

A PARASITOLOGICAL SURVEY OF THE CASCADE RED FOX
(*VULPES VULPES CASCADENSIS*) AND THE COYOTE (*CANIS LATRANS*) IN
MOUNT RAINIER NATIONAL PARK

by

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ABSTRACT

A parasitological survey of the Cascade red fox (*Vulpes vulpes cascadenis*) and the coyote (*Canis latrans*) in Mount Rainier National Park.

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Loss of biodiversity is widespread and increasing numbers of carnivores in North America are suffering from population decline and reduced distribution. The risk of extinction is reality for many of these species, predominately due to the consequences of human activities. The complexity of biodiversity loss has been linked to environmental alterations such as habitat loss and fragmentation, pollution, urbanization, and climate change. In addition, disease emergence among wildlife, including parasitism, is accelerating at an alarming rate. Parasites and pathogens often interact with other environmental stressors and cause species population decline. Species with small populations and low genetic diversity are at the greatest risk of extirpation. Thus the aim of this study was to identify parasitic helminths of the Cascade red fox (*Vulpes vulpes cascadenis*) and the sympatric coyote (*Canis latrans*) in the Mount Rainier National Park (MORA) of Washington State. Cascade red fox, an extremely rare mesocarnivore, has experienced a decline in population and a recent loss of genetic diversity. In order to understand the potential negative impact of parasitism in Cascade red fox, I begin by describing some common canine helminths. Further, I address the impact that multiple stressors play on immune function, the effect of climate change on parasitism, and the dynamics of coinfecting parasites. Additionally, I describe the impact of inbreeding in small wildlife populations and their consequent susceptibility to disease. To further understand the threats to Cascade red fox and to help identify mitigation strategies, it is necessary to also include parasite monitoring. For this study, helminths were identified and quantified through the use of fecal flotations on scats collected from trails in MORA. The knowledge gained from this research will provide a baseline to enhance future conservation efforts of Cascade red fox.

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To my late mother, Neo, who often said, “You can be whatever you want...it’s up to you”. Thank you for always pushing me to set goals and to go after my dreams.

CHAPTER 1

INTRODUCTION

Globally, biodiversity is diminishing and many species are facing imminent extinction (Lynch 1996; Lacy 1997; Aguirre and Tabor 2008). Of the more than 60,000 described vertebrate species worldwide, nearly one-fifth are threatened with extinction (Hoffman et al. 2010; Hoffman et al. 2018). Among these threatened species are carnivores. In North America alone, carnivores are experiencing a decline in abundance and contraction of range (Laliberte and Ripple 2004) as a result of direct or indirect consequences from human activities (Aguirre and Tabor 2008). Anthropogenic impacts, such as habitat destruction and fragmentation, pollution, globalization, and effects from climate change, can have independent, synergistic, additive, or antagonistic effects that contribute to degradation of the environment and loss of biodiversity (Pimm et al. 1995; Aguirre and Tabor 2008; Darling et al. 2009; Craig et al. 2017). Likely in response to a combination of environmental alterations, diseases and parasitism in wildlife have increased in recent decades, with disease transmission patterns changing among both human and animal populations (Dzszak et al. 2000; Aguirre and Tabor 2008; Aguirre 2009; Hollings et al. 2013; Weinstein and Lafferty 2015).

An increase of wildlife disease poses a challenge to endangered species conservation (Aguirre 2009; Pedersen et al. 2007; Smith et al. 2009). Species with small numbers of individuals are especially vulnerable to further population decline as a result of stochastic events such as disease outbreaks (Dzszak et al. 2000). Additionally, a loss of genetic variation is occurring among small wildlife populations due to genetic drift and inbreeding as a result of experiencing the aforementioned ecosystem threats (Lacy 1993;

Lacy 1997). Lower genetic variation can reduce an animal's immune system fitness and increase susceptibility to parasites and pathogens that may lead to increased morbidity or mortality (Lynch 1996).

One such species that is particularly vulnerable to disease and extirpation because of their small population and fragmented habitat is the mesocarnivore, Cascade red fox (*Vulpes vulpes cascadenis*; Akins 2017). The Cascade red fox (Figure 1.1) is endemic to the Cascade mountain range in Washington State at high elevations of alpine and subalpine habitats (Aubry 1983). Although Cascade red foxes once had a range that covered the entire Cascade Range in Washington and southern British Columbia, this subspecies is primarily found in the southern Cascades of Washington within Mount Rainier National Park (MORA; Figure 1.2) and parts of the Gifford Pinchot National Forest (Jenkins et al. 2014; Akins 2017). While little is known about Cascade red fox population abundance and distribution, through surveys previously conducted in MORA, areas in Mt. Baker-Snoqualmie National Forest, and areas in the Gifford Pinchot National Forest (Figure 1.3), researchers have determined that this species is rare (Aubry 1983; Jenkins et al. 2014; Akins et al. 2018).

The Red Fox

The wild canid species, red fox (*Vulpes vulpes*) is one of the most common and widely distributed terrestrial carnivores in the world (Voight 1987; Larivière and Pasitschniak-Arts 1996; Kamler and Ballard 2002; Statham et al. 2012). Indigenous red foxes of the western United States inhabit upper montane elevations and include the Cascade red fox (*Vulpes vulpes cascadenis*), the Sierra Nevada red fox (*Vulpes vulpes*

nectar), and the Rocky Mountain red fox (*Vulpes vulpes macroura*) (Aubry et al. 2009). A fourth native red fox subspecies, the Sacramento Valley red fox (*Vulpes vulpes patwin*) occurs in the lowlands of California (Sacks et al. 2010). These native red fox subspecies are genetically, morphologically, and ecologically distinct from nonnative red foxes (Aubry 1983; Aubry et al. 2009). The native montane red foxes are restricted to high elevations because of their adaptation to cold climates (Sacks et al. 2010). Based on historical and archeological records, these fox subspecies are descendants of red foxes that occupied areas south of the Wisconsin glaciers during the Pleistocene, where they likely became adapted to cold temperatures. Following the glacial retreat, the foxes moved into high elevations of the mountains with colder conditions, similar to temperatures during the glacial period (Aubry 1983).

While both native and nonnative red foxes are present throughout North America, the majority of the studies have been conducted on nonnative foxes because of their economic value as a furbearer (Aubry 1983). Originating from Europe, nonnative foxes were first introduced to the eastern United States in the 1700s, eventually expanding to the West in the 1900s through migration, introduction to the area by humans, and establishment of fur farms (Churcher 1959; Aubry 1983; Statham et al. 2012). In the Pacific Northwest, these red foxes live in lowland habitats and to date, there is no evidence of mixing between the introduced lowland and native montane populations (Aubry 1983; Akins et al. 2018).

Cascade Red Fox

The Cascade red fox is endemic to the Cascade mountain range in Washington State at high elevations of upper montane forests, alpine meadows, and subalpine parkland habitats (Aubry 1983). This subspecies relies on these specialized habitats for reproduction and foraging for a variety of rodents and lagomorphs (Aubry 1983). Cascade red fox lives mainly at elevations above 1,500m as first reported in 1927 (Taylor and Shaw 1927); Akins (2017) confirmed that while the foxes can range at elevations of around 1,000m to 3,200m, they are most likely found at elevations between 1,500m to 2,700m.

More than 35 years ago the Cascade red fox population was reported to be in decline with a threat of extinction (Aubry 1983) and this trend appears to have continued (Akins 2017). Based on genetic analyses of Cascade red fox scat collected from the southern Cascade Range, the current genetic effective population size, or the number of breeding individuals, is estimated to be 16 (Akins et al. 2018). The small genetic effective size of the Cascade red fox population is indicative that genetic diversity may decrease over time as a result of inbreeding (Akins et al. 2018).

It is unknown whether or not the entire population has suffered a recent serious decline or if habitat fragmentation has separated Cascade red fox subpopulations along the entire Cascade Range, resulting in low detectability and genetic isolation (Akins 2017). Currently, the genetic connectivity between populations in the southern Cascades appears to be intact, however, timber harvest and road construction could lead to additional habitat fragmentation isolating the populations to montane 'islands' (Akins et al. 2018). As such, the Cascade red fox is a candidate for listing as an endangered species

in Washington State, a United States Forest Service (USFS) Regional Forester's Sensitive Species, and a Species of Greatest Conservation Need on the Washington State Wildlife Action Plan (Jenkins et al. 2014; Akins et al. 2018). Additionally, it is considered "Critically Imperiled" by NatureServe (NatureServe 2017).

While the historic causes for Cascade red fox population decline are unknown, initial decline is likely attributed to the historically common practices of fur-trapping and poisoning of carnivores (Aubry 1983). More recently, the population has likely suffered as a result of anthropogenic effects such as habitat destruction, habitat fragmentation, and climate change (Akins 2017). Not only can climate change alter Cascade red fox habitat (Hansen et al. 2014), but it can also affect prey species that rely on the features of a subalpine meadow habitat (Akins 2017). The Cascade red fox requires a specialized ecosystem for survival, therefore, landscape alterations of these upper montane elevations may be detrimental to the population.

A vital component of wildlife conservation is recognizing species that may serve as indicators of ecological health. Many wild canid species fit the role of sentinel species because of their threatened or endangered status, specialized habitat requirements, and sensitivity to environmental perturbations (Aguirre 2009). Parasite monitoring in wild canids can provide insight to the state of health of the environment as well as indicate which species are most affected.

Despite their rarity, these foxes are sighted in developed areas of MORA, such as Paradise, because of food-conditioning behaviors and habituation that have occurred across several generations (Reese 2007; Jenkins et al. 2014). With close proximity to humans and increased exposure to vehicular traffic, the habituated foxes are in danger of

being struck and killed by vehicles. Additionally, they run a higher risk of acquiring diseases from domestic dogs that may be traveling with their owners. Wild canid-domestic dog interactions may also increase the likelihood of disease transmission in domestic dogs.

Anthropogenic landscape alterations may also facilitate the encroachment of coyotes (*Canis latrans*) into Cascade red fox habitat (Jenkins et al. 2014; Akins 2017). Historically confined to low elevations of the Great Plains, coyotes experienced a range expansion likely resulting from land use changes and the eradication of the gray wolf in the west during the early 1900s (Gompper 2002). Known to be dominant competitors as well as predators of foxes (Sargeant and Allen 1989; Levi and Wilmers 2012), coyotes are potential carriers of canine parasites and pathogens (Gompper et al. 2003; Bridger et al. 2009; Liccioloi et al. 2012; Redman et al. 2016). The likelihood for parasite spillover from coyotes into Cascade red fox populations increases with potential coyote range expansion and population growth (Telfer and Bown 2012; Weinstein and Lafferty 2015). Further, with isolation at high elevations, Cascade red fox is at risk for exposure to novel pathogens that may be the result of coyote association.

Wildlife species that require specialized ecosystems are more vulnerable to range contraction and population loss than species that have general habitat requirements or can easily adapt and expand their range in response to anthropogenic alterations (Parmesan 2006). Range expansion of nonnative, lowland red foxes are another concern because of the introduction of invasive parasites (Telfer and Bown 2012; Weinstein and Lafferty 2015), prey competition, and the possibility of hybridization with Cascade red fox (Aubry 1983; Kamler and Ballard 2002; Statham et al. 2012). While nonnative introgression does

not appear to have occurred for Cascade red fox in southern Washington because of landscape barriers (Aubry 1984), evidence suggests that future concern is warranted as the habitat alterations continue (Akins 2017). Unlike the west side of the Cascades, the landscape on the east side does not feature a natural buffer zone of uninhabited forests, thus introgression is most plausible in this area of the Cascade red fox range (Aubry 1984). To gain further insight into what may cause future Cascade red fox population decline requires an understanding of the complex features of parasitism. Addressing the potential negative effects that parasitism can have on a threatened wildlife population is necessary for successful management and conservation.

Parasites

Parasites are ubiquitous in all living systems, living on (ectoparasites) or in (endoparasites) another organism, or host, at its expense (Krull 1969; Taylor et al. 2016). Parasites generally cause harm to their hosts, however, the severity can range from a reduction of body condition to debilitating fatal disease (Taylor et al. 2016). The term parasite refers to both microparasites (e.g., bacteria, viruses, fungi) and macroparasites (e.g., arthropods, helminths) (Hatcher et al. 2012). All parasites require at least one host to complete their life cycles, while some parasites use multiple hosts (Taylor et al. 2016). A definitive host is one in which the final stage of development takes place, with the parasite reaching adulthood or sexually maturity (Krull 1969; Taylor et al. 2016). Parasites requiring more than a definitive host, will also have one or more intermediate hosts in order to develop into consecutive stages (Krull 1969; Taylor et al. 2016). Some intermediate hosts can also act as vectors (e.g., mosquitoes, ticks), spreading pathogenic

parasites from host to host (Taylor et al. 2016). Paratenic hosts are intermediate hosts that only serve as a transport to the definitive host; further parasitic development does not occur in these hosts (Taylor et al. 2016). While there is still a need for greater understanding of complex parasite-host interactions, it is clear that parasites serve important roles on long-term wildlife population dynamics (Daszak et al. 2000).

Helminths

Helminths are parasitic worms that can affect plants, animals, and humans (Taylor et al. 2016). Helminths of veterinary concern consist of two major phyla. Nematode worms, or roundworms, make up one of the phylum of helminths (Castro 1996; Ávila and Isaac 2013). The second phylum, Platyhelminths, or flat worms, consist of cestodes (tapeworms), and trematodes (flukes; Castro 1996; Ávila and Isaac 2013). While helminths in animals can affect a variety of organs, for the purpose of this study, I will focus on helminths that affect the gastrointestinal (GI) system of canids (e.g., domestic dogs, coyotes, foxes, wolves). Some of these helminths are also zoonotic in nature (Overgaauw and van Knapen 2000; Tackmann et al. 2001; Lee et al. 2010; Otranto et al. 2015; Ma et al. 2018). The life-cycle of helminths includes egg, larval, and adult stages (Castro 1996). Helminth eggs have environmentally resistant walls in order to withstand a wide variety of climatic conditions while developing. Infection of helminths can occur through transplacental, transcutaneous, or transmammary transmission, or by ingestion of a paratenic host, intermediate host, infective egg, or infective larvae (Bowman and Nelson 2014).

Diagnosing parasite infection in domestic animals commonly involves fecal analysis using a centrifugation fecal flotation method (Foreyt 2001; Dryden et al. 2005). In order to float and recover helminth eggs, the fecal flotation solution must have a higher specific gravity than the eggs (Dryden et al. 2005). Common solutions used in fecal flotations include sugar, magnesium sulfate, sodium chloride, and sodium nitrate (Dryden et al. 2005). For wild carnivores, parasitological surveys often rely on gross examination of intestinal material during necropsy (Richards et al. 1995; Wolfe et al. 2001; Saeed et al. 2006; Liccioli et al. 2012). However, fecal flotation is a more conservative approach to diagnosing intestinal parasites, especially for endangered species or when animal carcasses are not readily available (Liccioli et al. 2012). While fecal flotations may present limitations (e.g., eggs may not readily float) in accurately diagnosing certain helminth species, this method can provide important baseline information on the prevalence of parasitism. Fecal flotation using sugar solution is the most accurate diagnostic tool for nematodes over other helminths because their eggs easily float to the surface (Dryden et al. 2005).

Helminth infection is common in wild canids and environmental contamination with infective helminth stages is widespread (Bowman and Nelson 2014). Red foxes are known to carry a wide variety of helminths, including several of zoonotic significance (Dybing 2013). Further, changes in climatic events in terms of frequency and severity may increase the geographical range of parasites as well as affect the prevalence of infection in hosts (Morgan and Wall 2009; Polley and Thompson 2009). With climate change comes an increasing importance to understand parasite infection and impacts of

disease. Increased prevalence of parasitism is especially concerning for the fitness of threatened or endangered species and their long-term population dynamics.

Nematodes

Of the 561 species of nematodes that inhabit vertebrates (Anderson 1992), there are three main types of gastrointestinal worms common among canids in the Northwest including ascarids (*Toxocara canis*, *Toxascaris leonina*), hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), and whipworms (*Trichuris vulpis*) (Bowman and Nelson 2014; Otranto 2015). The basic life cycle for nematodes includes a series of four moults in which the larvae shed its cuticle (Taylor et al. 2016). The larval stages are indicated as L1, L2, L3, L4, and the immature adult worm as L5. Nematodes either undergo a direct life cycle (which is most common) where the infective stage directly enters its final and definitive host through ingestion or skin penetration or an indirect life cycle that requires an intermediate host for part of its development (Taylor et al. 2016; Figure 1.4).

