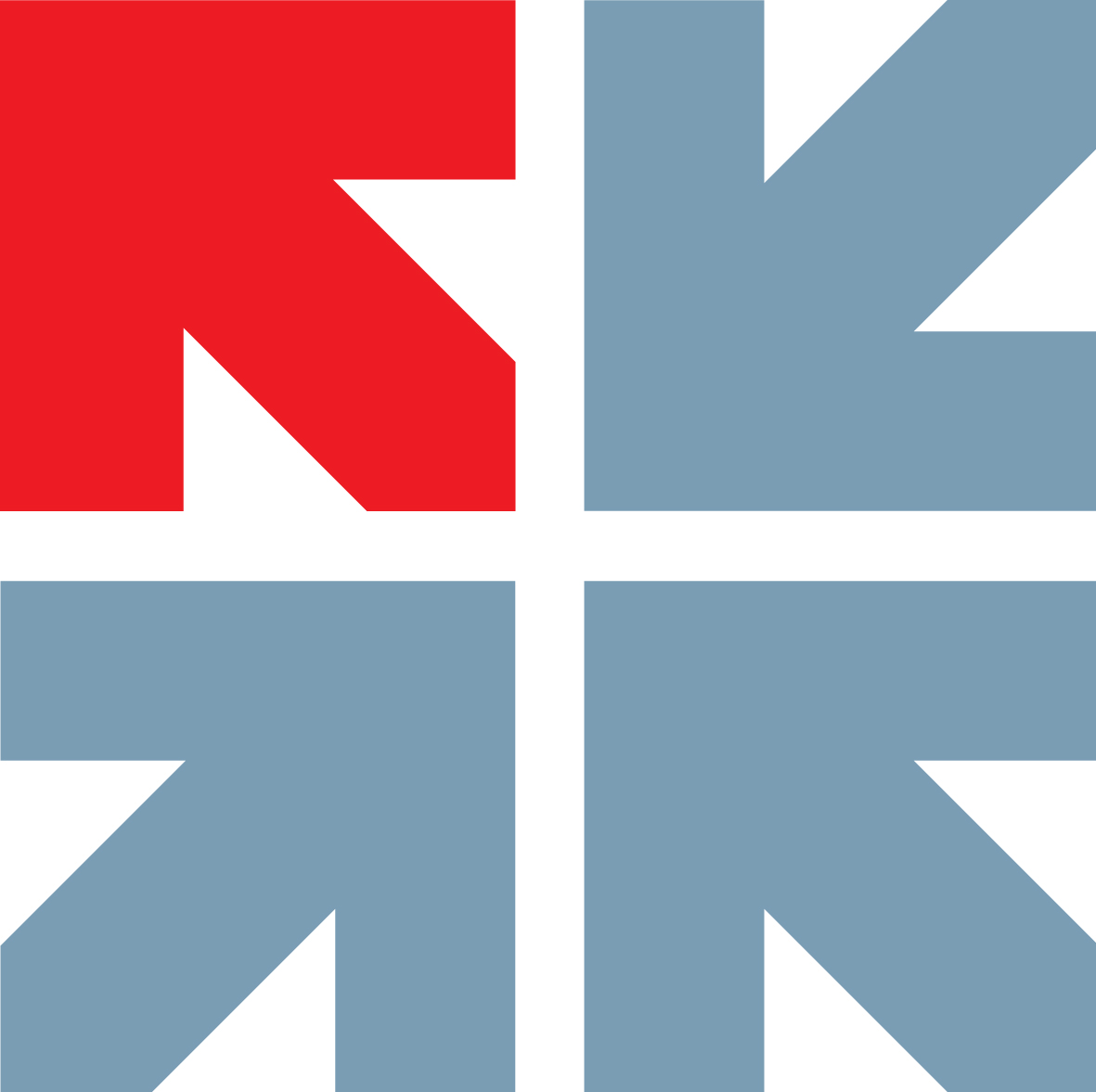
Master’s thesis



Investigating associations between intestinal microbiota and parasite load in Greenland sled dogs (*Canis lupus familiaris borealis*)  
Subtitle if you use one

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*Investigating associations between intestinal microbiota and parasite load in Greenland sled dogs (Canis lupus familiaris borealis)*

45 ECTS thesis submitted in partial fulfilment of a Master of Resource Management degree in Coastal and Marine Management at the University Centre of the Westfjords, Suðurgata 12, 400 Ísafjörður, Iceland

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**Declaration**

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

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Elsa Margaret Krook Brenner

**Abstract**

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# Foreword

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**Acronyms**

This chapter can include lists of acronyms and/or names of variables. Give the chapter a fitting name, e.g. Acronyms or Variables. You are not required to include this chapter.

GSD = Greenland sled dog

MAG = Metagenome-assembled genomes

DNA = Dioxyribose nucleic acid

Gb = Gigabases

CDV = Canine distemper virus

RNA = ribose nucleic acid

rRNA = ribosomal RNA

TSS = Total -sum scaling

POP = Persistent organic pollutant

DVM = Doctor of Veterinary Medicine

NMDS = Non-metric Multi-dimensional Scaling

# 1 Introduction

## 1.1 Overview

The Greenland sled dog (*Canis lupus familiaris borealis*, GSD) is a breed of working dog unique to Greenland, and the Greenland-Inuit people. Since the Inuit arrival in the twelfth century, GSDs have been an integral piece of Greenlandic culture, and the primary means of cross-ice transport for hunters (Fiocca, 2018). GSDs are a distinctly coastal animals: they are fed a diet of primarily marine mammals and fish – a reflection of the marine resources Greenlandic hunters provide their community (Harris et al., 2020). Consuming marine resources exposes GSDs to a variety of parasites and pathogens. These parasites may impact the dogs’ wellness and athletic performance, and some are *zoonotic*, meaning they can transmit from animals to humans. Assessing the impact of a parasitic burden on GSD health will contribute to a better understanding of disease transmission in the Arctic, as well as provide key insights into the management of GSDs: a key coastal resource in Greenland, and a population at risk of extinction (Sonne et al., 2020).

The digestive system of animals hosts a large and diverse community of organisms called the microbiome. The genes of these microbiota can affect the host organism physiologically and behaviorally (Jones et al., 2008). The intestinal microbiome of mammals is also highly correlated to mammalian health, and its composition can be influenced by the presence of parasites and pathogens (Ulusan Bagci & Caner, 2022). Likewise, changes in microbiome composition can impact host’s immune system, which can make the host more vulnerable to parasites (Ulusan Bagci & Caner, 2022). The intestinal microbiome can be passively monitored by the collection and analysis of fecal samples, making it a cost-effective and ethical means of investigating GSD health where access to veterinary care is scarce (Alberdi et al., 2019).

## 1.2 Research questions, aims & hypotheses

This thesis uses GSDs as a window into parasite transmission in the coastal Arctic, and aims to identify challenges associated with the management and husbandry of GSDs, and the consumption of raw marine resources. Data is drawn from fecal samples collected in the Fall of 2022 from Daneborg and Ittoqqortoormiit, East Greenland. Parasites were clinically identified and quantified by collaborating researchers, before I molecularly identified parasites, and characterized the intestinal microbiota using a *shotgun sequencing* approach. This thesis aims to answer the following research question through the exploration of three objectives:

How do different husbandry practices (including differing diet) impact the parasite load and gut microbiome of GSDs in East Greenland, and how can these findings be applied for coastal management?

**Objective one:** To explain the impact of differing management practices on the GSD microbiome, both in terms of the bacterial community function.

**Objective two:** To compare intestinal parasite detection using an untargeted metagenomic approach to traditional clinical methods applied in other GSD research.

**Objective three:** To apply the *One Health* framework to explore the role of GSD health in Arctic coastal community wellness.

2 Background

## 2.1 Qimmeq: a Greenlandic coastal resource

Sled dogs, or *Qimmeq* in Greenlandic, are a coastal resource of immense value— both cultural and economic— to Greenlandic people. The arrival of GSD to Greenland can be traced to the Inuit arrival to Greenland in the second half of the twelfth century (Ameen et al., 2019). Since the Inuit arrival to Greenland, GSD have been viewed as a natural resource rather than as a pet or as livestock (Sonne et al., 2020). The relationship between Greenlanders and GSDs bears many similarities with the relationship between dogs and humans across the circumpolar Arctic. Historically, Arctic dogs were used for polar bear hunting, pulling sleds to relocate nomadic communities, and crossing ice to access subsistence foods (Baker et al., 2022). Without dogs, anthropologists hypothesize that people would not have been able to inhabit the Arctic (Laugrand & Oosten, 2014; Morey, 2010; Strecker, 2018). Across Arctic Canada, Alaska and Greenland, sled dogs remain a vital cultural-keystone and means of winter transportation (Baker et al., 2022). Dogs provide services that modern technology lacks; unlike a snow scooter, dogs can sense when ice is too thin. Thus, GSDs persist as the most reliable form of Greenlandic-Arctic transit (Sonne et al., 2020). Modern GSDs remain an essential means of transportation for hunters, who provide traditional foods to remote communities (Fiocca, 2018). Groups of GSDs (packs) provide additional benefits to communities, such as acting as early-warning signals of predators (polar bears and wolves), and preserving culturally important hunting practices (Sonne et al., 2020). GSDs are highly adapted to live in the coastal Arctic: for decades Greenlanders, anthropologists, and explorers have described the GSD’s unique endurance, cold tolerance, and their ability to undergo long periods of food scarcity (Lyon, 2014; Gjaldbæk et al., 2021). These accounts are backed by genetic adaptations to cold temperatures and fat-rich diets (Sinding et al., 2020). The activity pattern of GSDs differs from other domestic dogs. Specifically, the breed undergoes long periods of inactivity in the summer months and extensive endurance activity in the colder months (Gjaldbæk et al., 2021). Individuals can undergo long periods of fasting, while maintaining a very high stamina, a trait that makes them the perfect tool for long journeys across ice (Lyon, 2014). GSDs also have morphological differences from other breeds of Arctic dogs—specifically a broader, larger stature (Ameen et al., 2019). Of the modern Arctic breeds, the GSD is the most ancient, having been genetically isolated until the period of European polar exploration in the 18th and 19th century (Sinding et al., 2020). Post-colonialism, GSDs have avoided breed mixing compared to other Arctic sled breeds (Sinding et al., 2020). This is partially due to the geographic isolation of Greenland, and partially due to Danish and Greenlandic efforts to preserve the breed due to its cultural importance (Government law no. 18, 30/10/1998 Chapter 6 §19).

