilts and common redshanks (*Limosa limosa*, *Himantopus himantopus*, and *Tringa totanus*, respectively; Santos *et al.* 2012), and waterfowl (Weigand, Ryu, Bozcek, Konstantinidis, & Santo Domingo, 2013). The genome of *Catellicoccus marimammalium* revealed that this bacterium encodes various functions such as nutrient transport and bile acid hydrolysis suggesting the possible symbiotic lifestyle of this species (Weigand et al., 2013). Possible beneficial effects for the host such as immune modulation and gut maturation have also been proposed for bacteria of this genus inhabiting the gut (Benskin et al., 2010).

High abundances of bacteria from the phylum Fusobacteria have also been observed in the guts of other seabirds such as gentoo and king penguins (*Pygoscelis papua* and *Aptenodytes patagonicus*, respectively; Dewar *et al.* 2013, 2014a), common diving petrels (*Pelecanoides*

urinatrix; Dewar *et al.* 2014b), gulls (J. Lu et al., 2008), vultures and carnivore mammals (Roggenbuck et al., 2014; Waite & Taylor, 2015), and even in humans (Potrykus, White, & Bearne, 2008). Despite the fact that bacteria from this phylum are known for being pathogenic, recently it has been observed that they could aid their host to metabolize nutrients (Roggenbuck et al., 2014; Waite & Taylor, 2015). Fusobacteria are known butyrate producers and can ferment amino acids and glucose (Dewar, Arnould, Krause, Trathan, et al., 2014; Dewar, Arnould, Krause, Dann, et al., 2014; Potrykus et al., 2008) and, in chickens, they boost the host immune system and adiposity (Dewar, Arnould, Krause, Dann, et al., 2014). The most important genus observed for this phylum was *Cetobacterium*. *Cetobacterium someare* isolated from the intestinal tract of freshwater fish were found to produce vitamin B_{12} and acetic acid which suggest the beneficial effects of this bacterium for its host (Tsuchiya, Sakata, & Sugita, 2007).

The genus *Breznakia* is relatively novel and not much is known about these bacteria but, this genus and others in the family Erysipelotrichaceae have been frequently isolated from the guts of mammals and insects making this family mostly comprised of inhabitants of animal intestinal tracts (Tegtmeier, Riese, Geissinger, Radek, & Brune, 2016). Many bacterial genera associated with opportunistic pathogens such as *Campylobacter*, *Helicobacter*, *Escherichia*/*Shigella*, *Corynebacterium*, *Mycobacterium*, *Neisseria* and *Ornithobacterium*, among others, were found in the murre gut. However, no risk of disease is believed to exist solely based of the presence of these genera as many of them contain representatives that have also been found in other healthy birds (Dewar et al., 2013; Dewar, Arnould, Krause, Trathan, et al., 2014; Grond et al., 2014; Santos et al., 2012; Vandamme et al., 1994). However, the presence of these taxa should continue to be monitored given that reverse zoonosis of various *Campylobacter* strains associated with humans has been detected in Antarctic seabirds (Cerdà-Cuéllar et al., 2019).

We observed differences between the gut microbiome of males and females in terms of phylogenetic diversity (Faith's PD; Fig. 3c) and qualitative community composition (unweighted UniFrac; Fig. 2b). These results are consistent with the social behaviour of murre mating pairs at Coats Island in which males stay in the colony during the day and leave their nest to feed at night while females feed during the day and go back to the colony during the night (Elliott et al., 2010). This leads to different types of prey being caught by each sex (Elliott et al., 2010). Additionally, females tend to be more risk-prone in terms of feeding strategies and have a greater loss of mass during chick-rearing than males (Elliott et al., 2010). The fact that a risk-prone diet could provide less consistent food sources compared to a risk-averse diet could help to explain why there was a smaller phylogenetic diversity for the females we studied. We also obtained significant differences in the composition of bacterial communities between reproductive stages with unweighted UniFrac (Fig. S5). This may occur because chick-rearing females have higher trophic levels than incubating females (Elliott et al., 2010) Prey composition changes throughout the breeding season, potentially leading to changes in the microbiome. Furthermore, unweighted UniFrac differences for reproductive stage should be taken with caution as the heterogeneity of multivariate dispersions in unbalanced designs (48 incubating vs 46 chick-rearing samples) causes PERMANOVA to become too liberal when there is a greater dispersion for the smallest group, as is the present case (Anderson & Walsh, 2013).

Despite the general patterns in taxonomical composition (Fig. S3), no consistent differences were observed between the two main diet types as red and yellow samples were significantly different only for the unweighted UniFrac PERMANOVA analyses (Fig. 2b). The color categories we established for this study represent the diet at a given moment in time (i.e. around 12 – 24 h before the sampling event) and thus, may not necessarily represent the overall

diet of the bird throughout the mating season which might shape the microbiome more than the diet of a single day. Daily diet variation can be clearly seen with some of the murres in the study which had one fecal color for the first sampling point and a different one for the second (e.g. sample 7 and 12 belong to the same bird and are red and yellow, respectively; Table S1). Indeed, even the most specialized murres occasionally capture prey types different than the ones they specialize in (Elliott et al., 2009) which could help to explain why the composition of the microbiome is not significantly different between the presumed arthropod and fish prey categories evaluated in this study. We can also see how the gut microbiome changes from individual to individual (Fig. 1 and Fig. S2), which is consistent with the idea that generalist populations consist of individuals with multiple degrees of specialization (Provencher et al., 2013). Our results corroborate the need to characterize diet for particular individuals instead of generalizing diets of populations and even species as a whole (Bolnick et al., 2003, 2002). The large variation in the proportion of ASVs from the genus *Catellicoccus* illustrates the various degrees of IPS that the murre colony at Coats Island has.