Ascarids

From the order Ascaridida, and superfamily Ascaridoidea, canids are commonly infected with *Toxocara canis* and *Toxascaris leonina*. On rare occasions they can be infected with the cat roundworm, *Toxocara cati*, or the raccoon roundworm, *Baylisascaris procyonosis* (Anderson 1992; Bowman and Nelson 2014).

Toxocara canis

Toxocara canis (Figure 1.5) is a ubiquitous nematode common among domestic and wild canids of zoonotic importance (Overgaauw and van Knapen 2000; Schnieder et al. 2011). *T. canis* requires its definitive canid host to complete its life cycle, however, humans can act as paratenic hosts with the parasite causing disease (Anderson 1992; Overgaauw and van Knapen 2000; Jenkins et al. 2011; Ávila and Isaac 2013; Bowman and Nelson 2014). Among foxes worldwide, the reported prevalence of *T. canis* infection is between 56% and 80% depending on the population examined (Richards et al. 1995; Overgaauw and van Knapen 2000; Saeed et al. 2006).

The life cycle of *T. canis* (Figure 1.6) is complex with four possible modes of infection (Taylor et al. 2016). The basic mode of transmission is through ingestion of embryonated eggs containing the infective L3 (Bruňaská et al. 1995; Nemzek et al. 2015; Taylor et al. 2016). Another route of infection is through ingestion of paratenic hosts (e.g., rodents, birds; Schnieder et al. 2011). In pups, infection occurs through transplacental or transmammary transmission (Schnieder et al. 2011; Taylor et al. 2016). The life cycle of *T. canis* within the host differs depending on the age of the animal (Schnieder et al. 2011). For canids older than six months, direct ingestion of embryonated eggs will result with the larvae invading the intestinal wall (Schnieder et al. 2011). A small percentage of the larvae will undergo tracheal migration, where the larvae will moult, get swallowed, and then develop into adult worms in the small intestine (Overgaauw and van Knapen 2000; Ávila and Isaac 2013). However, the majority of the larvae will continue to travel to the lungs and enter the pulmonary veins to the heart to be circulated out into the somatic tissues (Anderson 1992; Ávila and Isaac 2013). Once they

reach the tissues, the larvae will be trapped and encysted through an inflammatory response (Ávila and Isaac 2013), where they will remain dormant unless reactivated during pregnancy (Anderson 1992; Taylor et al. 2016).

Transplacental transmission is the primary route of infection in pups occurring when the larvae pass from the mother to the liver of the developing fetuses, where the larvae remain until birth (Overgaauw and van Knapen 2000; Schnieder et al. 2011; Taylor et al. 2016). Following birth, the larvae migrate to the lungs then get coughed up and swallowed where they will develop into adult worms in the small intestine. Newborn pups can also become infected through ingestion of larvae through their mother's milk when nursing (Overgaauw and van Knapen 2000; Schnieder et al. 2011). Transplacental transmission is a highly effective mode of infection for newborn pups; virtually all pups will be infected with *T. canis* if the mother is also infected (Ávila and Isaac, 2013). In juvenile canids (less than three months), direct ingestion of embryonated eggs will result in tracheal migration and ultimately adult worm development in the small intestine (Overgaauw and van Knapen 2000).

Transmission of *T. canis* via paratenic hosts results in the larvae developing into adults within the small intestine and does not involve tracheal or somatic migration (Schnieder et al. 2011). Environmental contamination takes place when canids infested with adult intestinal worms shed unembryonated eggs in their feces (Schnieder et al. 2011). Up to 200,000 eggs can be shed per day from one animal (Ávila and Isaac 2013). Eggs incubate in the soil and typically develop into the infective L3 within 2-6 weeks (Anderson 1992; Overgaauw and van Knapen 2000; Bowman and Nelson 2014; Otranto et al. 2015).

Clinical signs of disease from infection of *T. canis* are mostly seen in young pups. Symptoms can include diarrhea, failure to gain weight or grow, vomiting, poor haircoat, a pot-bellied appearance, abdominal discomfort, and reduced immune fitness (Lee et al. 2010; Bowman and Nelson 2014; Nemzek et al. 2015; Reinemeyer 2016). Pups with heavy worm burdens can also experience intestinal obstruction (Bowman and Nelson 2014) or intussusception (sliding of the intestine within itself; Nemzek et al. 2015). In other severe cases, neonatal pups may succumb to death due to pneumonia caused by large numbers of larvae migrating to the lungs (Lee et al. 2010; Bowman and Nelson 2014; Nemzek et al. 2015; Reinemeyer 2016; Taylor et al. 2016). Signs of pulmonary damage from migrating larvae include coughing, increased respiratory rate, and frothy nasal discharge (Taylor et al. 2016). Coinfection with other macroparasites and/or microparasites, stress, or malnutrition can also further complicate clinical disease.

With its ability to migrate in tissues, *T. canis* is thus a significant zoonotic agent of a variety of toxocariasis syndromes (Lee et al. 2010; Ma et al. 2018). Humans can become an accidental host when infective eggs are ingested from contaminated soil, contaminated raw vegetables, or from eating raw parts of paratenic hosts (e.g., chicken, pig, cow; Lee et al. 2010). In most cases, toxocariasis can remain asymptomatic, however, the disease can present as visceral larva migrans (VLM), ocular larva migrans (OLM), neural toxocariasis, or covert toxocariasis (mild non-specific infection) (Lee et al. 2010; Ma et al. 2018). Because humans are a dead-end host, the larvae cannot complete their life cycle and will instead migrate through various organs and tissues, resulting in an inflammatory immune response (Ma et al. 2018). Clinical symptoms, such as fever, headaches, coughing, skin conditions, and pain can occur (Ma et al. 2018). Both

VLM and OLM are more common in children likely because of their increased exposure to contaminated soil (Overgaauw and van Knapen 2000; Ma et al. 2018). Visual impairment is likely in the cases of OLM, because a dead worm in the eye can elicit an inflammatory response and induce granulomatous retinal lesions (Overgaauw and van Knapen 2000; Ma et al. 2018). Blindness is also possible. In rare cases, *T. canis* larvae can also migrate into the central nervous system of adults, causing neurotoxocarisis, which may lead to meningitis, encephalitis, or produce symptoms of headache and fever (Ma et al. 2018).

Toxascaris leonina

The definitive hosts for *Toxascaris leonina* (Figure 1.7) include species of canids (e.g., dogs, foxes, wolves) and felids (e.g., cats, jaguars, leopards, lynx) throughout most parts of the world. (Anderson 1992; Overgaauw and van Knapen 2000). Infection of *T. leonina* in canids is similar to that of *T. canis* except that the larvae do not migrate transplacentally nor transmammary (Overgaauw and van Knapen 2000). Transmission of *T. leonina* takes place from ingestion of infective eggs containing infective second-stage larvae or paratenic hosts with encysted L3 in their tissues (Overgaauw and van Knapen 2000; Taylor et al. 2016). Following ingestion of embryonated eggs (Figure 1.8), the larvae will penetrate the wall of the small intestine, where they will continue to develop (Anderson 1992; Overgaauw and van Knapen 2000). The larvae will mature into adult worms within the intestinal wall where they can remain for seven weeks and up to one year (Overgaauw and van Knapen 2000). In the environment, *T. leonina* eggs develop

within one week as compared to *T. canis* eggs which take up to four weeks (Taylor et al. 2016).

Clinical disease symptoms in juveniles are similar to *T. canis* infection ranging from unthriftiness to diarrhea, but tend to be milder (Taylor et al. 2016). Infection of *T. leonina* is often accompanied with *T. canis* infection. *T. leonina* is a potential zoonotic parasite, but there has been far less research on its ability to cause disease in humans (Overgaauw and van Knapen 2000).

Hookworms

The second most common nematode taxa among carnivores are collectively called hookworms and belong to the family Ancylostomatidae of the order Strongylida (Anderson 1992). Hookworms found in canids of the northern hemisphere include the species *Ancylostoma caninum* (Figure 1.10) and *Uncinaria stenocephala* (Anderson 1992; Overgaauw and van Knapen 2000; Bowman and Nelson 2014; Otranto et al. 2015). Hookworms earned their name because they “hook” their buccal capsules (mouthparts) to the intestinal mucosa of their hosts (Overgaauw and van Knapen 2000; Lefkaditis 2001; Otranto et al. 2015). Adult hookworms feed on blood from the capillaries they rupture when they penetrate the mucosa (Lefkaditis 2001).

Embryonated hookworm eggs are passed to the environment through the feces of hosts (Figure 1.9). An infected canid can pass millions of eggs in its feces daily for several weeks (Taylor et al. 2016). Under suitable moisture and temperature conditions (e.g., 23-33 degrees C), L1s will emerge from the eggs and feed on bacteria within the soil (Overgaauw and van Knapen 2000). Within two days they will molt into L2s and

four to five days later will molt into the infective L3 stage (Overgaauw and van Knapen 2000; Lefkaditis 2001; Otranto et al. 2015). Transmission of infective L3s to their canine host is either through ingestion of contaminated soil, paratenic host, or through cutaneous penetration (*A. caninum* only; Anderson 1992; Overgaauw and van Knapen 2000; Lefkaditis 2001; Bowman and Nelson 2014; Nemzek et al. 2015). Oral transmission of infective larvae of *U. stenocephala* and *A. caninum* enter the small intestine where they develop into adults (Lefkaditis 2001; Bowman and Nelson 2014; Nemzek et al. 2015).

Infection of *A. caninum* through penetration of the host's skin results in the larvae entering the circulatory system, where they are carried through the blood vessels, eventually reaching the lungs (Overgaauw and van Knapen 2000; Lefkaditis 2001). In the lungs, the L3s will develop into L4s and then undergo tracheal migration to be swallowed and end up in the small intestine of the host to develop into adult worms (Overgaauw and van Knapen 2000; Lefkaditis 2001; Taylor et al. 2016). Some of the third-stage larvae from the lungs will also migrate into the host's somatic tissues, including mammary glands in females, where they will arrest and lie dormant (Overgaauw and van Knapen 2000; Lefkaditis 2001). Pregnancy will reactivate the larvae from the infected mother's skeletal tissues and pass on to nursing pups through milk (Overgaauw and van Knapen 2000; Lefkaditis 2001; Bowman and Nelson 2014; Otranto et al. 2015; Taylor et al. 2016).

Pathogenicity of hookworms varies by the parasite species (Overgaauw and van Knapen 2000). For example, *U. stenocephala* pathogenicity is low, with symptoms of mild diarrhea and low-grade anemia usually only occurring in cases of heavy worm burden in young canids (Overgaauw and van Knapen 2000; Taylor et al. 2016). Whereas

in *A. caninum* infection, pathogenicity is one of the highest of all canine parasites (Overgaauw and van Knapen 2000). The main consequence of infection of *A. caninum* is anemia due to tissue damage and blood loss from the feeding parasites (Nemzek et al. 2015; Taylor et al. 2016). In pups with transmammary infection, blood loss can be so significant that it can lead to death as early as 2-3 weeks of age (Overgaauw and van Knapen 2000; Bowman and Nelson 2014; Taylor et al. 2016). Clinical signs in infected pups include ill thrift, anorexia, weight loss, stunted growth, bloody diarrhea, dehydration, and poor haircoat (Bowman and Nelson 2014; Taylor et al. 2016; Seguel and Gottdenker 2017). Disruption of the intestinal mucosa from lesions created by feeding hookworms can cause inflammation and inhibit proper nutrient absorption (Seguel and Gottdenker 2017). Hemorrhagic enteritis and anemia was observed in coyote neonates when experimentally inoculated with *A. caninum* (Radomski 1989). Additionally, *A. caninum* penetration of the skin of the host's feet can cause skin infections with clinical symptoms of moist eczema, pruritus, swelling, and ulceration (Overgaauw and van Knapen 2000; Taylor et al. 2016). Larval migration through the lungs can result in pneumonia and respiratory disease (Bowman and Nelson 2014; Taylor et al. 2016). In combination with anemia, these conditions can be debilitating, especially in young canids (Overgaauw and van Knapen 2000; Bowman and Nelson 2014). Coyote neonates inoculated with heavy burdens of *A. caninum* larvae (>500 larvae/kg), were observed with lung tissue damage, anemia, and subsequent death (Radomski 1989).

With its ability to penetrate the skin, *A. caninum* can also pose as a zoonotic threat to humans. Cutaneous larval migrans (CLM), a result of skin penetration, is a condition in which the hookworm larvae migrate through top layers of the skin over several weeks

causing skin eruptions and itching (Overgaauw and van Knapen 2000). Eventually the larvae will die and get reabsorbed by the host (Overgaauw and van Knapen 2000).

Throughout Europe, red foxes are known to be significant reservoirs of *U. stenocephala*. A study in Denmark by Willingham et al. (1996), reported prevalence of *U. stenocephala* infection in red foxes was 86%. In Europe, prevalence of hookworm infection in red foxes is between 38% and 78% depending on the population studied (Criado-Fornelio et al. 2000; Reperant et al. 2007; Stuart et al. 2013). *A. caninum* infection in endangered red wolves (*Canis rufus*) and sympatric coyotes in southeastern United States was detected in 94% of the individuals studied; with 32% prevalence of *U. stenocephala* infection (Brzeski et al. 2015).

Whipworms

Trichuris vulpis (Order: Enoplida, Family: Trichuridae) is a globally distributed whipworm (Figure 1.10) inhabiting the large intestine of domestic and wild canids (Blagburn 2008). Whipworm eggs enter the environment by passing through the feces of their host. The prepatent period (period of time from infection until mature adult parasites are producing eggs) is nearly three months, and thereafter, adult female whipworms only produce eggs intermittently (Blagburn 2008). Thus, diagnosis can be difficult because an animal may not shed eggs continuously, even while infected. *T. vulpis* eggs are extremely environmentally resistant and can remain viable in the soil for up to 7 years (Blagburn 2008). Under suitable conditions, the eggs will become infective by embryonating to L1 in the soil within 3-8 weeks (Traversa 2011; Taylor et al. 2016). Transmission is via oral route; the infective eggs will inhabit the intestinal glands to molt and eventually make

their way into the large intestine to develop into adults (Traversa 2011; Nemzek et al. 2015). Pathogenicity of whipworms is variable, with some infected animals living asymptotically, while others will suffer ill effects of high parasite burden. Adult whipworms feed on blood and other fluids while tunneling through the mucosa of the large intestine (Traversa 2011). Their action of feeding can cause inflammation and tissue damage of the cecum and colon. Pups are particularly vulnerable to suffering ill effects of *T. vulpis* infection and may exhibit reduced growth rate (Traversa 2011; Nemzek et al. 2015). Other clinical symptoms may include bloody and mucousy diarrhea, anemia, dehydration, and weight loss, and in severe cases could lead to death (Traversa 2011; Bowman and Nelson 2014; Nemzek et al. 2015). Whipworm infection can also decrease the host's ability to convert nutrients properly, which can reduce their immune function and make them more susceptible to acquiring secondary infections (Traversa 2011).

Cestodes

Common cestodes (tapeworms) found in wild canids include *Diplydium caninum* (Figure 1.11), *Taenia* spp., *Mesocestiodes* spp., and *Echinococcus* spp. (Little 2007; Bowman and Nelson 2014; Nemzek et al. 2015). All tapeworms have complex life cycles that require one or more intermediate hosts (e.g., fleas, lice, rodents) in order to transmit to carnivore definitive hosts (Little 2007; Nemzek et al. 2015; Taylor et al. 2016). Intermediate hosts become infected through ingestion of a tapeworm egg. Once the egg reaches the small intestine, larvae will hatch out of the egg, burrow into the intestinal wall, migrate to the liver via the blood, and then develop larval cysts in the tissues (Little 2007). Canids can become infected with tapeworms when they ingest the larval cysts of

intermediate hosts. Within the host's small intestine, proglottids (segments) of the tapeworms are formed. Gravid proglottids contain eggs, which are then spilled out into the lumen of the intestine that will be later passed out in the feces (Little 2007). The majority of tapeworm infections in canids are asymptomatic, however, intestinal blockage may occur in pups with heavy worm burdens (Bowman and Nelson 2014).