Since their arrival to the Arctic, GSDs have been fed hunted meat—from whatever source was locally available (Coltrain et al., 2004; Freuchen, 1921; Hantzsch, 1977; Jensen, 1961). Contemporary hunter-owned GSDs are fed a diet of primarily marine mammals (CITE). Sometimes this diet is supplemented with industrial food blends—though of relevance for this thesis, East Greenlandic hunters have shown preference for feeding their packs seal meat (Fiocca, 2018). Stable-isotope analysis has confirmed reports that local marine mammals are the primary dietary component of both modern and historic GSDs (Harris et al., 2020). It is important to note that GSD diet varies by settlement, with some feeding packs larger proportions of fish, or commercial dryfeed. Larger settlements often feed dogs a higher proportion of dryfeed than smaller settlements (personal communication, E. A.-Ranberg, QimmeqHealth project). In East Greenland, hunters tend to feed their packs primarily marine mammal meat, whereas in West Greenland hunters tend to feed their packs a higher percentage of fish (CITE). A diet of marine resources is not uncommon across all breeds of Arctic dogs— Indigenous communities in the circumpolar Arctic have a history of feeding their packs fish and marine mammals (Losey et al., 2018). Across Arctic Canada and Alaska, Indigenous peoples continue to feed their packs marine resources (CITE). Further, the diets of racing sled dogs and tourist packs across the circumpolar Arctic typically are supplemented by raw fish or seal (CITE). The diet of GSDs reflects the diet of the surrounding Greenlandic coastal community, meaning GSDs and Greenlanders can be exposed to many of the same challenges associated with consuming marine resources. This situates GSD in a unique position, linking terrestrial and marine wildlife, the environment, and humans.

For these reasons, GSDs have been identified as a *sentinel species* for monitoring Greenlander health (Sonne et al., 2020). Broadly speaking there is very little known about GSD health due to lack of research and regular veterinary care (Sonne et al., 2020). Of the research that has been conducted, a major focus is on exposure to heavy metals and Persistent organic pollutants (POPs)— materials that bioaccumulate in marine mammals and ultimately pose a threat to humans (Dietz et al., 2015; Letcher et al., 2010; Sonne et al., 2017). Such materials can be toxic when consumed and can negatively impact the mammalian immune system, neuro-endocrine function fertility, growth, development, and general fitness (Sonne et al., 2017). By monitoring GSD exposure to these toxic materials, researchers can infer accumulation rates in Greenlanders, and make recommendations on how communities can reduce their intake (Sonne et al., 2020). Other existing research on GSDs focuses on parasite exposure, zoonoses and epicootics (Table XX). As this thesis is being written, notably less work has been conducted regarding GSD parasite exposure than other aspects of veterinary health, and no research has been conducted on the GSD microbiome.

Table XX: existing research on parasites, zoonoses and epizootics in GSDs

|  |  |  |  |
| --- | --- | --- | --- |
| *citation* | *Sample type* | *location* | *disease* |
| Masterton and Lewis, 1955 | Muscle biopsy | Outbreak in Northeast Greenland, dogs from West Greenland | *Trichinella* spp |
| Roth 1949 | Muscle biopsy | West Greenland | *Trichinella* spp |
| Roth and Madsen, 1956 | Muscle biopsy  \*\* in German |  | *Trichinella* spp |
| Madsen, H. 1961 | \*\* no access | East and West Greenland | *Trichinella spiralis* |
| Moth-Lund, 1998 - Master’s thesis | tissue samples and gastrointestinal content  \*\* need access | West Greenland | *Uncinaria stenocephala,*  *Toxascaris leonina,*  *Trichinella nativa,*  *Mesocestoides lineatus,*  *Diphyllobothrium dendriticum* |
| Møller, 2007 | \*\* need access | East and West Greenland | *Trichinella* spp |
| Christiansen and Zareba, 2019 - Master’s thesis | Fecal  \*\* need access | West Greenland | *Uncinaria stenocephala,*  *Toxascaris leonina,*  *Diphyllobotrium* spp*,*  *Taeniidae,*  *Sarcocystis* spp*,*  *Cystoisospora canis* |
| Clausen and Larsen, 2021 - Master’s thesis | Fecal, Serum | West Greenland, Daneborg | *Cryptosporidium* spp*,*  *Giardia* spp*,*  *Trichinella* spp*,*  *Toxoplasma gondii* |
| Knudsen, 2022 - Master’s thesis | Fecal | West Greenland | *Giardia* spp*,*  *Cryptosporidium* spp |
| Klein – Ipsen and Rotovnik, 2023 – Master’s thesis | Fecal, serum, heart fluid | East Greenland, Daneborg | *Toxascaris leonina,*  *Trichinella spp,*  *Taenia spp,*  *Cryptosporidium spp,*  *Giardia spp,*  *Sarcocystis spp,*  *Toxoplasma gondii* |

### 2.1.1 GSD-linked Disease outbreak

Greenland has a history of GSD-linked disease outbreaks that impact multiple species at a time. Outbreaks of Rabies and Canine Distemper Virus (CDV) have historically caused major population decline in the GSD population, and can heavily impact hunters and entire communities who depend on strong, healthy packs for spring hunting trips (Andersen-Ranberg et al., 2019). In some cases, outbreaks have come close to causing the localized extinction of the breed (Andersen-Ranberg et al., 2019; Bohm et al., 1989). Historic outbreaks can be difficult do diagnose due to ambiguity of symptoms, and lack of formal reports. However the first generally accepted report of CDV was as early as 1861, and an “Eskimo dog disease,” with symptoms similar to rabies was reported in 1881 (Andersen-Ranberg et al., 2019; Colan, 1881). Outbreaks have frequently been described as zoonotic—spreading from GSDs to humans—or epizootic—spreading between animal species, but not to humans. A rabies outbreak in GSDs occurred in Egedesminde, in East Greenland, in 1960, during which one infected dog bit a child, who died of rabies (Lassen, 1962). This is the only confirmed case of rabies in Greenlandic people. Most reported cases of rabies and CDV come from the West coast of Greenland (Mansfield et al., 2006). The West coast is both more populated than the East, and better geographically linked to Canada. Studies of rabies have shown that epizootic diseases spread through terrestrial mammals flow from Canada to Greenland via Elsmere Island, highlighting the migration of reservoir species between the Canadian Arctic to Greenland where the two landmasses are closest (Hueffer et al., 2022). To get to East Greenland, reservoir species must either travel over the Greenland ice sheet or enter via the marine environment. As sea ice cover decreases in the Arctic, pathways for over-ice migration of terrestrial species disappear. This means that transmission pathways from the marine environment to the terrestrial environment will soon become much more relevant to the broader discussion of disease transmission in the Arctic. Disease surveillance is generally very difficult across the circumpolar Arctic due to the complicated nature of sample collection, and transportation from remote Arctic communities (Baker et al., 2021, 2022; Brook et al., 2010; Hueffer & Murphy, 2018; Sonne et al., 2017). Furthermore, managers have deemed other public health issues more pressing, and zoonoses are frequently overlooked due to the low number of confirmed human cases (Keatts et al., 2021).

Parasites have also been proven to have a negative influence on GSD wellness, though research on GSD parasites is limited (CITE). Three research papers and five master’s theses have investigated parasites in GSD packs. (Table XX). Studies tend to focus geographically on West Greenland or the settlement Daneborg. Studies also tend to use traditional forms of clinical diagnostics for parasites, including visual identification, diagnostic kits, and tissue samples. Few studies include any of the settlements in East Greenland, and no studies use an untargeted molecular approach or examined the microbiome.

Presently, there are no veterinarians working anywhere within the Sled Dog District of Greenland (Andersen-Ranberg et al., 2019).There is one official veterinarian in Greenland, who provides traveling services, and a second veterinarian responsible for the care of *Sirius* dogs, who makes routine trips to provide services (Klein-Ipsen & Rotovnik, 2023). This means that when disease outbreaks occur, professional response time can be extremely slow, and most of the time communities never receive responsive care (CITE). Almost all GSD healthcare falls under the responsibility of the hunter. Infrequently, packs have access to traveling veterinary care in the form of vaccination pop-ups, or other routine veterinary services. Vaccination programs have been shown to significantly reduce the incidence of rabies and CDV Greenland (Hueffer et al., 2022). This indicates that access to regular veterinary care can make a substantial change in disease occurrence, and reduce outbreaks. It should be noted that vaccination rates are very low across the circumpolar Arctic. In Arctic Canada, 63% of domestic dogs (including sled dogs) were reported as unvaccinated, and 40% of children had reported a dog bite (Brook et al., 2010; Hueffer & Murphy, 2018). Greenlandic veterinary services are irregularly provided in exchange for a hunter’s participation in veterinary research (Larsen & Clausen, 2021). It should be noted that these such exchanges introduce a source of bias. Dog owners who agree to participate in veterinary research may be more attentive to the health of their dogs, as owners of dogs in poor condition may be hesitant to showcase their husbandry practices. Similarly, the majority researchers who conduct such studies are Danish. The colonial history of Denmark and Greenland – which continues today – could influence dog owners against participating, or introduce skepticism and distrust.

The success of a pack relies on the combined performance of individual GSDs. Put simply, strong healthy dogs give hunters a wider range to hunt, increasing their access to food and bolstering community resilience. If an individual or a full pack were to fall ill, it would limit hunters’ ability to provide food for their community (Lassen, 1962). Often, these remote coastal communities have limited access to shipments, with pack ice making it impossible for the arrival of goods three seasons of the year (Fiocca, 2018). Effective management of GSD health is vital to the food security of Greenlandic settlements.