Differences among the other types of murre fecal samples could be observed in terms of alpha diversity and community composition suggesting that white samples are different from red, yellow, and, in some cases, green samples (Fig. 2 and Fig. 3). The role of intestinal microorganisms in uric acid recycling could explain the fact that white samples had a higher diversity and more even species distribution than fecal samples originating from arthropod and piscivorous diets. The microbial communities in the avian intestine that aid their host to utilize nutrients from their diets are mostly saccharolytic and, thus, only degrade compounds that the birds could metabolize themselves (Kohl, 2012). Uricolytic bacteria in bird guts, on the other hand, are much more active, fulfilling the function of uric acid recyclers. Introducing uric acid into their intestinal tract could

have direct benefits for the bird or indirect ones via the maintenance of the microbial community in their ceca (Vispo & Karasov, 1997). When the bird is protein-depleted, the use of the uric acid-derived ammonia for amino acid production could help to balance the nutrient deficiency in its diet (Karasawa, 1999). Given the social behaviour of murre mating pairs in which one of the individuals takes care of the nest for prolonged periods of time while its mate feeds, it is possible that the gut microbiome aids murres staying in their nests to compensate, to a certain extent, for their lack of food. Excreting nitrogen in the form of uric acid reduces water loss due to its low solubility in water (Thong-On et al., 2012). Besides the main role of uric acid in osmoregulation, it could also be important to maintain osmotic balance with the assistance of bacteria (Vispo & Karasov, 1997). When taken up the intestine by retrograde peristalsis, it is possible that some of the urinary water is reabsorbed in the ceca and microorganisms would degrade the remaining uric acid to prevent a build up of harmful nitrogenous waste in the ceca. Additionally, the produced ammonia, which is converted into glutamine, can be transported to the kidneys where the glutamine is cleaved, and the ammonia serves as a proton buffer. The resulting ammonium is discarded so recycling uric acid helps birds maintain osmotic balance while reducing the amount of nitrogen they are required to consume in their diet.

No apparent relationships between microbiome and stable isotope ratios could be detected. However, associations between diet and isotopic signatures such as $\delta^{34}S$, $\delta^{15}N$, and $\delta^{13}C$ have already been observed for trophic webs at Coats Island as well as for other seabird colonies (Elliott & Elliott, 2016; Góngora et al., 2018b; Woo et al., 2008). Our smaller sample size for stable isotope analysis (N = 17) compared to the total number of sampled birds (N = 36) and the number of fecal microbiomes sequenced and analyzed (N = 94) might help to explain why significant relationships were not obtained. Increasing sample size could help to validate the presumed correlations

originally detailed by BIOENV that were not supported by the Mantel tests. It must also be noted that the isotope ratios were measured in RBCs which have a slow turnover rate of months (Barger, Young, Will, Ito, & Kitaysky, 2016) compared to the expected turnover rate of days for fecal matter. This difference in the response rates for the two measurements could also explain the negative statistical results obtained when comparing microbiome composition and stable isotope ratios. Measuring isotopic signatures in plasma, rather than RBCs, is suggested for future studies given that plasma has a much faster turnover rate (approximately a week) than RBCs (Barger et al., 2016). Other molecular techniques could also be implemented in order to better characterize murre diet such as eDNA metabarcoding of prey genes found in murre gut samples (Leray et al., 2013) as well as other traditional techniques such as feeding watches. More specific diet categories could help to elucidate the high inter-individual diversity found in this study and explain if stable isotopes are a useful tool to describe changes in the gut microbiome of thick-billed murres.

3.6. Acknowledgements

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3.7. Supplementary Material

Figure S3.1. Sampling method used to collect fecal samples **(right)** and the three main types of fecal samples obtained from Thick-billed murres at Coats Island **(left)**. Based on coloration and consistency, samples are thought to belong to birds with a diet mostly consisting on: arthropods (red), uric acid (white), and fish (yellow).

