A COPROLOGICAL STUDY OF ENDOPARASITES IN THE ICELANDIC ARCTIC FOX USING TRADITIONAL AND MOLECULAR METHODS

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ABSTRACT

The Arctic fox (*Vulpes lagopus*) is the only land mammal native to the remote island of Iceland, which has a history of contending with endemic parasites in canines. This dissertation utilises fox carcasses from storage at the Icelandic Institute of Natural History to survey endoparasitic fauna, and comments on the suitability of traditional and molecular methods for the identification of endoparasite species and estimation of egg burden.

A total of 129 Arctic fox carcasses were analysed using traditional microscopy (Apacor Mini Parasep®) and molecular techniques (real-time PCR). Examination revealed 66.9% (n=77) of foxes had an endoparasitic infection; *Capillaria aerophila* 6.9% (n=8), *Diphyllobothrium latum* 6.9% (n=8), *Toxascaris leonina* 36.4% (n=41), *Toxocara canis* 9.3% (n=12), *Toxoplasma gondii* 4.7% (n=6), *Eimeria* or *Isospora* 1.6% (n=2), and *Cryptocotyle lingua* 0.8% (n=1). All samples positive by PCR were detected by microscopy.

The dissertation suggests that coprological analysis of frozen faeces using bright-field microscopy and real-time PCR for the detection of parasites in wild Arctic fox populations in Iceland can be achieved despite DNA degradation, interference with PCR inhibitors, and poor sample quality.

Declaration

The data presented in this thesis was obtained in collaboration with Náttúrufræðistofnun Islands. Full access to the foxhunting data was granted for the purpose of this study, courtesy of the Environment Agency of Iceland and the Icelandic Ministry for the Environment and Natural Resources. The obtaining, preparation, and analysis of all faecal samples (macroscopy, microscopy, and real-time PCR) are my own work under the supervision and assistance of Ester Rut Unnsteinsdóttir who has contributed the other data sets (jaw length, weight, age, etc) for this thesis. The data analysis and interpretation are entirely my own work. I certify that this thesis is my own work, except where indicated by reference, and the work presented in it has not been submitted in support of another degree or qualification from this or any other university or academic institution.

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1. Introduction

1.1 Arctic Foxes in Iceland

The sub-Arctic island of Iceland (64.9631° N, 19.0208° W) is home to a population of approximately 10,000 Arctic foxes (*Vulpes lagopus*) - a small canine species that typically inhabits low productivity Arctic and northern tundra habitats (Hersteinsson and Macdonald, 1996). The population was the sole terrestrial species abandoned on the island after the ice from the last ice age receded during the Holocene (Geffen et al., 2007) and has thrived for millennia in an environment bereft of predator and intraspecies competition. Due to the harsh weather conditions, volcanic activity, and geographical isolation, the island lacks terrestrial biodiversity. Therefore, Arctic foxes of Iceland have a unique diet, and associated exposure to trophically transmitted parasites, as they cannot rely on the cyclic populations of lemmings as observed on mainland Europe (Hersteinsson and Macdonald, 1996), instead predominantly relying on aquatic and avian prey species. Prey availability fluctuates and the Arctic foxes take advantage when sources of food are plentiful during summer and autumn to store fat to be used during the winter (Fuglei and Øritsland, 1999).

In Iceland, the Arctic fox occupies both coastal and inland ecotypes (Hersteinsson and Macdonald, 1996) and shows both opportunistic (Elmhagen et al., 2000) and individual dietary specialization habits (Angerbjörn et al., 1994). This specialization has meant the influence of the marine habitat and migrating

birds on the coastal ecotype foxes is considered much stronger, while inland foxes rely more on terrestrial species and resident birds (Hersteinsson, 1996; Dalerum et al., 2012).

As the Arctic foxes in Iceland are dependent on terrestrial, avian, and marine prey species (Angerbjörn et al., 1994), their population size, structure and dynamics are affected by events occurring in these ecosystems (Pálsson et al., 2015).

1.2 A Species in Decline

Population data for the Arctic foxes of Iceland is determined using hunting statistics collected by the Environment Agency of Iceland and the Wildlife Management Institute. The species is culled legally throughout all seasons, including during denning. The stable hunting initiative and reliable records since 1958 mean the total number of adult foxes culled annually can indicate the size of the total population (Unstteindottir et al., 2016). A decline in the population was observed in Iceland from 1958 until the 1970s. However, it has since been increasing: a fluctuation that has been attributed to the dynamics of fox prey populations and climate (Unstteindottir et al., 2016).

The conservation status of the Arctic fox is recognised as good, except for the Scandinavian mainland population where the species is classified as endangered. While the Icelandic Arctic fox population reached 10,000 individuals in 2013 (Pálsson et al., 2015), such high numbers are atypical for Scandinavia, with the species recognised as extinct in Finland, endangered in Sweden, and critically endangered in Norway (Landa et al., 2017). It is estimated that the Arctic fox population in Norway, Sweden and Finland combined is less than 200 individuals (Dalén et al., 2006). It is thought that these populations were sensitive to variation in demography and environmental change, and had undergone several disease outbreaks (Loison, Strand & Linnell, 2001) after the initial dramatic decline in the late 1800s and early

1900s attributed to over-hunting (Tannerfeldt et al., 1998). However, even after the introduction of legislation protecting the Arctic fox in the early 20th century, the species continued to decline (Herfindal et al., 2010).

The population density of foxes in Iceland varies across the country, with their highest concentration of the species in the Westfjords (Pálsson et al., 2015) where the extensive coastline provides an abundance of nesting coastal bird species and a nature reserve prohibits hunting. The fox is the apex predator on the island, and there is little competition or threat other than the hunting initiative in the country. However, the population of Arctic foxes in the country saw a sudden decline in numbers in 2014, after several decades of uninterrupted growth. At this time, many foxes were found dead and the few breeding pairs could successfully raise offspring (Ester Unnsteinsdottir, pers. comm.).

The factor(s) responsible for this unsuccessful year for the foxes has yet to be established, but it has been suggested by researchers at Náttúrufræðistofnun that climate, pollutants, or the condition of food, as well as a series of particularly harsh winters, overhunting, and associated decrease in prey, could be contributing to the one-third decrease, possibly as a multifactorial combination (Ester Unnsteinsdottir, pers. comm.). It has been suggested that this could be a short stochastic fluctuation as a result of the species reaching the normal carrying capacity of the land (Ester. Unnsteinsdottir, pers. comm; (Unnsteinsdottir et al., 2016). Disease may also be implicated in the decline but there has been no speculation on whether this may be genetic, viral, bacteria, or parasitic (Ester. Unnsteinsdottir, pers. comm.).

The potential of a disease in the population of foxes in Iceland is concerning and potentially damaging. The Icelandic population of Arctic foxes have a history of segregation from other members of their species inhabiting mainland Scandinavia which has led to a degree of habitat specialisation (Norén et al.,

2009). This isolation is known to be a risk factor for population decline compared to non-island populations as the species are potentially vulnerable and unable to adapt to rapid environmental changes (Wikelski et al., 2004).

Their vulnerability has become particularly noticeable during the last century following habitat changes resulting from extensive anthropogenic upheaval. Ecological change as a consequence of human settlements has been further compounded by both hemispheric and regional climate change (Mellows et al., 2012). This activity is known to alter parasitic transmission and trophic interactions (Dobson et al., 2015), and can contribute to the emergence or re-emergence of parasitic diseases (Short , Caminade & Thomas, 2017).

1.3 Parasitology in Iceland

Parasitological studies are not currently part of the health screening of the carcasses arriving at Náttúrufræðistofnun Íslands due to financial restrictions and limited resources for routine testing. However, as the stored carcasses are already used in a wide range of studies in various fields, establishing simple methods to include as part of monitoring could provide access to a reservoir of data regarding intestinal parasites.

Iceland's ecosystem is being reshaped by the increase in the amount of imported animals and migratory birds, a quickly growing human population, and the effects of global warming. These factors have the potential to change parasitic and host interactions, potentially increasing the distribution and transmission of endoparasites. As a result, the use of fox carcasses for parasitological studies is a subject of increasing interest.

Surveillance of parasites in the wild population may increase understanding of population anomalies such as the sharp decline in fox numbers in 2014, as well as the associated reports describing small cubs, sterile vixens, and fewer burrows compared to previous years (Ester. Unnsteinsdottir, pers. comm.). Additionally, large-scale studies that seek to assess the presence, distribution, host associations or numerical occurrence of parasites in hosts in high latitude environments are lacking on a global scale. This is partly due to the limited historical data that means it is challenging to establish baseline prevalence, and therefore it is difficult to measure trends or changing patterns by a retrospective study (Ester. Unnsteinsdottir, pers. comm.).

The impact of parasitic infestations of wild animals extends beyond detrimental health effects for the host, as they have the potential to serve as a reservoir of infection that could be a threat not only to humans but also other wildlife and agriculture (Rhyan and Spraker, 2010). Endoparasitic infection from a human health perspective can cause a range of problems, from mild discomfort and deficiencies as the parasite consumes vital nutrients to a more serious impact on morbidity and mortality (Haque, 2007). The consequences of intestinal parasite outbreaks are known to cause serious economic, social, and health impacts throughout the world. This includes impaired growth in children, both physically and mentally, as well as impaired educational achievement and slow economic development (Haque, 2007).

As endoparasites influence the dynamics and regulation of host populations, and mediate competition among conspecific hosts (Price, 1988), infections have the potential to substantially decrease the population of Arctic foxes. Such an event could damage the ecosystem immeasurably by significantly contributing to population decline with the risk of reducing the species to extinction (De Castro & Bolker, 2004).

1.4 Trophic Interactions and Parasite Transmission in Iceland

Parasites comprise over 40-50% of the organisms on the planet and are integral to all ecosystems (Dobson et al. 2008). They are heavily involved with over 75% of trophic links within food webs (Lafferty et al., 2006). The spread of parasites relies on links between hosts, either transmitting vertically from maternal parent to offspring or horizontally by faecal-oral or food-oral routes (Antonovics et al., 2017). Therefore, circulation of parasites is based on specific pathways within the environment connecting individuals. In the Arctic and sub-Arctic, predator-prey interactions dominate trophic links. Therefore parasite life cycles and transmission are influenced directly by predator and prey species populations (Hoberg, 2013), as well as the food habits and behaviour of hosts, including migration and response to ecological conditions. (e.g. (Mueller et al., 1967), Muzzafar 2009).

Arctic foxes in Iceland are opportunistic omnivores subsisting predominantly on an avian and aquatic prey, a diet which is supplemented by scavenging carrion and microtine terrestrial species (Hersteinsson and Macdonald, 1996). Each of these sources provides a potential route for parasite transmission into the foxes. These trophic interactions with other species are responsible for the distribution and spread of endoparasites in the country.

1.4.1 Aquatic

Trematodes, (such as *Plagiorchis elegans* and *Cryptocotyle* sp.) and acanthocephalans (such as *Polymorphus* sp.) are associated with marine environments (Elmore et al., 2013). Marine fish parasites are usually generalists for both intermediate and definitive hosts (Polyanski, 1961) and often indiscriminately infect paratenic and transport hosts (Marcogliese, 1995). This generalistic approach to finding a host means many endoparasites can infect a wide range of oceanic, avian, and terrestrial species that prey upon the fishes including invertebrate predators such as foxes that hunt and scavenge on the shoreline (Bush et

al., 1993). Crustaceans are one of the most common intermediate hosts in parasite life cycles (Marcogliese, 2002). In Arctic ecosystems amphipods are a particularly vital link in parasite transmission where they are commonly preyed upon by fish and seabirds (Weslawski and Kwasniewski, 1990) which are also prey species for the Arctic fox.

1.4.2 Avian

Arctic foxes also rely on avian sources for prey, including juveniles, adults, and eggs. Icelandic birds on the island carry gastrointestinal parasites, many of which can cross species boundaries (Carrera-Játiva at al., 2018). Their migratory status allows them to carry pathogens across borders (both short and long distance), and the introduction of microorganisms in this way has been shown to be harmful to vertebrates (Hubálek, 2004). Additionally, migratory birds are at a higher susceptibility to parasites due to the physically demanding migratory process impacting the resistance of their immune system (Feare, 2007). Avian life carry and transmit *Giardia, Coccidia,* and *Sarcocystis,* as well as a variety of nematodes and cestodes such as *Capillaria* sp. (Read, 1991).

1.4.3 Terrestrial

The Icelandic fox population is derived from a combination of Greenlandic and northwestern European communities and it was not until humans arrived to the island during the 9th Century that other species were introduced into the country, either intentionally or accidentally (Smith, 1995). These introductions included companion dogs (*Canis lupus familiaris*) and cats (*Felis catus*), horses (*Equus ferus caballus*), field mice (*Apodemus sylvaticus*), and house mice (*Mus musculus*). Since these first foreign species were introduced, American mink (*Mustela vison*) and the Spanish slug (*Arion lusitanicus*) have also arrived to the country (Callow, 2006). These non-native species have transformed the natural ecosystem of Iceland

with some acting as novel prey species for the foxes, but also serving as vectors of zoonotic disease and facilitating parasite transmission either through ingestion or contamination via faeces.

Arctic foxes do not primarily rely on the few terrestrial species in Iceland, but will occasionally feed on mice, cats and mink when other food sources are limited. The presence of these species mean *Toxoplasma gondii* is able to complete its life cycle in Iceland, and the foxes will prey on these species which may provide a potential transmission route. Mink have been known to carry species including *Trichinella* sp., *Taenia* sp., and *Crenosoma* sp. (Shimalov and Shimalov, 2001).

1.5 Diet of the Arctic Fox in Coastal and Inland Ecotypes

The Arctic foxes of Iceland are diverse in their food foraging and hunting behaviour, and they adapt their diet in response to changes in food availability. This has been observed on the island in several instances, including the year the foxes of the coastal habitat of Ofeigsfjörður switched from consuming black crowberries in the autumn of 1978 to kelp-fly larvae and pupae the following year as the cold weather reduced the vegetation (Hersteinsson & Macdonald, 1996). Coastal foxes rely more on migrating seabirds nesting in the cliffs, while inland foxes subsist on resident birds that nest on the ground.

The diet of the Arctic fox in Iceland being obtained from various sources provides an opportune transmission route for parasites in wildlife. Understanding the host species and their interactions can assist in directing studies towards parasites of interest.

1.5.1 Coastal

Coastal foxes are known to have a more varied diet than their inland counterparts, and predominantly sustain themselves on seabirds. For example, the birds kittiwake (*Rissa tridactyla*) and eiders (*Somateria* sp), and fish including Arctic cod and lumpfish (*Gadus saida* and *Cyclopterus lumpus*), as well as

scavenged marine mammals such as the harbour seal (*Phoca vitulina*). Arctic foxes are also known to feed on invertebrates inhabiting the littoral zone of the coast such as Asteroidea, Porifera, and Nudibranchs (Pálsson et al., 2015), many of which are involved in helminth life cycles (Marcogliese, 2002). When food is scarce in their coastal habitat due to seasonal changes in nesting bird species, the foxes can move hundreds of kilometres to hunt inland (Ester. Unnsteinsdottir, pers. comm.).

The Atlantic cod is a common fish species in the shallow waters around Iceland and may be pivotal in understanding parasitic interactions in both terrestrial and oceanic environments, as it provides a link to the top predators in the food chain (Hemmingsen and MacKenzie, 2001). It predates on fish, crustaceans, and polychaetes, among other species, and collects a diverse number of parasites throughout its life (Marcogliese, 2002) as marine parasites tend to be long-lived (Campbell, 1983). A total of 107 different parasites have been reported in cod, 83 of which are generalists. Cod acts as intermediate, paratenic or definitive host to these parasites, with eight species requiring marine mammals to mature (Marcogliese, 2002). The generalist nature of the parasites harboured in the Atlantic cod offer a trophic route from marine species into terrestrial animals.