### 2.1.2 Current GSD Management

Sled Dogs can only be legally kept in the Sled Dog District of Greenland, which is defined by Greenlandic law as: “the area from and including Sisimiut Municipality, Kangerlussuaq and northwards to and including Qaanaap Municipality, Greenland National Park and Ittoqqortoormiit Municipality and Ammassallip Municipality” (Government law no. 18, 30/10/1998 chapter 1§3) (Figure XX). Within the Sled Dog District, people cannot keep any other domestic pets. According to chapter 3 §7: “It is prohibited to keep and bring together dogs and cats in places that may present a health risk to the surroundings or may be considered unsuitable for domestic animals.” The specific regulations relevant to the management of GSDs laid out in law no. 18 include:

* All dogs over six months old must be kept on a chain (Chapter 3 §9)
* Stray dogs can be euthanized without liability to the owner (Chapter 3 §9)
* Biting dogs must be immediately euthanized (Chapter 3 §10)
* Anyone who keeps dogs and cats must ensure that they are treated with care, including that they are fed, watered and looked after considering their physiological, behavioral and health needs in accordance with recognized practical and scientific experience (Chapter 5 §15)
* Dogs must be inspected at least once per day (Chapter 5 §15)
* Operative interventions that may cause suffering to dogs or cats, apart from insignificant pain of a transient nature, may only be carried out by a veterinarian, unless the intervention is urgent (Chapter 5 §18)
* The Sled Dog Districts are reserved for the Greenlandic Sled Dog, and all other importation of dogs into the Sled Dog Districts are forbidden (Chapter 6 §19)
* Sled dogs that have been taken out of the sled dog districts may only be reintroduced with special permission from the National Government, and the importation of Greenlandic sled dogs into the other districts is prohibited (Chapter 6 §19)
* The National Government may initiate monitoring of dogs, including collection and registration of data, taking of samples. Owners must provide the necessary assistance free of charge for examinations and taking samples and for other measures implemented in accordance with the law (Chapter 7 §21)

These regulations broadly fit into three categories of laws: those protecting humans who interact with domestic dogs, those protecting welfare of dogs, and those designed to protect and preserve the GSD breed. Notably, no chapters within law 18 reference the diet and nutritional health of dogs. Chapters 5 calls on veterinary professionals as the sole persons legally suitable to administer health services to dogs, yet there are currently no permanent veterinarians practicing in the Sled Dog District (CITE). Thus, in practice, GSDs do not receive regular access to veterinary care. Instead, hunters are responsible for the care of their packs. Chapter 7 calls for monitoring and research into GSD health for the preservation and wellness of the breed; yet significant research gaps exist in general health status and baseline data for the breed.

It is common practice for puppies to range free, until they reach six months of age and legally have to be chained (Government law no. 18, 30/10/1998 chapter 3 §9). At six months of age, hunters will decide if a puppy is suitable for their pack, and those deemed extraneous will be euthanized (Fiocca, 2018). Similarly, working GSDs who fall ill, who are let off their chain, or who are deemed to be too old to work are typically euthanized and replaced by a younger dog (Fiocca, 2018). Euthanasia of puppies is the responsibility of the hunter or a designated “dog killer,” who is responsible for the euthanasia of old GSDs, loose GSDs, and ill GSDs (Fiocca, 2018). The designated “dog killer” is often responsible for other aspects of GSD health, including the administering of vaccines (Fiocca, 2018). Notably, a 2022 study on the benefits of veterinary care in the remote Arctic found one of the major benefits of a community veterinarian to be access to humane and sanitary euthanasia (Baker et al., 2022). The presence of corpses in the environment may attract predators and scavengers, who may bring with them new parasites and zoonoses. Similarly, any human interaction with GSD corpses may expose the community to parasites and zoonoses residing in the body.

Law number 18 also does not specify how dog yards should be kept sanitary. Chapter 5 §15 specifies that dogs must be inspected a minimum of once per day, fed and watered. The law never specifies how much space dogs should have, how regularly their enclosure should be cleaned, or a minimum cleanliness standard. SECTION ABOUT HOW SANITATION IS MAJOR REDUCER OF DISEASE HERE.

Currently, many dog yards in Greenland are situated outside of the settlements, physically removing GSDs from the houses and non-hunting population. In some communities, dogs are chained outside of the town in the summer – when they are not being used – and moved closer to town in the winter – for ease of access (Fiocca, 2018). This is the case for Ittoqqortoormiit. Historically, GSDs were chained within settlements, and were a central cultural component of the community. The migration of dog yards out of the town represents a cultural shift in Greenlanders relationship with the GSD (CITE).

## Zoonoses, epizootics, and parasites in the coastal Arctic

In this section I define zoonoses, epizootics, parasites before explaining their relevance to the coastal Arctic. I then give more in depth descriptions of specific examples relevant to this thesis (sections 2.2.1 – 2.2.5). Examples are organized by their taxonomic classification, starting with *viruses*, then moving to *Bacteria,* *Protozoa*, *Nematoda*, and concluding with *Cestoda*.

*Zoonoses* are infectious diseases that can be transmitted from animals to humans. An estimated 60% of all human infectious diseases found are classified as zoonoses (Jones et al., 2008). Many zoonoses are *parasites*, meaning the infecting organism lives by consuming its nutrients from another host organism. The most devastating pandemics in human history—including the Black Death (*Yersinia pestis*), Spanish Flu (A/H1N1), HIV/AIDs (*Lentivirus* spp.) and Covid-19 (SARS-CoV-2)—are zoonotic in origin (K. E. Jones et al., 2008; Keatts et al., 2021). Currently the dynamics and extent of Arctic zoonoses and parasites are poorly understood, and likely under-reported (Keatts et al., 2021). Modeling has indicated that the emergence of a global scale pandemic from the Arctic is extremely unlikely, due to the region’s relative isolation, cold conditions, and sparse population (Keatts et al., 2021). Nevertheless, zoonotic infections pose a significant risk to Arctic coastal communities who rely on the extraction of animal resources for their wellbeing (CITE). Global climate change is predicted to increase the range of zoonotic diseases, and the Arctic is experiencing climate change at over twice the rate of the rest of the globe (Rantanen et al., 2022). The consumption of wildlife has been linked to several global-scale zoonotic outbreaks over time; including HIV/AIDs, Ebola, and Avian Flu and most recently Covid-19. The Arctic region is at an increased risk from zoonoses, as many Arctic Indigenous communities tend to rely on harvested wildlife for a large portion of their diet (CITE). In Greenland specifically, settlements receive infrequent shipments of food, and rely greatly on hunted game—specifically fish and marine mammals (CITE). For these reasons the monitoring and management of harvested foods is essential for community health. Sled dogs have been identified as an ideal monitoring tool for studying zoonoses across the circumpolar Arctic, as they are evenly distributed and are fed a diet that closely resembles those of Northerners (Sonne et al., 2020). *Carnivora* carry the most parasites of any mammalian group, and *Canidae* harbor the greatest diversity within the group (Han et al., 2021). In humans, individuals who rely on hunted game for their primary source of protein have a higher prevalence of intestinal parasites than those reliant on domestic sources of protein (Rubel et al., 2020). Within the Arctic there are 3943 known host-parasite interactions across bird and mammal populations (Watson, 2020). Of these, humans and dogs have been identified to harbor 454 and 80 parasites respectively, including: bacteria, helminths, protozoa, and viruses (Watson, 2020).

The primary pathways for GSD exposure to parasites can be direct or indirect. An example of direct transmission is through the consumption of meat containing a parasite (CITE). Examples of indirect transmission include the presence of fresh game attracting other potential host species, such as Arctic foxes, polar bears and wolves, and persistent presence in the environment (Andersen-Ranberg et al., 2019; Han et al., 2021; Sonne et al., 2020). As GSDs and Greenlandic hunting communities consume the same marine resources, any parasites present in the GSD intestine may also impact the human population.

Previous studies investigating the relationship between sled dogs and zoonoses have been conducted in the Canadian and Alaskan Arctic, but at present there are no circumpolar or Greenland-specific studies using sled dogs to investigate zoonoses harbored in the Arctic (Jenkins et al., 2013; Salb et al., 2008; Sonne et al., 2017). Investigating the zoonotic parasites present in GSD provides key insights for management of human and environmental health in coastal Greenland.

### 2.2.1 Viruses

#### 2.2.1.1 Rabies and Canine distemper virus

Both Rabies and CDV are highly contagious, often fatal viral diseases that can infect mammals. Rabies is a neurological virus, whereas CDV attacks the respiratory and gastrointestinal systems. Both diseases have been known to attack the spinal cord. The two diseases exhibit very similar symptoms, and can have devastating impacts on sled dog packs. Rabies is zoonotic, and CDV is epizootic. It is extremely unlikely that GSDs would be exposed to Rabies or CDV by ingesting marine resources. However, both diseases are described in this section due to their historic relevance to GSD health, the success of past management programs to limit incidence, and their relevance to novel management strategies.

Rabies (family *Rhabdoviridae* genus *Lyssavirus*) has been described in Greenland since 1881, when an “Eskimo dog disease,” with symptoms like rabies was reported to spread regionally, including infecting Arctic foxes (Colan, 1881). Rabies can have devastating impacts on Arctic sled dogs. In one three-month outbreak in 1960, over one thousand GSDs died (Lassen, 1962). Such large outbreaks can have devastating impacts on Greenlandic communities, including increasing the threat of famine due to the inaccessibility of hunting without packs of GSDs (Lassen, 1962; Peplinski, 1996). To date, there has only been one confirmed case of Rabies in East Greenland, in 1960. In this outbreak, an infected GSD bit a child, who died from symptoms twelve weeks later (Lassen, 1962). Rabies is most frequently passed through the saliva of an infected individual biting another, but can be passed through ingestion of infected tissue (CITE). Rabies vaccination programs have been shown to significantly reduce the occurrence of Rabies in sled dogs (Hueffer et al., 2022). However vaccination rates in the Arctic are extremely low, due to the lack of available veterinary services (CITE). The Arctic fox is considered to be the primary resevoir of rabies in the Arctic, and domestic dogs – including sled dogs – are considered to be the most significant link to humans (Pamperin et al., 2008; Raundrup et al., 2015). Due to the relatively high number of studies on rabies in the Arctic, rabies has been identified as an ideal model disease for understanding changing zoonotic disease dynamics in the context of the warming Arctic (Huettmann et al., 2017).