Diagnosing tapeworm infection through fecal flotation alone can be challenging and unreliable because proglottids are not consistently shed nor are they uniformly distributed in fecal material (Little 2007; Liccioli et al. 2012; Bowman and Nelson 2014). Furthermore, to diagnose tapeworm infection through fecal flotation, it is necessary for proglottids to release their eggs in order for them to float.

Echinococcus multilocaris

E. multilocaris is commonly referred to as the fox tapeworm, because it is often found in red foxes in Europe, North America, and Asia (König and Romig 2010). *E. multilocaris* is a zoonotic parasite that may cause alveolar echinococcosis (AE) in humans (Tackmann et al. 2001; König and Romig 2010; Bowman and Nelson 2014; Massolo et al. 2014; Otranto et al 2015). AE has serious medical implications that requires intensive, lifelong medication (König and Romig 2010; Otranto et al. 2015). There is limited research on *E. multilocaris* distribution, ecology, and epidemiology within North America (Massolo et al. 2014; Otranto et al. 2015). However, *E. multilocaris* appears to be expanding in range with increased prevalence of infection in wild canids across Europe, Canada, and the United States (Bowman and Nelson 2014; Massolo et al. 2014; Otranto et al. 2015; Taylor et al. 2016). Increased prevalence in wild

canids will cause an increase in environmental contamination. Because of its high zoonotic potential, understanding the dynamics of *E. multilocaris* in wild canid populations is important for future prevention of transmission to domestic dogs and humans.

Trematodes

Commonly called flukes, trematodes have been detected in wild canids throughout the world, but to a lesser degree than nematodes and cestodes (Custer and Pence 1981; Aubry 1983; Dibble et al. 1983; Richards et al. 1995; Wolfe et al. 2001). Flukes in the northwest include species of *Alaria* and *Nanophyetus salmincola* (Bowman and Nelson 2014). The life cycle of flukes is complex, requiring more than one intermediate host before infecting their definitive hosts via oral route. Infection of *Alaria* spp. usually does not produce clinical disease, however, infection of *N. salmincola* in canids can cause illness commonly referred to as salmon poisoning disease, with symptoms of vomiting, diarrhea, swollen lymph nodes, and fever (Bowman and Nelson 2014). *N. salmincola* itself is virtually harmless, however, when the fluke is parasitized by the rickettsial organism, *Neorickettsia helminthoeca*, the result is extreme disease (Krull 1969; Taylor et al. 2016). Transmission of *N. salmincola* is through ingestion of metacercariae present in salmonid fish in the coastal Pacific Northwest (Bowman and Nelson 2014). Infection of *N. salmincola* has been detected in lowland red foxes in Washington State, however, given the location of salmonid prey, Cascade red fox is an unlikely candidate for contracting the rickettsial parasite (Aubry 1983).

Multiple Stressors on Immune Function

The combined effects of parasites and multiple stressors can have serious negative effects on the health of a variety of wildlife species (Lafferty and Kuris 1999; Marcogliese and Pietroock 2011; Cable et al. 2017). Parasites are ubiquitous and induce stress in hosts regardless if hosts are showing symptoms or not (Lafferty and Kuris 1999; Marcogliese and Pietroock 2011). Parasitism in conjunction with an additional stressor, be it natural or anthropogenic, may exacerbate the negative impact on animal health (Acevedo-Whitehouse and Duffus 2009). Alternatively, the opposite interaction of certain parasites decreasing may occur when associated with other stressors (Lafferty and Kuris 1999; Marcogliese et al. 2009; Marcogliese and Pietroock 2011). The primary focus for this paper is to evaluate the occurrence of parasites in a critically imperiled species who face a multitude of stressors. This information will allow managers to assess the potential risk parasites may have on Cascade red fox populations at Mount Rainier National Park so they can take management action to minimize negative health effects and mortality.

Stressors can be any type of abiotic or biotic event or stimuli that elicits stress in an organism. Natural stress factors include parasite infection, food availability, predation, UV radiation, salinity, population density, and temperature (Marcogliese and Pietroock 2011). However, environmental changes involving UV radiation, temperature, and salinity can also be an effect of anthropogenic climate change (Marcogliese and Pietroock 2011). Other anthropogenic stressors include habitat destruction, fragmentation, invasive species, and contaminants (Lafferty and Kuris 1999; Acevedo-Whitehouse and Duffus 2009; Cable et al. 2017). Multiple studies conclude that parasitism and other concurrent

stressors can have more drastic effects on host health than just the presence of one stressor (Blaustein and Kiesecker 2002; Beldomenico et al. 2008; Marcogliese and Pietroock 2011). Animals under environmental stress may experience reduced immunity, thereby decreasing their ability to resist or tolerate parasite infection (Acevedo-Whitehouse and Duffus 2009). In turn, the added effect of parasitism may further jeopardize host condition that increases susceptibility to additional parasites or pathogens (Beldomenico and Begon 2009).

The majority of research on combined effects of multiple stressors has been conducted in laboratory, however, species in the wild have provided evidence that stressful conditions can enhance pathogenicity of parasites (Pedersen and Greives 2008; Christin et al. 2009; Gilbertson et al. 2003; Marcogliese et al. 2010; Marcogliese and Pietroock 2011). For example, a study on yellow perch (*Perca flavescens*) examined from the St. Lawrence River, Quebec, Canada indicated that pathogenic effects of perch helminths increased in polluted sites. The perch with contaminant exposure experienced immunosuppression, limiting their tolerance to parasite infection (Marcogliese et al. 2010). Another example of increased pathogenicity of parasites is demonstrated in fish that have been contaminated by crude oil (Khan 1990). The toxicity of oil suppresses fish immune functions causing increased prevalence and intensity of ciliated protozoan parasites (Khan 1990) such as *Ichthyophthirius multifiliis* that cause the debilitating “white spot disease” (Petty and Francis-Floyd 2018). Further evidence of the negative impact of multiple stressors on parasite infection and disease is exemplified in many of the declining amphibian species around the world (Christin et al. 2009; Gilbertson et al. 2003; Blaustein et al. 2012).

In terms of an entire host population, the interaction of parasitism in combination with other stressors follows similar outcomes as seen in individual hosts; as an increase or decrease in pathogenicity (Lafferty and Kuris 1999; Acevedo-Whitehouse and Duffus 2009). This is an important complexity to understand when studying Cascade red fox. This population already experiences several stressors including, but not limited to, habitat fragmentation, low genetic effective size, small population, changes to winter food availability, and human interaction. Exposure to these abiotic or biotic stressors can compromise their defense mechanisms, and with the added effect of parasitism, Cascade red fox may be pushed further to the threat of population loss and consequently extirpation.

Climate Change and Parasitism

Human-induced climate change is having negative effects on the planet's ecosystems, biodiversity, organism demography, and complex ecosystem processes (IPCC 2007). Features of climate change include increasing global temperatures, a shift in precipitation patterns, and an increase in severity and frequency of extreme weather conditions (Patz et al. 2000; Polley and Thompson 2009; Polley et al. 2010; Morley and Lewis 2014). These climatic changes are influencing parasite and host (intermediate and definitive) distribution, parasite life-cycle rates, parasite transmission, pathogenicity, disease prevalence, host-parasite dynamics, and host immunology (Polley and Thompson 2009; Polley et al. 2010; Jenkins et al. 2011; Blaustein et al. 2012). Moreover, there are gaps in knowledge regarding the effects of extreme temperature variations on thermal

stress of animal hosts and how this may influence host-parasite interactions (Morley and Lewis 2014).

Previous studies on the effect of climate change on zoonotic parasites and diseases transmitted via vector (e.g., mosquitoes, ticks) may provide a model-based scenario for predicting climate-based patterns of other types of parasitism prevalent in wildlife (Polley et al. 2010). For example, warming temperatures are increasing the abundance of ticks and altering their distribution patterns (Dantas-Torres et al. 2012; Estrada-Peña et al. 2012; Ostfeld and Bruner 2015). Increasing precipitation is another environmental condition that is playing a significant role in tick range expansion (Estrada-Peña et al. 2012; Ostfeld and Bruner 2015). Together, these features of climate change have created an ideal situation for tick survival. The temperate region of the northern hemisphere is becoming more favorable for ticks due to adequate moisture and warmer climate (Ostfeld and Bruner 2015). Increased distribution and abundance of tick populations are increasing the incidence of tick-borne diseases (e.g., Lyme disease, ehrlichiosis, anaplasmosis) in animals and humans (Dantas-Torres et al. 2012; Estrada-Peña et al. 2012; Ostfeld and Bruner 2015). Furthermore, range expansion of ticks to new areas carries the risk of introducing nonnative pathogens to naive hosts whose immune systems have not had prior exposure.

Parasites that expand in range along with their hosts can have detrimental effects in naïve hosts in these new locations, eliciting disease that isn't normally noticed (Telfer and Bown 2012). Regions that previously had unsuitable conditions to support a parasite and its host may now offer the ideal habitat. An increase in temperature in higher latitudes or higher altitudes may influence host migration routes as well as promote

introduction of nonnative species (Polley and Thompson 2009; Polley et al. 2010; Atehmengo and Nnagbo 2014).

The impact that climate change is having on the Canadian Arctic is one of the greatest in North America. In addition to increased temperatures, precipitation, and severe weather events, this region appears to have a higher incidence of helminth parasitism among individuals of indigenous communities than people of the developed areas of Canada (Jenkins et al. 2011). Over the past 60 years, this region has experienced a warming trend of 1.8-2.3°C that may be facilitating the northern movement of *T. canis* and other zoonotic parasites that were historically unable to become established (Jenkins et al. 2011). Similarly, *E. multilocaris* is expanding in range as well as its host preference (Polley and Thompson 2009; Jenkins et al. 2011). Further, increased precipitation and temperatures in the north will likely increase rodent intermediate hosts, consequently influencing *E. multilocaris* transmission (Jenkins et al. 2011). Wildlife species in the north that are adapted to cold environments, much like Cascade red fox, can be studied to help understand and predict the climate change influences on parasitism.

In the case for Cascade red fox, the potential for range expansion of coyotes to higher elevations could result in increased parasitism and consequent disease. Historically, coyotes have been absent from the mountains (Quinn 1997) due to their lack of adaptation to heavy snow (Perrine 2005). However, warming events that result in a decrease in annual montane snowfall and changes to snow pack could facilitate upward coyote movement (Akins 2017). While hard to predict given the complexities, an introduction of exotic parasites could have deleterious effects on Cascade red fox.

Warmer climate also has the potential to increase the rate that parasites develop and become infective (Blaustein et al. 2012; Atehmengo and Nnagbo 2014). Thus, increasing the likelihood that a host will have higher parasite burdens as well as an increase in the number of parasites available for transmission from the environment (Polley and Thompson 2009; Polley et al. 2010). Hosts with high parasite loads are more susceptible to developing clinical symptoms and possible co-infection of additional macroparasites and/or microparasites. Survival rates may increase for parasites that normally perish in cold climate (Polley et al. 2010). However, the opposite may occur for other parasites that have a lower heat tolerance (Jenkins et al. 2011; Morley and Lewis 2014). The effects of climate change on parasitism is difficult to predict due to the complex ecosystem-parasite-host dynamics. However, careful monitoring of host-parasite systems can provide insight to help guide mitigation and prevention of disease.

Coinfection Dynamics

Concurrent infection of multiple parasite species commonly occurs in most wild animals. Coinfection can occur with multiple macroparasites (e.g., helminths, arthropods), multiple microparasites (e.g., viruses, bacteria, protozoa), or a combination of both. Coinfection of parasite species within a host can be simultaneous, or the presence of one parasite can cause host immunosuppression that facilitates subsequent parasite infection (Ezenwa et al. 2010; Bordes and Morand 2011; Ezenwa and Jolles 2011; Budischak et al. 2012; Vanmourin et al. 2015; Ezenwa 2016). Alternatively, coinfection interactions can also have inhibitory mechanisms that result in cross-immunity or resource competition between parasites (Bordes and Morand 2011; Ezenwa and Jolles

2011). Recent studies have documented the impact of helminth infection on a wildlife host's immune system, dynamics of coinfecting microparasites, and the progression and severity of disease (Ezenwa et al. 2010; Ezenwa and Jolles 2011; Budischak et al. 2012; Broughton 2017). Given the ubiquity of helminths among wild canids, it is paramount to understand intracellular microparasite coinfection, especially when managing a threatened or endangered species.

Helminths can impair a host's immune response to secondary microparasitic infection (Ezenwa et al. 2010; Bordes and Morand 2011; Ezenwa and Jolles 2011; Ezenwa 2016). When an animal is coinfecting with helminths and microparasites, the immune system responds by activating T-helper cells type 1 (Th1) and type 2 (Th2) to the presence of each parasite respectively (Mosmann and Sad 1996). Th1 cells produce specific cytokines (e.g., Interleukin-4) that promote immune mechanisms to fight against helminths, while Th2 cells produce cytokines (e.g., interferon- γ) that promote effector mechanisms that target intracellular microparasites (Mosmann and Sad 1996). Both types of cytokines mutually inhibit one another simultaneously, thus an animal with prior helminth infection is more susceptible to acquiring secondary microparasite infection (Ezenwa et al. 2010; Bordes and Morand 2011; Ezenwa and Jolles 2011; Vanmourin et al. 2015; Ezenwa 2016). Helminths can also stimulate a host's regulatory T cells (T_{REG}) that will produce immunoregulatory cytokines that lead to suppression of Th1 and Th2 activity (Ezenwa et al. 2010; Ezenwa and Jolles 2011). Consequently, helminth coinfection can increase microparasite transmission, pathogenicity, and persistence of disease. Further, coinfection dynamics can depress recovery rates and increase disease-induced mortality (Ezenwa et al. 2010; Ezenwa and Jolles 2011).

Several studies have shown interactions between coinfecting parasites (Ezenwa et al. 2010; Ezenwa and Jolles 2011; Budischak et al. 2012; Broughton 2017). For instance, associations between gastrointestinal nematode infection and bovine tuberculosis (*Mycobacterium bovis*, TB) in free-ranging African buffalo (*Syncerus caffer*) at Hluhluwe-iMfolozi Park, South Africa demonstrated that nematode infection suppressed host Th1 response, increasing susceptibility and progression of TB infection (Ezenwa et al 2010). Further, African buffaloes with helminth-TB coinfection showed reduced body condition and accelerated mortality as compared to those who had a single infection (Ezenwa et al 2010). Nearly 73% of wild lions (*Panthera leo*) in Kruger National Park, South Africa, are infected with feline immunodeficiency virus (FIV) and coinfecting with gastrointestinal parasites and tick-borne hemoparasites (Broughton 2017). Immunosuppression as a result of FIV infection played a significant role in overall parasite species richness and abundance, consequently leading to further FIV disease progression. Another study showed three-week delay in vaccinia viral clearance was observed in mice who were coinfecting with the trematode *Schistosoma mansoni* versus rapid viral clearance in mice infected with vaccinia alone (Actor et al. 1993). A breeding colony of Purple martins (*Progne subis*) coinfecting with the hematozoan, *Haemoproteus prognei*, and an unidentified filarial nematode, although rare among the population (8%), resulted in mortality 90% of the time (Davidar and Morton 2006).

Wild canids worldwide are notorious for harboring a broad spectrum of concurrent infections of both endoparasites and ectoparasites (Dibble et al. 1983; Richards et al. 1995; Miller et al. 1998; Wolfe et al. 2001; Dalimi et al. 2006; Saeed et al. 2006; Figueiredo et al. 2016; Hermosilla et al. 2017). While most infected canids can live

without clinical symptoms, those with heavy burdens of parasites or those who experience additional stressors may be more susceptible to morbidity and mortality.

External stress from altered climatic conditions and habitat modification can increase the prevalence of coinfection in wildlife hosts. Additionally, physiological stressors, such as malnourishment and poor body condition, can increase susceptibility of acquiring coinfecting parasites as well as exacerbate the effects from coinfection (Vanmourin et al. 2015).