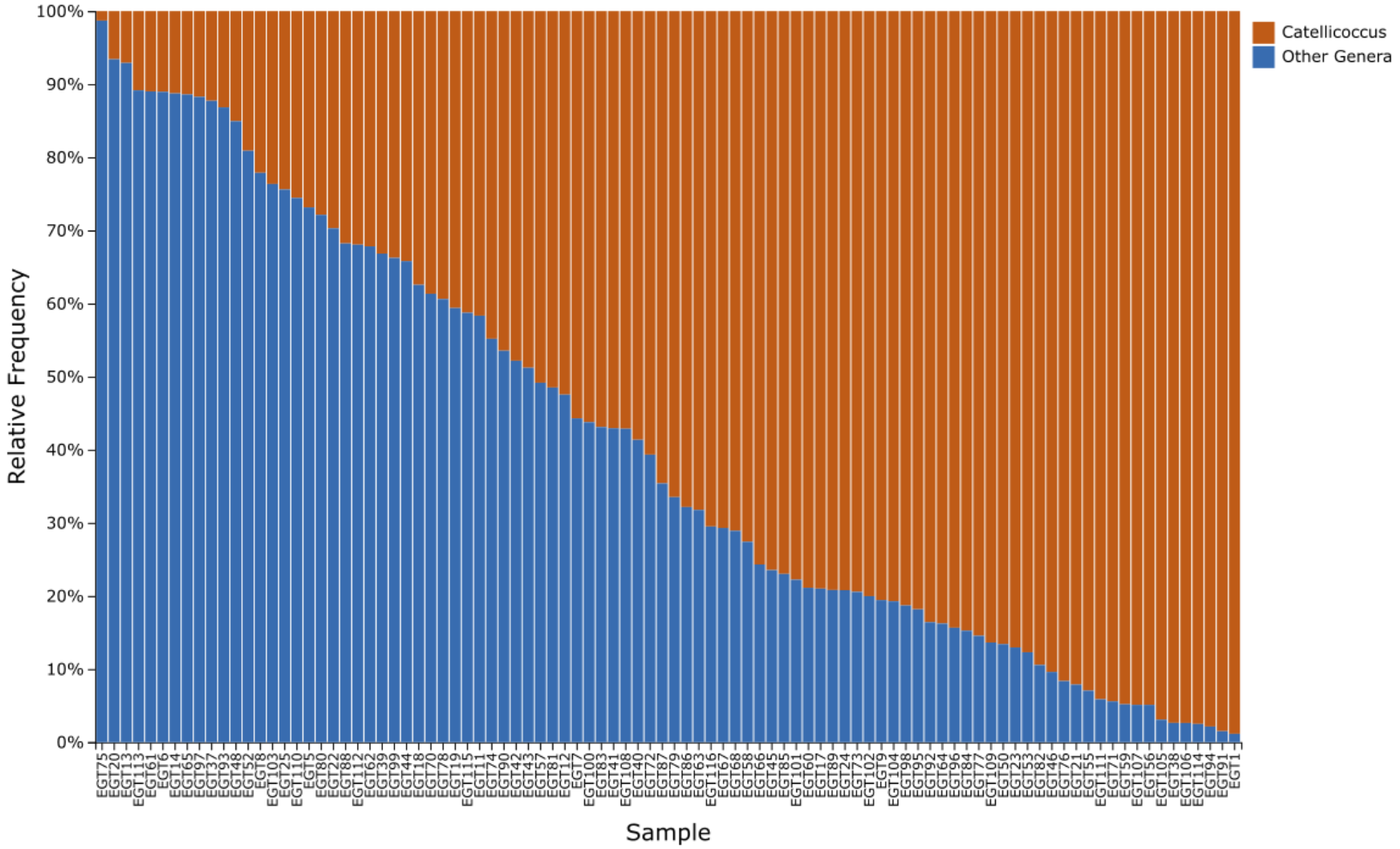

Figure S3. 2. Taxonomic classification at the Genus level of the ASVs obtained from murre fecal samples showing a strong influence of bacteria belonging to the genus Catellicoccus compared to all other genera.

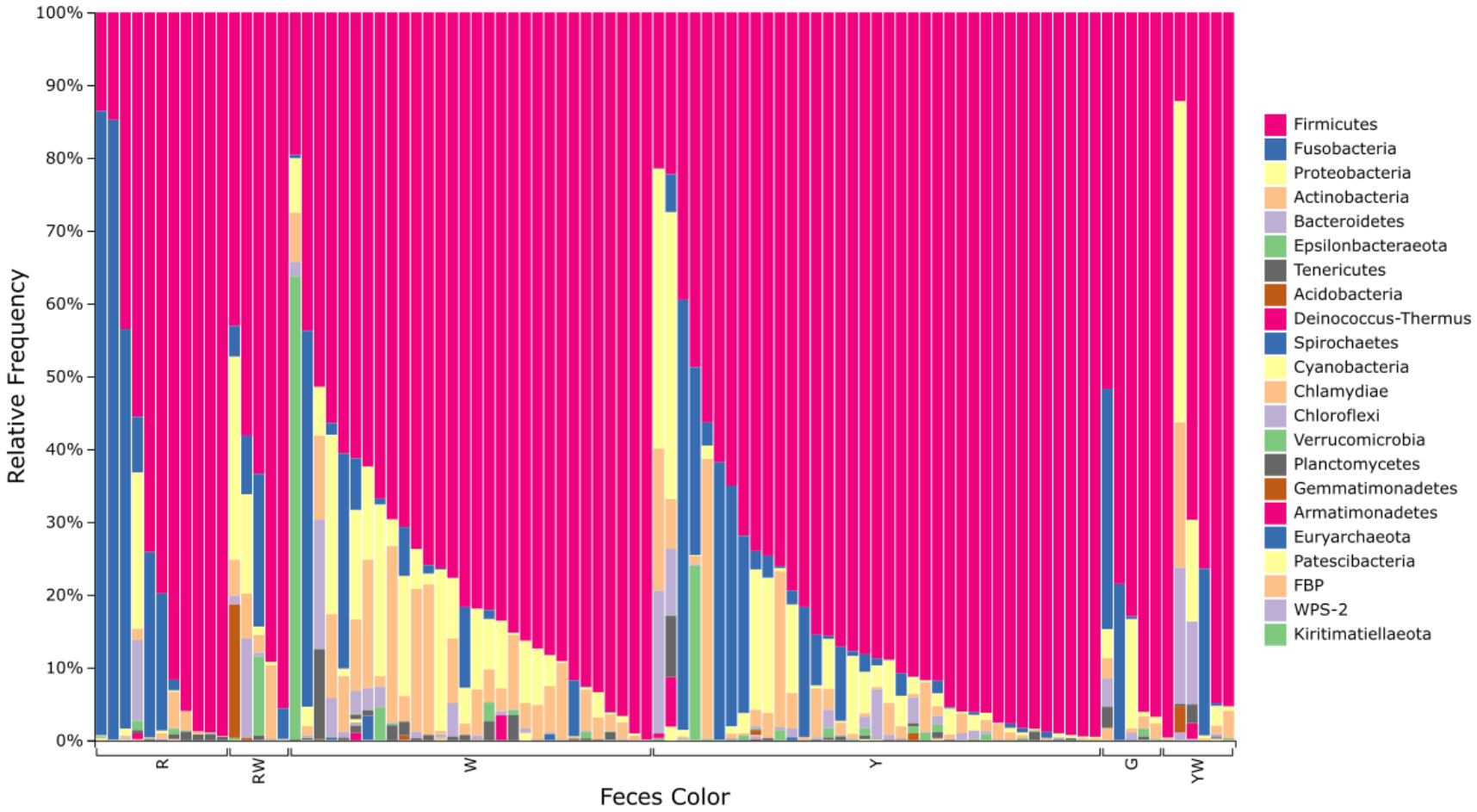

Figure S3. 3. Taxonomic classification at the Phylum level of the ASVs obtained from murre fecal samples grouped by fecal sample colors showing general patterns in taxonomical composition from each category.