1.5.2 Inland

Foxes inhabiting environments inland subsist primarily on birds, particularly ptarmigan (*Lagopus mutus*) in winter and migratory birds including passerine (*Passeriformes*), geese (*Anserini*) and waders (*Charadriiformes*) in the summer. They also feed on invertebrates (Hersteinsson and Macdonald, 1996) and freshwater fish. Arctic foxes are also known to predate on domestic sheep (*Ovis domesticus*), particularly lambs, and will scavenge reindeer (*Rangifer tarandus*) carcass when there is an opportunity (Pálsson et al., 2015).

1.6 The Introduction and Impact of Parasitic Species in the Arctic Fox

Iceland has a history of contending with endemic parasites introduced by migratory birds and imported species, as well as the proliferation of native species and contamination of food products. These have been known to affect livestock, wildlife, and the human population (Akuffo, 2003).

The introduction of foreign parasitic species into livestock has already been demonstrated in Iceland. This includes the free-living zoonotic nematode *Halicephalobus gingivalis*, which is fatal in horses (Henneke et al., 2014). Previously unrecorded in Iceland, *H. gingivalis* is believed to have been recently introduced (Eydal et al., 2012).

There has only been one comprehensive study on the endoparasites of the Icelandic Arctic fox. Skírnisson et al. (1993) searched for parasites in 50 foxes by passing the gastrointestinal faeces through a 100 μm mesh. The study used the entire contents of the small intestines, cecum, and colon, and used a stereoscope to observe samples for counting and identification. Helminths were cleared in lactophenol and stained with carmalum (Coil, Pritchard and Kruse, 1983). The study found a significant difference between coastal and inland ecotypes, with coastal foxes harbouring a higher number of different species in the intestinal tract. Additionally, 11 species were reported in Iceland for the first time. The observed species were: *Eimeria* sp. or *Isospora* sp. (in 4%), *Cryptocotyle lingua* (24%), *Plagiorchis elegans* (4%), *Brachylaemus* sp. (12%), *Tristriata* sp. (10%) *Spelotrema* sp. (8%), *Mescocestoides canislagopodis* (72%), *Schistocephalus solidus* (2%), *Diphyllobothrium dendriticum* (4%), *Toxascaris leonina* (50%), *Toxocara canis* (2%), *Uncinaria stenocephala* (4%), *Capillaria aerophila* (6%), *Polymorphus meyeri* (8%), and

Corynosoma hadweni (2%). Many of the species of interest selected for this dissertation are based upon the findings of this study.

1.7 Human Parasites in Iceland

The parasitic infections that circulate the ecosystem are not necessarily contained solely within wild populations, as these infectious diseases are also transmissible to humans. *Sarcoptes scabiei* and the tapeworm Echinococcus have been among the most concerning parasitic outbreaks in humans in Iceland (Akuffo, 2003). The ectoparasite *Sarcoptes scabiei* has been endemic since inhabitants first arrived to Iceland. Abundance has fluctuated dramatically in humans as demonstrated by the 1,569 cases reported in 1941, followed by just eight reports in 1961, and an annual average of around 426 cases in the 1980s (Akuffo, 2003). *Echinococcus granulosus* is recognised as the most dangerous parasite to have infected humans in Iceland, as the country once had the highest prevalence of human hydatid disease anywhere in the world (Kristjánsdóttir and Collins, 2010).

Human gastrointestinal parasites were routinely monitored between 1973 to 1999 at the Institute for Experimental Pathology at Keldur, University of Iceland using the formalin-ethyl acetate concentration method. This monitoring of approximately 12,750 faecal samples found that 10% of the surveyed population were infected with protozoan oocysts, helminth eggs, or larvae, and identified *Giardia lamblia*, *Cryptosporidium parvum*, *Toxoplasma gondii*, and *Toxocara canis* as endemic parasites (Skirnisson, 2003). Since 1999, gastrointestinal parasites in humans have been surveyed at Landspítali (National University Hospital in Reykjavik).

While human parasites have some degree of regular monitoring, since the previous study on the parasites of the Icelandic Arctic fox almost two decades ago, there has been no more research in this area. During this time, Iceland has seen huge changes to the country's culture, climate, and infrastructure, with more homes being built outside of the city, more Icelandic citizens travelling abroad, and record numbers of companion pets being bought into the country. These factors, amongst others, may be having an impact on the species, distribution, and abundance, of various parasites harboured by the Arctic fox. Additionally, previous studies in the country have not used PCR and microscopy, and while the use of frozen carcasses as a method of parasitological surveying has been considered, there have been few formal projects attempting it.

2. Aims and Objectives

Aim: This dissertation seeks to identify and quantify the endoparasitic species living in the gastrointestinal tract of the Arctic fox in Iceland.

Objectives:

- Extract faecal samples from 130 Arctic fox carcasses;
- Use one traditional method (sedimentation) and one molecular method (polymerase chain reaction) to identify and quantify endoparasitic species and explore the efficacy of each method in regards to frozen faeces;
- Quantify species abundance, burden, and richness according to sex, age, and ecotype;
- Discuss any observed changes in the parasitic fauna over the last two decades and compare the findings with those of Skírnisson et al. (1993).

Methods

3.1 Sample Group

Whole carcasses of foxes were collected from hunters around Iceland (see Figure 1) and dissected in accordance with Pall Hersteinsson's methods (Hersteinsson 1984). Intestines were removed from -20^{O}C and defrosted for this dissertation.

For each carcass, hunters completed a standardised form to include details on: (1) the location of kill, (2) date of kill, and (3) if the fox was killed within or outside a den. Data regarding colour morph and litter size was then added at Náttúrufræðistofnun Íslands.

The whole carcass was weighed, entire skulls were removed and boiled in water for a minimum of 1.5 hours to remove flesh, and the maximum length of the lower jaw was measured as an indication of complete body length. Rump fat thickness of the subcutaneous layer above the musculus erector spinae was measured by hand using a vernier calliper at a location 2 cm to the right from the vertebral column and 6 cm from the pelvic bone. Mandibular canines were extracted and analysed at Matson's Laboratory in the US to be aged using cementum counting methods. Uteri were removed and placental scars counted to estimate the fertility of vixens (Strand et al., 1995).

The candidate specimens (n = 380) for this study were selected at random from those culled in Iceland by shotgun or rifle hunting methods between 2 June 2005 and 20 March 2017 (see Table 1). Preliminary dissection and extraction of faeces were performed on these samples. Of these, 296 carcasses could not be transported to the United Kingdom for further analysis due to import problems and so were excluded

(see Appendix 1 for the list of carcasses which were transported to the United Kingdom). A further 55 foxes were excluded from the study due to poor quality of faecal samples, including individuals without any material in the gastrointestinal tract, a severe level of decomposition, or obstructions due to plastic or glass that had prevented food passing from the stomach.

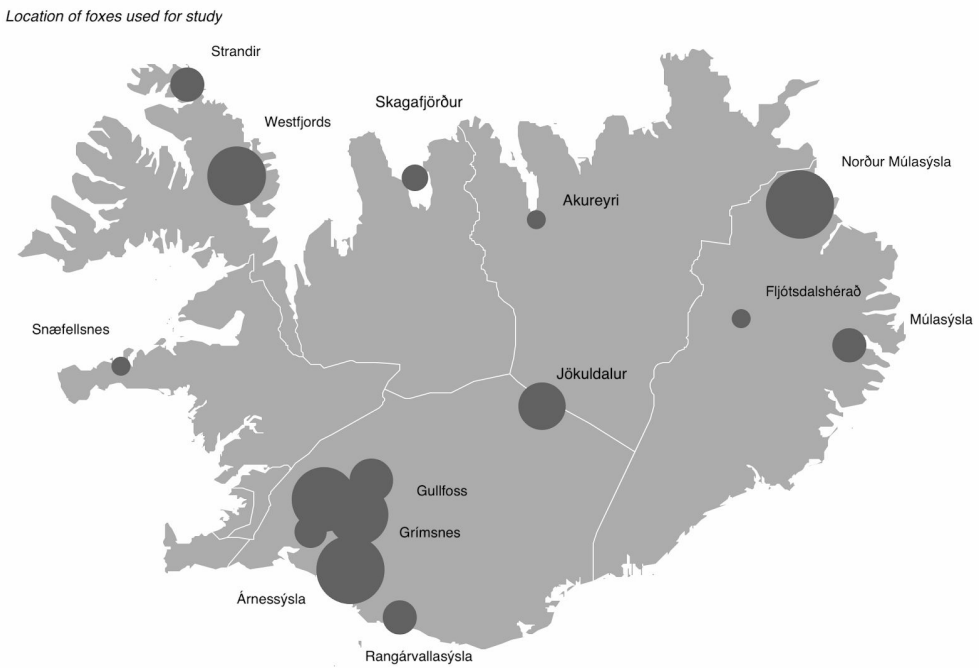

Figure 1: Location of culled foxes obtained for study. Size of dots correspond to sample size (n=129).

A sample of 129 foxes from 2005 (n=1), 2006 (n=1), 2007 (n=1), 2008 (n=5), 2009 (n=60), 2010 (n=60), 2011

(n=60), 2013 (n=2), 2015 (n=1), 2016 (n=15) and 2017 (n=12). Of the individuals involved in this study were, 39.53% (n = 51) were female and 59.68% (n = 77) were male. The mean age of individual foxes used in the study was 1.4 months. Sample group were of both blue (n=75) and white (n=47) colour morphs (see Figure 3) and were killed throughout the year (see Figure 2) with most culled during the winter (n=82), but also spring (n=19), summer (n=5), and autumn (n=22). A minority of vixens had been breeding the previous year (n = 12) and five were lactating or carrying offspring. Lower jaw lengths were measured with an average for foxes less than one year of age 86.38 cm, one year 89.72 cm, and two year 94.05 cm.

Table 1: Number of foxes in final sample group killed per year (n=129).

Year of kill	Number of foxes
2005	1
2006	1
2007	1
2008	5
2009	60
2010	60
2011	60
2013	2
2015	1
2016	15
2017	12

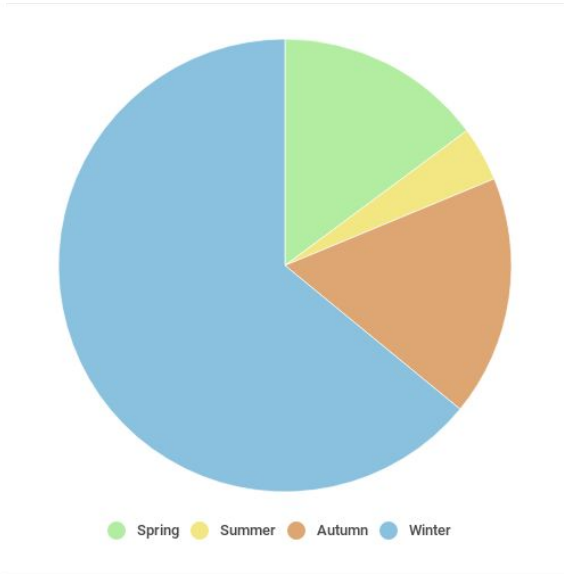

Figure 2: Seasons in which foxes of final sample group were culled (n=129).

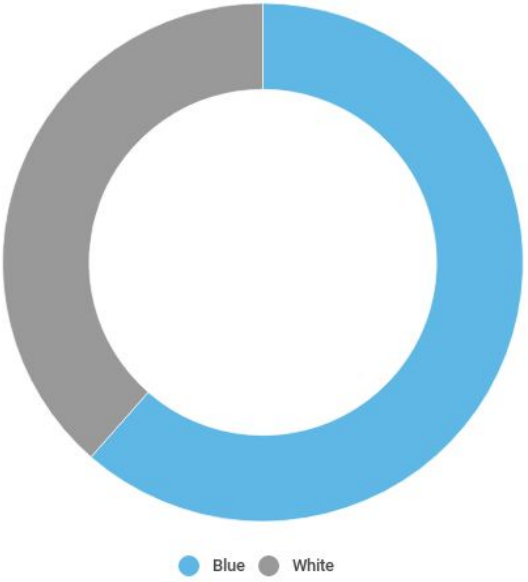

Figure 3: Sex and colour morph of final sample group (n=129).

3.2 Sample Collection

Gastrointestinal tract was thawed for one hour and two grams of gastrointestinal material was removed from across the length of the intestines. Samples were stored in an eppendorf and homogenised with 2 ml sterilized normal saline using a vortex machine.

3.3 Parasites of Interest

This study selected the following parasites of interest based on a combination of clinical relevance to human health and threats to livestock or wild populations. Species were selected that had been previously identified as persisting in Iceland, either within foxes (Skirnisson et al., 1993) or other species (Skirnisson et al., 2016; Petersen and Haraldsson, 1993; Smaradottir and Skirnisson, 1996; Sigurdarson, 2010; Skirnisson, et al., 1993), or within Arctic foxes outside of Iceland (Elmore et al., 2013; Friesen et al., 2015) (see Table 2). These parasites of interest included three different types of intestinal parasite - cestoda; *Diphyllobothrium* sp., *Echinococcus* sp., *Mesocestoides canislagopus*, *Taenia ovis*, nematoda; *Capillaria aerophila*, *Strongyloides stercoralis*, *Toxascaris leonina*, *Toxocara canis*, *Trichinella* sp., and protozoa; *Cryptosporidium* sp., *Giardia* sp., *Toxoplasma gondii*.

Table 2: A list of target species for this study with information on zoonosis, status within Iceland, host species, and distribution.

Species	Description
Capillaria aerophila	A lungworm species selected as a parasite of interest for this study as is known to be prevalent in the Icelandic Arctic foxes (Skirnisson et al., 1993), and potential to cause fatal disease in canines (Traversa et al., 2011).
Cryptosporidium sp.	A common species of parasitic protozoa (Hilmarsdottir et al., 2011) which has been identified as contaminating playground sandboxes in the city (Smaradottir and Skirnisson, 1996), infected farmed foxes (Elmore et al., 2013), and livestock (Skirnisson et al., 1993). Selected as a parasite of interest for this study as can be fatal in all hosts, and has been identified as causative agent in human disease (Eydal et al., 1990).
Diphyllobothrium sp.	This tapeworm species selected as a parasite of interest for this study as has been previously recorded in Iceland (Skirnisson et al., 1993) and can cause fatal infections – Diphyllobothriasis - in humans (Kutcha et al., 2013).
Echinoccocus sp.	Selected for this study as was once a serious public health concern in Iceland (Sigurdarson, 2010) though has been considered eradicated since 1979 from all species in the country (Beard 1973; Skirnisson et al., 2003). The causative agent of hydatidosis, a debilitating or fatal disease in many hosts, is still present in the Arctic and mainland Scandinavia, and there is a risk of reintroduction.
Giardia sp.	A common protozoa causing acute diarrhoea that has been reported in humans in Iceland (Hilmarsdottir et al., 2011) and present in other populations of Arctic fox (Elmore et al., 2013). Selected as a parasite of interest in this study as has not yet been recoded in Icelandic Arcitc foxes though has been found in children's public play areas and thought to be spread by companion animals (Smaradottir and Skirnisson, 1996).
Mesocestoides canislagopus	Mesocestoides was selected as a parasite of interest due to the high prevalence of the tapeworm in foxes in previous studies in Iceland (Skirnisson et al., 1993) and the objective of this study to comment on changes in parasitic abundance over the last two decades.
Strongyloides stercoralis	This parasite was chosen as it has been recorded in quarantined domestic dogs being imported into Iceland, though has not been found in the wild (Akuffo, 2003) though has been recoded in one Icelandic household dog (Skirnisson, 2012) which suggests it may have spread into the country. S. stercoralis may be moving between Arctic foxes and dogs during interactions in the countryside.
Taenia ovis	A recently recorded species infecting Icelandic sheep (Skirnisson, 2017) which can cause meat to be downgraded or rejected at abattoir (Erickson, 2018). This species has been selected for study as foxes are known to scavenge adult sheep carcasses and predate on young lambs (Hersteinsson and Macdonald, 1996) which may cause transmission of the parasite to the foxes.
Toxascaris leonina	Chosen as a parasite of interest in this study due to its risk to human health (predominantly, nutritional deficiency; Kaleva and Raynova, 2015) and high prevalence already recorded in Icelandic Arctic foxes of up to 50% (Skirnisson et al., 1993).
Toxocara canis	This zoonotic nematode has been selected as a parasite of interest as it has been recorded in Icelandic companion dogs (Zhu et al., 2015) and can cause serious human disease included blindness in young children, myelitis, and epilepsy (Finstere and Auer, 2007). Additionally, the parasite has been recorded once before in the Icelandic Arctic fox (Skirnisson et al., 1993).
Toxoplasma gondii	A protozoan parasite selected for study as it has been recorded in humans in Iceland (Birgisdottir et al., 2006), and is common in Arctic foxes outside of Iceland. Additionally, T. gondii can cause severe disease and death in immunocompromised immune systems (Hoshino et al., 2014).
Trichella sp.	Trichinella sp. have been reported in high abundance in the waters surround Iceland, and has been selected for this study as it has yet to be recorded within the country itself despite the movement of infected polar bears and seals onto the land.