Inbreeding Depression and Disease Susceptibility

Small and isolated wildlife populations often suffer from loss of genetic diversity as a result of genetic drift and inbreeding effects (Lacy 1997). Inbreeding leads to a reduction of individual heterozygosity and increased risk of fixed recessive or deleterious alleles within in the population (Charlesworth and Charlesworth 1987; Lynch 1996; Lacy 1997; Frankham 1998). Fragmented populations can experience restricted gene flow thus promoting a reduction of fitness components collectively termed inbreeding depression (Charlesworth and Charlesworth 1987; Templeton et al. 1990; Lynch 1996; Lacy 1997). Characteristics of inbreeding depression at an individual level include decreased fecundity, higher mortality, slower growth rates, high frequency of developmental defects, decreased adaptation to stressors, and lower immunocompetence (Lacy 1997). Small populations that experience loss of genetic variation have slower population growth and impaired ability to adapt to environmental change. Therefore, endangered species are extremely vulnerable to extinction from stochastic events (Lynch 1996; Lacy 1997; Frankham 1998; Keller and Waller 2002).

Genetic diversity in wildlife populations plays an essential role in immune capabilities including parasite and pathogen recognition and resistance (Smith et al. 2009). In particular, the most important genetic component of the vertebrate immune system involves a group of polymorphic genes that make up the major histocompatibility complex (MHC). MHC genes encode for proteins on the surface of cells and present them to T-lymphocytes to initiate an immune response to the presence of pathogens or parasites (Penn et al. 2002; Kurtz et al. 2004; Sommer 2005). Several studies have reported the importance of MHC heterozygosity in a variety of vertebrate species in regards to enhanced parasite recognition and ability to cope with parasite infection (Penn et al. 2002; Acevedo-Whitehouse et al. 2003; Kurtz et al. 2004; Hawley et al. 2005; Sommer 2005; Acevedo-Whitehouse et al. 2006; Luikart et al. 2008). For example, an association between reduced heterozygosity and increased parasitism with lungworms (*Protostrongylus* spp.) was demonstrated in a bottlenecked population of bighorn sheep (*Ovis aries*; Luikart et al. 2008). Inbred California sea lions (*Zalophus californianus*) correlated to a high susceptibility of helminth infection, increased parasite diversity, and longer recovery from disease. Similarly, an additional study on California sea lion pups revealed that genetic homozygosity was strongly associated with hookworm-related lesions, a weakened immune response and ability to clear hookworm infection, and mortality (Acevedo-Whitehouse et al. 2006). The results from these studies have important future implications for threatened wildlife populations with small populations.

CONCLUSION

For the purpose of this research and future conservation management, it is critical to understand the impact that decreased immune fitness may have on a small population, such as Cascade red fox. Inbreeding that leads to a loss of immune fitness can make a species extremely vulnerable to pathogens, and in turn, may cause further population decline. In species that are already genetically immunocompromised, the added stress of parasitism may weaken their ability to fight off potentially fatal pathogens. Cascade red fox habitat at mid-elevations has been shown to be highly fragmented due to timber harvest, thus reducing the connectivity between populations (Akins 2017). Any loss in connectivity could result in reduction of gene flow and consequent genetic diversity, which is necessary to maintain viability of this threatened species (Akins 2017). Furthermore, with a genetic effective population size of 16 individuals, and a predicted loss in heterozygosity of 25% per 10 generations, canine parasites and diseases may have a detrimental impact on the small Cascade red fox population (Akins et al. 2018).

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Figure 1.1 Female Cascade red fox (*Vulpes vulpes cascadenis*) in Mount Rainier National Park; detected approximately 1600m outside (westside) of Paradise. Photo by Jessica Brown.



Figure 1.2 Mount Rainier National Park; view from above Paradise. Photo by Jessica Brown.

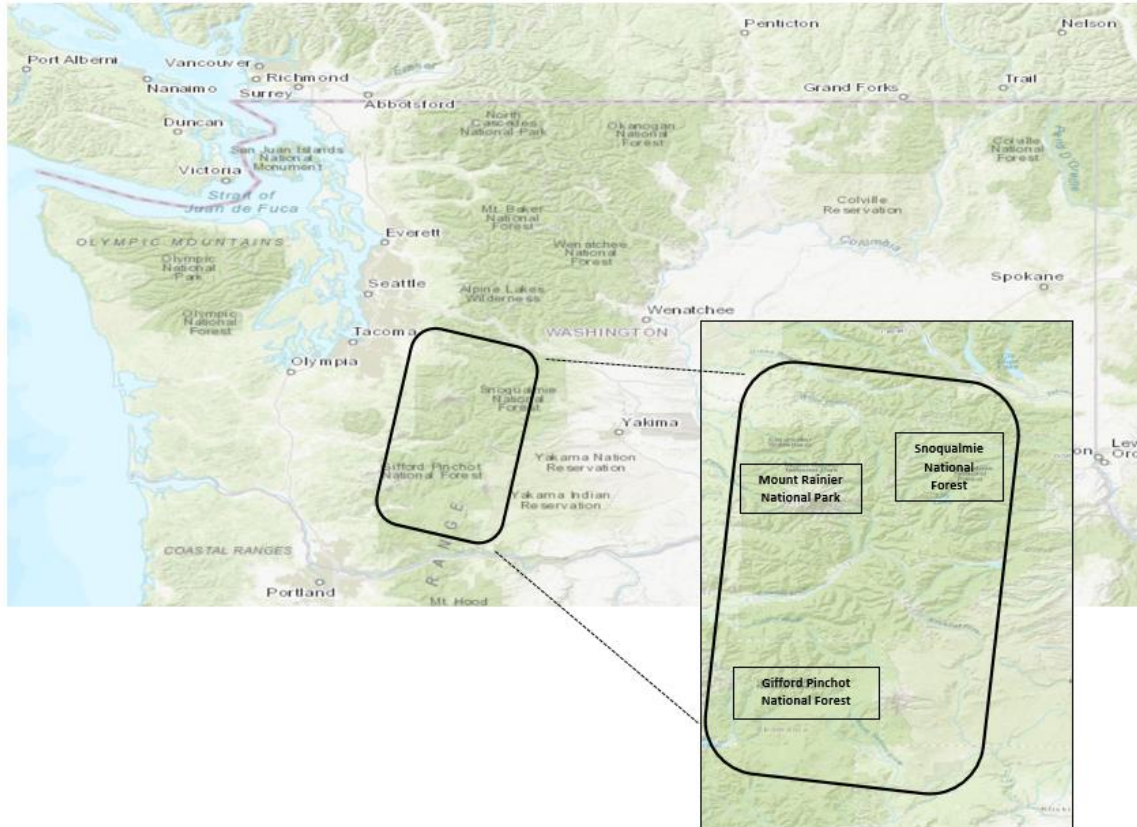


Figure 1.3 Map of Washington State with close-up (inset) of southern Cascade Range (Mount Rainier National Park, Snoqualmie National Forest, Gifford Pinchot National Forest) where Cascade red fox (*Vulpes vulpes cascadensis*) has been mainly detected (Aubry 1983; Akins et al. 2018). Map created by Jessica Brown. Base map courtesy of ArcGIS.

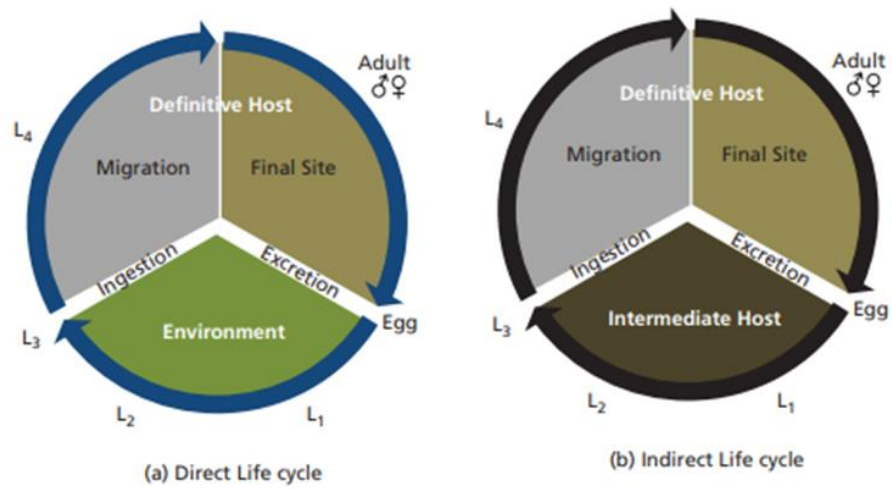


Figure 1.4 Basic life cycle diagrams for nematodes: (a) direct and (b) indirect (Taylor et al. 2016).

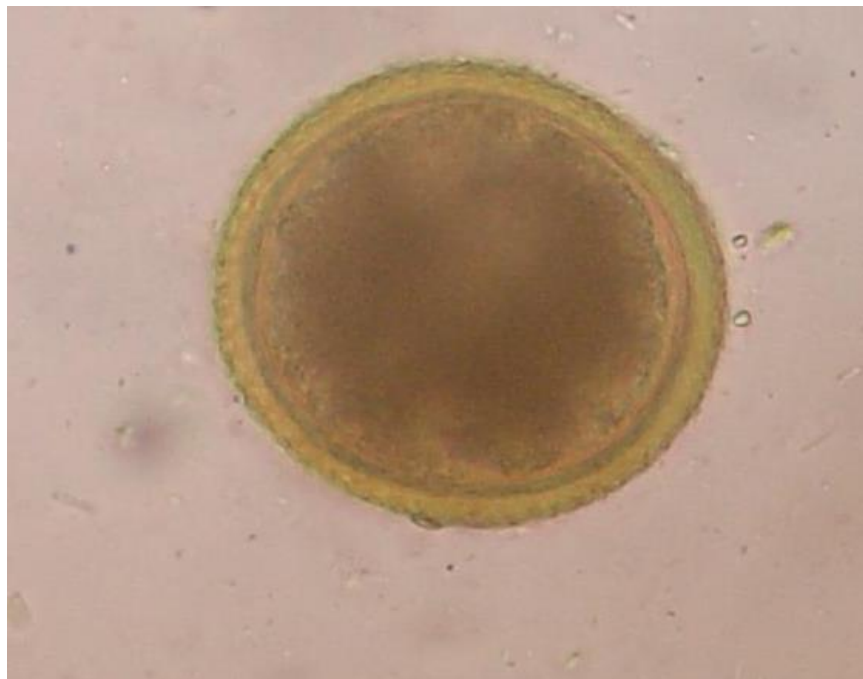


Figure 1.5 Microscopic view of *Toxocara canis* egg. Photo courtesy of Mills 2006, Wikimedia Commons.

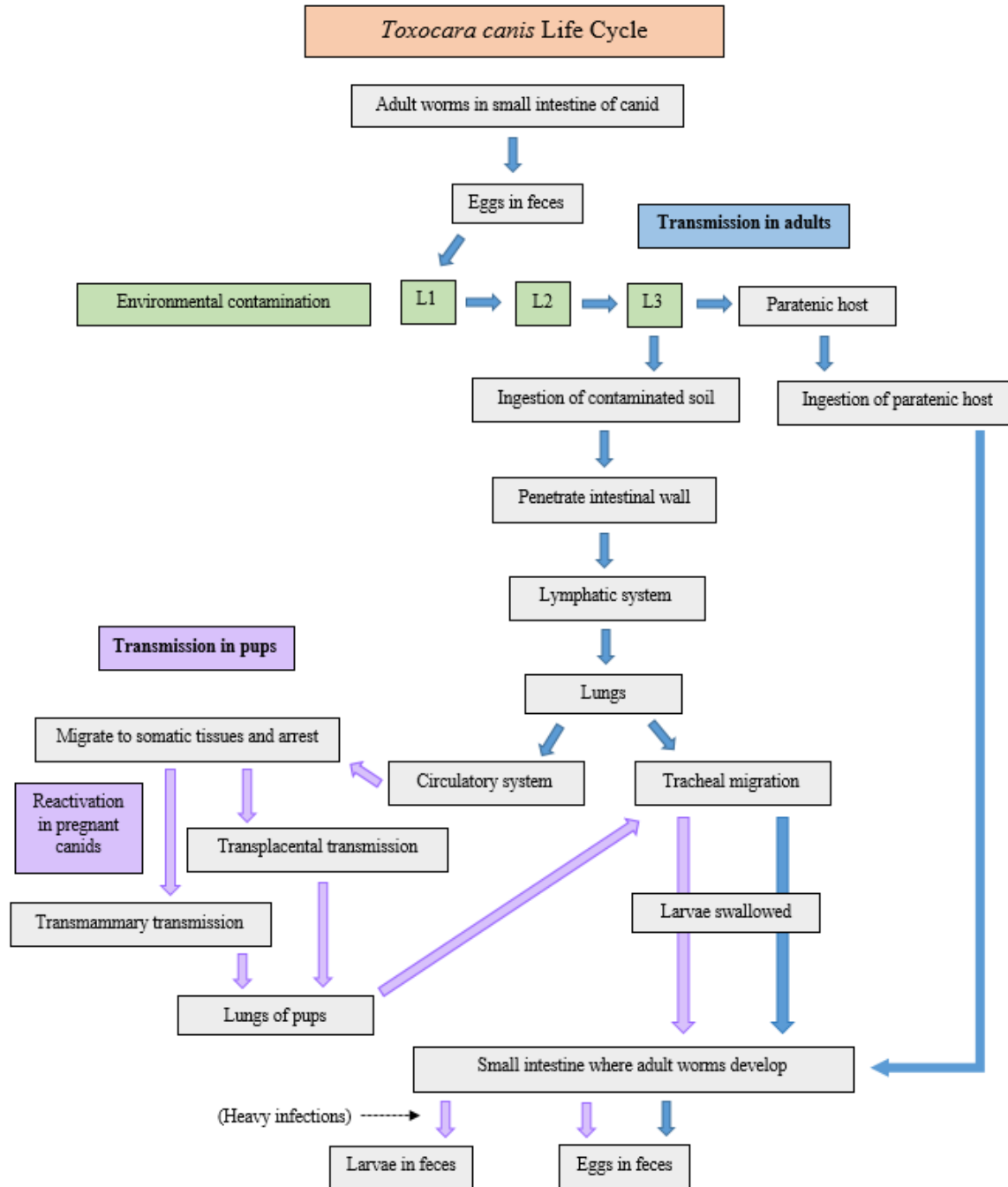


Figure 1.6 Life cycle diagram of the ascarid, *Toxocara canis* in canids (Bruňaská et al. 1995; Schnieder et al. 2011; Nemzek et al. 2015; Taylor et al. 2016). Purple shading represents parasite life cycle in pups younger than six months of age; blue shading represents parasite life cycle in canids older than six months of age. Diagram created by Jessica Brown.



Figure 1.7 Microscopic view (100x magnification) of *Toxascaris leonina* egg. Photo by Jessica Brown.

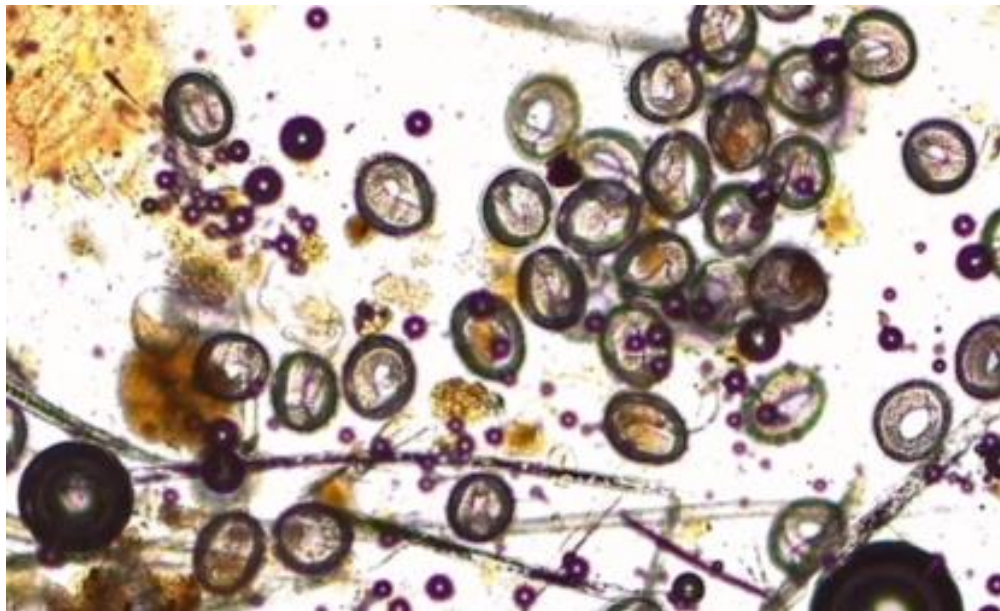


Figure 1.8 Embryonated *Toxascaris leonina* eggs under 40x magnification; very heavy parasite load. Photo by Jessica Brown.