CDV is the most frequent epizootic outbreak in Greenland (CITE). Reservoirs of CDV are Arctic foxes, Arctic wolves, and polar bears (CITE). Similar to Rabies, large outbreaks could have catastrophic impacts on communities, as hunters heavily rely on packs for access to subsistence foods (Kuhl, 2011; Tester, 2011). It can be difficult to differentiate between historic accounts of CDV and Rabies; most studies that consider historic outbreaks rely on descriptions of the spread of symptoms. Notably, recorded symptoms of CDV in GSDs differ from symptoms in other domestic dogs (Andersen-Ranberg et al., 2019). Infected GSDs have consistent reports of showing few-to-no prior symptoms before exhibiting major neurological deficits or sudden death. Most CDV outbreaks occur when GSDs come into close contact with Arctic foxes and wolves. Most frequently these interactions occur in the spring, during hunting season, when fresh meat attracts other large Arctic predators (Andersen-Ranberg et al., 2019). There are other strains of Distemper Virus, including Phocine Distemper Virus (PDV), which infects pinnipeds.

### 2.2.2 Bacteria

#### 2.2.2.1 Bacteroides

*Bacteroides* is a genus of obligate anaerobic bacteria commonly found in the mammalian gastrointestinal microbiome. *Bacteroides* play a major role in the breakdown of animal fats and proteins, and are therefore associated with a carnivorous diet (Xu et al., 2007). Most species of *Bacteroides* are associated with a healthy gut microbiome in carnivores (Pilla & Suchodolski, 2020). Certain species of *Bacteroides* are opportunistic pathogens, and can lead to serious infection in humans, and has been the cause of conditions including appendicitis and meningitis (Wexler, 2007; Zafar & Saier, 2021). The most clinically important species is *Bacteroides fragilis,* which is associated with a variety of gastrointestinal infections and abscesses, and when untreated in humans has a 60% mortality rate (Wexler, 2007). In dogs, *Bacteroides* is one of the most common infection-causing bacteria reported by veterinarians (Tsuyuki et al., 2020). *Bacteroides* is found in the canine oral microbiome, and commonly reported as zoonotically transmitted in dog-bite wound infections (Bailie et al., 1978; Goldstein, 1980; Goldstein et al., 1984; Sturgeon et al., 2013).

In the Arctic context, *Bacteroides* has been studied in the gastrointestinal microbiome of a few Arctic mammals, including: captive mink, Arctic foxes, and Arctic ground squirrels, as well as wild Svalbard reindeer (Mathiesen et al., 1987; Smura et al., 2016; Stevenson et al., 2014). *Bacteroides* has never been examined in the context of zoonotic infection in the Arctic.

#### 2.2.2.2 Campylobacter

*Campylobacter* is a genus of bacteria commonly associated with gastrointestinal infection in humans. Over 12 species of *Campylobacter* are associated with bacterial gastroenteritis and diarrheal disease (Blaser, 1997; EUFSA). *Campylobacter* infection is zoonotic, with the pathway for transmission being fecal-oral transmission, contaminated foods, and consuming raw meat (Moore et al., 2005). *Campylobacter* is a common cause of gastrointestinal problems in dogs, as it is in humans, and has previously been observed in sled dogs (McKenzie et al., 2010; Skirrow, 1981).

In the Arctic, *Campylobacter* has been identified as an emerging zoonotic threat, with climate change predicted to increase the range and spread of the bacteria (Reed et al., 2003). In one study of dogs housed outside in the Canadian Arctic, zoonotic species of *Campylobacter* were observed in >75% of fecal samples- indicating that *Campylobacter* has a high zoonotic potential (Thompson et al., 2010).

#### 2.2.2.3 Clostridium

*Clostridium* is a genus of anaerobic bacteria that can cause serious infection, including botulism tetanus, and gangrene (Britannica, 2022). A major risk factor for *Clostridium* infection is the ingestion of raw meat, and the main pathway for infection is fecal-oral (Rasetti-Escargueil et al., 2019). In dogs, *Clostridium* infection can be acute, and fatal (Trusiano et al., 2022).

In the Arctic, *Clostridium* has been observed in polar bears, Arctic foxes, hooded seals, and at seal-butchering sites in the Canadian Arctic (Aschfalk & Muller, 2001; Leclair et al., 2013; Weese et al., 2019). The Arctic Council has listed botulism a major risk associated with the preparation of traditional foods (AMAP, 2015). In a review of food-borne botulism in Canada, 92% of botulism patients were Inuit from the Canadian Arctic, and 59% of cases were linked to the consumption of raw, traditional foods (seal, walrus, whale) (Hauschild & Gauvreau, 1985). *Clostridium* outbreaks have been reported in Greenland— the first confirmed outbreak of botulism in North Greenland, which was linked to the consumption of raw eider duck (Hammer et al., 2015). *Clostridium* has been previously observed in sled dog feces, and associated with dysbiosis (McKenzie et al., 2010; Tysnes et al., 2020).

#### 2.2.2.4 Enterococcus B

*Enterococcus* is a genus of anaerobic bacteria that can cause difficult to treat infections. Twelve species of *Enterococcus* can infect humans (Murray, 1990). In the United States, *Enterococci* rank second in the number of reported hospital infections, followed by *Streptococcus* (Section 2.2.2.9) (NIH, 2022). *Enterococcus* can be zoonotic, and exposure pathways include through the ingestion of raw meat, as well as fecal-oral transmission (Bortolaia & Guardabassi, 2023). Dogs have been previously identified as reservoirs for *Enterococcus* human infection (Damborg et al., 2009; Kubašová et al., 2017). The presence of dog feces in the environment has been identified as a public health concern due to the presence of *Enterococcus* (Cinquepalmi et al., 2012).

*Enterococcus* has been observed in Arctic environments (Hernandez, 2014). Most studies of *Enterococcus* focus on antibiotic-resistant genes (which have also been observed in the Arctic) rather than the bacteria’s zoonotic potential (Damborg et al., 2009; Hernández & González-Acuña, 2016; Kalinowska et al., 2021). Studies have found *Enterococcus* with zoonotic potential in semi-domestic Arctic reindeer (Kemper et al., 2006).

#### 2.2.2.5 Escherichia coli

*E. coli* is an anaerobic bacterium, very commonly found in the gastrointestinal microbiome of mammals. Most strains of *E. coli* are harmless, though some can be pathogenic, leading to *E. coli*’s reputation as the major bacterial cause of food poisoning (CDC, n.d.). A major pathway for transmission of the pathogenic strains is the fecal-oral route. Because of *E. coli*’s prevalence in feces, it is an indicator for fecal contamination in the environment (Russell & Jarvis, 2001). *E. coli* is regularly observed in dogs, both with asymptomatic transmission, and where the dogs are exhibiting symptoms of pathogenic *E. coli* (Garcia et al., 2010; Marks et al., 2011; Salgado-Caxito et al., 2021; Schaufler et al., 2015).

*E. coli* is a known bacterial pathogen in sled dogs, and zoonotic transmission of *E. coli* has been observed in sled dogs in the Alaskan Arctic, where they were linked to an outbreak of pathogenic *E. coli* (McAlister et al., 1965; McKenzie et al., 2010). Elsewhere in the Arctic, *E. coli* has been observed in a wide variety of wildlife, including: Svalbard reindeer, Barnacle geese, a variety of seals, and harbor porpoises (Mohamed Hatha et al., 2013; Saab et al., 2023; Sunde et al., 2021; Wunschimann et al., 2001). In Greenlandic people, *E. coli* is considered an “invasive” bacterial pathogen, and is the second most frequently reported infection (Meyer et al., 2008).

#### 2.2.2.6 Fusobacterium

*Fusobacterium* is a genus of anaerobic bacteria associated with bacterial infections. Unlike other anaerobic bacteria discussed above, *Fusobacterium* is not clinically considered a typical bacterium in the human gastrointestinal microbiome, and is always considered pathogenic (Aliyu et al., 2004). *Fusobacterium* is associated with oral infections – including dental plaque — and gastrointestinal infections – including ulcerative colitis (Sturgeon et al., 2013; Su et al., 2020). In dogs, *Fusobacterium* is not uncommon to find in the gastrointestinal and oral microbiome (Sturgeon et al., 2013). In fact, *Fusobacterium* is associated with a healthy gut microbiome in dogs, though it is associated with an unhealthy oral microbiome (Pilla & Suchodolski, 2020; Sturgeon et al., 2013). The primary zoonotic pathway for *Fusobacterium* infection is through dog bites (Bailie et al., 1978). *Fusobacterium* has been previously observed in sled dog feces (Tysnes et al., 2020).

In the Arctic, *Fusobacterium* has been observed in a variety of different hosts, including migratory shorebirds, Russian Arctic reindeer, Arctic foxes, and a variety of seals (Glad et al., n.d.; Sen et al., 2019; Smura et al., 2016; Tarlavin et al., 2021). Notably for this thesis, a 2013 study found that marine carnivores have a significantly higher average relative abundance of *Fusobacterium* in their gut microbiome than terrestrial carnivores (Nelson et al., 2013). The phyla *Fusobacterium* is considered one of the major bacterial phyla observed among polar animals (Abirami et al., 2023).

#### 2.2.2.7 Helicobacter

*Helicobacter* is a genus of bacteria associated with stomach ulcers and infections and in some cases stomach cancer in humans (Brown, 2000; Yamaoka & DeBakey, 2008). The most clinically important species is *Helicobacter pylori*, which infects up to 50% of the human population, though 90% of all infections are asymptomatic (Hooi et al., 2017). *Helicobacter* is not uncommon in the canine gastrointestinal microbiome, though *H. pylori* is unusual (Neiger & Simpson, 2000). Yet zoonotic potential from non-*pylori* *Helicobacter* does exist, and little is known about zoonotic pathways and significance of the bacterium (Neiger & Simpson, 2000). A 2021 study was the first to find that *H. pylori* can be transmitted between domestic dogs and people (Kubota‐Aizawa et al., 2021). *Helicobacter* spp. were found in biopsy samples of sled dogs with gastric diseases (Ritchey et al., 2011). Non-*pylori Helicobacters* are of increasing relevance to both human and veterinary health- and pathways for zoonotic transmission include fecal-oral, and through uncooked infected meat (Strauss-Ayali & Simpson, 1999; Taillieu et al., 2022).