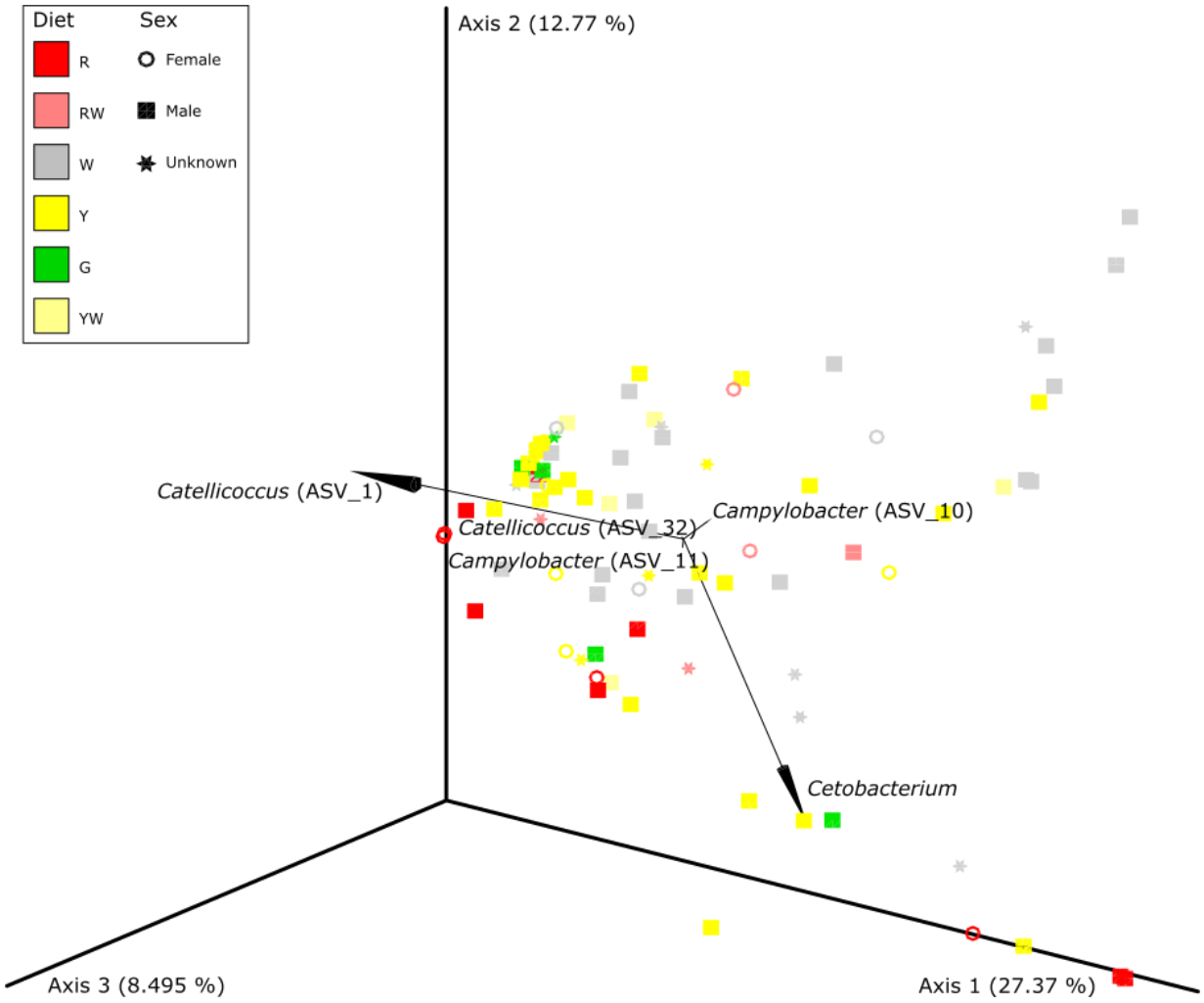

Figure S3.4. Principal Coordinates Analysis (PCoA) plots for the gut community composition using Bray-Curtis dissimilarities. R = Red; RW = Red and white; W = White; Y = Yellow; G = Green; YW = Yellow and white. Biplots show the taxonomy of the ASVs with the top 5 effects on the community composition with position of the arrow indicating the direction of the effect.