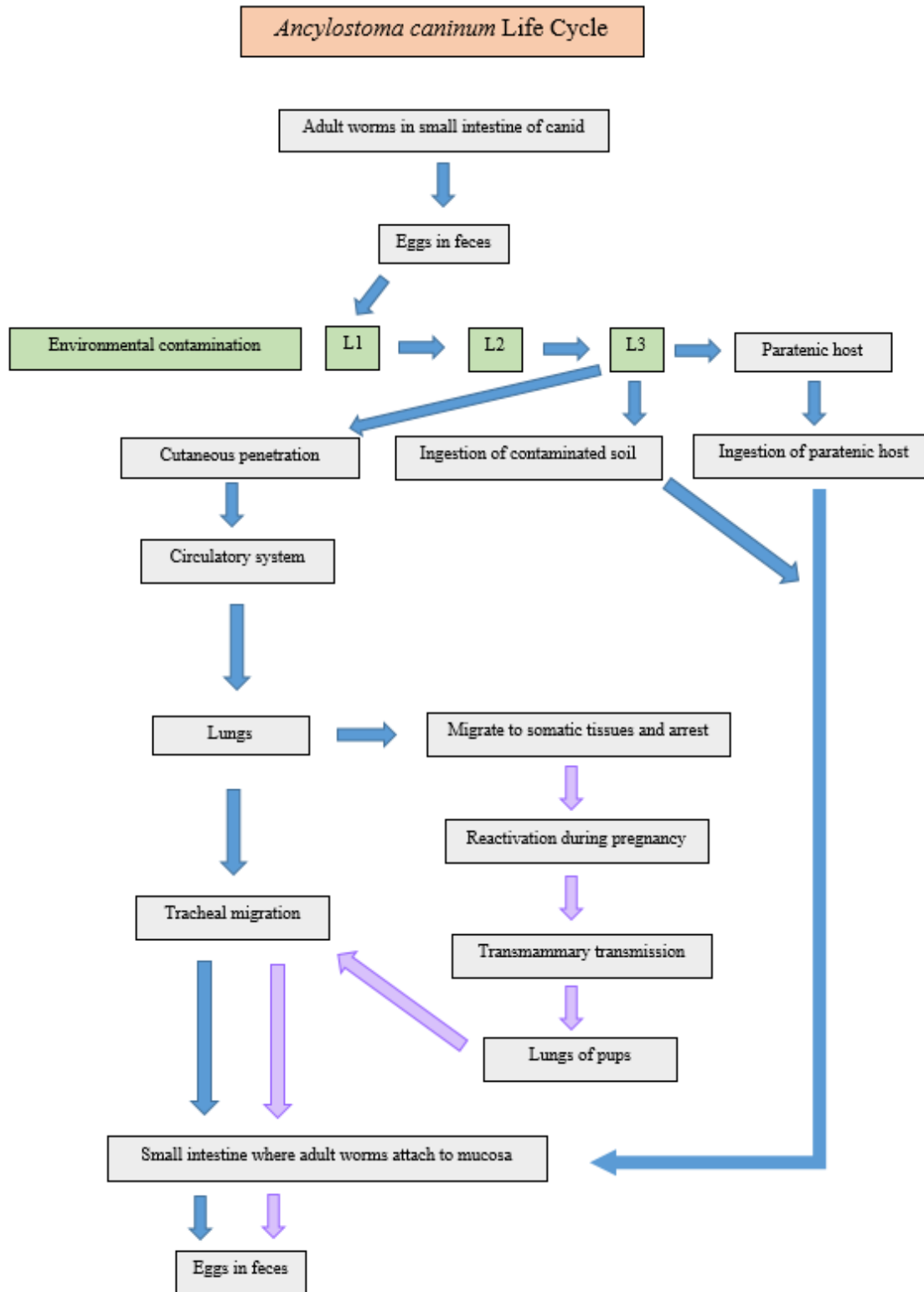


Figure 1.9 Life cycle of the hookworm, *Ancylostoma caninum* (Lefkaditis 2001). Purple shading represents parasite life cycle in pups; blue shading represents parasite life cycle in adult canids. Diagram by Jessica Brown.



Figure 1.10 Microscopic view (40x magnification) of hookworm, *Ancylostoma caninum* (blue circle) and whipworm, *Trichuris vulpis* (red arrow). Photo by Jessica Brown.

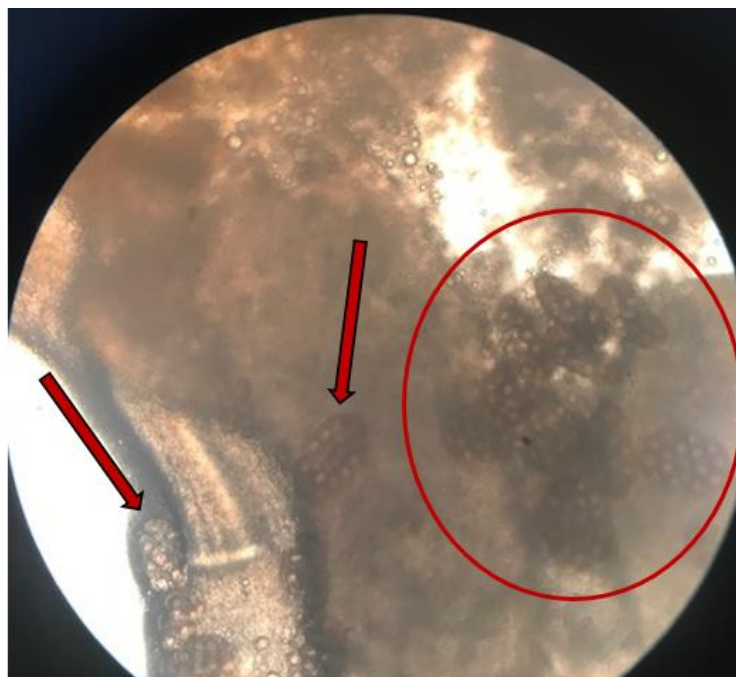


Figure 1.11 Microscopic view (40x magnification) of *Diplydium caninum* proglottid with several eggs (red arrows and inside red circle). Photo by Jessica Brown

CHAPTER 2:

A PARASITOLOGICAL SURVEY OF THE CASCADE RED FOX
(*VULPES VULPES CASCADENSIS*) AND THE COYOTE (*CANIS LATRANS*)
IN MOUNT RAINIER NATIONAL PARK

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ABSTRACT

Parasites are ubiquitous in wildlife and can pose a significant risk to population dynamics of threatened and endangered species. In this study, I determined the prevalence of gastrointestinal helminth parasites in two wild canids of Mount Rainier National Park located in Washington State: the Cascade red fox (*Vulpes vulpes cascadenensis*) and the coyote (*Canis latrans*). Fecal flotations were performed on a total of 128 scats (92 fox, 36 coyote) collected between July and August 2017 during a mesocarnivore scat survey. Overall prevalence of helminth infection in Cascade red fox was 25%, with three helminth species identified: *Toxocara canis* (13%), *Toxascaris leonina* (13%), and *Ancylostoma caninum* in one individual. The prevalence of overall helminth infection was less in coyotes at 14% with two species identified: *Toxocara canis* (11%), and *Toxascaris leonina* (6%). Results were compared to previous data recorded from fecal flotations performed on Cascade red fox scat ($n=40$) collected in 2016. Findings revealed an overall helminth prevalence of 45% with four types of helminths identified: *T. canis* (18%), *T. leonina* (23%), *A. caninum* (13%), and a Taeniid species recovered from one individual. To my knowledge, this is the first fecal analysis study of helminth fauna for Cascade red fox and coyotes in this location. My results show that both canids carry parasite species with potential pathogenicity as well as those of zoonotic concern. Going forward with this baseline data, I recommend continued studies with a more comprehensive approach to further understand the impacts of parasitism in a changing world.

INTRODUCTION

Across the world, the loss of biodiversity is increasing and more species are becoming threatened or endangered (Pimm et al. 1995; Lynch 1996; Lacy 1997; Aguirre and Tabor, 2008). In particular, Cascade red fox (*Vulpes vulpes cascadensis*), a rare species, has experienced recent population decline likely as a result of anthropogenic effects (Akins et al. 2018). While the exact causes behind Cascade red fox population decline is unknown, initial decline is likely attributed to the historically common practices of fur-trapping and poisoning of carnivores (Aubry 1983; Laliberte and Ripple 2004). The only native red fox in Washington State, this montane fox subspecies is adapted to live at high elevations of alpine and subalpine habitats in the Cascade mountain range (Aubry 1983). Their entire range once spanned throughout the entire Cascade Range in Washington from southern British Columbia, Canada, through southern Washington State, however, recent surveys suggest that their distribution is limited to the southern Cascades of Washington in Mount Rainier National Park (MORA) and parts of Gifford Pinchot National Park (Jenkins et al. 2014; Akins et al. 2018). The rarity of Cascade red fox and the fragmentation of its specialized montane habitat makes this species particularly vulnerable to adverse effects of parasitism and consequent extirpation (Akins 2017).

Anthropogenic activities such as habitat destruction and fragmentation, pollution, urbanization, globalization, and global climate change are contributing to an increased prevalence of infectious disease and parasitism among wildlife (Dzszak et al. 2000; Aguirre and Tabor 2008). The dynamics of disease transmission are changing with altered ecosystems and closer contact between humans, domestic animals, and wildlife

(Dazsak et al. 2000; Aguirre and Tabor 2008; Aguirre 2009; Hollings et al. 2013). The consequences can be devastating and cause further population decline in threatened and endangered species already facing a number of environmental stressors (Dazsak et al. 2000), such as Cascade red fox. Red foxes worldwide are notorious for harboring a broad spectrum of canine gastrointestinal helminths, including several of zoonotic significance (Dibble et al. 1983; Richards et al. 1995; Wolfe et al. 2001; Dalimi et al. 2006; Saeed et al. 2006; Dybing, 2013). While many helminths do not produce clinical disease, nonetheless, parasites induce stress in their hosts (Lafferty and Kuris 1999). Additional stressors, such as adverse environmental conditions, may reduce a host's immune fitness, consequently increasing the level of pathogenicity of parasite infections (Beldomenico and Begon 2009; Marcogliese and Pietroock 2011). A loss of genetic variation is plausible for Cascade red fox due to the likelihood of inbreeding in this small, fragmented population (Lacy 1993; Lacy 1997). Low genetic variation may negatively impact their immune fitness and increase susceptibility to parasites with consequences of increased morbidity and mortality (Lynch 1996).

Cascade red fox also faces the threat of potential encroachment of the coyote (*Canis latrans*) into their habitat (Jenkins et al. 2014; Akins 2017). While historically absent from high elevations (Quinn 1997) due to an inability to adapt to heavy snow conditions (Perrine 2005), the coyotes' range could expand as a result of landscape changes and warming climate (Akins 2017). Coyotes are known to carry a variety of canine parasites (Radomski 1989; Foster et al. 2003; Gompper et al. 2003; Bridger et al. 2009; Liccioli et al. 2012; Redman et al. 2016), and as such, future parasite spillover into the Cascade red fox population is likely if these two species eventually share habitat.

Coyotes may also introduce exotic parasites into a naïve population that has ultimately lived in isolation from other canids.

Previous research on helminth infection in Cascade red fox is limited, with only one study examining the helminth fauna from gastrointestinal contents of 13 deceased individuals (Aubry 1983). As for coyotes in MORA, I have not uncovered any previous research. Thus, the aim of this study is to provide baseline data on the prevalence and intensity of helminth infection in Cascade red fox and coyotes of MORA. This study provides a starting point for understanding the impacts of parasites and disease on the threatened Cascade red fox.

METHODS

Study area

The area of focus for the study covered 750 km² in Mount Rainier National Park (MORA) of the southern Washington Cascade Range (Figure 2.1). A mesocarnivore survey team, consisting of up to eight field-trained technicians, surveyed several hiking trails in the summer of 2016 and 2017 within the park at elevations ranging from 750 to 3000 meters. Landscape types included low elevation forests, subalpine forests, subalpine parklands, and alpine grasslands (Akins, 2017). The trail system of MORA is frequented by hikers during the summer months, with Paradise having the highest number of human visitors (MORA 2017). Despite their rarity, Cascade red fox is often sighted in developed areas of MORA, such as Paradise, because of food-conditioning behaviors and habituation that have occurred across several generations (Reese 2007; Jenkins et al. 2014).

Sample collection

Between July and August 2017, feces from Cascade red fox ($n=92$) and coyote ($n=36$) were collected during scat trail surveys conducted by the MORA mesocarnivore survey team (Figure 2.2). For the prior year, Cascade red fox ($n=40$) feces were collected by the survey team during June and September 2016 (Figure 2.3). The surveys involved scanning the trail edges (especially in grass and shrubs), along boulders, and logs and stumps while hiking. All surveys involved one to two people and typically began at a trailhead with the odometer of a GPS starting at zero. When a scat was located and determined to be from either Cascade red fox or coyote, the GPS waypoints (latitude, longitude, and elevation) were recorded in a field notebook or data form as well as on a plastic collection bag and vial. Prior to collection, a photograph was taken of the scat sample with vial and label, and GPS showing the waypoints (Figure 2.4). In addition to recording the waypoints, each scat was assigned a field ID consisting of the date, species, and number of scat found for the day. The scat was collected and placed into the labeled plastic bag and labeled vial. At the end of the daily survey, all of the vials had 95% ethanol added to them and all samples (vials and bags) were then placed in a -20C freezer.

Following collection, each sample was separated into three subsamples and labeled accordingly (Figure 2.5). Samples were transported from MORA on ice to The Evergreen State College laboratory to store in the refrigerator (at 10 degrees C) until examination.

Laboratory analyses

Parasite assays were performed on each fecal sample following the centrifugation fecal flotation technique using a sugar saturation solution (Foreyt 2001; Dryden et al. 2005). Centrifugation is the preferred method for fecal analysis because of its consistency in recovering most intestinal helminth eggs and protozoan oocysts with the least amount of false-negatives (Dryden et al. 2005; Dryden and Payne 2010; Liccioli et al. 2012). In order to detect parasite eggs in a fecal solution and float them to the surface, the specific gravity (SG) must be more than the eggs themselves. The sugar solution has an SG of 1.27, whereas most parasite eggs and larvae have a SG of 1.05 to 1.23 (Dryden et al. 2005). Centrifugation provides a more consistent recovery as compared to passive flotation methods.

The parasite analysis was performed on six samples at a time and begun by placing approximately one to two grams of fecal material from each sample into individual small paper cups (Figure 2.6). A new paper cup was used for each sample. To each paper cup, 10 mL of sugar solution was added and mixed thoroughly using a metal lab spatula, making sure to break down dry feces to create a fecal suspension. The suspension was then strained through a clean tea strainer into a clean paper cup, poured into a 15 mL test tube, and placed into the centrifuge. The spatula and tea strainer were disinfected with a 10% bleach solution in between samples to avoid cross-contamination. Additional sugar solution was added to each test tube allowing for approximately one inch of space from the top. The six tubes were centrifuged at 1400 RPM for five minutes. Following centrifugation, the test tubes were removed and placed in a test tube rack. I added additional sugar solution to each tube to create a slight meniscus at the top, added

coverslips to each, and let them stand for 10 minutes to allow for egg/larvae flotation (Figure 2.7). For evaluation, I placed one of the coverslips, liquid side down, onto a glass slide. I systematically examined the slide at 10X magnification using a compound microscope and used 40X magnification as needed to confirm results when parasite eggs were detected. Helminth eggs and larvae were identified (Foreyt 2001), quantified with a scoring system for determining the parasite intensity (low, moderate, heavy, very heavy) (Table 2.1), and recorded. Fecal examinations for the samples collected in 2016 were conducted by a veterinary student from the University of Wisconsin-Madison; results were provided by MORA.

Data analyses

The data was compiled into a spreadsheet using Microsoft Excel 2013 to summarize the following: Date collected, geographic location of the collected scats (GPS waypoints and elevation), parasite detection (yes or no), and parasite load (low, moderate, heavy, very heavy) for each parasite species detected.