*Helicobacter* is a major threat to Arctic communities, particularly Indigenous Arctic communities. In studies in Alaska, Alaska natives are three times as likely to be seropositive for *Helicobacter pylori* than non-native Alaskans (Parkinson et al., 2015). In the Canadian Arctic, the task force *CANHelp* was established to address the high levels of infection, and early diagnosis and effective treatment are areas of significant research (Cheung et al., 2008; Goodman et al., 2008). In Greenland, the seroprevalence of *Helicobacter pylori* is 58% (Parkinson et al., 2015).

In Arctic wildlife, *Helicobacter* has been observed in Atlantic white-sided dolphins, and beluga whales (Harper et al., 2002).

#### 2.2.2.8 Klebsiella pneumoniae

*K. pneumoniae* is a species of anaerobic bacteria that can be pathogenic and cause bacterial pneumonia when aspirated. In addition to pneumonia, gastrointestinal diseases and infected wounds are possible sites of *K. pneumoniae* infection. Notably, diabetes and alcoholism – both of which are very common in the Arctic, including Greenland – are risk factors of *K. pneumoniae* infection, meaning the Greenlandic population is at a higher risk of infection. As a pathogen, *K. pneumoniae* has a mortality rate of 50% (Setiawan et al., 2022). *K. pneumoniae* is commonly found in the oral, dermal, and gastrointestinal microbiota. It is only when aspirated that is poses a pathogenic risk (Li et al., 2014). *K. pneumoniae* can be pathogenic in dogs, causing pneumonia, and gastrointestinal problems, both of which can be fatal (Olson et al., 1985; Roberts et al., 2000). Previous research has identified the zoonotic potential of *K. pneumoniae* passing from dogs to humans where the two species are in close contact (Santaniello et al., n.d.).

*K. pneumoniae* has been identified in soil across the circumpolar Arctic-- though notably less so in Greenland and Svalbard than in other Arctic nations (Prakash et al., n.d.). Though it has not been well recorded in Arctic wildlife, the bacterium has been found in sea lions, and is now a common cause of sea lion pup death (Jang et al., 2010). *K. pneumoniae* has also been observed in migratory birds, including gulls in the Alaskan Arctic (Bonnedahl et al., 2014).

#### 2.2.2.9 Streptococcus

*Streptococcus* is a genus of bacteria responsible for a wide variety of bacterial infections. Though *Streptococcus* is a normal part of the human oral microbiome, it is also an opportunistic pathogen. Different species of *Streptococcus* can cause bacterial pneumonia, meningitis, conjunctivitis, “strep throat,” and skin infections (Spellerberg & Brandt, 2015). *Streptococcus* is the most frequently reported invasive bacterial infection in Greenland (Meyer et al., 2008). *Streptococcus* has many of the same clinical symptoms in dogs as it does humans (Lamm et al., 2010). In a 2013 clinical report, *Streptococcus* was found to be the cause of infection for sled dogs with hemorrhagic pneumonia (Jaeger et al., 2013). Several species of *Streptococcus* can be zoonotic, and there are multiple reports of *Streptococcus* being transmitted to humans via close contact with dogs (Abbott et al., 2010; Zahlanie et al., 2019). The species *S. zooepidemicus* is of emerging zoonotic importance in dogs (Priestnall & Erles, 2011). Other zoonotically important species include *S. phocae,* with is a major bacterial pathogen in large number of marine mammals (Barratclough et al., 2023; Taurisano et al., 2018).

### 2.2.3 Protozoa

#### 2.2.3.1 Cryptosporidium spp.

*Cryptosporidium* is a parasite infamous as one of the most abundant protozoan parasites related to enteric diseases, globally. The parasite has 30 known species, and can infect mammals, fish, birds, and amphibians. Different species of *Cryptosporidium* have different levels of sensitivity. For instance, some species can jump between birds and mammals, whereas *C. canis* – the species most commonly diagnosed in dogs—is primarily identified in canids (CITE). Some species of *Cryptosporidium* are zoonotic (CITE). *C. canis* is not generally considered zoonotic, but has been seen to infect those with immunodeficiencies, and children (Xiao et al., 2007). In both dogs and humans, the primary clinical symptom of *Cryptosporidium* infection is diarrhea; though it should be noted that most canine infections are asymptomatic, and many human cases go unreported do to underdiagnoses (CITE). Gastrointestinal bacterial infection is a comorbidity, and may increase the severity of *Cryptosporidium* infections (Beugnet et al., 2018).

*Cryptosporidium* has been identified in sled dogs, Arctic wolves and Arctic foxes, caribou, ringed seals, and bowhead whales across the circumpolar Arctic (Elmore et al., 2013; Julien et al., 2019; Myšková et al., 2021; Schurer et al., 2016) (CITE). Within Greenland, *Cryptosporidium* has been found in GSDs across the sled dog district (Klein-Ipsen & Rotovnik, 2023; Knudsen, 2022; Larsen & Clausen, 2021).

#### 2.2.3.2 Giardia spp.

*Giardia* is one of the most abundant protozoan parasites related to enteric diseases globally (CITE). There are nine species of *Giardia*, which form multiple genotypes—called *assemblages—*which exhibit differential host preference (Deplazes et al., 2016). Dogs are potential hosts for four *Giardia* assemblages, two of which are zoonotic (Sprong et al., 2009). When excreted, *Giardia* spp. show resistance to environmental factors, including being cold tolerant (Kutz et al., 2008). The primary zoonotic transmission pathways for *Giardia* spp. are fecal-oral transmission from close contact with infected animals, and through contaminated water (Hunter & Thompson, 2005). In both humans and dogs, the primary symptom of *Giardia* infection is diarrhea—though infection can range from asymptomatic to acute. A dated study of *Giardiasis* in Greenland found the Greenlandic population to have a 7% prevalence (Babbott et al., 1961). Like *Cryptosporidium*, *Giardia* is likely underdiagnosed and underreported. A 2019 study in Iqaluit, Nunavut, Canada found that sled dogs hosted both zoonotic and non-zoonotic *Giardia* assemblages, and that sled dogs were significantly more prone to *Giardia* than other types of domestic dogs (Julien et al., 2019).

Across the Arctic, *Giardia* has been observed in Arctic wolves, sled dogs, Arctic foxes, boreal caribou, muskoxen, a variety of seals, and the bowhead whale (Elmore et al., 2013; Hamnes et al., 2007; Julien et al., 2019; Myšková et al., 2021; Schurer et al., 2016). *Giardia* has been well described in GSDs across the sled dog district of Greenland (Klein-Ipsen & Rotovnik, 2023; Knudsen, 2022; Larsen & Clausen, 2021).

#### 2.2.3.3 Sarcocystis spp.

*Sarcosystis* has a global distribution, and has been described in mammals, birds, fish and reptiles (CITE). 21 species of *Sarcosystis* have been identified in fecal samples from dogs, though species infecting dogs typically do not have zoonotic potential (Dubey et al., 2015).

In Arctic wildlife, *Sarcocycsis* has been observed in hunted harbor porpoises in Greenland (Wunschimann et al., 2001). *Sarcosystis* has been described in GSDs in West Greenland, East Greenland (Klein-Ipsen & Rotovnik, 2023). In past master’s thesis research done on the same cohort of GSDs, *Sarcosystis* was only found in dogs fed marine resources (Klein-Ipsen & Rotovnik, 2023).

#### 2.2.3.4 Toxoplasma gondii

*T. gondii* is cited as one of the top-ten foodborne parasites with the greatest global impact (FAO, 2014). The definitive host of *Toxoplasma gondii* is felids, yet *T. gondii* has been well described outside of the range felids, and the *T. gondii* lifecycle can persist without a local felid population (Dubey et al., 2009). The parasite has reached global distribution, and other mammals – including humans, dogs, polar bears and marine mammals—can act as intermediate hosts (Bachand et al., 2019; Dubey, 2016; Oksanen et al., 2009). An estimated 33% of the human population is infected by *T. gondii* (Deplazes et al., 2016). Oocytes must sporulate to become infectious, and sporulation requires incubation temperatures >10C, which can be challenging in Arctic conditions (Deplazes et al., 2016). Yet once sporulated, oocytes are very resistant to freezing and drying (Dubey, 1998). Outside of the range of felids, *T. gondii* is transmitted primarily through the ingestion of cysts in tissue from an infected host. This means that ingestion of raw meat is a major risk-factor for *T. gondii* exposure (Jung & Skinner, 2017). Once infected, the parasite can remain latent, and potentially re-activated if the host immune system weakens (Deplazes et al., 2016). Human infection is often asymptomatic, though infection in pregnant women can lead to severe pregnancy complications (Deplazes et al., 2016). Canine infection is more frequently symptomatic, and may include respiratory problems, nervous system problems, and digestive problems (Beugnet et al., 2018).

Antibodies for *T. gondii* have been detected in a wide variety of Arctic wildlife, including: polar bears, walruses, ringed seals, harbor seals and hooded seals (Measures et al., 2004). In two 2019 studies in the Canadian Arctic, researchers found 20-30% of the local ringed seal population to be infected (Bachand et al., 2019; Reiling et al., 2019). These results indicate that *T. gondii* can pose both a direct and secondary threat to food security. Marine mammals are an important source of country foods for Indigenous Arctic communities. Infected marine mammals could potentially pass to humans via ingestion of infected meat (direct threat), or could diminish locally available food (secondary threat). Studies in the Canadian Arctic have determined that the consumption of raw food and marine mammals are risk factors for *Toxoplasmosis,* the disease caused by *T. gondii* (Goyette et al., 2014).