3.4 Microscope Identification

Apacor Mini Parasep® was used to concentrate eggs, larvae and cysts. The enclosed system, an advanced version of the Ridley-Allen formalin-ether sedimentation technique, was used with approximately one gram of faeces. Samples were mixed with 6 mL of 10% formalin in water in the mixing chamber and two drops of Triton-X to aid in the emulsification of the sample, and the mixing chamber was screwed into the rest of the system before vortexing for 15 seconds. The Parasep was inverted for homogenisation of the faeces samples to filter through the 425 μm gauze during centrifugation at 1200 *g* for three minutes. Both fatty plug and formalin supernatant were discarded and 4 x 5 μl samples were taken from the deposit at the bottom of the system. Samples were examined under the microscope: one stained with iodine, the other unstained with saline, and each ran in duplicate. Ova were counted for each coverslip using 100x objective, covering the slide in approximately 15 overlapping fields. Smaller protozoa were searched for using 400x lens. Photographs were taken using a magnification of 400x.

Ova were identified using the Center for Disease Control diagnostics resources for morphological identification. Ascarid eggs were distinguished by the appearance of their shell; *Toxascaris leonina* identified by a lamellated shell, and *Toxocara canis* identified by a distinctive granular texture.

3.5 DNA Extraction

Genomic DNA was extracted from the faecal samples using a modified QIAamp DNA Stool Mini Kit (Qiagen) with an extended lysis time to increase nematode egg degradation. 200 μg of stool was emulsified in a 1.4 ml buffer in a microcentrifuge tube and vortexed until fully homogenised. The protocol was modified by heating the sample for 15 minutes at 95°C, rather than 5 minutes at 70°C, to better lyse

parasite ova, cysts, oocysts and larvae, and therefore increase DNA yield. The manufacturer protocol was then followed until the wash stage after incubation which was repeated once in an attempt to reduce salt content as recommended by Qiagen (pers. comm. Qiagen, 2016). The protocol was then followed for the remainder of extraction and eluted DNA was stored at -80 °C.

3.6 Primer Design and PCR

Table 3: Primers selected for use in qPCR.

Parasite	Forward primer and reverse primer	Probe	Reference	Target gene	Genbank
Cestoda	5'- TAGGTCGACCCGCTGAAYTTAAGC-3'	5'- CAAGTACCGTGAGGGAAAGTTG-3'	Olson et al., 2003,	28S rRNA	AM931032.1
	5'- GCTATCCTGAGGGAAACTTCG - 3'				
Cryptosporidium	5'-AACTTCACGTGTGTTTGCCAAT -3'	5'- CATATGAAGTTATAGGGATACCAG-3'	Mejia et al., 2013	DNA J Like Protein	AF279916.1
	5'-CCAATCACAGAATCATCAGAATCG-3'				
Echinococcus	5'-CTGTGATCTTGGTGTAGTAGTTGAGATTT-3'	5'-TGGTCTGTTCGACCTTTTTAGCCTCCAT-3'	Knapp et al., 2014	rrnL	AB208545.1
	5'-GGCTTACGCCGGTCTTAACTC-3'				
Giardia	5'-CATGCATGCCCGCTCA -3'	5'-AGGACAACGGTTGCAC-3'	Mejia et al., 2013	16S rRNA	AJ293299.1
	5'-AGCGGTGTCCGGCTAGC-3'				
Mesocestoides	5' - TCTATAGTATGTTTAGGGAGTAGTGT- 3'	5' TCCAACAGTAAACATGTGGTGCCCTCA-3	Per. comms. Eva Myskova	18S rRNA	AY426257.1
	5' - ACGACAGGACCCATCACAAT- 3'				
Strongloides	5'- GAATTCCAAGTAAACGTAAGTCATTAGC -3'	5'-ACACACCGGCCGTCGCTGC-3'	Sultana et al., 2013	18SrRNA	AF279916.1
	5'-TGCCTCTGGATATTGCTCAGTTC -3'				
Taenia ovis	5' - GACCGATGGTGAATCTGGGT- 3'	5'-TGGTGGTACTGCACTCCACCGCA-3'	Designed	ITSm	KU639651.1
	5' - AGGCACAGTAGAAGTCGACAA- 3'				
T. leonina	5'- GGCTAAGCCATGCATGTC-3'	5'-AAACCGCGAACGGCTCAT-3'	Designed	18S rRNA	AB110034
	5'- ACTTGATAGACACGTCGCC-3'				
T. canis	5'- GCGCCAATTTATGGAATGTGAT-3'	5'-FAM-CCATTACCACACCAGCATAGCTCACCGA-3'	Durant et al., 2012	ITS2	AB110034
	5'- GAGCAAACGACAGCSATTTCTT -3'				
T. gondii	5'-TCCCCTCTGCTGGCGAAAAGT-3'	5'-TCTGTGCAACTTTGGTGTATTCGCAG-3'	El-Geddawi et al., 2016	B1 Gene	AB110034
	5'-AGCGTTCGTGGTCAACTATCGATTG-3'				
T. spiralis	5'- TGTGTGTATGATGAACAACGCG-3'	5'-TTTATTAAATGATCAGTGTTGAGGTGCTGTTGTGAT-3'	Designed	16S ITS	AJ293299.1
	5'- TCATTGTCACTTGCCACACTGA-3'				

Primers and probes were designed by using Primer3 (http://primer3.ut.ee) using default parameters (18-22 bp, spanning exon junctions, 40% - 60% GC content). NCBI's nucleotide Basic Local Alignment Search Tool (BLAST: https://blast.ncbi.nlm.nih.gov) was used to run multiple alignments (Appendix 2) to check target genes for specificity using FASTA format of the nucleotide sequence.

PCR assays described in previously published studies were used in most of this projects assays, though some were designed for the purpose of this study when species specific primers could not be found in the literature.

These primers were tested using the BLAST algorithm for 100% query coverage and maximum identity. Designed primers were run using cDNA in 1 uL, and tested against three concentrations (2, 5, 10 pmoles / uL) and melt curve peaks were analysed to determine primer concentration. Primers in 1pmoles / uL concentration were used in the assays described in this study. A sample-free control was run to test for primer dimer, and product used on a 2.5% agarose gel and compared to expected size of product.

Each set of primers and probes was synthesized by Sigma Aldrich, and all assays were conducted in 96-well optical plates (Applied Biosystems).

TaqMan probes (Sigma Aldrich) were labelled with fluorophore 6-carboxyfluorescein (FAM) and quencher tetramethylrhodamine (TAMRA) or Black Hole Quencher-1 (BHQ-1). PCR reactions were carried out in a total volume of 25 µl using reagents from Invitrogen (preparing master mix using 0.15 µl of Taq DNA polymerase (1.25 units/50 µl PCR), and 2.5 µl of 10X buffer, 10 mM 2.5 µl dNTPs and 2.5 µl 50mM $MgCl_2$), with 2 µl template, 10 µM 2.5µl of each primer and 7.85 µl purified water. All reactions were carried out using AriaMx Real-Time PCR System (Agilent Technologies, Inc) and all cycles included a 95 °C phase for two minutes for polymerase activation. The standard thermocycler conditions set for these assays was an initial denaturation at 95°C for 30 seconds, 95 °C for 15 seconds, and a final extension for 5 minutes at 68 °C for 30 cycles.

Reagents including Taq were confirmed to work as expected during other research in the same laboratories (Sally Cutler, pers. comm.). The reaction plates were loaded with 14.65 µl of master mixes for each target. The template and controls were added according to plate setup, accounting for duplicates of both no template controls and positive controls of the target organism.

Where possible, samples were run alongside duplicate positive controls using known positive extracted DNA for the target species to check primers were amplifying correctly (*Mesocestoides, Toxocara canis, Giardia, Echinococcus* and *Trichinella*). All positive controls used the assays were extracted DNA obtained from the University of South Bohemia in České Budějovice (by courtesy of Eva Myšková) or from the University of East London (by courtesy of David Giuliano). Positive controls were not included in some assays due to financial restrictions and limited resources. The results were considered negative if cycle threshold (Ct) values were > 25. Nanodrop results revealed a good yield with an average of 16.56ng/ul (see Appendix 4).

Table 4: Cycle conditions for PCR assays deviating from standard settings

Target	Thermocycler conditions
Echinococcus sp.	45 cycles of 95 °C for 15 s and 60 °C for 60 s
Strongyloides stercoralis	40 cycles of 95 °C for 15 s and 60 °C for 30 s
Toxocara canis	45 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 5 s
Toxoplasma gondii	40 cycles of 95 °C for 15 s and 60 °C for 60 s

3.7 Statistical Analysis

Two sample z-testing was used for all comparisons between demographics (i.e. age, sex, location, ecotype) to compare whether the average difference between the two groups in each case is significant. This test was used for statistical analysis as the two groups are always independent from one another, and the data can be assumed to be a normal distribution.

4. Ethics Statement

Arctic foxes are culled in Iceland as part of an initiative to protect rare bird species and livestock. All foxes in this study were carcasses that were sacrificed under this government drive and are stored and studied as part of a population dynamics study that has been ongoing since 1979.

5. Results

The results of this dissertation revealed more positive results by microscopy (n=86) for foxes infected with target endoparasitic species compared to molecular methods (n=22).

5.1 Parasite Richness and Prevalence

Table 5: Parasitic abundance using microscopic and molecular methods. Dashes indicate that parasites were not tested with molecular methods.

	Microscope	Molecular
Capillaria aerophila	**9**	-
Cryptosporidium	0	0
Diphyllobothrium	**9**	-
Echinococcus multicularis	0	0
Giardia	**0**	**0**
Mesocestoides	0	12
Strongloides	**0**	**0**
Taenia ovs	0	0
Toxascaris leonina	**47**	**8**
Toxocara canis	12	2
Toxoplasma gondii	**6**	**0**
Trichinella spiralis	0	0
Eimeria / Isospora	**2**	-
Cryptocotyle lingua	1	-

All 129 faecal samples were analysed by microscopy, and 109 of these were selected at random for PCR analysis (See Appendix 1). Eight intestinal parasites were identified in the gastrointestinal tract of the 129 foxes in the sample group (Table 5). Examination revealed 66.9% (n=86) of foxes had an endoparasitic infection: *Capillaria aerophila* 6.9% (n=9), *Diphyllobothrium latum* 6.9% (n=9), *Mesocestoides* 9.3%

(n=12), *Toxascaris leonina* 36.4% (n=47), *Toxocara canis* 9.3% (n=12), *Toxoplasma gondii* 4.7% (n=6), *Eimeria* or *Isospora* 1.6% (n=2), and *Cryptocotyle lingua* 0.8% (n=1).

Two of the three molecular positives were a subset of those detected by microscopy, while one species (*Mescocestoides* sp.) was detected by molecular but not microscopy methods. The remaining six species were negative for both molecular and traditional methods. Neither microscopy or molecular method detected any results for *Cryptosporidium* sp., *Echinococcus* sp., *Giardia* sp., *Strongyloides stercoralis*., *Taenia ovis*, or *Trichinella* sp..

5.2 Molecular Results

Analysis by PCR revealed 20.2% of 109 foxes had an endoparasitic infection (see Appendix 3): *Mesocestoides* sp., 11% (n=12), *Toxascaris leonina* 7.4% (n=8), and *Toxocara canis* 1.8 % (n=2) (see Table 5). Half of the positive controls were successfully amplified (see Table 6).

Table 6: Amplification of results and positive controls suggesting if primers were working

	Positive results	Amplification of positive control
Cryptosporidium	No	Yes
Echinococcus	No	No
Giardia	No	No
Mesocestoides	Yes	Yes
T. ovis	No	-
T. leonina	Yes	-
T. canis	Yes	Yes
T. gondii	No	Yes
T. spiralis	No	No

5.3 Overall Parasite Abundance

Toxocara leonina was found to be the most abundant species inhabiting the Arctic fox, with 36.4% of individuals infected with the parasite, more than three times the number of the second most abundant, *Toxocara canis* (9.30%) (Table 5; Figure 4). *Diphyllobothrium latum*, *Capillaria aerophila*, and Toxoplasma were present in many fewer individuals, with abundance rates of 7-8% respectively

Parasitic species appears to be very similar across both sexes, with similar infection rates for both female and male foxes (Figure 13). Two sample z testing revealed no statistical difference.

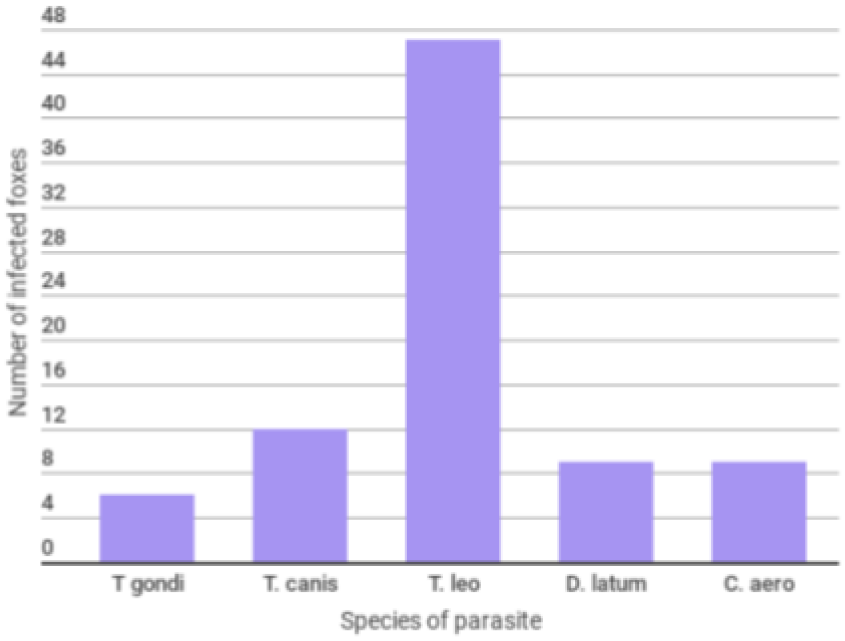

Figure 4: Five most abundant parasites (*Toxoplasma gondii, Toxocara canis, Toxascaris leonina, Diphyllobothrium latum*, and *Capillaria aerophila*) as measured by microscopy (n=82).

5.4 Parasite Intensity

The numbers of conspecific parasites living in an infected host, calculated as the average of the four replicates of ova count per coverslip (i.e. mean parasite intensity), were *Capillaria aerophila* (3.6), *Diphyllobothrium latum* (12.7), *Toxocara leonina* (12.9), *Toxascaris canis* (13.6), and *Toxoplasma gondii* (0.79). The following sections discuss the variation of the four most abundant of these by sex and age.

5.4.1 Intensity of *Toxascaris leonina* Estimated by Egg Count

In the 47 foxes in which *Toxascaris leonina* were found, subadult foxes (≤ one year of age) had a wider variation of parasitic intensities than adults for both sexes, with ranges of around 2-32 egg counts per fox (Figure 5). Adult males had a narrower variation (interquartile range 15-22) and higher median (20) than the three other groups, showing a systematically higher burden of parasitic infection.