Maps were generated with ArcGIS to show the location of each collected Cascade red fox scat for 2016 (Figure 2.8), 2017 (Figure 2.9), and coyote scat for 2017 (Figure 2.10). In addition, I created a map showing the combination of Cascade red fox scat from 2016 and 2016, as well as the coyote scat collected in 2017 (Figure 2.11). I then created a map showing the location of scats positive for helminth infection and color coded the points according to the parasite diversity; 2016 Cascade red fox scat (Figure 2.12), 2017 coyote scat (Figure 2.13), and 2017 Cascade red fox scat (Figure 2.14). In addition, I

made a map with a close-up section near Paradise to show a detailed view of the 2017 Cascade red fox scats with helminth infection (Figure 2.15).

RESULTS

From a total of 40 Cascade red fox fecal samples collected in 2016, 18 (45%) were infected by four different helminth parasites (Table 2.2). Seven fecal samples were infected with the ascarid, *Toxocara canis* (18%; low parasite load, $n=4$; moderate, $n=1$; heavy, $n=1$; very heavy, $n=1$), and 9 samples were infected with the ascarid, *Toxascaris leonina* (23%; low parasite load, $n=2$; moderate, $n=2$; heavy, $n=1$; very heavy, $n=3$). Five of the samples were infected with the hookworm, *Ancylostoma caninum* (13%), all of which showed a low parasite burden. Two of the fecal samples were infected with two species of helminths including one with the genus *Taenia*. From a total of 128 fecal samples collected in 2017, 28 samples (Cascade red fox = 23 [25%]; coyote = 5 [14%]) were infected with three different helminths (Table 2.2). Twelve red fox feces were infected with *T. canis* (13%; low parasite load, $n=7$; moderate, $n=2$; heavy, $n=2$; very heavy, $n=1$), and 12 samples were infected with *T. leonina* (13%; low parasite load, $n=7$; moderate, $n=1$; heavy, $n=1$; very heavy, $n=3$). Two samples were infected with two species of helminths with one of those having the only detection of *A. caninum*. Four coyote fecal samples were infected with *T. canis* (11%; low parasite load, $n=3$; moderate, $n=1$), and two samples were infected with a low parasite burden of *T. leonina* (Table 2.2). Only one sample was infected with the both ascarids.

DISCUSSION

I detected four helminth species in Cascade red fox and coyote scats collected in

2016 and 2017. All of the helminths identified are consistent with findings from previous wild canid studies worldwide (Richards et al. 1995; Wolfe et al. 2001; Gompper et al. 2003; Saeed et al. 2006; Liccioloi et al. 2012; Figueiredo et al. 2016; Redman et al. 2016). The present study demonstrated lower parasite diversity with four types of helminths across the two canid species as compared to other studies that have identified up to 19 species (Richards et al. 1995; Gompper et al. 2003; Saeed et al. 2006), however, the majority of the studies examined gastrointestinal contents rather than scats alone.

This study showed that overall prevalence of parasitism in Cascade red fox scat was 45% ($n=18$) in 2016, and 25% ($n=23$) in 2017. Compared with studies on lowland red foxes (Richards et al. 1995; Wolfe et al. 2001; Saeed et al. 2006) and the Cascade red fox study (Aubry 1983), the results in my study show incidence of parasitism to be rather low. Similarly, the prevalence of helminths detected in coyote scat (14%, $n=5$) was also less than other studies with prevalence reported between 38% and 93% (Gompper et al. 2003; Bridger et al. 2009; Liccioloi et al. 2012; Redman et al. 2016). The majority of all the Cascade red fox and coyote fecal samples were infected with only one species of helminth, which was consistent with similar studies (Gompper et al. 2003; Figueiredo et al. 2016). However, Saeed et al. (2006) reported that most of the red foxes in the study were infected with multiple parasites, with the greatest proportion infected with three.

All of the helminths detected in the 2017 scat samples, and three of the four from 2016 were nematodes: *Toxocara canis*, *Toxascaris leonina*, and *Ancylostoma caninum*. While nematodes are easily recovered with the centrifugation fecal flotation using sugar solution (Dryden et al. 2005), the true prevalence in this study may be underrepresented. For the 2017 samples, both ascarids, *T. canis* and *T. leonina* were prevalent in 13% of

foxes, and in 6% of coyotes. *T. canis* and *T. leonina* had a prevalence of 18% and 23% respectively for the 2016 samples. The reported prevalence of *T. canis* infection in red foxes worldwide is between 56% and 80% depending on the population examined (Richards et al. 1994; Overgaauw and van Knapen 2000; Saeed et al. 2006). Aubry (1983) reported prevalence of ascarid infection in 77% of the Cascade red foxes examined. Hosts can be infected with *T. canis* or *T. leonina* directly through ingestion of infective eggs or paratenic hosts (Overgaauw and van Knapen, 2000; Schnieder et al. 2011; Taylor et al. 2016). Of the two ascarids detected in this study, *T. canis* is most commonly found in young canids due to its ability to be transmitted transplacentally (Ávila and Isaac 2013) and transmammary (Overgaauw and van Knapen 2000; Schnieder et al. 2011). This ascarid is also the most pathogenic in pups, potentially causing such symptoms as diarrhea, failure to gain weight or grow, vomiting, poor haircoat, a pot-bellied appearance, abdominal discomfort, and reduced immune fitness (Lee et al. 2010; Bowman and Nelson 2014; Nemzek et al. 2015; Reinemeyer 2016). With its ability to migrate, *T. canis* is also an important zoonotic parasite that can cause a variety of toxocariasis syndromes in humans including visceral larva migrans and ocular larva migrans (Lee et al. 2010; Ma et al. 2018).

The hookworm, *A. caninum* was found in only one of the 2017 fox scats and none of the coyote scats, however, it was detected in 13% of the samples in 2016. Hookworms such as *A. caninum* and *Uncinaria stenocephala*, are the second most common nematode found in canids of the northern hemisphere (Anderson 1992; Overgaauw and van Knapen 2000; Bowman and Nelson 2014; Otranto et al. 2015). There was no detection of *U. stenocephala* in the current study, however, it had been reported as a prevalence of 85%

(Aubry 1983). Adult hookworms hook their buccal capsules to their hosts' intestinal mucosa and feed on the ruptured capillary blood (Lefkaditis 2001). Pathogenicity of hookworms varies among species with *A. caninum* causing some of the most severe symptoms of all canine helminths (Overgaauw and van Knapen 2000). The most dramatic consequence of *A. caninum* infection in pups is anemia, however, other symptoms include ill thrift, anorexia, weight loss, stunted growth, bloody diarrhea, dehydration, and poor haircoat (Bowman and Nelson 2014; Nemzek et al. 2015; Taylor et al. 2016; Seguel and Gottdenker 2017). Transmission of *A. caninum* to the canine host is either through ingestion of contaminated soil, paratenic host, cutaneous penetration, or in the case of nursing pups via milk (Anderson 1992; Overgaauw and van Knapen 2000; Lefkaditis 2001; Bowman and Nelson 2014; Nemzek et al. 2015). The ability of penetrating the skin makes this parasite a significant zoonotic threat with the potential of cutaneous larval migrans (Overgaauw and van Knapen 2000). Throughout Europe, red foxes are known to be significant reservoirs of *U. stenocephala*, with prevalence between 38% and 86% depending on the population studied (Willingham et al. 1996; Criado-Fornelio et al. 2000; Reperant et al. 2007; Stuart et al. 2013). Endangered red wolves (*Canis rufus*) and sympatric coyotes in southeastern United States had a prevalence of 94% for *A. caninum* infection, and 32% prevalence of *U. stenocephala* infection (Brzeski et al., 2015).

Cestodes (Taeniid species) were detected in one sample from 2016 and none were detected in 2017. Cestodes are challenging to recover through fecal flotation alone because proglottids are not uniformly distributed in fecal material nor are they consistently shed from the host, and in order to float, the proglottids would need to

release their eggs (Little 2007; Liccioli et al. 2012; Bowman and Nelson 2014). Detection of cestodes in the prior Cascade red fox study was successful with gross examination of intestines, with prevalence of *Diplydium caninum* in 15%, *Mesocestoides* spp. in 77%, and *Taenia* spp. in 31% (Aubry 1983). One cestode commonly found in red foxes around the globe, *Echinococcus multilocaris*, has significant zoonotic importance because of its potential to cause the serious disease, alveolar echinococcosis (AE) in humans (Tackmann et al. 2001; König and Romig 2010; Bowman and Nelson 2014; Massolo et al. 2014; Otranto et al. 2015). *E. multilocaris* is expanding in range with increased wildlife infection, likely due to a warming climate (Bowman and Nelson 2014; Massolo et al. 2014; Otranto et al. 2015; Taylor et al. 2016). While *E. multilocaris* has not yet been detected in Cascade red fox or sympatric coyotes, future monitoring for this parasite is paramount for prevention of transmission to domestic dogs and humans who visit MORA. Throughout the scat survey, the survey team encountered several domestic dog feces. Not only are visiting pets potential reservoirs of parasites, but they themselves may be exposed to parasites and pathogens from wildlife, including those of zoonotic nature.

Limitations

This research was met with several limitations that may provide some explanation as to the low number of helminth diversity and lower parasitism prevalence as compared to results of similar studies. First, there was a high variability in the condition of the scats from fresh and moist to dry and overwintered. This presents a couple of problems, such as, the potential for contamination from soil-dwelling parasite species, as well as increase the likelihood for degradation of parasites due to time and environmental exposure.

While some helminth species eggs can survive through extreme freezing, the morphology of other species may be altered (Liccioli et al. 2012). Secondly, my parasite results may not be a true representation of the Cascade red fox and coyote populations as a whole. Scats may have been missed during the survey because of low visibility, or the actual location was off trail. In addition, without genetic testing, it is unknown as to whether or not some samples came from the same animal. Some of the scat samples were also quite small, thereby not providing an ample amount to examine. Next, the limited duration of the survey to the summer months may pose a problem due to the seasonality of some parasite life cycles and their sensitivity to temperature and weather conditions. Further, hosts may not always shed eggs in their feces despite their helminth infection because there are times when egg excretion is greatly reduced (Liccioli et al. 2012). Screening of helminths was conducted with non-invasive methods of scat collection and fecal analysis based on their suitability for studying threatened and endangered wildlife populations. Fresh fecal collection directly from the animal would likely produce higher accuracy of helminth recovery. However, it has been noted by other authors that fecal flotation underestimates the level of certain species of helminths (Willingham et al. 1996; Criado-Fornelio et al. 2000; Martínez-Carrasco et al. 2007). In regards to freezing, the initial deep freeze of the scat samples may have also caused degradation, and in the future, samples should only be kept in refrigeration for a short duration prior to analysis.

CONCLUSION

A fundamental part of wildlife conservation is recognizing species that may serve as indicators of ecological health. Many wild canid species fit the role of sentinel species because of their threatened or endangered status, specialized habitat requirements, and sensitivity to environmental perturbations (Aguirre 2009). Parasite monitoring in wild canids can provide insight to the state of health of the environment as well as indicate which species are most affected. Knowledge gained in this study about the helminth fauna of Cascade red fox and coyotes in Mount Rainier National Park provides important information to inform their conservation. From the time of the first examination of helminth fauna of Cascade red fox in 1983 until present day, the ecosystem of MORA has undergone many alterations and has thus increased the foxes' risk of disease. Climate change is altering parasite life cycles and host-parasite dynamics, and it may promote range expansion of nonnative parasite species (Polley and Thompson 2009; Polley et al. 2010; Atehmengo and Nnagbo 2014). Therefore, routine monitoring for the presence of parasites in Cascade red fox is vital to the survival of this small, rare species.

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Table 2.1 Scoring system for determining parasite load during microscopic fecal analysis.

Parasite load		Number of eggs per slide
+	Low	1-4
++	Moderate	5-10
+++	Heavy	11-20
++++	Very heavy	<20

Table 2.2 Number of positive samples and prevalence of helminths found in feces collected in Mount Rainier National Park.

Canid species	Helminth species	Number positive	Prevalence (%)
2016			
Cascade red fox (n=36) <i>Vulpes vulpes cascadenis</i>	<i>Toxocara canis</i>	7	18
	<i>Toxascaris leonina</i>	9	23
	<i>Ancylostoma caninum</i>	4	13
	<i>Taeniia</i> sp.	1	3
2017			
Cascade red fox (n=92) <i>Vulpes vulpes cascadenis</i>	<i>Toxocara canis</i>	12	13
	<i>Toxascaris leonina</i>	12	13
	<i>Ancylostoma caninum</i>	1	1
Coyote (n=36) <i>Canis latrans</i>	<i>Toxocara canis</i>	4	11
	<i>Toxascaris leonina</i>	2	6



Figure 2.1 Map of Washington State and boundary of Mount Rainier National Park. Map created by Jessica Brown using ArcGIS online; World Imagery base map; boundary feature from National Park Service; USA state boundary from ESRI.

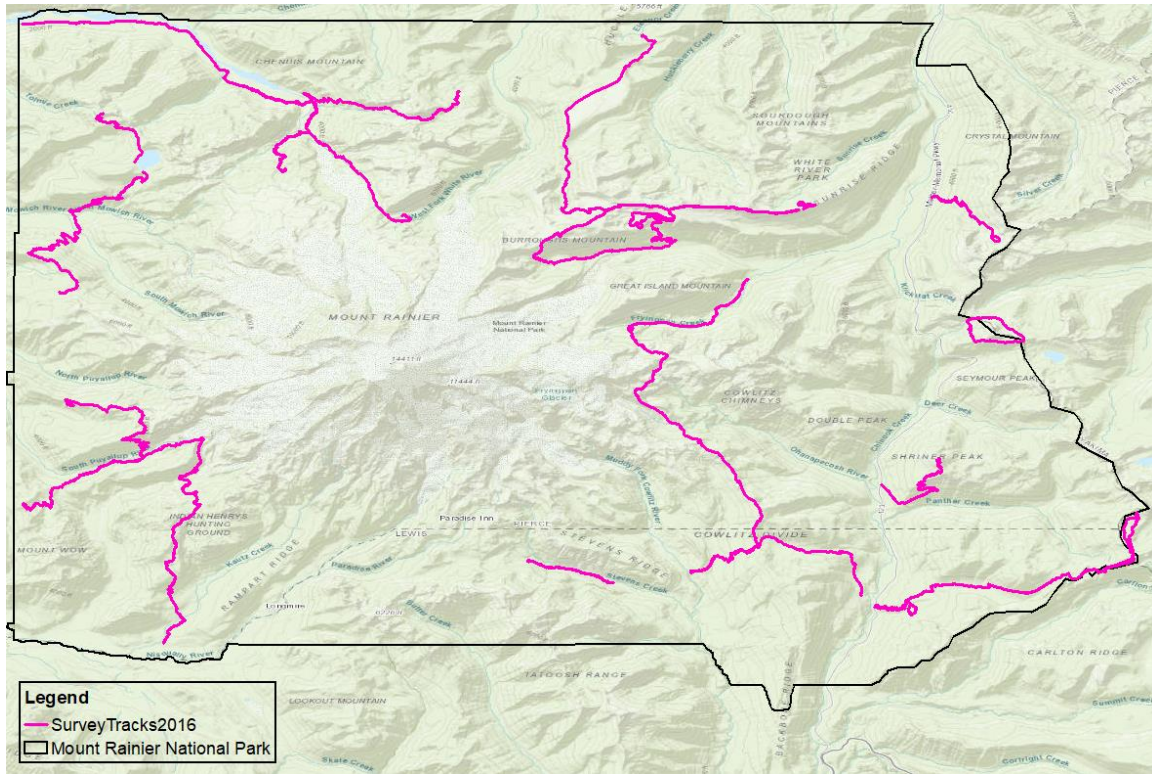


Figure 2.2 Trails hiked and surveyed in Mount Rainier National Park (MORA) by scat survey crew in 2016. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

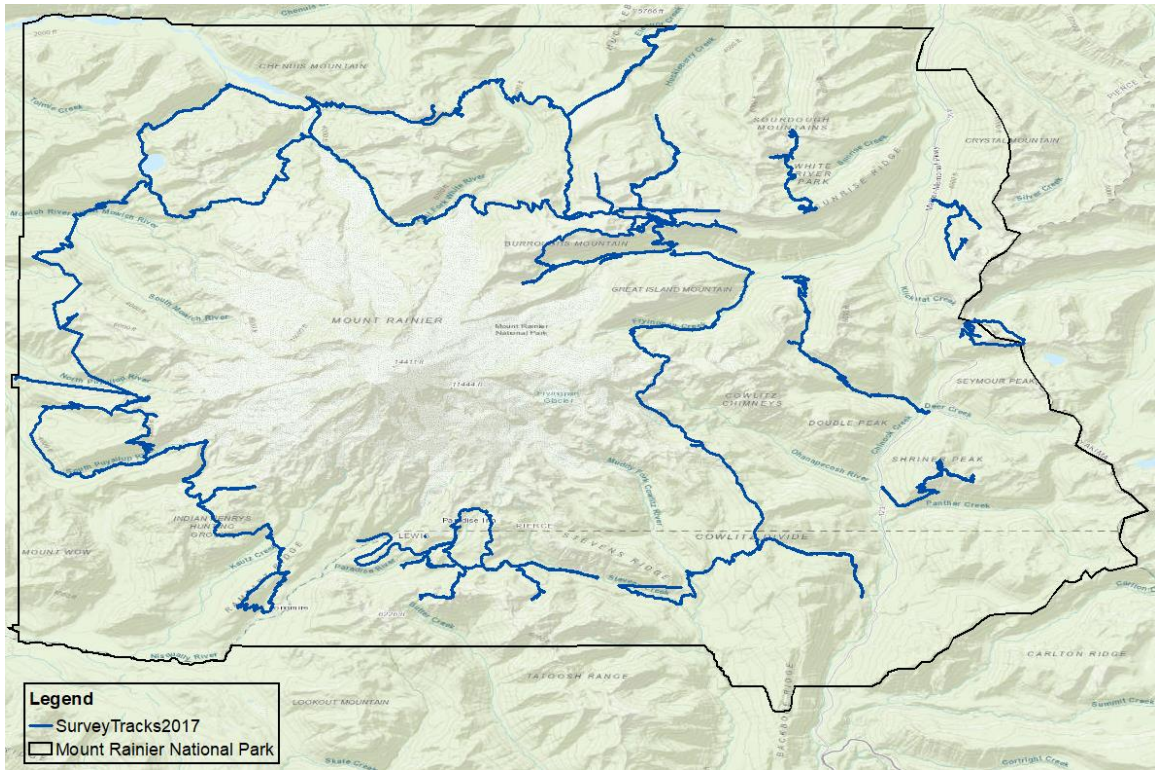


Figure 2.3 Trails hiked and surveyed in Mount Rainier National Park (MORA) scat survey crew in 2017. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.