Prevelance of *T. gondii* in humans in the Arctic can be as high as 60% (Nunavik), which is double the global average, and significantly higher than the North American average (10-20%) (Blanchet, 2008; J. L. Jones et al., 2018; Messier et al., 2009). These figures led to *T. gondii* being named the most important parasite to control for public health in the North American Arctic (Hotez 2010). Notably, there are no felids present in Greenland, meaning that GSDs had to have been exposed through an intermediate host.

*Toxoplasma gondii* has been observed across the sled dog district of Greenland (Klein-Ipsen & Rotovnik, 2023; Larsen & Clausen, 2021). In past master’s thesis research done on the same cohort of GSDs, *T. gondii* was found only in dogs fed marine resources, and found to relatively high proportions (39.5%, n=43) (Klein-Ipsen & Rotovnik, 2023).

### 2.2.4 Nematoda

#### 2.2.4.1 Toxacaris leonine

*Tocacaris leonine* has been observed in GSDs and Arctic wolves across the sled dog district of Greenland (Klein-Ipsen & Rotovnik, 2023; Christiansen and Zareba, 2019; Moth-Lund, 1998, Marquard-Petersen, 1997, Andreassen et al., 2017; Kapel and Nansen, 1996; Rausch et al., 1983). *T. leonina* is a nematode with global distribution, estimated to have a 2.9% prevalence in all dogs worldwide (Rostami et al., 2020). The most common route of infection in GSDs is through the ingestion of eggs (CITE). Though some human infections have been recorded, *T. leonina* is generally not considered to be zoonotic (CITE). However, it remains relevant to GSD management, as infected dogs may exhibit symptoms of potbelly, diarrhea and depression—all of which may reduce their ability to work, or could increase the potential for other parasites or diseasses to spread (CITE). In past master‘s thesis research done on the same cohort of GSDs, *T. leonina* was found only in dogs fed marine resources(Klein-Ipsen & Rotovnik, 2023).

#### 2.2.4.2 Trichinella spp.

*Trichinella* spp. is a roundworm, that infects muscle tissue. *Trichinella* is typically acquired from ingesting raw or undercooked meats, and the main routes of infection are scavenging behavior and predation (Deplazes et al., 2016). There are nine known species of *Trichinella*, only *T. nativa* has been previously observed in Greenland (Kapel, 1997). *Trichinella* infection is often asymptomatic in animals, and symptomatic in humans, with allergic reactions and persistent muscle weakness as the two primary symptoms (Beugnet et al., 2018). When dogs do express symptoms, it is typically muscle pain and weakness.

Past outbreaks of *Trichinella* in Greenlandic people have been linked to the ingestion of walrus, beluga whale and polar bear meat (Møller et al., 2010). *Trichinella* has been observed in both East and West Greenland, and described in the Arctic fox, polar bear, gray wolf, walrus, and a variety of seals (Oksanen et al., 2018, 2022). *Trichinella* was first described in GSDs as far back as 1949 (Masterton & Lewis, 1955). In past master’s thesis research done on the same cohort of GSDs, *Trichinella* infection was found to be significantly higher in GSDs fed marine resources (100%) than GSDs fed dryfeed (20%) (Klein-Ipsen & Rotovnik, 2023).

#### 2.2.4.3 Uncinaria stenocephala

*U. stenocephala* has been found in GSDs on the West Coast of Greenland—where 81% of dogs were infected (Moth-Lund, 1998). *U. stenocephala* has not been observed in GSDs since Moth-Lund’s thesis.

### 2.2.5 Cestoda

#### 2.2.5.1 Echinococcus

The zoonotic parasite *Echinococcus* is a genus of cestode tapeworm associated with ungulate (caribou and reindeer) herding (Rausch, 2003). *Echinococcus* considered one of the top-ten most important food-borne parasite globally (FAO, 2014). *Echinococcus* is transmitted through the ingestion of infected meat, or through adult worms or eggs free in the environment—usually from feces (Kolapo et al., 2022). Canids are the definitive host of *Echinococcus,* and dogs who consume harvested wildlife and then interact with humans are the primary mode of zoonotic transmission. Certain strains of *Echinococcus* were once endemic to the Arctic, and sled dogs are potential hosts for all Arctic *Echinococcus* species. However, once snowmobiles were introduced as an alternative mode of transportation to sled dogs, human *Echinococcus* infections decreased, with the highest prevalence of *Echinococcus* outbreaks correlating with the presence of sled dogs, and the highest frequency of human infections in Arctic Indigenous communities (Gilbert et al., 2010). Presently, there have been no confirmed cases of *Echinococcus* in Greenland, though there is the opportunity for migration. The reservoirs species of *Echinococcus* in the Arctic is the collard lemming. Lemmings have a highly variable population in Greenland, which is largely spillover from the Canadian Arctic, through Elsmere Island. In years when the lemming population is high, there is a chance for spillover into Greenland (Moth-Lund, 1998). *Echinococcus* is described here due to its management relevance (Kolapo et al., 2022).

#### 2.2.5.2 Tania spp.

*Taenia* has been observed in Arctic foxes and GSDs across Greenland (Christiansen and Zareba 2019, (Andreassen et al., 2017; Klein-Ipsen & Rotovnik, 2023). In past master’s thesis research done on the same cohort of GSDs, *Tania* was only found in dogs fed marine resources (Klein-Ipsen & Rotovnik, 2023).

## 2.3 The Microbiome

*Microbiota* is the term for the sum of all the different microscopic fungi, viruses, bacteria and protozoa living inside another living being (O’Hara & Shanahan, 2006). The composition and diversity of the intestinal microbiota play an important role in mammal health, including a defensive role against some parasites (Bär et al., 2015).

### 2.3.1 Parasites and the intestinal microbiota

Many common parasites that enter the host via ingestion inhabit the mucus layer of the intestine, where they are surrounded by the intestinal microbiota (Ulusan Bagci & Caner, 2022). In one 2020 study of the human microbiome, microbiota community composition was found to determine the presence or absence of a parasitic worm with 80% accuracy (Rubel et al., 2020). The presence of parasites can have long-term effects on the microbiota community composition and structure, even after the parasitic infection has been eliminated from the host (Wensaas et al., 2012). The complex interplay between intestinal parasites and intestinal microbiota is still being untangled. However, understanding how these two factors may impact one another, and the role an animal’s diet plays in intestinal health are vital to determining best practices for managing pack fitness and informing feeding regimens.

### 2.3.2 sled dog intestinal microbiota

In other breeds of sled dogs, the composition of the intestinal microbiota community has been linked to athletic performance (Thornton et al., 2021; Tysnes et al., 2020). Human and canine microbiota share a distinct similarity, therefore studies investigating canine microbiota serve the dual function of benefitting research on human health (Coelho et al., 2018).

## 2.4 Molecular sequencing

In this section I briefly outline the molecular tools applied in this thesis. The purpose of this section is to define the key concepts of molecular analyses, and explain their relevance to this thesis. Specific protocol can be referenced in the Methods section (3.4 – 3.6).

### 2.4.1 Overview of shotgun sequencing

*High-throughput sequencing* is the broad term for determining the content of genetic molecules (DNA or RNA). High-throughput sequencing has many applications, including being used to identify microscopic organisms within a sample. Unlike other forms of high-throughput sequencing, which break DNA or RNA from samples at set points in the molecular chain to search for specific sequences, *shotgun sequencing* breaks nucleic acids at random points to fragments of varying sizes, and the results are then checked against a reference database to determine where fragments in a sample overlap with known sequences (CITE). Shotgun sequencing has been compared to shredding multiple copies of a book, and then reassembling the pieces using overlapping text to read the story (https://www.genome.gov/genetics-glossary/Shotgun-Sequencing).

### 2.4.2 Benefits of shotgun sequencing for this thesis

Another form of high-throughput sequencing – 16S rRNA sequencing — examines only one gene, the 16S gene on ribosomal RNA (rRNA). The 16S gene is used because it is consistently conserved in all bacteria, which makes gene splicing possible. The 16S gene also contains a variable region, which allows researchers to identify distinct organisms from just one gene. Many intestinal microbiota studies use 16S rRNA sequencing because it allows researchers to identify numerous organisms present in the gut bacterial microbiota community (Cox et al., 2013). However, this thesis applies a different type of high-throughput sequencing: shotgun sequencing.

For the purposes of this study, shotgun sequencing provides many benefits over 16S sequencing. The most relevant benefit is that shotgun sequencing is *untargeted,* so provides a broader picture of community diversity by allowing the researcher to identify the presence of organisms they may not be actively searching for with unique primers (Wylezich et al., 2020). Shotgun sequencing further allows researchers to determine the functional capacity of a microbial community (Deusch et al., 2014; Quince et al., 2017). Shotgun sequencing of fecal samples has been successfully applied to determine microbiota community composition and diversity, as well as to detect parasites in mammals (Alberdi et al., 2019; Rubel et al., 2020; Wylezich et al., 2020).

## 2.5 The *One Health* Framework

3 Methods

## 3.1 Study sites

In this section I outline the two study locations discussed in this thesis: the town of Ittoqqortoormiit, and the Danish military base in Daneborg. Both towns are located in East Greenland, and maintain large populations of GSDs (Figure XX).

### 3.1.1 Ittoqqortoormiit

Ittoqqortoormiit (70°29′07″N, 21°58′00″W) is a town inhabited by 353 people and 180 sled dogs (Statistics Greenland, 2023, 2020). It is considered one of the most remote villages in the Greenland, and in the world (Visit Greenland, 2023). The nearest airport is Nerlerit Inaat Airport, 38km away. Nerlerit Inaat is only serviced by Norlandair, and flights come weekly from Reykjavik and Akureyri in Iceland, with frequent cancellations due to weather (Norlandair, 2023). From Nerlerit Inaat, transfer to Ittoqqortoormiit is possible by boat—when the sea is ice-free— helicopter, or snow scooter (Visit Greenland, 2023). Goods come to Ittoqqortoormiit on infrequent shipments from Denmark by Royal Arctic Line (CITE).