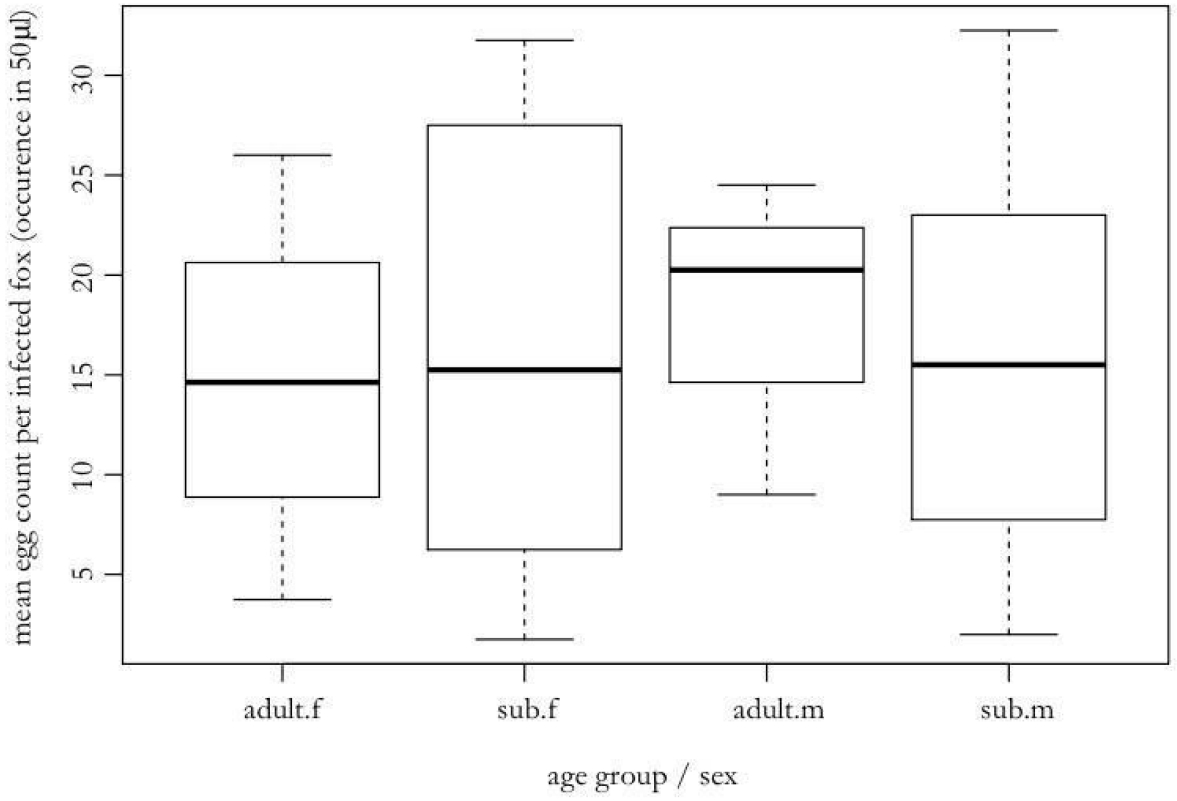

Figure 5: Mean egg counts of *Toxascaris leonina* in infected foxes. Box and whiskers show median, interquartile range and full data range.

5.4.2 Intensity of *Toxocara canis* in Infected Foxes

Toxocara canis was found in 12 foxes, though was completely absent from adult males (Figure 6). As for *Toxascaris leonina*, subadults had wider variation in parasitic intensities than adults, particularly subadult males (egg count range 3-24). Conversely, the variation was smaller (range 13-20) and median higher in adult female foxes (18) than the other groups, i.e. a higher parasitic burden in adult females rather than adult males.

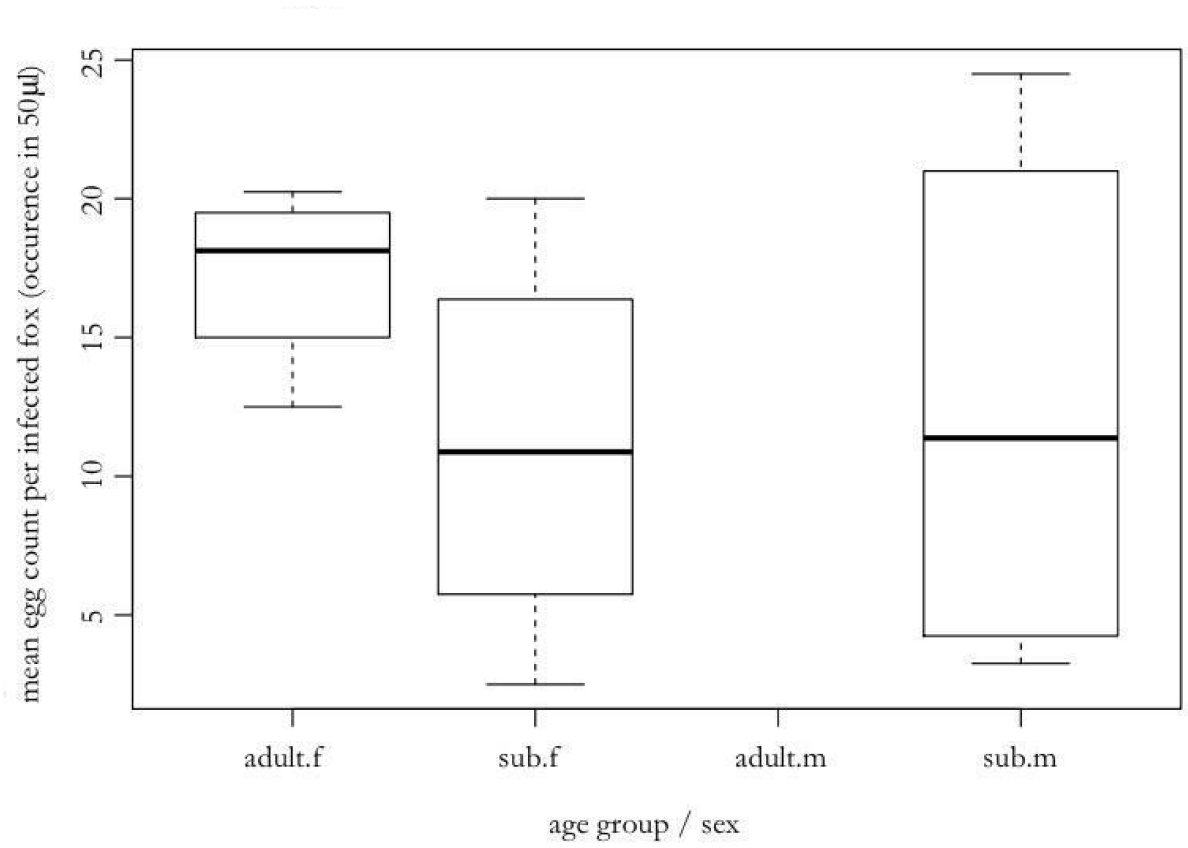

Figure 6: Mean egg count of *Toxocara canis* in infected foxes. Box and whiskers show median, interquartile range and full data range.

5.4.3 Intensity of *Diphyllobothrium latum* in Infected Foxes

Diphyllobothrium sp. were found in 9 foxes: it was not observed in any adult females, and only in two younger females with an average egg count of 5 (Figure 7). *Diphyllobothrium latum* egg counts were both higher (median 15 and 20 for adults and subadults respectively) and more variable (from n=1 / n=2 to n=29 / n=25 for adults and subadults) in male foxes.

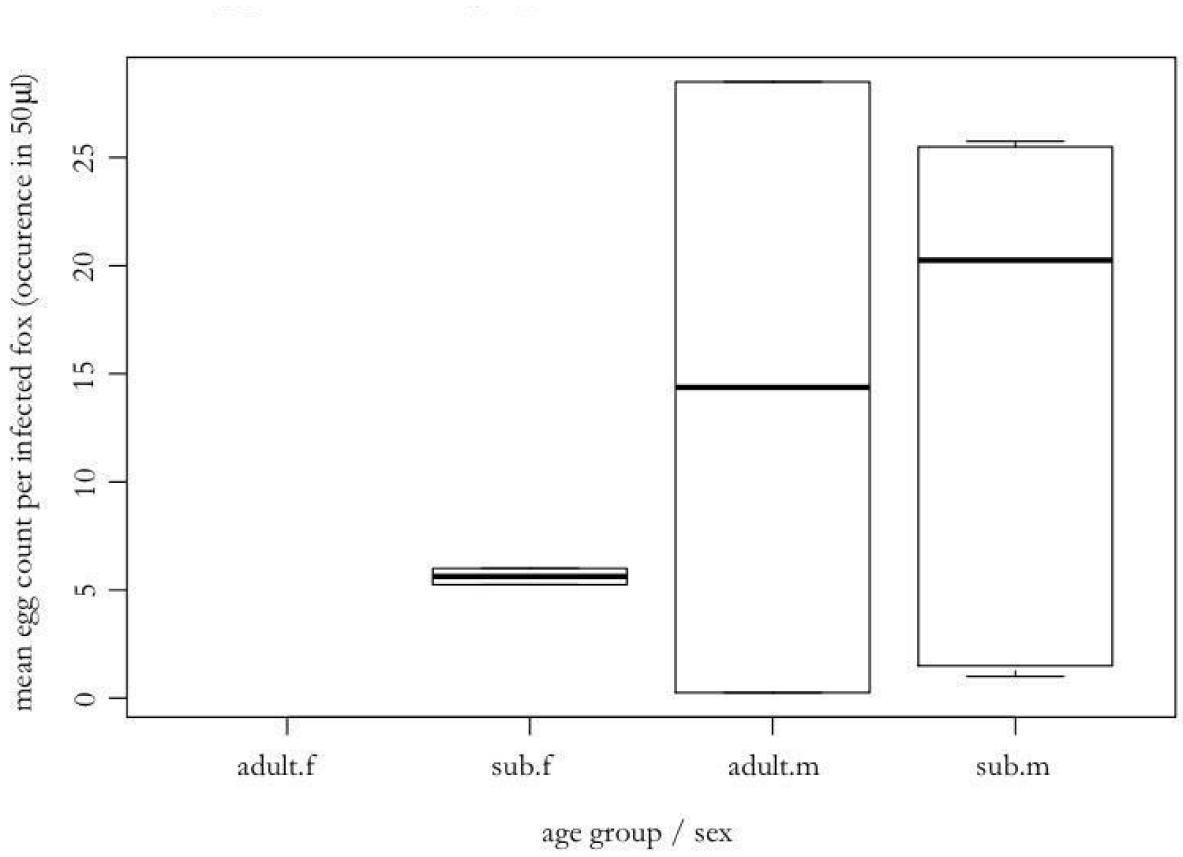

Figure 7: Mean egg counts of *Diphyllobothrium latum* in infected foxes. Box and whiskers show median, interquartile range and full data range. No eggs were detected in adult females.

5.4.4 Intensity of *Capillaria aerophila* in Infected Foxes

Capillaria aerophila eggs were found in 9 foxes. Mean egg counts were lower than for the previous three species discussed (Figure 8), with the greatest variation and highest count in subadult males (maximum = 8).

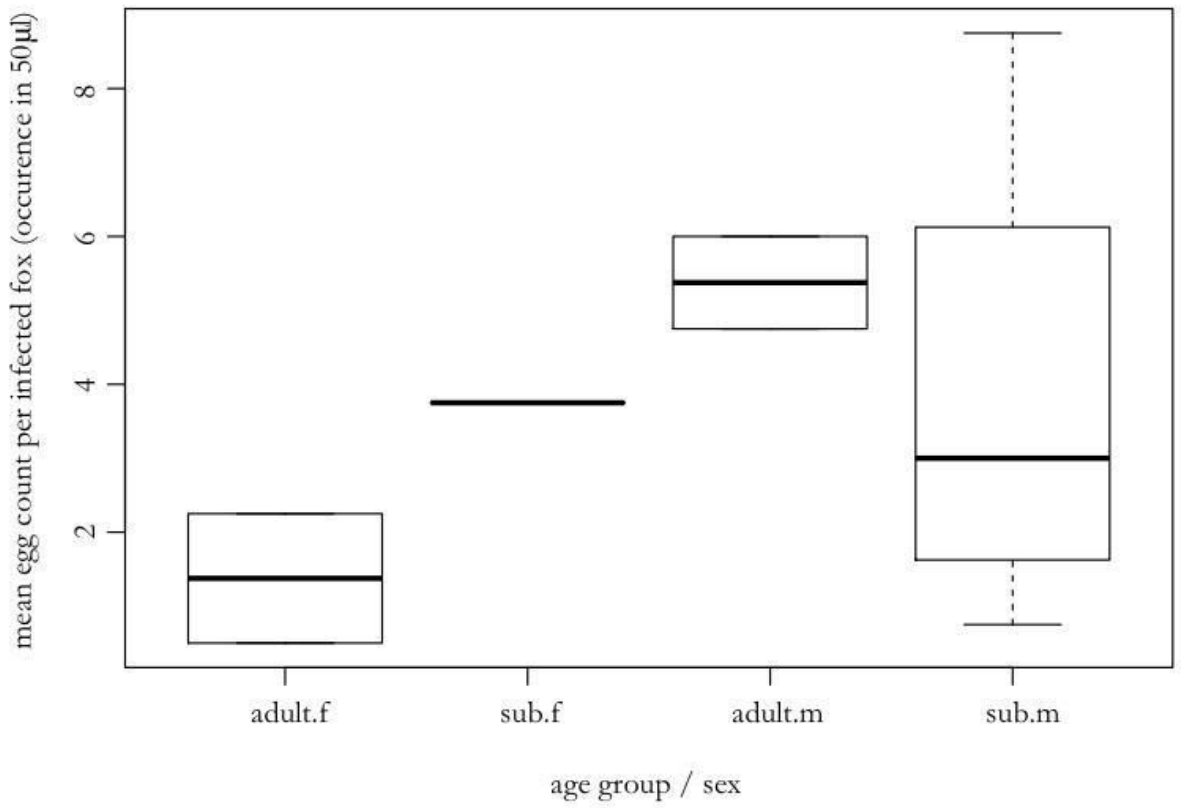

Figure 8: Mean egg count of *Capillaria aerophila* in infected foxes. Box and whiskers show median, interquartile range and full data range.

The microscope method used in the project allowed for the photographing of parasitic eggs to help with the accuracy of identification. These images have been included here and discussed in later sections of this thesis.

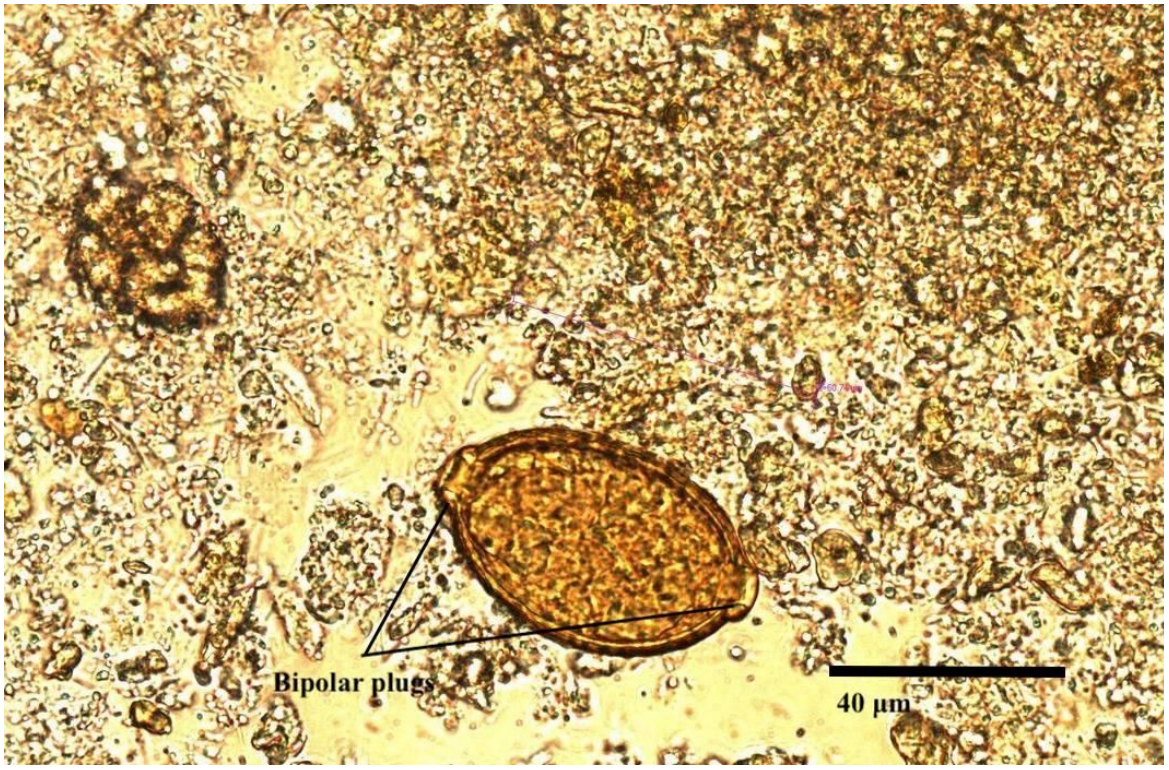

Figure 9: Microscope image of *Capillaria aerophila* with visible bipolar plugs from sample 11025. Magnification is 400x.