Figure 2.4 GPS showing coordinates and elevation of a coyote scat located in Mount Rainier National Park; 2017. Photo by Jessica Brown.



Figure 2.5 Cascade red fox scat samples collected in 2017 from Mount Rainier National Park. Bags are labeled with field ID, coordinates, and elevation where collected. Photo by Jessica Brown.

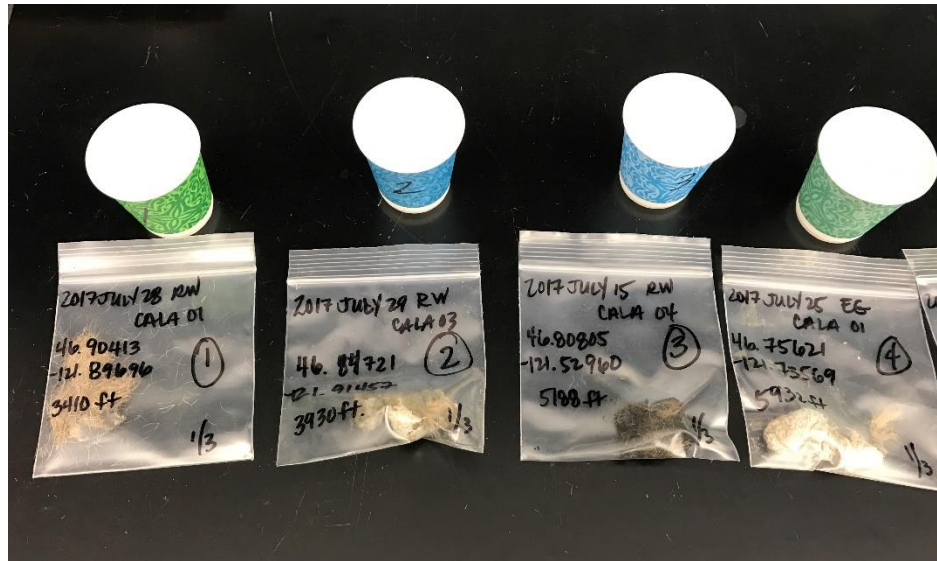


Figure 2.6 The first step of the fecal flotation process: Coyote fecal samples and the designated paper cups. Photo by Jessica Brown.



Figure 2.7 Test tubes with coverslips during the final phase of fecal flotation. Photo by Jessica Brown.

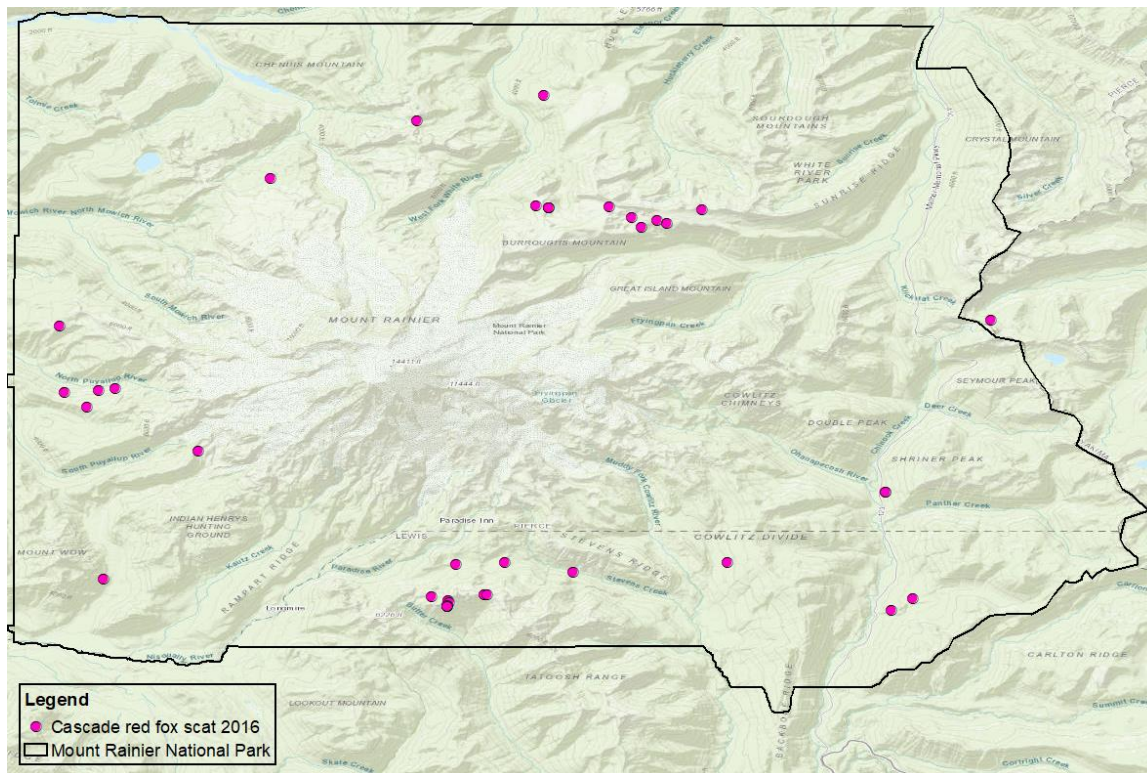


Figure 2.8 Map showing locations of Cascade red fox scat collected in 2016 by mesocarnivore survey team in Mount Rainier National Park (MORA). Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

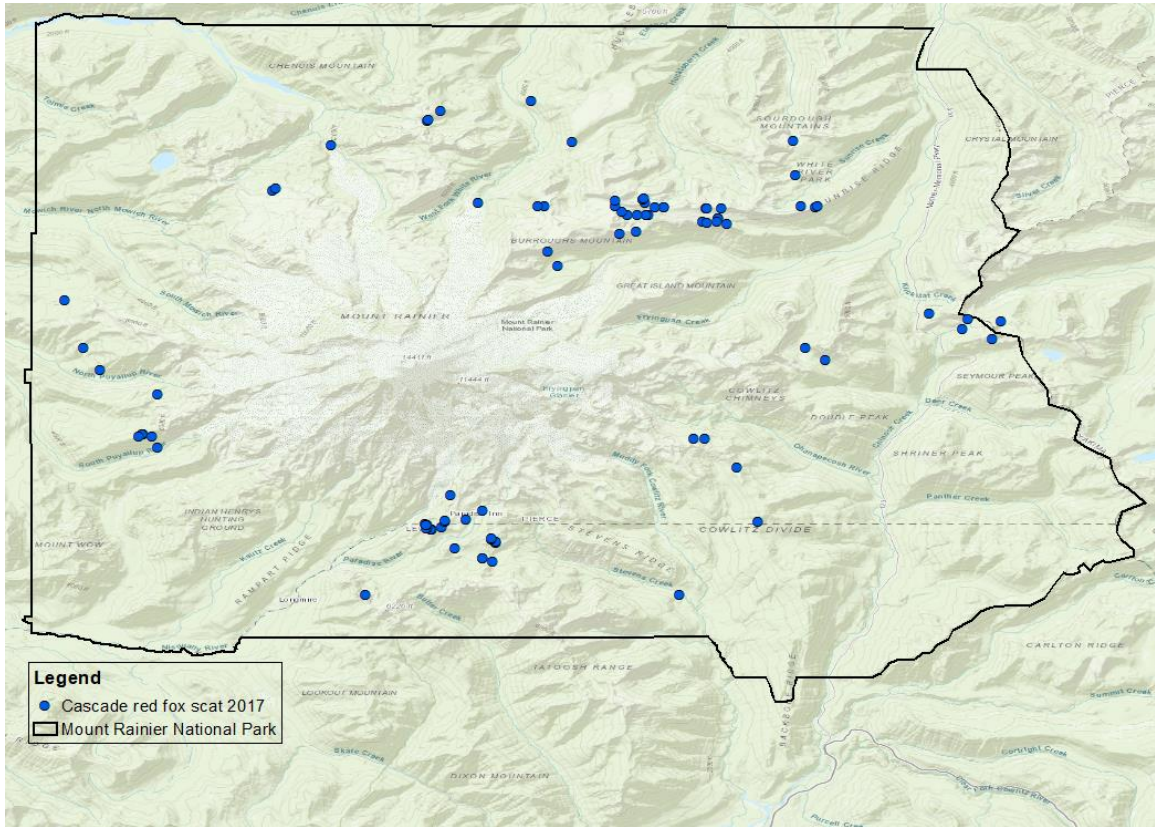


Figure 2.9 Map showing locations of Cascade red fox scat collected in 2017 by mesocarnivore survey team in Mount Rainier National Park (MORA). Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

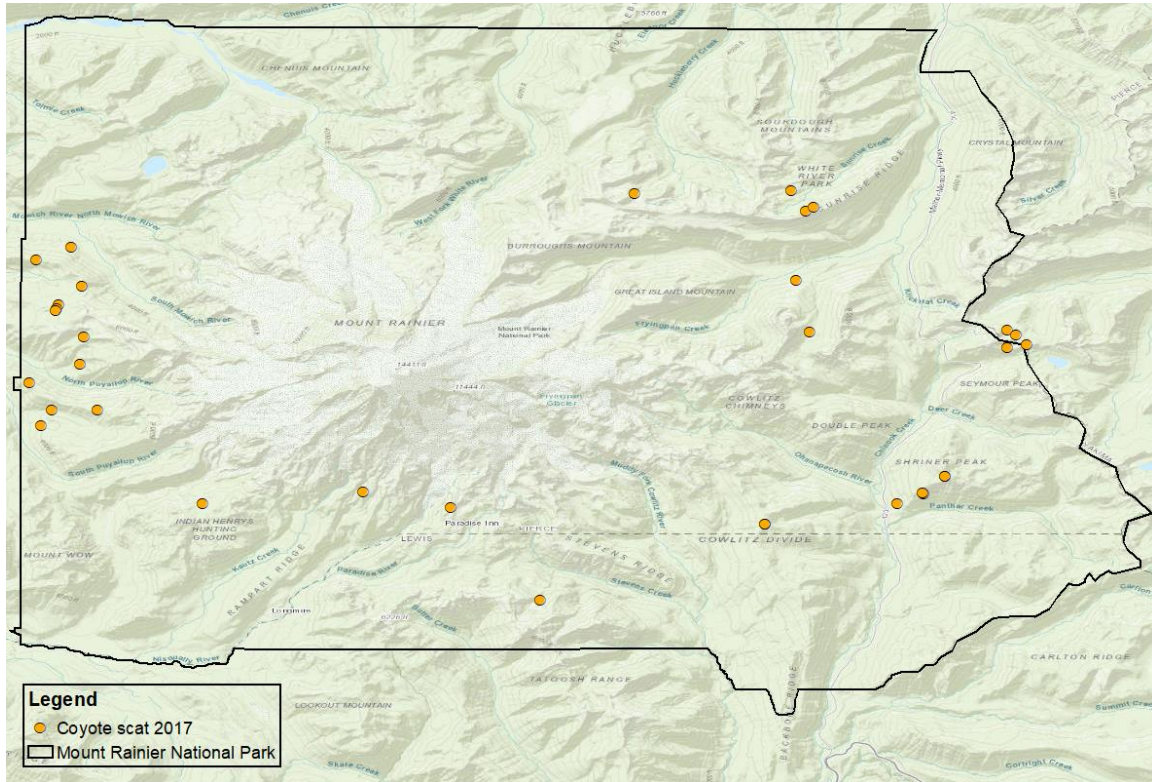


Figure 2.10 Map showing locations of coyote scat collected in 2017 by mesocarnivore survey team in Mount Rainier National Park (MORA). Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

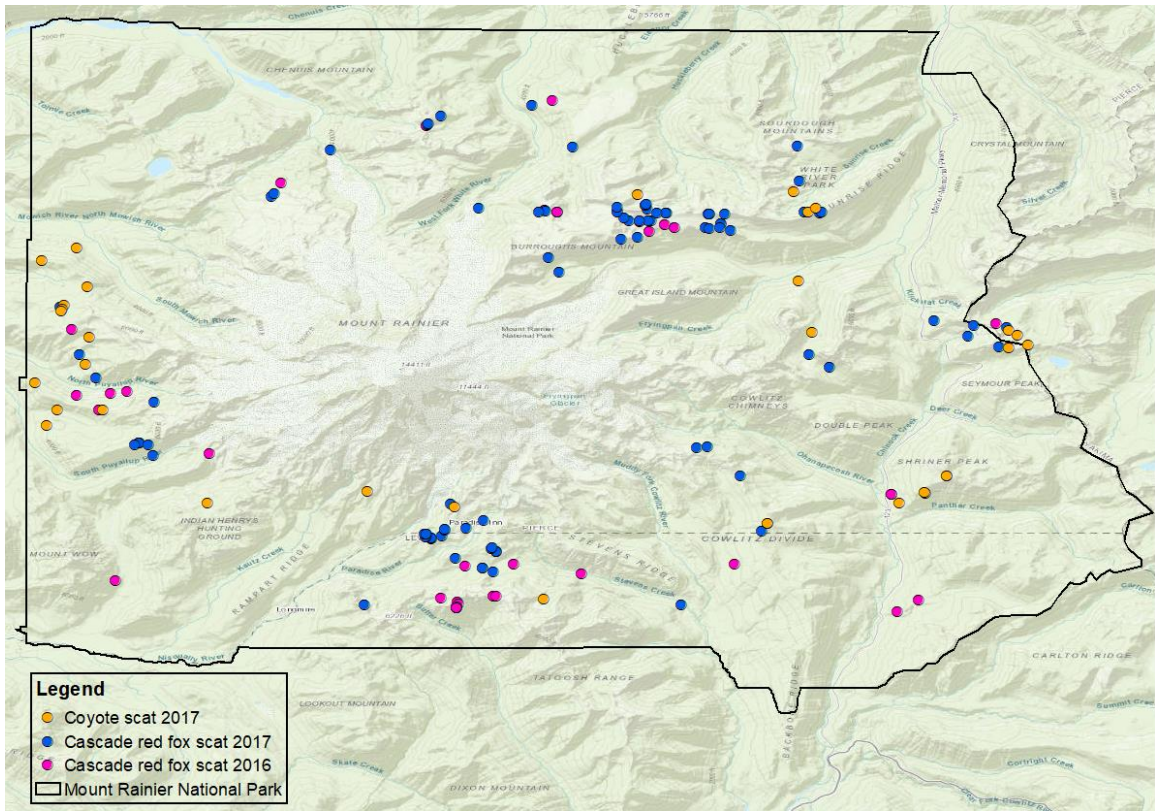


Figure 2.11 Map showing locations of Cascade red fox scat 2016 (pink), Cascade red fox scat 2017 (blue) and coyote scat 2017 (orange) collected by mesocarnivore survey team in Mount Rainier National Park (MORA). Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