Ittoqqortoormiit falls within the sled dog district of Greenland, where sled dogs are the only domestic animals allowed (Government law no. 18, 30/10/1998 chapter 1§3). Ittoqqortoormiit has an established hunting community, where hunters use GSDs to access the ice edge to hunt. Dogs in Ittoqqortoormiit are predominantly used for hunting, cross ice transport, predator warning, and a small but growing dog sled tourism industry (Visit Greenland, 2023). Dogs older than six months are kept chained, in groups of 6-12 individuals. Presently, dogs are kept out of town: including at the municipal waste dump (Klein-Ipsen & Rotovnik, 2023). Collaborating researchers communicated that dogs in Ittoqqortoormiit were fed hunted game (seal, narwhal, polar bear, muskoxen, fish) occasionally supplemented by commercial dryfeed (Klein-Ipsen & Rotovnik, 2023). Dogs are fed every 2-3 days, and legally have to be monitored once daily (CITE). Ittoqqortoormiit dogs do not receive veterinary care, and had never been dewormed; however approximately every three years the Ittoqqortoormiit dogs are vaccinated against Rabies and CDV (CITE).

Ittoqqortoormiit was selected to be one of my two study sites primarily due to its GSD population being fed primarily marine resources. Furthermore, most GSD research has been conducted on populations in West Greenland, leaving a gap in the literature regarding East Greenlandic dogs, who are geographically isolated from their West Greenlandic counterparts. Meaningful to this project, a master’s thesis on the occurrence of endoparasites in Ittoqqortoormiit GSDs was submitted in February 2023, and the two authors collaborated with this project by sharing their data, and collecting samples in Ittoqqortoormiit (Klein-Ipsen & Rotovnik, 2023).

### 3.1.2 Daneborg

Daneborg (74°18'N, 20°14'W) is a military base located in Northeast Greenland, and it is home to the *Sirius* sled dog patrol (Sirius). Sirius was founded in 1941 as a special force branch of the Royal Danish Navy. Today, Sirius remains in operation under the Joint Arctic Command, with the primary objective of maintaining Danish sovereignty and policing North and Northeast Greenland (Forsvaret, 2020). The population of Daneborg is dynamic and very small, though all have a direct link to Sirius (CITE).

Currently Sirius maintains 78 GSDs—in six sled teams, each with 13 dogs (CITE). Teams are named SH 1-7 (six is omitted, due to tradition). Sirius dogs have markedly different management than hunter-owned packs, including those in Ittoqqortoormiit. Dogs in Daneborg are exclusively kept for their use in Sirius patrol exercises (Forsvaret, 2020). Teams are kept separate from one another, in fenced enclosures, with the exception of puppies and their mothers, who are kept separately (CITE). Collaborating researchers communicated that in working months, Sirius dogs are fed high fat, high calorie food specifically designed for working sled dogs (pemmican) supplemented by dry fish every 2-3 days, and pig fat every other week, and occasionally by raw seal fat (Klein-Ipsen & Rotovnik, 2023). In non-working months, Sirius dogs are fed *Royal Canin* dryfeed, supplemented by dry fish every 2-3 days, and pig or seal fat if they are not doing well (Personal communication, Klein-Ipsen and Rotovnik, 2022). Sirius dogs are constantly monitored, and regularly receive veterinary care. Dogs receive annual prophylactic deworming treatment, twice annual veterinary checks from a designated veterinarian, and soldiers have the possibility to contact a veterinarian for emergency care and are trained in basic canine first aid (CITE).

Daneborg was selected as my second study site due to different feeding regimens than the Ittoqqortoormiit cohort. Sirius dogs are not regularly fed marine resources. Sirius dogs are completely isolated from both East Greenland dogs, and West Greenland dogs. Further, Sirius has a history of participating in GSD health studies, and contributed to this study by contributing samples to be used in both this project, and other master’s theses (Klein-Ipsen & Rotovnik, 2023; Clausen & Larsen, 2021).

## 3.2 Sample collection

Samples from both Ittoqqortoormiit and Daneborg were collected by collaborating researchers in the Fall of 2022, following a standardized protocol outlined by the Earth Hologenome Initiative (EHI) (CITE).

In Ittoqqortoormiit, 38 fecal samples were collected in September 2022 by collaborating master’s students Pernille Klein-Ipsen, MSc and Rosalina Rotovnik, MSc. Participating dogs were selected based on owner’s willingness to participate in the research. In Daneborg, 44 fecal samples were collected in August 2022 by Sirius veterinarian Lone Lykke Hansen, DVM. Selection of dogs for participation in the study was arbitrary, and depended on which dogs defecated while researchers were present. Dogs with severe diarrhea were excluded from this study.

A piece of the inner part of the feces was taken, and placed into a sterile collection tube, filled with preservation buffer (reagent from © 2023 Zymo Research Corporation. ISO 9001:2015 Certified) and sealed. The sealed tube was then homogenized by flicking the sides to mix the contents. For the *Sirius* dogs, samples were refrigerated within 30 minutes of collection (Personal communication, Klein-Ipsen and Rotovnik, 2023). In Ittoqqortoormiit, Metadata on the location, sex and estimated age of each dog was collected. In Ittoqqortoormiit, researchers also recorded notes on fecal consistency, appearance, and their body condition. In Daneborg, the dog’s team was also recorded.

Samples were then transported back to the University of Copenhagen Globe Institute for long term storage. EHI protocol calls for samples to be frozen at -20C within 14 days of collection, and kept as cold as possible—avoiding temperature fluctuation—while in transit (EHI Protocol, Personal communication, Azipurua, 2022). The Daneborg samples were transported to Copenhagen through Aalborg in an insulated sample transport box. Samples were kept insulated, but unrefrigerated, from the evening of September 9, 2022 – the morning of September 12, 2022 (Personal communication, R. Rotovnik, 2022). Samples were then refrigerated from September 12-15, at which point they were frozen at -20C for long term storage. The Ittoqqortoormiit samples were kept at 5C for 1-3 hours after collection, before processing in the field (Personal Communication, Klein-Ipsen and Rotovnik, 2022). Samples were then refrigerated until transport back to Copenhagen, at which point they were kept in an insulated sample transport box for 24 hours. Samples were then refrigerated, and placed into long term storage at -20C.

GET SAMPLE COLLECTION PROTOCOL FROM OSTAIZKA.

## 3.3 External analysis: clinical identification of parasites

For their master’s thesis, Klein-Ipsen and Rotovnik identified parasites from GSD fecal samples, blood serum, and heart fluid from GSDs in Ittoqqortoormiit and Daneborg (Klein-Ipsen & Rotovnik, 2023). For the analysis of their fecal samples, they applied the Concentration McMaster Technique (Roepstorff & Nansen, 1998). Concentration McMaster is a fecal flotation test, in which solution is added to samples, causing eggs and sporocysts to float, making it possible to visually identify any parasites. Immunofluorescence Assays were also preformed to test for *Cryptosporidium* spp and *Giardia* spp(CeLLabs, Crypto/Giardia Cel IF Test). This thesis uses duplicate samples of the ones investigated by Klein-Ipsen and Rotonvik (n=29 Ittoqqortoormiit, n=26 Daneborg) and agreed to share their results. Thus, in any discussion of comparison between molecular techniques and clinical identification, the later comes from their findings. Three samples from Daneborg examined in this thesis were excluded by Klein-Ipsen and Rotovnik.

## 3.4 Laboratory preparation of fecal samples

## 3.5 Genome resolved metagenomics of the microbiome

In this section I outline the steps followed to analyze metagenomic sequencing data resulting from the laboratory procedure described in section 3.4. This process consists of running R scripts developed by the EHI, adapted to be applied to this thesis. Annotated R markdown files of the code described in this section are located in the Appendix. The code for sections 3.5.1 – 3.5.2 can be found in Appendix XX. The code for sections 3.5.3 can be found in Appendix XY. The version control for all R packages can be found in Appendix XZ.

### 3.5.1 Forming Metagenome-assembled genomes

Once the sequencing data was delivered to the EHI, it was run through an R (4.3.1) pipeline developed for processing metagenomic samples (Appendix ##). Sequencing data was first filtered for quality (threshold = ##), before host (GSD) DNA and unmapped reads were removed from the dataset (CITE). The remaining metagenomic data were in the form of metagenome-assembled genomes (MAGs). MAGs are created by clustering together groups of reads that come from the same genome. These groups are called *assemblies* (thus the A in MAG) and form draft genomes with which the rest of the metagenomic analyses are performed. Functionally, a MAG represents a single taxon. Once MAGs were assigned, a minimum genome-coverage filter was applied (threshold = ##) (CITE). The function of the filter is to offset inflation of diversity calculations caused by the analysis itself by removing MAGs with a low number of mapped reads. A minimum coverage of 0.3 as established in CITE was applied (CITE). MAG data was then normalized using total-sum scaling (TSS) to correct differences in bacterial genome sizes. TSS normalization takes each individual count, and divides it by the total number of counts for each sample, thus making a relative abundance value on a scale of 0-1, and making it possible to compare MAGs regardless of the size of the bacterial genome.

The sequencing depth—or number of times a base is represented in any given sequence—was then assessed for mapped MAGs. Mean sequencing depth, and depth range were subsequently calculated. Sequencing depth provides insight into the accuracy of an individual sequence. The greater the depth, the more confidently you can determine a specific base at a particular position (CITE).

After MAGs were filtered normalized, and sequencing depth was assessed, the next step was to visualize the relative abundance of MAGs for each individual sample. A stacked bar plot was created using *ggplot*, and samples were grouped by cohort. The R package *phyloseq* was then used to calculate summary statistics at the phylum level (McMurdie & Holmes, 2013).

### 3.5.2 Diversity calculations

Hill numbers are a unified measure of biological diversity, represented by the following equation, such that =the order of diversity (any positive integer)richness and relative abundance (a value between 0-1):

In R, hill numbers can be calculated using the package *hilldiv* (Alberdi, Thomas, et al., 2019). In this thesis, hill numbers were applied to measure the alpha diversity within each sample: neutral diversity, phylogenetic diversity, and functional diversity (section 3.5.3). Neutral diversity represents species richness—also called Shannon diversity (Shannon, 1948). The mean and standard deviation was calculated for alpha diversity in each cohort, and box and whisker plots for neutral and phylogenetic diversity were constructed using *ggplot*.