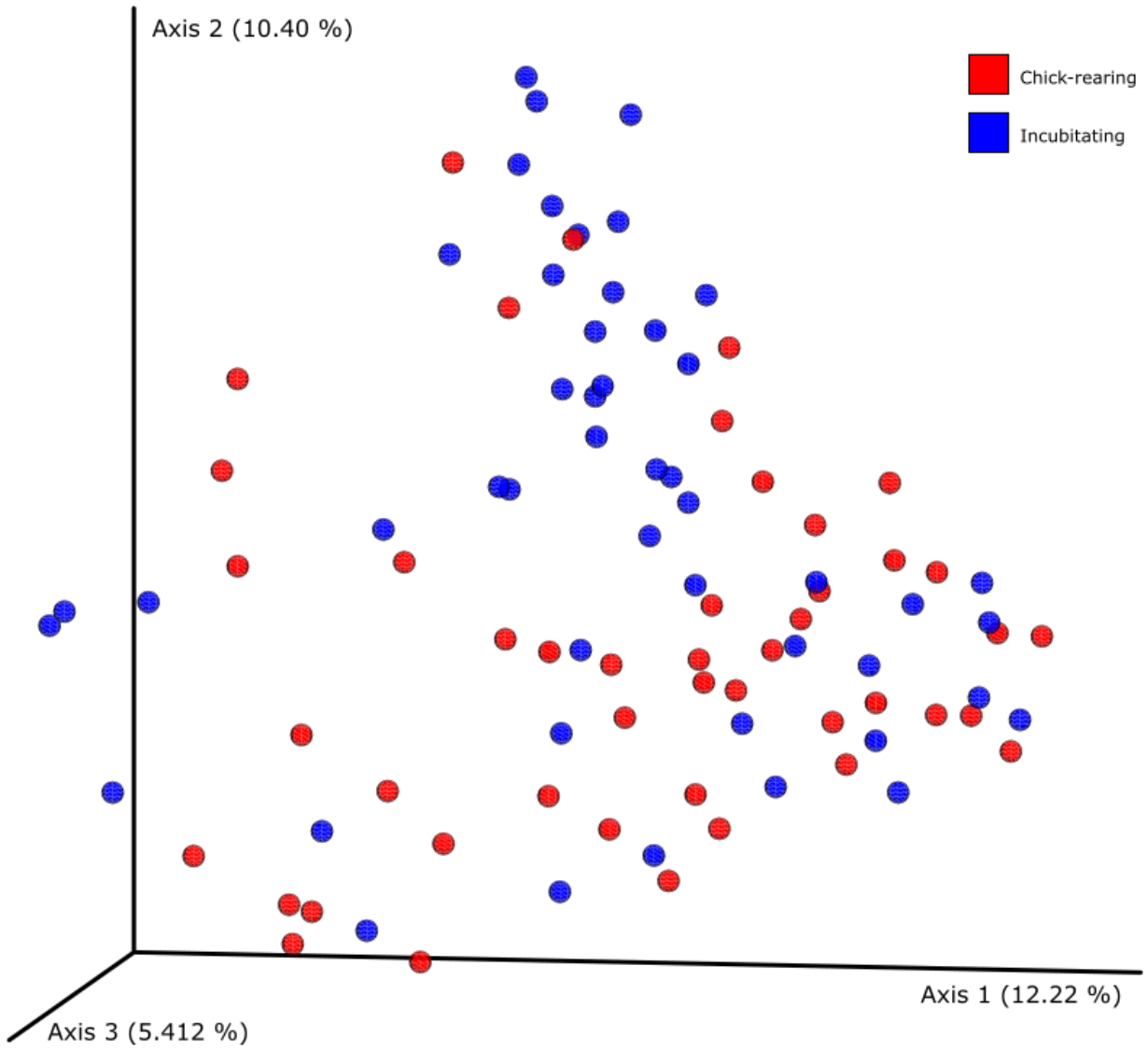

Figure S3.5. Principal Coordinates Analysis (PCoA) plots for the gut community composition using the unweighted UniFrac metrics with samples separated by reproductive stage.