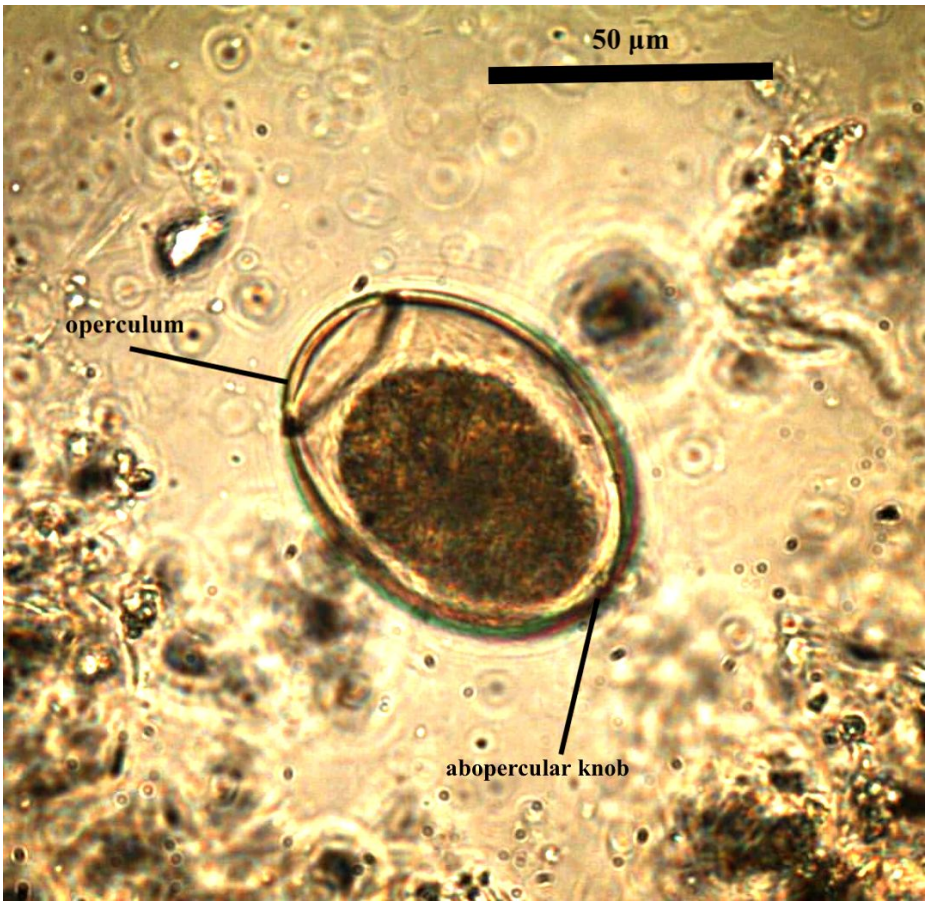

Figure 10: Photograph of microscope slide of *Diphyllobothrium* sp egg with visible operculum and abopercular knob from sample 8801. Magnification is 400x.

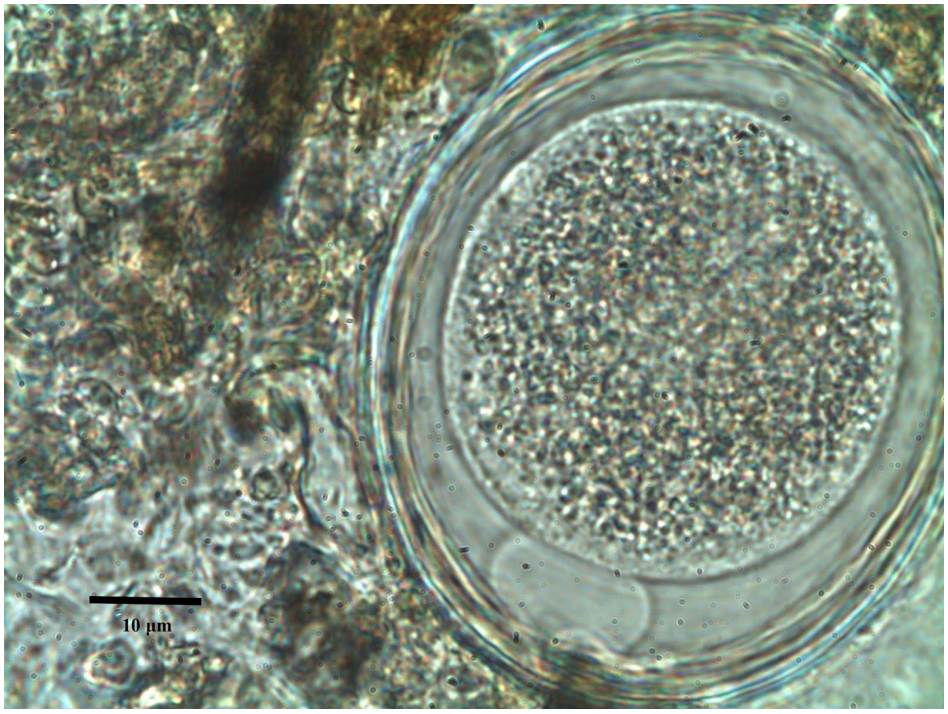

Figure 11: Photograph of *Toxascaris leonina* from sample number 8840. Magnification is 400x.

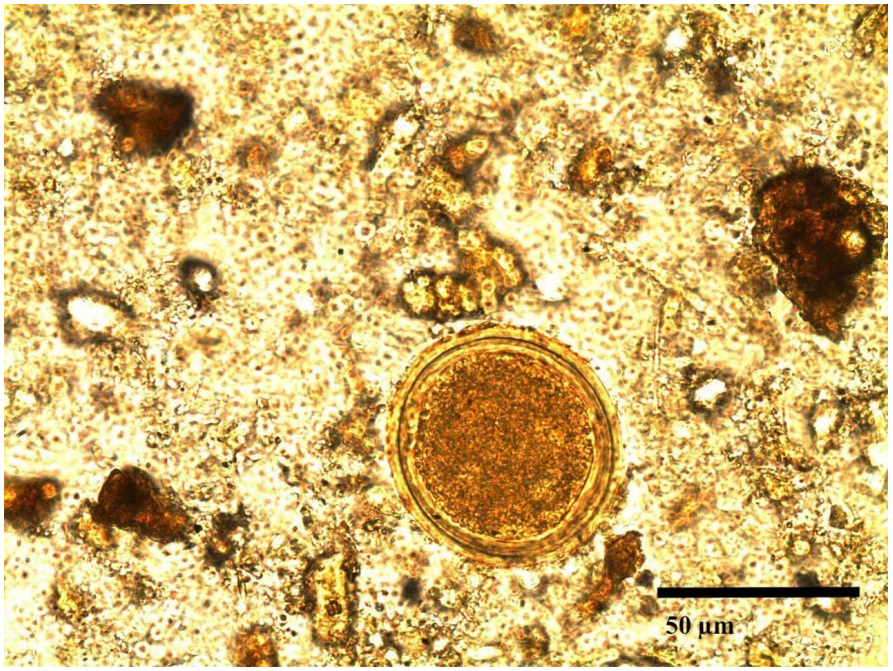

Figure 12: Photograph of *Toxocara canis* from sample number 10938. Magnification is 400x.

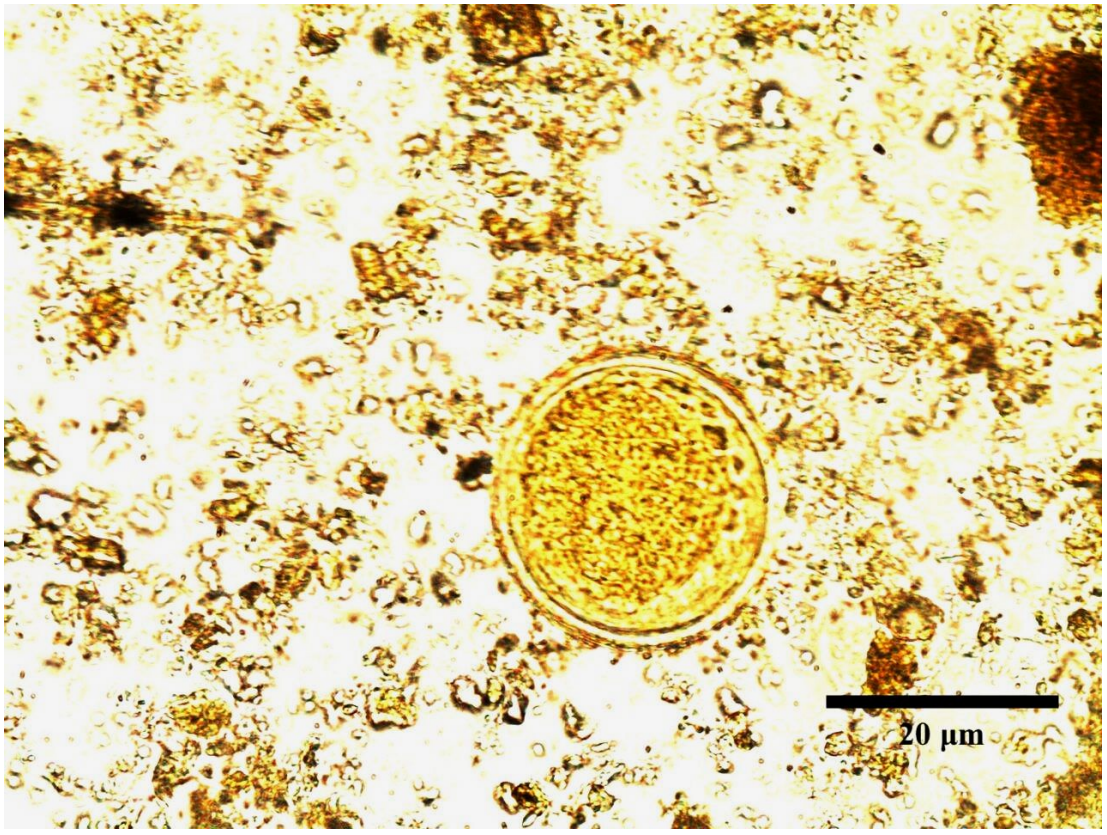

Figure 13: Photograph of *Toxoplasma gondii* from sample number 8904. Magnification is 400x.

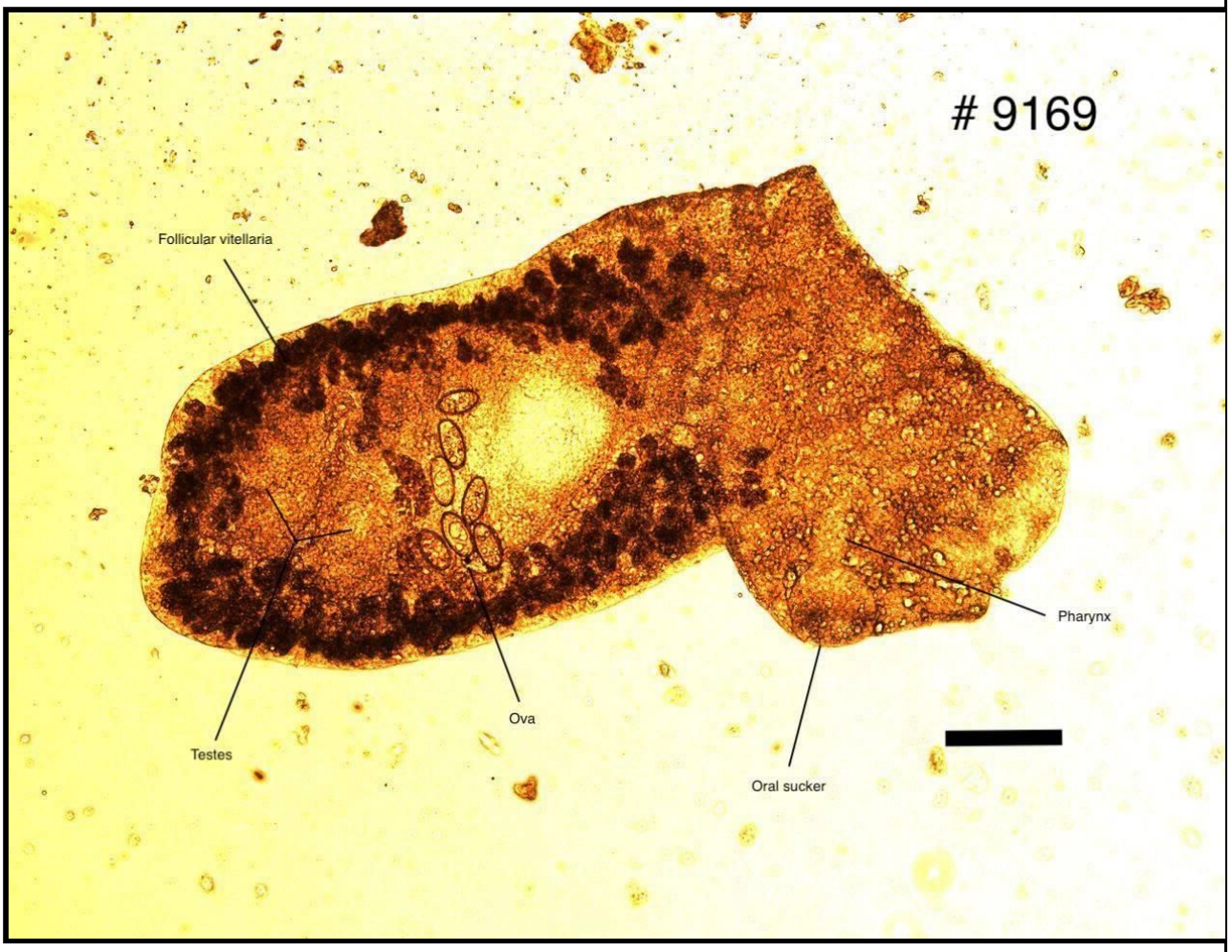

Figure 14: Microscope image of an adult *Cryptocotyle* sp. from sample number 8887. Magnification is 40x.

5.5 Co-infection

Co-infection was observed in 11.6% of the infected foxes, with 0.7% (n=1) infected with three intestinal parasites or more. Co-infection occurred at a higher rate in samples infected with *Toxascaris leonina*. Of the 47 foxes infected with *Toxascaris leonina*, 26 (63.4%) were single species infections while the remaining were infected with one or more species, predominantly *Toxocara canis* (12.2%), *Diphyllobothrium* (9.8%), and *Toxoplasma gondii* (9.8%) (see Figure 15).