Beta diversity, which compares samples against each other, to compare the overall diversity of the Daneborg and Ittoqqortoormiit cohorts. Beta diversity was initially calculated in R using the *hill\_taxa\_parti\_pairwise* function of *hilldiv*. The resulting beta diversity values were then visualized on a Non-metric Multi-dimensional Scaling (NMDS) plot created with *ggplot,* to assess the clustering of samples. NMDS plots are a representative visualization of the dissimilarity of multiple samples, considering multiple variables, rank order, and ordination. It allows for the simplistic visualization of multidimensional space on a two-axis plot and is particularly valuable for understanding the separation between communities, like the microbiome. The ultimate goal of an NMDS plot is to visualize patterns and clustering; in this thesis NMDS was applied to understand how microbiome communities differed between cohorts of GSDs. A permutation test for homogeneity of multivariate dispersions was run using the function *permutest*, when the permutation test resulted in a p-value > 0.05, the data was deemed suitable for further multivariate analysis (Anderson, 2006). A MANOVA test was run in R to test for statistical significance, using the function *adonis* from the ecology tool package, *vegan* (Dixon, 2003). MANOVA stands for “multivariate analysis of variance,” and is an appropriate statistical test for understanding the significance of variance in ecological communities, as described by McArdle and Anderson (McArdle & Anderson, 2001).

### 3.5.3 Evaluating the functional diversity of the microbiome

This is next R, I believe

## 3.6 Shotgun of potential parasites\*\*?

## 3.7 Statistical Analysis

## 3.8 Ethical considerations

4 Results

## 4.1 The GSD fecal microbiome

### 4.1.1 Community assessment

A total of 333.7l gigabases (Gb) of metagenomic data was sequenced from 58 GSD fecal samples (29 = Daneborg, 29 = Ittoqqortoormiit). 293.14 Gb were mapped to 555 MAGs. Prokaryotic DNA comprised 92.51% of metagenomic data, while GSD DNA represented 2.01%, and 5.48% was left unmapped. The total mapped sequencing depth of the metagenomic analysis was 266.02 Gb (x̄=4.59 Gb). The quantity of MAGs, GSD DNA, unmapped reads, and discarded poor quality reads for each individual sample is visualized in figure XX.

Twelve different phyla of bacteria were identified, X% were identified to the species level, and the remaining X% were identified to the genus level (Table XX). The relative abundance of these twelve phyla across each sample is plotted in figure XY.

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| --- |
|  |
| *Figure XX: In this figure shows the sequencing depth, and classifications of sequenced data. The quantity of data (in Gb) is on the vertical axis, and individual samples run along the horizontal axis.* |

|  |
| --- |
|  |
| *Figure XY: This figure shows the taxonomic composition of samples. Relative abundance of taxa is on the vertical axis, and individual samples are on the horizonal axis.* |

### 4.#.## Diversity calculations

#### Alpha diversity

The average neutral diversity for Daneborg and Ittoqqortoormiit respectively were, 93.53 19.99, and 92.7335.48. For the phylogenetic diversity, the means were 47.946.34 (Daneborg) and 50.49 (Ittoqqortoormiit) (Table XY). Figure XZ shows the neutral and phylogenetic diversity of each individual sample, more on that here…

Table XY: This table shows the mean and standard deviation for two alpha diversity metrics, in both cohorts of data. Both neutral diversity and phylogenetic diversity are shown.

|  |  |  |  |
| --- | --- | --- | --- |
| **Location** | **Diversity metric** | **Mean** | **Standard deviation** |
| Daneborg | neutral | 98.53 | 19.99 |
| phylogenetic | 47.94 | 6.34 |
| Ittoqqortoormiit | neutral | 92.73 | 35.48 |
| phylogenetic | 50.49 | 12.75 |

|  |  |
| --- | --- |
|  |  |
| *Figure XZ: These box plot shows the Alpha diversity of individual samples, separated by sample location. The neutral alpha diversity is show in (a) and the phylogenetic diversity is shown in (b).* | |

#### Beta diversity

Two distinct clusters formed based on cohort location in the NMDS plot for beta diversity (Figure XA). The result of the permutation test for homogeneity of the beta diversity resulted in p=0.537. In this case, a p-value of greater than 0.05 indicates that the data is suitable for multivariate analysis, so *adonis* was run. *Adonis* indicated that the clustering was statistically significant, and that cohort location explained 12% of variance in the data (p=0.001, R2=0.122).

|  |
| --- |
|  |
| *Figure XA: This NMDS shows beta diversity clustering.*(p=0.001, R2=0.122). |

5 Discussion

## 5.X Management

- botulism in the Arctic increased after the introduction of plastic wrapping 🡪 created anaerobic conditions for bacteria to thrive

- once snowmobiles were introduced as an alternative mode of transportation to sled dogs, human *Echinococcus* infections decreased, with the highest prevalence of *Echinococcus* outbreaks correlating with the presence of sled dogs, and the highest frequency of human infections in Arctic Indigenous communities (Gilbert et al., 2010).

-Dogs housed in municipal waste dump may introduce a cyclical transmission pathway, with the dogs getting infected by bacteria / pathogens from human feces, then reinfecting humans

* + May also be transmission pathway to the marine environment 🡪 especially for bacteria

-Investigating dog / parasite relationships is also beneficial for understanding the pathways for domestication that happened in the north -> understanding how the dog / human relationship evoloved over time (Losey et al., 2018)

6 Conclusion

Acknowledgements

This chapter should include thanks to those who have supported the research financially, with facilities or work, e.g. funds, companies, advisors and others that have in any way assisted, including friends and family, if applicable. Acknowledgements should be on an odd page (right page).

# Formatting information

Heading 1 is a chapter heading. Heading one should be bold in 20 pt Tahoma. Spacing before should be 54 pt and spacing after should be 12 pt. The first page should be an odd page (right page) and new chapters always start on an odd page. If Heading 1 is directly before Heading 2, there should be 24 pt total spacing between the headings.

All headings should have the same font.

The body should be written in Times New Roman, 12 pt and 1½ line space. There should be 12 pt spacing after each paragraph.

All text should be in single colour; black. Exceptions are made within pictures or figures. Hyperlinks should not be used in text, but if an exception it should simply be as other text, not blue and underlined.

You should have 2.5 cm margins on top and outside (not where the spine is). On the inside (by the spine) you should add 0.5 cm (gutter) to have the total of 3.0 cm margin on the inside. The bottom margin should be 3.0 cm from the bottom and up to the text, but 1.5 cm from the bottom and to the page number.

The page numbers should start from one on the first page of the first chapter with Arabic numerals. The page numbers should be by the outer edge and at the bottom of the page, but the roman numerals are centered. References and appendices are numbered the same way as the thesis body.

You can use *italics* moderately to emphasise words/text. Use **bolds** very sparingly. Do not use underlines.

Use footnotes sparingly. They should be numbered and at the bottom of the page which they refer to.

Number and refer to formulas as is applicable.

## Heading 2

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### Heading 3

Heading 3 is the last numbered subheading. It should be in 12 pt Tahoma. Spacing before should be 18 pt.

#### Heading 4, 12 pt Tahoma, no chapter numbers, is not shown in Table of Contents, 12 pt spacing before and 6 pt below.

Do not use other headings than from 1-4.

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Carefully follow the template for the cover or back pages and don’t add any picture/logo and don’t take away. Never put a picture on the cover page.

You should always mention collaborators and sponsors in your thesis, but you should do that under Acknowledgments.

On the spine the author’s name, a short title and the year should be printed in one line in Tahoma.

# Lists

#### Bulleted list

Here below is an example of a bulleted list. You are allowed to decrease the space between bullets, e.g. that there’s only 0 pt between bullets, but you should always keep 12 pt spacing before the first bullet.

* Number 1;
* Number 2;
* Number 3.

If the first line below the list continues the earlier paragraph, you should not have spacing between.

# Figures and tables

This chapter gives examples of how to use pictures and tables and how to refer to them.

## Figures

Captions should be located beneath the figures and should be in italics..



*Figure 3.1 Example of a caption (beneath)*

It’s important to define figures with “paragraph format”: “keep with next” so that you don’t sever the connection between the figure and the caption. You may centre the figure (and caption) on the page.

You can insert the caption automatically with References – Insert Caption.

You can also use the caption function to insert captions for tables. The table caption should also be in italics. The text should be above the table. Do not use 1 ½ line spacing in the table. There’s a rule of thumb regarding table design = keep it simple! You may centre the table, but then the caption should start from the table’s left edge.

*Table 3.1 Example of a table (above).*

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You should input a line beneath the tables.

Generally you shouldn’t use spacing beneath the table. Figures and tables should appear in-text as they are referenced, not in the end of chapters.

# Other notes

Do refer to master’s thesis directions in the student handbook and general guidelines regarding structure (IMRAD) of thesis. We don’t require a specific format, but the thesis should include an introduction that frames the thesis and topic well (with aims and research questions), a background section that goes more in depth into the literature, methods, results, discussion and conclusion. Remember the conclusion or the discussion should have management implications. Some theses combine the results and discussion, others create different chapters, some have an executive summary for managers as the first chapter. Consult your advisor and program director if you have any questions.

Master’s thesis should be printed on A4 paper.

The thesis should be printed on both sides of the page and each chapter should start on the right side of the fold.

The printer is an eco-friendly printshop, and all prints are with the eco-standard of the Nordic Swan eco-label.

Students are responsible for correct formatting of the thesis. Common mistakes noticed by the printer include:

- Wrong page size (UsLetter not A4)

- Incorrect set up for double side printing

- Very low quality of images

- Table of content is not correct (pages numbers not matching, missing items. etc)

# References

The main heading for References should be included in the table of contents. It should be as heading 1, only without a chapter number. As other main headings it should start at a new page and on an odd (right) page. References should be single spaced with a hanging indent.

Use the APA format for references and when citing.

**Appendix A**

Appendix A should always contain the student’s ethics clearance letter from the Masters Program Committee.

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