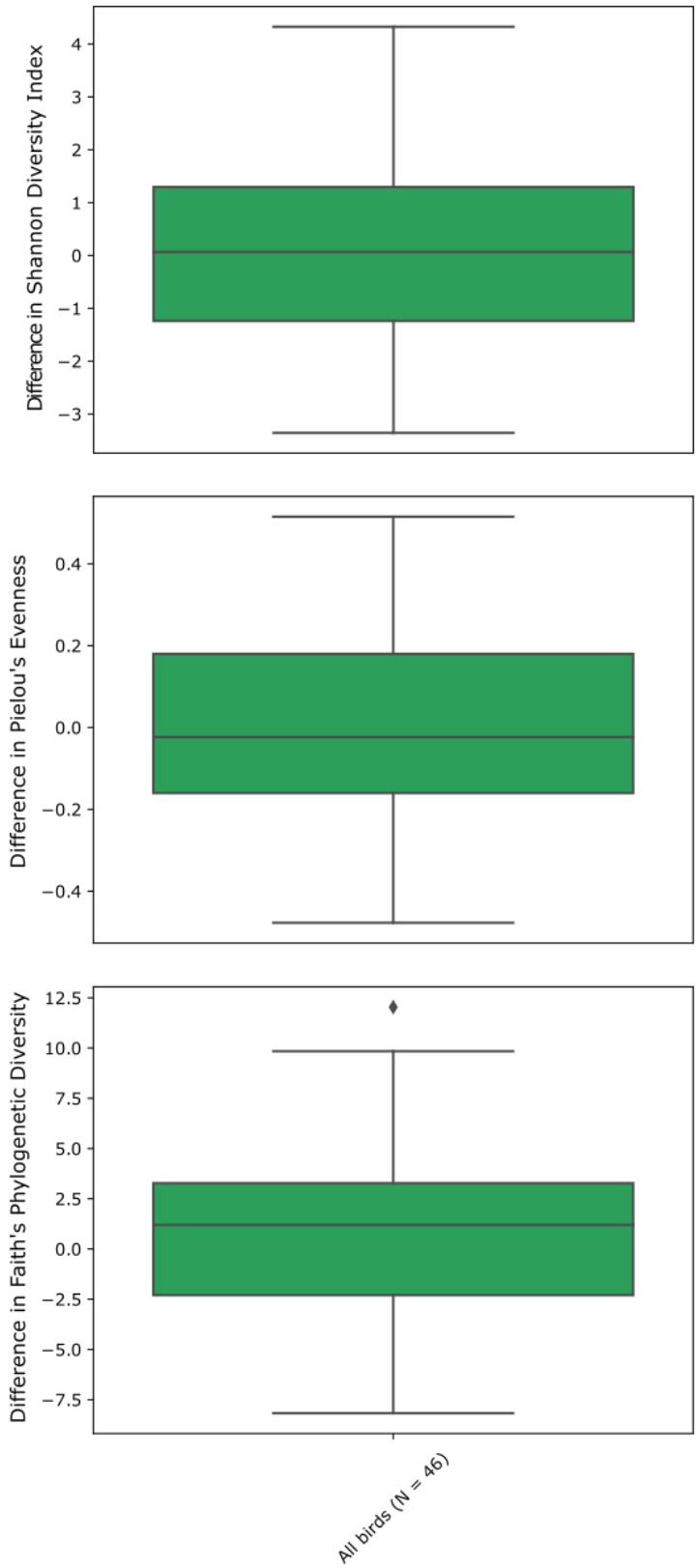

Figure S3. 6. Differences in alpha diversity measurements between samples from the same bird in the two studied time points.

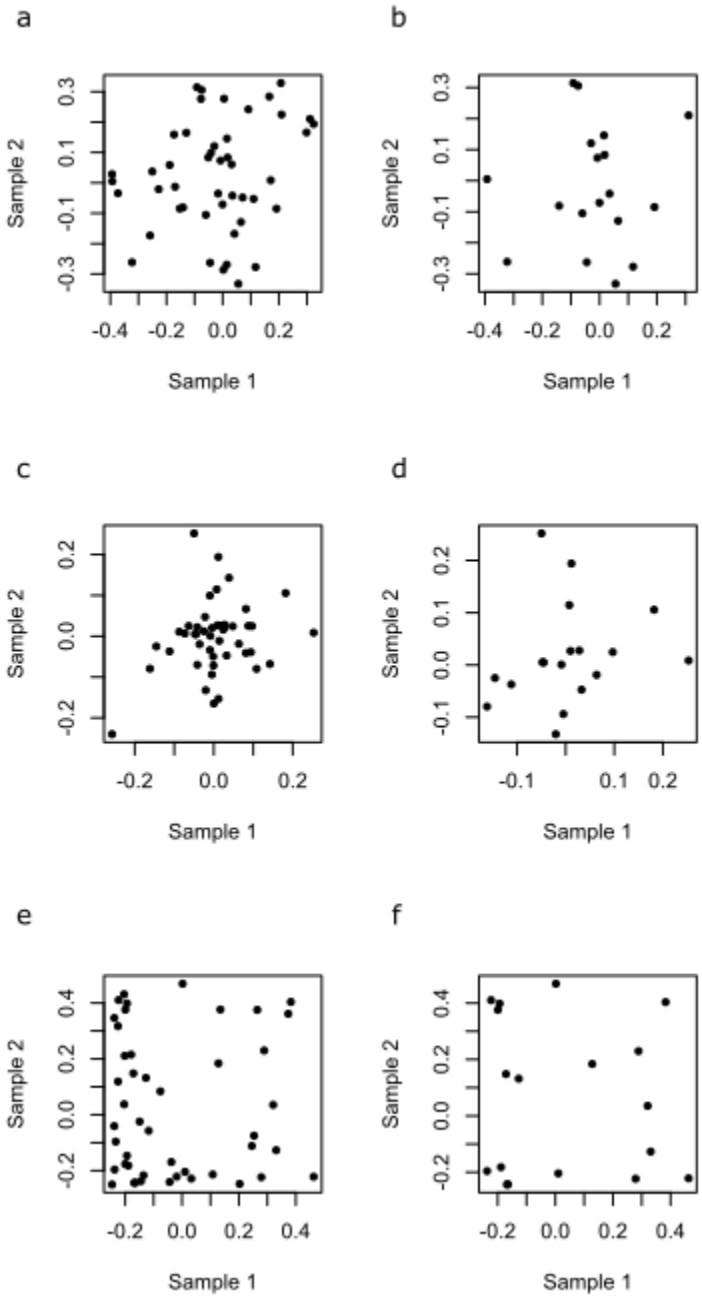

Figure S3. 7. Comparison of the value of the first PCoA axis of each bird between sampling points for the unweighted **(a, b)** and weighted **(c, d)** UniFrac metrics, and Bray-Curtis dissimilarities **(e, f)**.

Table S3.1. Metadata for the various Thick-billed murre samples collected at Coats Island.