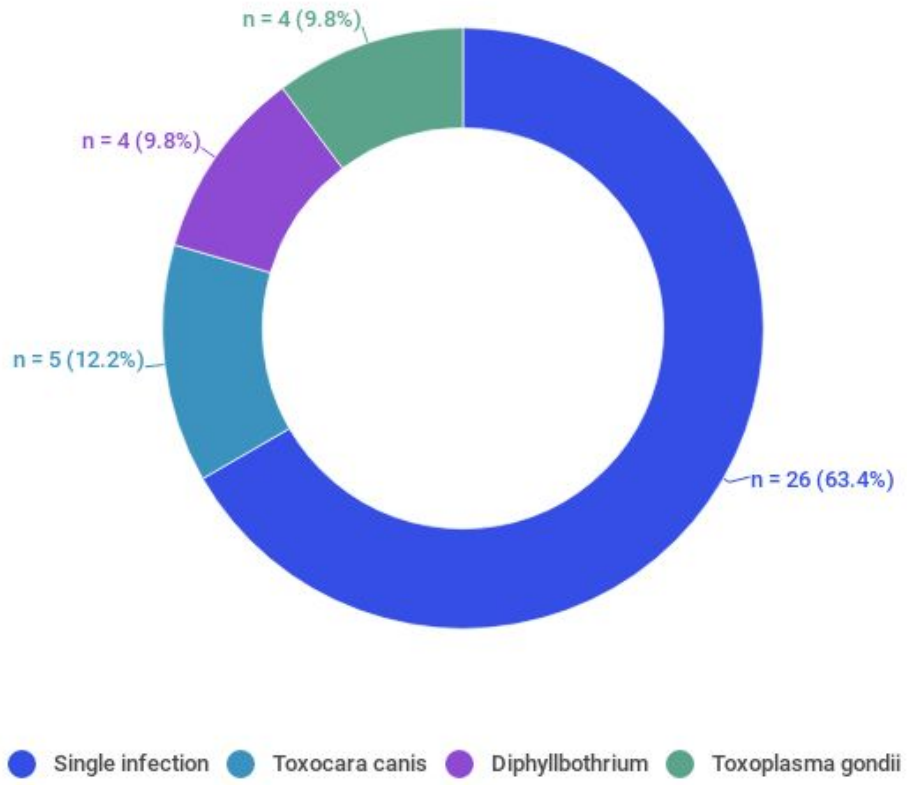

Figure 15: Abundance of coinfection (multiple species of endoparasites inhabiting a single host) in foxes infected with *Toxascaris leonina* (n=39).

6. Discussion

6.1 General Abundance, Richness, and Intensity

In general, parasite abundance and richness were lower than found by Skirnisson et al. (1993). This is particularly unexpected because of the focus in this dissertation on detecting eggs, rather than adults, and the use of molecular methods, rather than microscopy. It is also unexpected as climatic effects and human

activities (including an increase in immigration and importation of domestic animals) are being observed to increase parasitic abundance around the world. This is predominantly caused by the global temperature which has increased, supporting the survival and spread of parasites as well as increasing host range (Short et al., 2017) by causing faster larvae development in helminths which decreases their time to infectivity and the increased precipitation levels which may be preventing desiccation of eggs or larvae and increasing the survival rates of these endoparasites (Weaver, Hawdon & Hoberg, 2010).

Despite these factors, a large decrease was observed in the abundance of target parasitic species in comparison to Skirnisson et al. (1993). The largest decrease of which was observed in *Mesocestoides* sp. The reasons for this difference are unclear but may be as a result of the cumulative effect of legislation introduced to Iceland in the 1980s for the eradication of *Echinococcus* sp.. However, large natural variation could not be ruled out until further studies are completed.

This study saw the extraction of 130 faecal samples from individual Arctic fox carcasses, and successfully achieved the projects aims to use one traditional and one molecular method to identify and quantify the species present, those PCR was found to be less accurate in this instance than microscopy. Additionally, the species abundance, burden, and richness were all analysed according to sex, age, and ecotype and successfully determined the differences and similarites between these demographics. Comment and comparison of this study to the previous study (Skírnisson et al., 1993) was also successful, and the differences discussed below (see Table 6).

6.2 Limitations of Study

These differences could be a result of a genuine decline or fluctuation in the abundance and richness of target species, or else limitations of the methods, such as missing obscured, distorted, and ruptured ova as a result of working with frozen faeces. These are various limitations of the study methods which are discussed here.

The carcasses had undergone several freeze-thaw incidents during multiple transits and removal from chest freezers for evisceration, meaning ice crystals had caused distortion. It is known that nematode eggs from cryogenically stored faeces have a dramatically reduced egg count in comparison with fresh samples, with empty and ruptured eggs observed in samples after just several days of freezing (Van Wyk and Van Wyk, 2002). While some eggs are adapted and more robust against the effects of freezing, it is possible that as a long term storage solution it may be affecting egg counts of all parasites of interest in this study. Other studies have shown that PCR using long-term frozen urine as the source of DNA template does lead to more false negatives than using fresh urine as the temperature affects both the performance of DNA extraction and decreases the sensitivity of PCR (Fernández-Soto at al., 2013). This may be the case with the frozen faeces used for this dissertation.

While the Nanodrop results revealed a good yield with an average of 16.56ng/ul (see Appendix 4), these results can not confirm, though the DNA is certainly present, if it may have suffered fragmentation damage as observed in other studies (Thomson et al., 2009) which may have impacted the efficiency of the PCR analysis. More likely, however, is that the DNA extracted from the faecal samples was not pure. The 260/280 ratio for the majority of samples were much lower than the ideal 1.8 (mode = 1.48, median = 1.62) (see Appendix 4) which indicates the presence of protein or other contaminants which could have

impacted the molecular assays. Therefore, the results may have been hindered due to the presence of faecal inhibitors such as complex polysaccharides, bile salts, lipids, and urage (Tabassam et al., 2012; Rådström et al., 2004). In addition to the difficulties of PCR inhibition due to the quality of DNA, other difficulties arose during extraction. Arctic fox gastrointestinal material and faeces contain an extensive amount of hair, feathers, plant material, and bones, that makes DNA extraction severely limited and in some cases not possible. The culmination of these factors have potentially reduced the efficacy of PCR methods in this study.

While PCR was not as sensitive as hoped for this dissertation,there is some value to knowing that this molecular method can be used in parasitological studies where there are no other options. It was not known before this dissertation the degree to which molecular methods could successfully identify parasites in frozen faecal samples that had been stored for a significant number of years. The obtention of some positive samples suggests the method is possible, and the techniques may be suitable for at least partial detection in environments where only degraded or frozen faecal samples are available.

In addition to the usual challenges of identifying endoparasites using molecular methods, the analysis of these samples using microscopy were susceptible to further difficulties. There are no studies quantifying the efficacy of detecting parasites in gastrointestinal material using bright field microscopy, so it is possible that morphology may differ from established morphological identification guides; i.e some eggs might have been dismissed as artefacts. Eggs may be underdeveloped in the intestines prior to being passed in the faeces, and telediagnostic guides are based upon passed faecal samples, which may also lead to misidentification or non-identification of eggs. Additionally, the particularly debris-laden faecal samples may have obscured ruptured eggs. As discussed above, the effect of freezing also appears to be

detrimental to eggs, causing rupturing, distorting, or discolouring. Despite these challenges this dissertation suggests microscopy is more valuable than molecular methods for parasitological studies involving frozen faeces, as PCR did not detect many of the positives identified using morphological methods.

PCR was carried out for the detection of all parasites of interest with the exception of *Capillaria aerophila* and *Diphyllobothrium latum* (see Table 5) due to budget and time constraints. This method was at least partially successful in detecting *Mesocestoides* sp., *Toxascaris leonina*, and *Toxocara canis* in the faecal samples but failed to detect *Toxoplasma gondii* in the samples that had been confirmed as positive by microscopy (see Table 5). Additionally, while PCR successfully amplified four of the positive controls (*Cryptosporidium* sp., *Mesocestoides* sp., *Toxoplasma gondii*, and *Toxocara canis*), the method failed to amplify *Trichinella* sp., *Giardia* sp., or *Echinococcus* sp. (see Table 5).

All primers were checked for specificity using Primer BLAST (see Appendix 2) and showed no signs of amplifying any non-target genes (see Appendix 2). However, some PCR assays did not return positives, even when predicted by positive microscopy results and others did not amplify even positive control.

Of the positive controls not amplified in PCR experiments, two of these (*Echinococcus* sp., and *Giardia* sp.,) may have been because this dissertation relied on the specific and method optimisation of the primers and thermocycler conditions that had been previously described in study and may not have been effective (Knapp et al., 2014; Melja et al., 2013). However, as the positive control for *Echinococcus* sp. had an extremely low yield of 0.35 ng/ul (see Appendix 4), this may have been responsible for the lack of amplification of the controls.

In one case, the lack of positive results may have been a result of assays not amplifying due to incorrectly assigned thermocycler settings due to user error. The *Taenia* sp., primers had an extremely low TM of 55 °C but were mistakenly heated to 65°C (see Table 4) which was likely to have prevented the annealing stage of the PCR cycle. This may have accounted for the failure of the assays to amplify, but as there was no positive results in the microscopy either for this species, it could have been negative in any case as the species as it has not been recorded in the Icelandic Arctic fox previously. This was also the case for *Strongyloides* sp., which was also not detected in microscopy or molecular methods, and was not run with positive control to confirm a robust negative (see Table 6). T*oxocara leonina* also did not have a positive control but was successfully identified in samples confirmed as positive in microscopy, so it can be assumed to be working correctly.

Trichinella primers did not amplify the positive control (see Appendix 3), which may be attributed to the probe being too long (35 base pairs) for qPCR. There was only one species of intestinal parasite (*Mesocestoides* sp.,) that was detected by molecular methods and not microscopy, which was expected as the species releases proglottids and not ova in faeces and therefore cannot be observed.

Given the greater success of the microscopy methods, and the additional information this method provides by estimating parasitic intensity as determined by egg count, the remainder of this discussion focuses on these results.

6.2 Parasite Species and Burden Differences in Location

For the purposes of ecological studies, Iceland has historically been divided by the East and the West (Figure 16), with foxes in the Eastern area considered 'inland' inhabiting and the West 'coastal' inhabiting. This division defines the coastline in the west which is 1.5 times longer than in the east (3884

km vs 1985 km), despite only being a quarter of the area of the country (20,850 km^2 vs 82,150 km^2). The habitats are vastly different, and the prey species for the foxes varies within these areas. This is reflected in the differing levels of heavy metals accumulated in the bodies of Western vs Eastern Arctic foxes, with recent research suggesting that mercury is present in high levels in the fox population in the coastal West and almost absent in the inland foxes in the East (Bocharova et al., 2013). The fox population is clearly genetically subdivided between the two regions (Norén et al., 2009), and there is an observed pattern of disease distribution including rabies and the ear canker mite (*Otodectes cynotis*) (Gunnarsson et al., 1991).

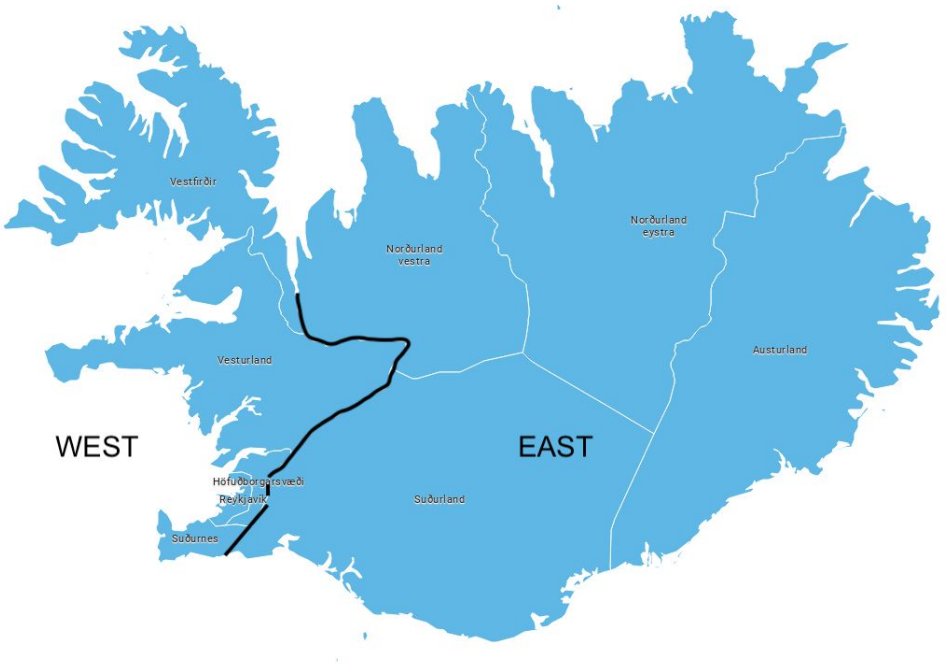

Figure 16: Geographical locations of the West and East testing areas. Most species of parasite detected were observed across all regions, with the exception of the species with the lowest abundance (*Toxoplasma gondii*) which was only detected in Vesturland and Austurland.

Often, parasites require specific environmental conditions, or ingestion by an intermediate host, which could affect their distribution: particularly between inland and coastal habitats, which differ in temperature, weather conditions, vegetation, and terrain, causing differences in feeding and breeding behaviour. This may have an impact on the distribution and occurrence of intermediate or paratenic hosts.

Migration of the foxes is hindered by a narrow isthmus present between the Westfjords and the rest of the island, as well as the extensive networks of sheep-proof fences that have separated 25 generations of foxes between the north west and central locations of the island. Glacial rivers are thought to disrupt the movement of foxes between the eastern and central areas during the summer months but foxes are able to cross in late winter when the rivers are frozen or shallow enough to cross (Noren et al., 2009). It is thought that these various factors may restrict movement of foxes causing them to become genetically and behaviourally distinct from each other (Noren et al., 2009).

The environment between the west and east of Iceland also differs, with the western area having a high proportion of productive shoreline that provides a variety of marine species as prey for the foxes. With these different food sources, it would be expected that there would also be different parasites present in the intestinal tract. Feeding behaviour can influence the exposure to prey in infectious stages (Despommier, 2003) and so if behavioural differences are observed as a result of the differing environments, a change may be seen between the eastern and western foxes.

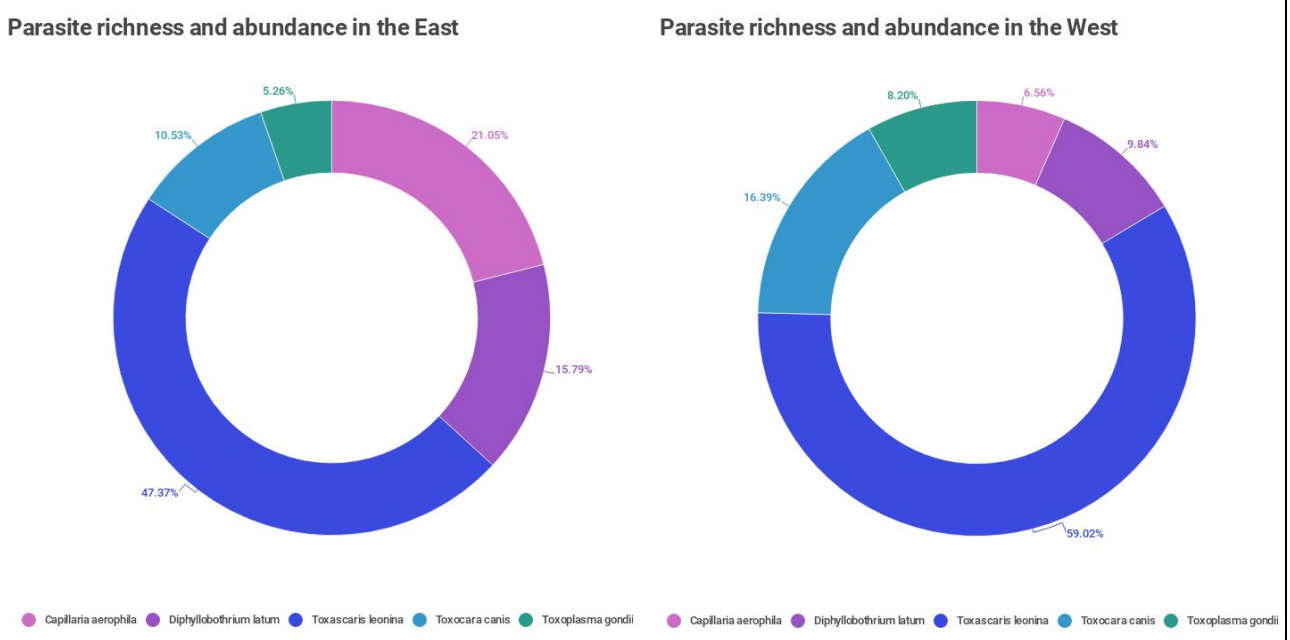

Figure 17: Parasitic richness and abundance according to geographical location, East (inland) vs West (coastal)

Despite the differences between the East (inland) and West (coastal) ecotypes, parasite abundance does not significantly differ (see Appendix 4). Only one species of parasite was determined to differ significantly (p value = 0.028, z value = 2.3): the marked reduction in the percentage of *Capillaria aerophila* in Eastern foxes (see Figure 17).