SampleID	BirdID	Date	Sample	Color	d13C	%C	d15N	%N	d34S	%S	Sex	Status
EGT1	99676429	10/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT100	99658331	28/07/2017	1	Y	-20.75	42.4	15.52	13.17	18.04	0.66	N/A	Chick
EGT101	117639797	28/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	F	Chick
EGT102	99679694	28/07/2017	1	W	-20.34	48.2	15.27	15.4	19.49	0.69	M	Chick
EGT103	99656411	28/07/2017	1	Y	-20.66	48.2	14.11	15	17.03	0.72	M	Chick
EGT104	99680764	28/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT105	118606010	28/07/2017	1	W	-20.43	49.9	15.59	15.3	19.57	0.64	F	Chick
EGT106	99653374	28/07/2017	1	R	-21.14	48.2	15.33	13.89	16.45	0.74	F	Chick
EGT107	99653374	01/08/2017	2	R	-21.14	48.2	15.33	13.89	16.45	0.74	F	Chick
EGT108	99680540	01/08/2017	2	W	-20.76	48.7	15.63	14.95	19.99	0.72	F	Chick
EGT109	99656411	02/08/2017	2	W	-20.66	48.2	14.11	15	17.03	0.72	M	Chick
EGT11	99670720	06/07/2017	2	Y	-20.31	48.8	13.83	15.2	18.69	0.61	M	Incubitating
EGT110	117639823	01/08/2017	2	RW	-20.35	48.7	15.45	14.9	19.68	0.65	F	Chick
EGT111	99679694	01/08/2017	2	Y	-20.34	48.2	15.27	15.4	19.49	0.69	F	Chick
EGT112	99658331	01/08/2017	2	RW	-20.75	42.4	15.52	13.17	18.04	0.66	N/A	Chick
EGT113	99680764	01/08/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT114	118606010	01/08/2017	2	Y	-20.43	49.9	15.59	15.3	19.57	0.64	F	Chick
EGT115	99676921	01/08/2017	2	R	-20.35	49	16.09	15.4	19.44	0.71	F	Chick
EGT116	117639797	01/08/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	F	Chick
EGT12	99686990	07/07/2017	2	Y	-20.44	49.7	14.67	15.3	19.57	0.82	M	Incubitating
EGT13	99667063	09/07/2017	2	R	-20.58	48.2	14.29	15.1	17.24	0.73	M	Incubitating
EGT14	99686180	09/07/2017	2	Y	-20.67	47.8	14.39	14.86	19.61	0.71	M	Incubitating
EGT17	99676429	12/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT18	117639776	12/07/2017	2	W	-20.97	50.1	14.71	14.52	19.28	0.74	N/A	Incubitating
EGT19	99676337	06/07/2017	1	Y	-20.39	49	15.07	15.3	18.74	0.72	F	Incubitating
EGT20	99676338	06/07/2017	1	R	-20.42	49	14.14	15.5	18.59	0.72	M	Incubitating
EGT21	99696862	12/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT22	99667218	10/07/2017	1	Y	-20.35	48.3	14.86	15.1	17.91	0.8	M	Incubitating

EGT23	117639773	10/07/2017	1	Y	-20.23	50.1	15.12	15.5	18.99	0.73	N/A	Incubitating
EGT24	99676338	10/07/2017	2	R	-20.42	49	14.14	15.5	18.59	0.72	M	Incubitating
EGT25	117639773	12/07/2017	2	W	-20.23	50.1	15.12	15.5	18.99	0.73	N/A	Incubitating
EGT37	99697231	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Chick
EGT38	99683053	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT39	99676555	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT40	99699833	17/07/2017	2	YG	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT41	99687257	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT42	99600376	15/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT43	118600092	17/07/2017	2	YW	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT44	99689936	17/07/2017	2	YW	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT45	99659671	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT46	99664156	17/07/2017	2	YG	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Chick
EGT48	99687257	14/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT5	117639776	10/07/2017	1	W	-20.97	50.1	14.71	14.52	19.28	0.74	N/A	Incubitating
EGT50	118607286	17/07/2017	2	YG	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT52	99683053	14/07/2017	1	YG	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT53	99684228	15/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT55	118600400	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Chick
EGT56	99689234	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT57	99667218	13/07/2017	2	W	-20.35	48.3	14.86	15.1	17.91	0.8	M	Incubitating
EGT58	99659671	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT59	99683052	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT6	99670720	03/07/2017	1	Y	-20.31	48.8	13.83	15.2	18.69	0.61	M	Incubitating
EGT60	99680514	17/07/2017	2	RW	N/A	N/A	N/A	N/A	N/A	N/A	F	Chick
EGT61	99689147	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT62	99684228	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT63	118607294	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT64	99670801	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT65	99689936	15/07/2017	1	RW	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT66	99699835	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick

EGT67	99689234	14/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT68	118600092	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT7	99686990	03/07/2017	1	R	-20.44	49.7	14.67	15.3	19.57	0.82	M	Incubitating
EGT70	118607294	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT71	118607286	14/07/2017	1	R	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT72	118607282	14/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT73	99665084	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT74	118600400	14/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT75	99689147	14/07/2017	1	YW	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT76	99676614	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT77	99676337	13/07/2017	2	Y	-20.39	49	15.07	15.3	18.74	0.72	F	Incubitating
EGT78	118607282	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT79	99683052	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT8	99667063	03/07/2017	1	R	-20.58	48.2	14.29	15.1	17.24	0.73	M	Incubitating
EGT80	99680748	14/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT81	99699837	15/07/2017	1	YG	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT82	99699832	15/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT83	99699835	15/07/2017	1	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT84	99600376	17/07/2017	2	R	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT85	99699833	15/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT86	99670801	15/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT87	99665084	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT88	118607287	17/07/2017	2	W	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT89	99680514	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	F	Incubitating
EGT9	99686180	03/07/2017	1	Y	-20.67	47.8	14.39	14.86	19.61	0.71	M	Incubitating
EGT90	99664156	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT91	99699837	17/07/2017	2	YG	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT92	118607287	14/07/2017	1	YW	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT93	99676555	14/07/2017	1	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Incubitating
EGT94	99699832	17/07/2017	2	Y	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick
EGT95	99676614	17/07/2017	2	YW	N/A	N/A	N/A	N/A	N/A	N/A	M	Chick

EGT96	99697231	15/07/2017	1	RW	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incubitating
EGT97	99676921	28/07/2017	1	R	-20.35	49	16.09	15.4	19.44	0.71	F	Chick
EGT98	117639823	28/07/2017	1	W	-20.35	48.7	15.45	14.9	19.68	0.65	F	Chick
EGT99	99680540	28/07/2017	1	W	-20.76	48.7	15.63	14.95	19.99	0.72	F	Chick