This difference could be attributed to the more densely fox populated area contributing to the transmission of the parasite between conspecific hosts as *Capillaria aerophila* has a direct life cycle. However, earthworms have been suggested as being involved in their life cycle as an intermediate host. A higher abundance of earthworms in the West, or a stronger reliance on the species as prey, could account for the difference in number of foxes infected with the species. While there are vast numbers of species

nesting on the coast, there are limited avian species in land. This may mean that foxes inhabiting inland areas are more reliant on earthworms as prey.

6.3 Parasite Species and Burden Differences in Age and Sex

Age can be a major influence on the parasites present in hosts, and load of parasites often increases with age due to continued exposure over the course of their lifetime (Rollinson and Stothard, 2009). On the other hand, subadult immune systems would be weaker, implying a greater parasitic load. There may be no relationship with age, as the rate of acquisition of parasites and parasite mortality often balances out in individuals, particularly when host immune defences are effective at decreasing the establishment, growth and survival of parasitic species (Rollinson and Stothard, 2009).

Arctic foxes reach sexual maturity within their first year of life. The sample group for this study were divided by subadults (≤ one year of age) and adults (> one year of age). A marked difference in the intensity of endoparasitic eggs infecting the adults and subadults was observed (Section 5.3): both *Toxocara leonina* and *Toxascaris canis* showed wider variation in subadults than adults. This may reflect the lower age (lower minimum), reduced ability to fend off infection (higher maximum), and a greater diversity in immune response (greater spread). The median egg count was higher in adult males for *Toxocara leonina* and in adult females for *Toxascaris canis*, potentially indicating the influence of increased environmental exposure to parasites.

It was expected that the results would also show a difference in species between subadults and adults due to changes in diet throughout an individual fox's life. For example, kits may feed on intermediate hosts that their parents bring to the den, such as microtine rodents which are vital for the life cycle of cestodes.

(Rausch and Fay, 1988; Loos-Frank, 2000), whereas adult foxes are more likely to feed on cached eggs which do not carry endoparasites.

There may also be a difference in the living proximity of different aged foxes, with kits staying close to the den and living closely with their siblings compared to full adults. It is known that ascarid nematodes, as they are transmitted via contamination of food and ingestion of faeces (Okulewicz et al., 2012), have an increased transmission of the ova in the presence of increased host density (Arneberg et al., 1998). Subadults live in closer proximity as they share a den with their parents and siblings for the first few months of their lives, and will stay close for their first year (Ester. Unnsteinsdottir, Pers. comm.).

However, this dissertation did not find any statistical difference between adults and subadults which suggests that these dietary and behavioural difference between the age groups might not be noticeably impacting their parasitic fauna (see Figure 12). The sight increased percentage of adult foxes infected with *Capillaria aerophila* might be indicative that these foxes are feeding more on the intermediate host, earthworms, than younger foxes.

This dissertation also reports very little differences in parasite richness and abundance between sexes (see Figure 19). Vertebrates commonly exhibit sex-biased parasite loads as many species evolve sex-based dietary, behavioural, morphological, and physiological differences. In particular, these differences can predispose males to greater parasitic infection as a result of testosterone suppressing the immune system (Folstad and Karter, 1992). However, Arctic foxes are observed to have low sexual dimorphism, are socially monogamous, and exhibit low sexual selection with both parents investing similarly in

offspring-rearing (Friesen et al., 2015). No differences between diet, foraging behaviour, or home range size have been reported in previous studies (Angerbjörn et al., 1994). The species richness for both cestodes and nematodes was the same, and the abundances similar, across both adults and subadults (see Figure 18). Two sample z-testing suggests that none of the observed species abundances between subadult and adult foxes were statistically relevant (Appendix 4). The increased number of adult foxes infected with *Capillaria aerophila* was closest to being significant (p value = 0.0827, z value = 1.7). This may be as a result of adult foxes coming into more contact with faeces of infected foxes as they travel further, or if they are ingesting the intermediate host, earthworms.

The only notable difference between the sexes is the frequency of *Toxocara canis* species which was found at a much higher rate (15.7% compared to 5.6%) in female foxes which was close to being statistically relevant (p value = 0.0519 , z value = 1.9, see Appendix 4). This difference could be attributed to a difference in hunting tactics, or perhaps lowered immunity due to energy expenditure of birthing offspring.

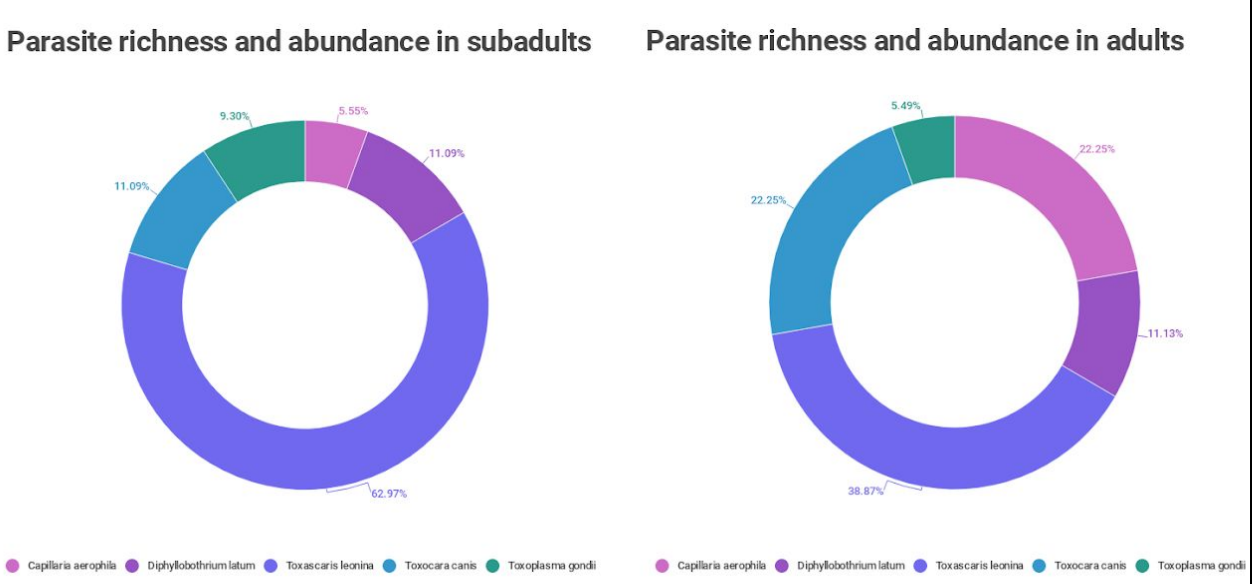

Figure 18: Parasite richness and abundance for adults (> 1 year) and subadults (≤ 1 year).

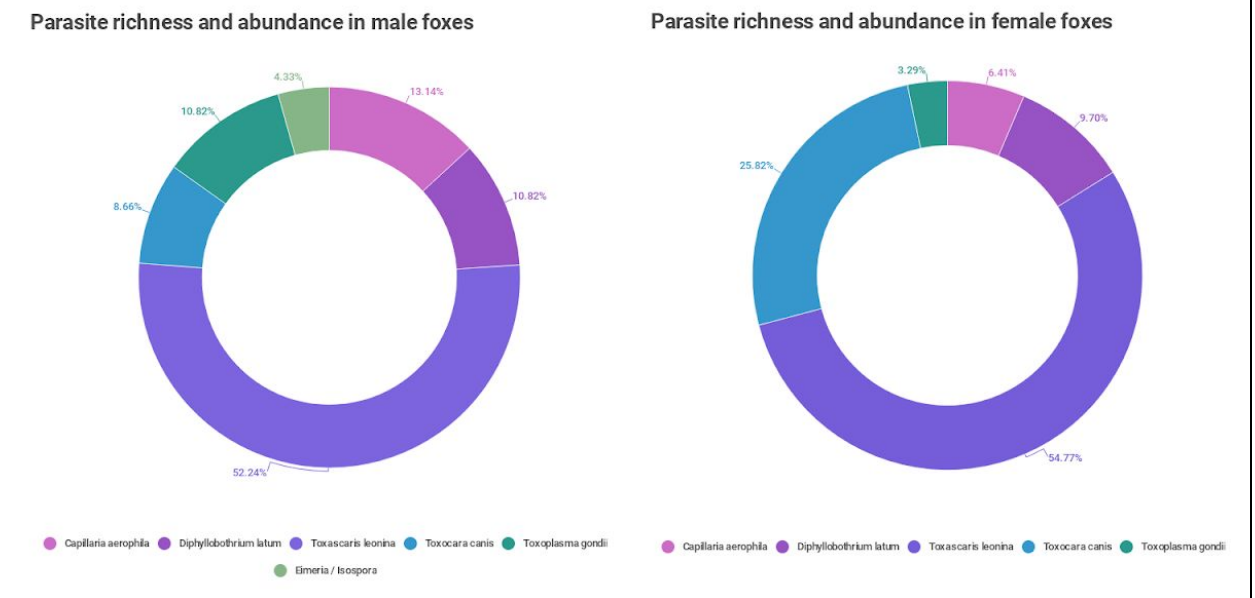

Figure 19: Parasite richness and abundance in male and female foxes.

6.4 Macroscopic Identification

Macroscopic identification was attempted for this project but the freeze-thaw cycles meant the adult worms were often 'unrecognisable mush' as described by Shoop et al. (1987) and identifying features including scolexes were not found attached to the specimens.

6.5 Parasites of Interest

6.5.1 *Capillaria aerophila*

This dissertation reports a prevalence of 6.9 % (n=9) of *Capillaria aerophila* in microscopic analysis, which is in agreement with previous studies in Iceland that investigated the endoparasites of Arctic fox carcasses taken from the same areas of the country as the present study (Skírnisson et al., 1993). The previous study found adult *Capillaria aerophila* in both coastal foxes (n=1) and inland foxes (n=2), in a sample size of 50 giving an abundance rate of 6 %. The larger sample size of this dissertation, coupled with a similar percentage abundance in previous studies, suggests this may be reflective of the fox community in Iceland. The lack of ringed thickening when using 1000x magnification at the base of the bipolar plugs suggested this was not *Trichuris vulpis* (Traversa et al., 2011) (see Figure 9).

6.5.2 *Diphyllobothrium* sp.

The previous study on the endoparasites of the Arctic fox identified *Diphyllobothrium dendriticum* (Skirnisson et al., 1994); a species that was not found in this study. Instead, *Diphyllobothrium latum* was found in similar numbers in microscopy (see Figure 10). It is possible that the parasitic fauna of the Icelandic foxes has changed over the last two decades, and *Diphyllobothrium latum* is now more

prevalent, or the adults identified in the previous study were misidentified as another species within the genus.

Additionally, the difference could be attributed to the differences in methodology; where this dissertation describes methods seeking to identify eggs and ova, the previous study used techniques to identify adult tapeworms by sieving and washing faeces. Confirmatory tests were not carried out by either project. The presence of this species in the Arctic foxes suggest they have been infected by eating the infected musculature or viscera of fish hosting the plerocercoids life stage of the species (Kuchta et al., 2013).

6.5.3 *Echinococcus multilocularis*

The results of this study seem to be in accordance with current data that suggests *Echinococcus multilocularis* has been eradicated from Iceland. There were no evidence of *Echinococcus* sp. observed during morphology or real-time PCR. The molecular methods on this occasion lacked a positive control but followed methods and used primers according to protocols in other studies can be considered robust as the primers had previously successful in other studies (Sultana et al., 2013; Durant et al., 2012; El-Geddawi et al., 2016).

Morphological analysis also revealed no positive samples. Though difficulties can arise as *Echinococcus* sp., are morphologically identical to other *Taenia* sp. there were no eggs identified from either genus. Therefore, a confident negative result across all samples can be considered. The findings of this study support previous observations (Skirnisson et al., 1993) that *Echinococcus* sp. are unlikely to be inhabiting the Arctic foxes in Iceland and that the species has been eradicated as a result of education and legislation governing domestic dogs.

6.5.4 *Giardia* sp.

Giardia was not detected by either microscopy or molecular methods. There was no *Giardia* sp. control but as their design came from another study (Mejia et al., 2013) which did see successful amplification of the target gene, it can be assumed the primers were working correctly.

At least one previous study (Erlandsen et al., 1990) has reported that freezing and thawing of *Giardia* sp. cysts may render them useless in bright field microscopy detection as both the trophozoites and the cyst wall are warped or destroyed within 1 - 2 freeze-thaw cycles making morphological characteristics distorted beyond recognition. It is possible that using the fox carcasses for this study meant *Giardia* sp. was undetectable, rather than absent, due to this distortion. However, the additional lack of positives in PCR provides more robust evidence that *Giardia* sp. may not be prevalent, or even present, in the Arctic foxes.

6.5.5 *Mesocestoides* sp.

Mesocestoides sp. were not observed at microscopy, likely because the species develop into adults in the definitive hosts and shed gravid proglottids containing oncospheres within the parauterine organ (Skirnisson et al., 2016) rather than as ova as seen in other species.

Mesocestoides sp. were detected at PCR with a prevalence of 9.3% (n = 12). This is considerably lower than the previous parasitological studies in Iceland that reported a much higher prevalence at 72% (n = 36) in 50 wild foxes (Skirnisson et al., 1993). The results of this study might be as a result of a genuine decline or fluctuation, or may be due to the limitations of the methods as previously discussed.

Additionally, the previous study in Iceland took foxes hunted from areas in the western and northern areas of the country from Thingvellir to Eyjafjordur (Skirnisson et al., 1993) and all, with the exception of two, were culled during summer months. These factors may cause some disparity between the two

studies, as the current project used a larger sample size, from almost all areas of Iceland, and across all seasons, and the fox carcasses had been in frozen storage for a much longer period of time.

Extracted DNA from known positive samples of *Mesocestoides canislagopus* were obtained for this molecular study to use as positive controls and were successfully amplified, all with similar Ct values of between 13 and 14 (see Appendix 3). Therefore, with such robust results from molecular methods, the much lower than expected prevalence in this dissertation could be attributed to a difference in the prevalence of *Mesocetoides* sp. over the last 25 years. It could be suggested that the prevalence of *Mesocestoides* sp. has decreased as a result of more modernised husbandry practices and the further implemented strategies that saw the eradication of *Echinococcus* sp.. It could also be hypothesised that this change has occurred because of a change in the population of the, yet unknown, arthropod intermediate host or second intermediate host (mice and frogs), but more work would be required involving a larger number of samples spanning the years to determine this.

6.5.6 *Strongyloides stercoralis*

Strongyloides stercoralis was not detected in this dissertation, either by molecular or microscopic methods. No positive control was obtained for the PCR assays but as these primers have been used in several previous studies it can be assumed they were working correctly. As the species has not been recorded in Icelandic Arctic foxes previously, this result supports previous findings.