Chapter 4. Conclusion

Seabirds are important monitors of various aspects of the environments they inhabit and breed in. They can help to assess oceanic fish stocks (Brisson-Curadeau, Patterson, Whelan, Lazarus, & Elliott, 2017) and monitor how contaminant levels change throughout the years (Elliott & Elliott, 2013). Given the rapid changes in Arctic ecosystems in recent years due to the adverse effects of climate change, and the ubiquity of thick-billed murres throughout the Arctic, understanding aspects of their life history can provide background for the use of murres to monitor changes in the environment. IPS is a concept that is not widely considered by ecologists due to the 'tyranny of the Golden Mean' (Williams, 2008). Ecologists often prefer to increase sample sizes so as to conclude average traits of populations or species as a whole. Such analyses allow insight to provide general models based on population averages without considering individual variation. However, intra-specific variation can have important ecological effects that should also be studied (Bolnick et al., 2011; Elliott et al., 2009).

There are two aspects that clearly show the importance of studying inter-individual variation. For the study of Hg accumulation in food webs it is normally assumed that trophic level is the most important factor determining the movement of Hg through the food web (Elliott & Elliott, 2016). Thus, considering IPS in that scenario is already important because specialist individuals tend to feed on higher trophic levels (Woo et al., 2008) and, thus, would consume larger amounts Hg. Here, we incorporate another aspect to be taken into consideration to better understand the relationship between IPS and Hg, namely Hg methylation and a method to indirectly quantify Hg methylation, $\delta^{34}S$. In Chapter 2 we showed that throughout a typical trophic web occurring at Coats Island, $\delta^{34}S$ complements the information provided by trophic level regarding Hg bioaccumulation and, in some cases, better explains Hg levels. If we associate the

information provided by $\delta^{34}S$ with IPS, it would be possible to say that if murres at Coats Island were to specialize on organisms that have relatively low $\delta^{34}S$ levels, those individuals would presumably consume higher amounts of Hg than what is expected based on trophic level biomagnification. However, there are still some aspects of the relationship between Hg methylation and accumulation that must be clarified. An excess of sulfide reduces the amount of Hg available for methylation (Benoit, Gilmour, Mason, & Heyes, 1999) and this can help to explain why Hg levels in the studied fish and invertebrates decreased with $\delta^{34}S$ (sulfide abundance hypothesis). However, other studies have supported the sulfate availability hypothesis is also valid for other organisms in other sites. The relationship between $\delta^{34}S$ and Hg should continue to be studied in the future so that these two hypotheses can be reconciled.

In Chapter 3, we observed that differences in feeding habits due to IPS, sex or reproductive stage can cause differences in terms of the abundance of specific bacterial taxa or diversity and composition of the overall gut microbial community. Based on the taxonomy of some of the most important bacteria we identified, it is possible that the gut microbiome has been shaped to help the host to process the different food items it is selecting and consuming. Different feeding behaviours in murres tend to provide similar energy intakes for males and females (Elliott et al., 2010), and tend to provide less energy for prey specialists (Woo et al., 2008), and so future studies could help to understand if gut microorganisms are helping birds to maximize the nutrients they obtain in terms other than energy consumption. However, a limitation of our results for the chapter is that we were not able to characterize diet in a more detailed way. We recommend increasing the sample size for the stable isotope analysis and using plasma samples, in addition red blood cells, to determine whether there is a relationship between those signatures at different time scales and the microbiome. We also recommend using prey eDNA obtained while sequencing the microbial 16S

samples to determine diet composition. Using the information obtained by these additional methods, we could obtain a description of murre diet at different time scales which could allow us to see if the murre gut microbiome is mostly affected by changes in diet on a short (eDNA), medium (plasma), or long (red blood cells) time scale.

Chapters 2 and 3 showed the importance of combining the knowledge from different disciplines and how understanding the underlying microbiology occurring in this system may be helpful to describe processes in animal physiology and ecotoxicology that are not explained using the more traditional approaches. We showed that bacteria can be both detrimental, as in the case of Hg methylation by SRB, and beneficial, as in the case of gut symbionts. Continuing to study these systems with other approaches such as isolation of the most important bacteria or metagenomic and metatranscriptomic surveys should be performed in the future to continue to understand the new aspects of the environments where these microorganisms inhabit. Isolating some of the most important bacteria in murre feces, such as those belonging to the *Catellicoccus*, *Cetobacterium* or *Breznakia* genera or the bacteria responsible for Hg methylation in oceans surrounding Coats Island, could tell us more about the physiology of these bacteria and their roles in the bird gut and marine sediments. Given that some of these bacteria cannot be grown under laboratory conditions, assembling their genomes based on metagenomes, and measuring the expression of genes in those genomes with metatranscriptomes, would also allow understanding of the metabolic pathways that are being used.

Another future direction that could be applied to the systems we studied in this thesis would be to link the three main topics we treated: the thick-billed murre, Hg accumulation, and bacteria. This could be done with the evaluation of Hg methylation or demethylation occurring inside the murre gut. A meta-analysis of published metagenomes looking for Hg methylation genes in

different environments found that Hg methylation genes are not present in vertebrate gut metagenomes, but only domesticated animals were included so it is possible that there are different patterns occurring for wild birds (Podar et al., 2015). Hg demethylation genes have been detected in the guts of isopods (Lapanje et al., 2010) and it has been suggested that probiotic bacteria could reduce Hg accumulation in humans (Bisanz et al., 2014). We recommend the study of Hg demethylation in the murre gut in the future.

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