If there was a failure of these methods to detect *Strongyloides* sp. eggs that were present in the faeces, it has come as a result of freezing the samples or due the low egg output of the genus. Storage by freezing faecal samples is known to decrease the recovery of canine *Strongyloides* sp. (Schurer et al., 2014), as they are known to have thin shells and can rupture within 12 hours of storage at -20°C (Marcilla et al., 2012).

Additionally, *Strongyloides* sp. egg output is notoriously low and irregular larval output which may have been responsible for no positive samples (Repetto et al., 2010). As a result, the lack of any positives may not accurately reflect the prevalence of the species, and it is possible that *Strongyloides* sp. are persisting in the fox population but have gone unnoticed in this study.

6.5.7 *Taenia ovis*

Taenia ovis was not detected using either traditional or molecular methods. Negative results would be expected as, at the time of study, Taenia sp. had not been reported in Iceland, (though recent reports from abattoirs suggest there have been some cysts discovered in sheep hearts, these have yet to be confirmed). This is possibly a reflection on the good husbandry practises mentioned previously to eradicate *Echinococcus* sp., as the life cycles of the two species are remarkably similar and therefore controlled by the same methods.

6.5.8 *Toxascaris leonina*

Toxascaris leonina was detected by microscopy in 36.4% (n=47) (see Figure 11) of the total sample group and at much lower prevalence using PCR methods at 5.4% (n=7). This result is unexpected as PCR is generally more sensitive. There were no positive controls obtained for this species, but the samples were amplified with similar Ct values, suggesting they were amplifying the intended target. As with *Mesocestoides* sp. the microscope data revealed an abundance of *Toxascaris leonina* which was slightly less than expected based on the previous work that found a prevalence of 50% in 50 foxes. This could be as a result of the same mechanisms potentially limiting the infection rate of *Mesocestoides* sp..

6.5.9 *Toxocara canis*

Toxocara canis was found at a higher prevalence in microscopy at 9.3% (n=12) (see Figure 12) compared to PCR where the species was detected in just 1.55% (n=2). There were no positive controls of extracted DNA from known *Toxocara canis* samples to use in this PCR, but all results were confirmed by microscopy. As there were significantly more positive results detected at microscopy rather than when using PCR, it suggests that either not all samples were successfully amplified, perhaps due to the quality of DNA. It might also be that the PCR was more accurate and some misidentification had occurred in the microscopy, though without further testing it is difficult to say definitively.

Toxocara canis and *Toxascaris leonina* have the same life cycle, therefore it is expected that similar patterns will show for both species in regards to distribution. For both species, puppies (and kits) are a major source of environmental contamination, where eggs live in the soil and are transmitted via direct ingestion or ingestion of paratenic hosts. The increased frequency in foxes infected with *Toxocara canis* and *Toxascaris leonina* may reflect an increase in the population of small mammals, such as rabbits, inland. Additionally, there is evidence that birds that feed primarily on the ground (such as ptarmigan) can serve as paratenic hosts and transmit eggs by carrying them on their feet and beaks which could be responsible for their spread (Despommier, 2003). As coastal foxes rely more on the ocean for their sources of prey, this could explain why a higher portion of inland foxes are infected with these parasites species particularly.

6.5.10 *Toxoplasma gondii*

The presence of *Toxoplasma gondii (*see Figure 13) suggests the Arctic foxes are ingesting oocysts from infected birds, although the abundance observed in microscopy was low at only 4.7% (n=6). Unlike the red fox (*Vulpes vulpes*), the Arctic fox rarely frequents the city so they are likely obtaining infection from a wild source rather than from domestic cats or rabbits as contact with urban animals is limited. However,

it is unusual to find oocysts in definitive hosts; it would be expected that these oocysts would have generally transformed into tachyzoites shortly after ingestion by the definitive host and disseminate through the body (Długońska, 2014). This suggests that the oocysts must be either dead incidental ova ingested with prey, or potentially were about to continue their life cycle as normal in the new host but died after the animal was culled. Cannibalism is considered common in Arctic foxes, with adults eating their young shortly after a kit's death from malnutrition or disease (Sklepkovych, 2018), so it is possible transmission can be occurring between members of the population. These numbers are all much lower than studies in other populations of Arctic foxes in Svalbard, Norway, which found a seropositive abundance of 43% (Prestrud et al., 2007) using a direct agglutination test for antibodies against *Toxoplasma gondii*. However, as this test will also detect previous exposure to the parasite as well as active infection, this may be reflected in the higher abundance statistics.

Migratory birds have been established both as a prey species for the Arctic foxes and an important factor of *Toxoplasma gondii* mobilisation around the world (Dubey, 2002). Some studies consider cats to be crucial to *Toxoplasma gondii* epidemiology, but several Arctic examples prove that it can thrive even in the absence of this species.

6.5.11 *Trichinella* sp.

Trichinella sp. were not identified in either microscopy or PCR, but the negatives from PCR can not be considered robust negatives. The extracted DNA from *Trichinella* sp. which was used to test the designed primers did not amplify, suggesting there was a flaw with their design.

Additionally, female *Trichinella* sp. individuals release larvae rather than ova which migrate into the striated muscles (Mitreva, 2006). Therefore, they only inhabit the intestines briefly and do not excrete ova that could be detected in microscopy when observing faeces. It is likely that even with correctly working

primers, these assays would have also have been negative due to looking at parasites in the gut as opposed to tissue or blood.

6.5.12 *Cryptocotyle lingua*

This species was not selected as a parasite of interest for this study, but special mention is deserved as its presence in Iceland is surprising. Though reported previously (Skirnisson et al., 1993) with an abundance of 9.3%, *Cryptocotyle lingua's* usual intermediate hosts are the brackish-water and seawater snails *Littorina littorea* and *Littorina rudis,* which do not seem to be present in Iceland (Galaktionov and Skirnisson, 2000). Additionally, *Cryptocotyle lingua* larval stages have not been reported in the coast around Iceland. As the cercariae are released and encyst in salt-water fish without the presence of the host snails, these findings suggest that the fox has been infected by eating one of the usual definitive hosts; fish-eating migratory seabirds such as terns (*Sterninae* sp.) and gulls (*Laridae* sp.).

The adult worm is generally between 1.29mm and 1.49mm long by 0.59mm to 0.77mm wide and the one sample identified in this dissertation is notably smaller (see Figure 14). Experimental infection of these worms in cats have shown no clinical symptoms, despite growth between villi of intestine (Bowman, 2018). The photograph taken of the slide shows an adult *Cryptocotyle lingua* using 10x lens on bright field microscope measuring approximately 550 um x 150 um.

It can be identified as such confidently due to recognisable aspects of the anatomy such as the shape of the follicular vitellaria and oral sucker, and location of the testes and ova as observed in reference guides (see Figure 25). It is expected that this specimen was found in sedimentation methods after scraping the decomposing gastrointestinal lumen of the intestines and introducing some of this tissue into the sedimentation method.

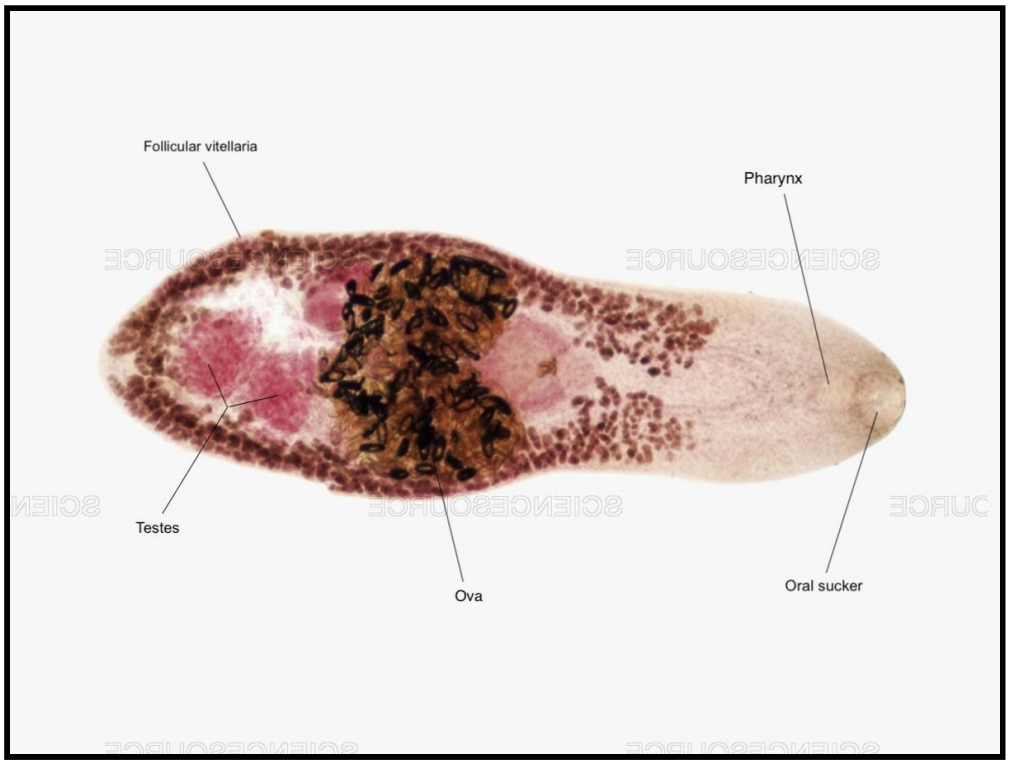

Figure 20: Reference image of Cryptocotyle (CDC). Magnification is 400x.

6.6 Co-infection

While there is little information on the mechanisms of *Toxocara leonina* and its impact on the immune system, the high level of coinfection with other helminthic parasites suggests that the nematode may be evading, suppressing, or altering the immune system as observed in other species in the phylum Nematoda (Cooper and Eleftherianos, 2016). This immunomodulatory action may be causing the weakening of the host's ability to resist colonisation by other parasites. Alternatively, it can be hypothesized that there are prey species within Iceland that are hosting multiple parasites that are being

passed onto the foxes, or the *Toxocara leonina* infections are persisting over a significant portion of the foxes' lifespan while it accumulates other species from various prey species.

Toxocara sp. are also known to have a particularly outstanding ability to survive in host tissue of intermediate hosts by using excretory-secretory products to modulate the host immune response and by shedding a specialised mucin surface coat in response to adherence of antibodies (Maizels, 2013). Arctic foxes may be almost continuously exposed to the species from prey animals causing a high infection rate and subsequent coinfection. It would be typical of *Toxocara leonina* to be using similar immune defense mechanisms as observed across the genus. Repeatedly observing *Toxocara leonina* with other species suggests there may be immunomodulatory behaviour or other means of facilitation occurring rather than being purely incidental.

This project could have benefited from using an endogenous control to confirm the quality of the PCR template DNA, such as using species specific primers targeted at an Arctic fox gene. While this was not possible due to financial and time constraints, these extra assays would have provided additional information to determine if the PCR had been affected by the poor quality of the sample. Other methods that could have been used to test the quality of the DNA include using a spike control which, if amplified by PCR, would have also been able to demonstrate extraction from the faecal samples themselves were possible.

7. Conclusions

This dissertation suggests that coprological analysis of frozen faeces for the detection of parasites in wild Arctic fox populations in Iceland is possible, in contrast to previous suggestions that parasites in frozen carcasses are rendered completely unusable for research (Shoop et al., 1987). Additionally, this dissertation has shown it is possible to utilise DNA extraction kits in combination with real-time PCR to achieve some degree of success for the molecular detection of *Mesocestoides, Toxocara canis,* and *Toxascaris leonina* from gastrointestinal material samples from cryogenically stored foxes.

These non-invasive sampling methods allowed a retrospective study spanning over one decade using carcasses that had been pre-collected and stored as part of the long-term population dynamic studies at the institution. Poorly preserved biological tissues are an important source of DNA for a wide range of zoonotic investigation and can provide a wealth of information that would be otherwise impossible or unethical to obtain. The use of frozen faeces from culled foxes allows the avoidance of ethical implications and facilitates retrospective studies, and by using multiple methods in parallel can provide an overview of the endoparasitological fauna of the Arctic foxes in Iceland.

The methods described have been particularly valuable in a low resource and remote environment, and have demonstrated the ability to recover both DNA and helminthic eggs from frozen and highly degraded samples of gastrointestinal material.

The results have shown statistically significant geographical differences in abundance of *Capillaria aerophila,* with a higher abundance observed in the east. No statistically significant differences were found between sexes or age groups in this sample of 129 foxes, although there was some indication of greater variation in parasitic intensity in subadults. The findings from this dissertation also support the view that Iceland remains Echinococcus free. Overall abundance and richness statistics were lower than

found by Skirnisson et al. (1993), which could be due to a combination of legislative measures to prevent parasitic spread and the methodological limitations discussed above. However, these results have supported the previous observation of *Cryptocotyle lingua*, confirming the species is able to establish infection in Arctic foxes.

In comparison to the Skirnisson et al., (1993) there have been significant differences between both the species identified, and their abundance (see Table 6), most notably the absence of *Mestocestoides* sp. in the present study using either method, and the increase in percentage of infected foxes with *Diphyllobothrium* sp. The abundance of *Capillaria aerophila*, *Toxascaris leonina*, and *Toxocara canis* are relatively consistent across both studies, accounting for the greater sample size of this study. Once unusual result from Skirnisson et al. (1993) was confirmed in this dissertation. They had observed *Cryptocotyle lingua* but described it as an anomalous result because they judged it unlikely to successfully survive and reproduce in Icelandic foxes. One fox in the sample here was found to be infected, supporting this previous studies findings. The specimen was gravid (see Section 6.5).

Table 6 Comparison of studies

	Results from Skirnisson et al., 1993			Results of current study		
	Coastal foxes (n=28)	Inland foxes (n=22)	Total (n=50)	Costal foxes (n=61)	Inland foxes (n=19)	Total (n=80)
Sporazoa						
Eimeria sp. or Isospora sp.	2 (7)	0 (0)	2 (4)	0 (0)	(0)	0 (0)
Toxoplasma gondii	0 (0)	0 (0)	0 (0	5 (8)	1 (5)	6 (5)
Trematoda						
Cryptocotyle lingua	12 (43)	0 (0)	12 (24)	1 (0)	0 (0)	0 (0)
Cestoda						
Mesocestoides sp.	23 (83)	13 (59)	36 (72)	0 (0)	0 (0)	0 (0)
Diphyllobothrium dendriticum / latum	2 (7)	0 (0)	2 (4)	6 (10)	3 (15)	9 (11)
Nematoda						
Capillaria aerophila	1 (4)	2 (9)	3 (6)	4 (7)	4 (21)	8 (10)
Toxascaris leonina	17 (61)	8 (36)	25 (5)	36 (59)	9 (47)	45 (56)
Toxocara canis	0 (0)	1 (5)	1 (2)	10 (16)	2 (10)	12 (15)

The parasites of the Arctic fox in Iceland remains a largely under researched subject. While this project has provided preliminary investigation into the most suitable methods using the facilities available, lack of resources and financial restrictions of Icelandic institutes has left a large body of potential data unused. Future investigations in the endoparasitological fauna of the Icelandic Arctic fox should consider further refining the methods described here, and focusing on finding ways to optimize the use of the frozen fox carcassess stored at *Náttúrufræðistofnun Íslands* to gain the most amount of data possible. Particularly, these carcasses can be used to monitor potential risks to human health and native fauna as the country

sees a record number of imported domestic companion animals, and as climate change causes the environment to become more hospitable to foreign parasitic species.

Optimally, future endoparasitological investigations from frozen Arctic fox carcasses should occur within several months to prevent egg distortion. Further research using the foxes stored at Náttúrufræðistofnun Íslands for investigation of parasites should consider storing faeces immediately upon arrival of the carcass. In addition to the standard -20 ^{O}C aliquots, 10% formalin should also be considered for good preservation of helminth egg morphology, larvae, protozoan cysts, and to use with sedimentation / floatation methods. However, formalin causes cross-linking and can fragments DNA, making it redundant for use in PCR. It is therefore important to consider additional preservation in PVA as it offers complementary advantages: allowing for the preservation of protozoan trophozoites and cysts morphology and is suitable for later molecular or microscopic work (CDC, 2016).

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