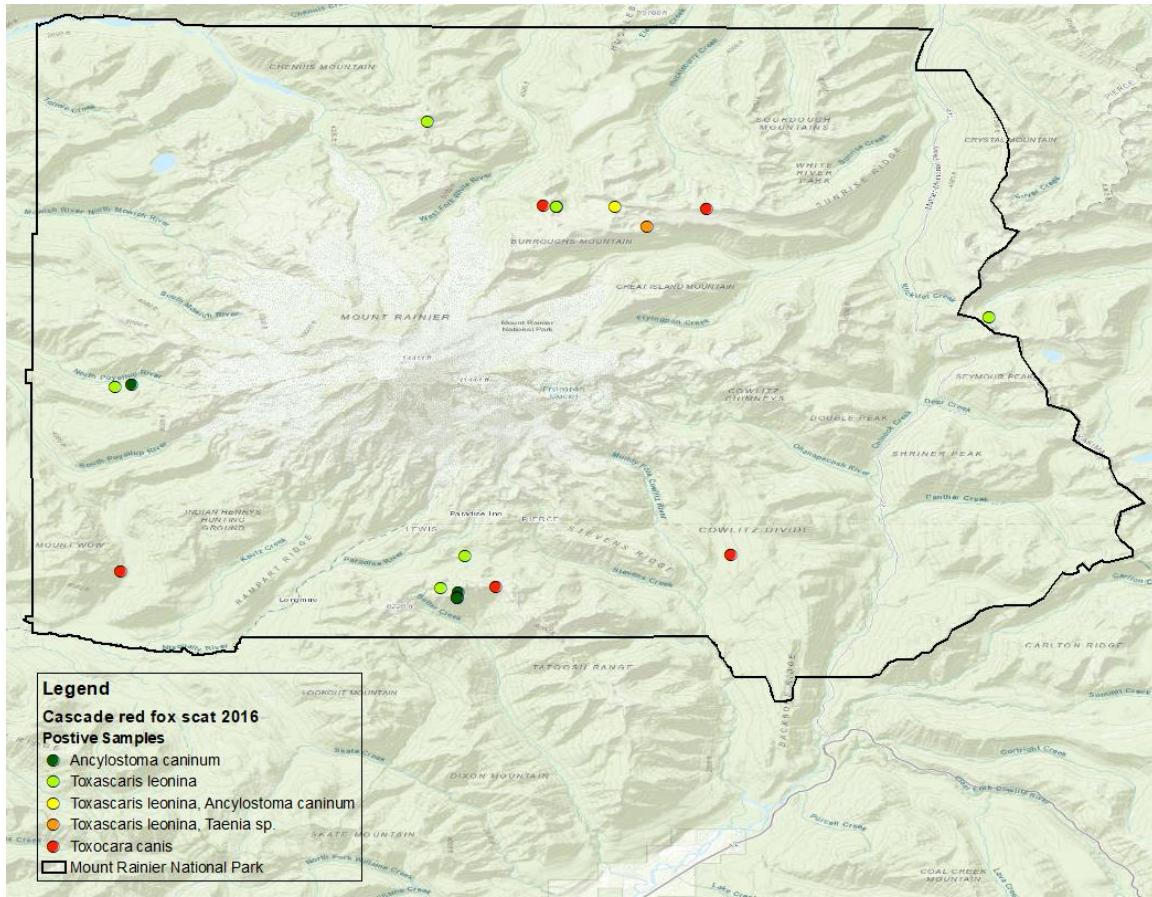


Figure 2.12 Map showing the location of each Cascade red fox scat with helminth infection for 2016 in Mount Rainier National Park (MORA). The scats are color coded based on the helminth detected and the parasite load. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

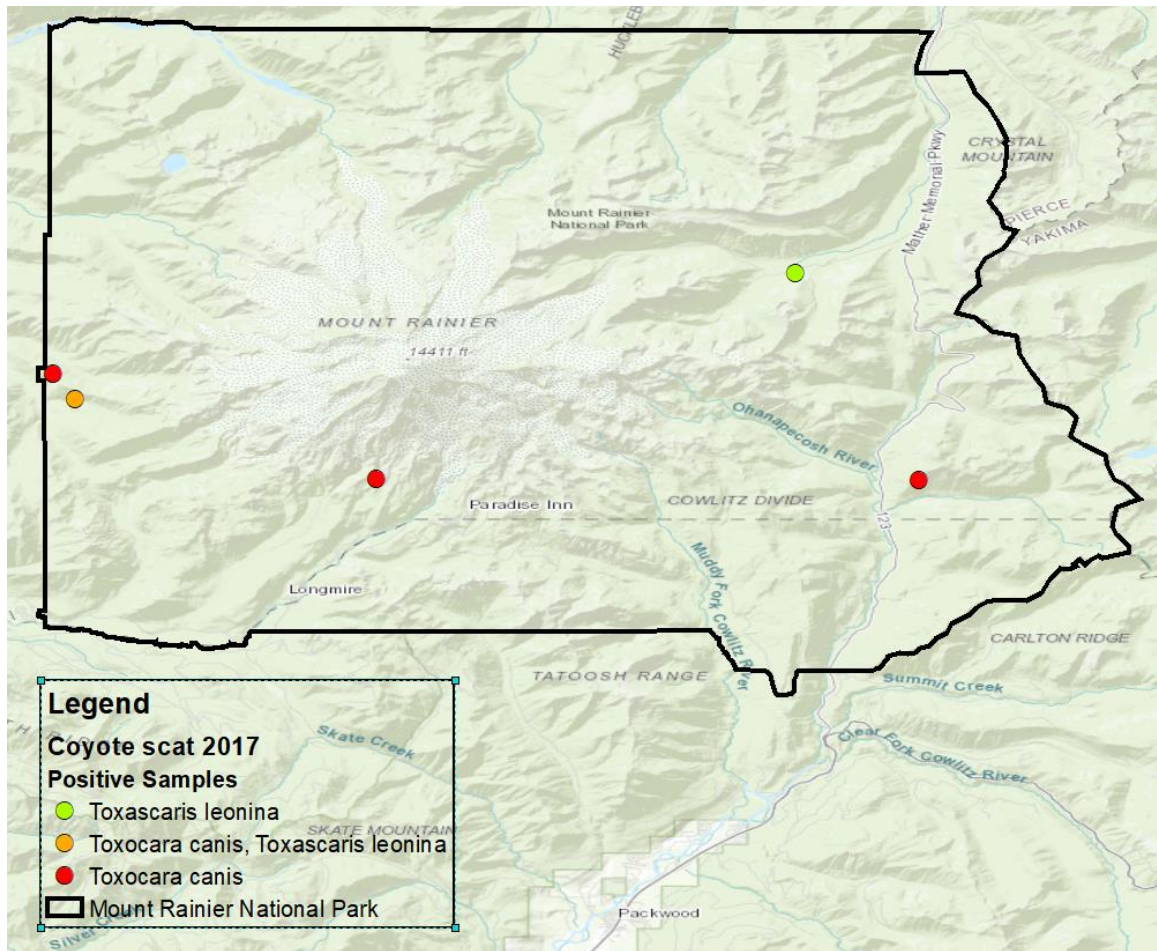


Figure 2.13 Map showing the location of each coyote scat with helminth infection for 2017 in Mount Rainier National Park (MORA). The scats are color coded based on the helminth detected and the parasite load. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

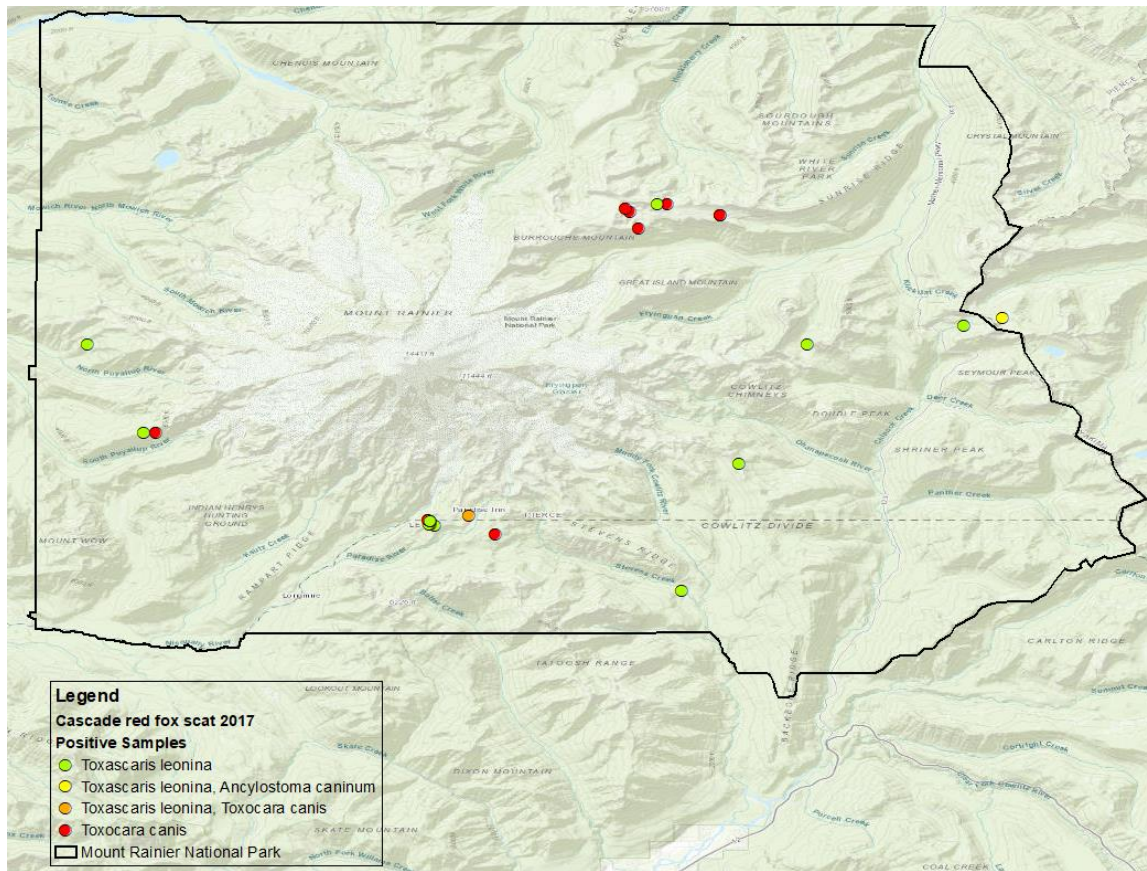


Figure 2.14 Map showing the location of each Cascade red fox scat with helminth infection for 2017 in Mount Rainier National Park (MORA). The scats are color coded based on the helminth detected and the parasite load. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

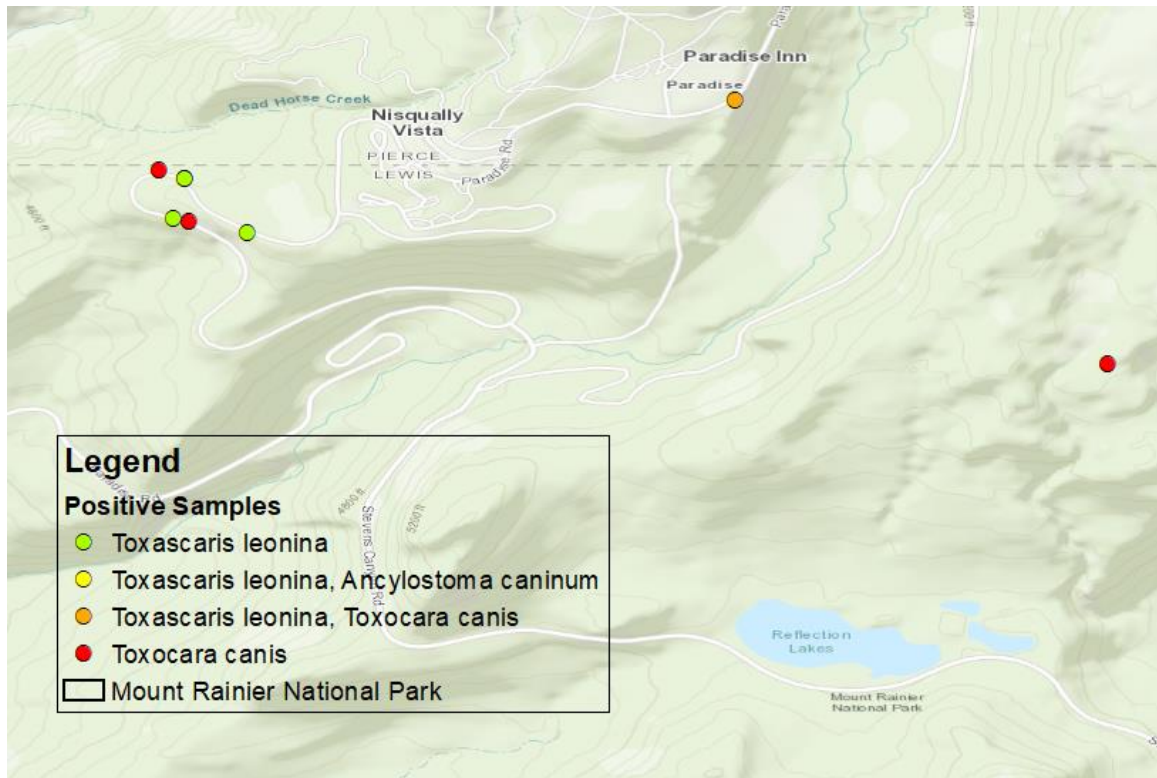


Figure 2.15 Close-up view of the Paradise area, Mount Rainier National Park (MORA), showing the location of each Cascade red fox scat with helminth infection for 2017. The scats are color coded based on the helminth detected and the parasite load. Map created by Jessica Brown using ArcGIS online; coordinates provided from MORA; World Imagery base map; National Park Service boundary feature.

CHAPTER 3

SUMMARY AND CONCLUSION

Carnivores in North America are experiencing population declines and a decrease in their range, mainly as a result of human activities (Laliberte and Ripple 2004) such as habitat loss and degradation, habitat fragmentation, pollution, urbanization, and climate change (Pimm et al. 1995; Aguirre and Tabor 2008). These environmental alterations are also having an impact on disease emergence and transmission of parasites among wildlife species and humans (Dazsak et al. 2000; Aguirre and Tabor 2008; Aguirre 2009; Hollings et al. 2013). Threatened or endangered wildlife species with small populations are particularly vulnerable to the consequences of disease and parasitism, potentially causing further population decline (Dazsak et al. 2000). Further, disease transmission is changing as a result of closer contact between humans, domestic animals, and wildlife (Dazsak et al. 2000; Aguirre and Tabor 2008; Aguirre 2009; Hollings et al. 2013). Cascade red fox (*Vulpes vulpes cascadensis*), the only native red fox in Washington State, is one such mesocarnivore that has experienced recent population decline likely attributed to human impact (Akins et al. 2018). Historically, Cascade red fox could be found at high elevations throughout the entire Cascade Range, however, more recently they have only been detected in the southern Cascades (Akins et al. 2018), specifically in the Mount Rainier National Park (MORA) and parts of Gifford Pinchot National Park (Jenkins et al. 2014). With their small population, this montane red fox subspecies faces multiple threats and is susceptible to further population decline should disease become a threat. Cascade red fox is also showing a loss in genetic diversity which can lead to inbreeding (Akins et

al. 2018). Inbreeding has been linked to a reduction in an individual's fitness including the ability to resist many parasites and pathogens (Charlesworth and Charlesworth 1987; Templeton et al. 1990; Lynch 1996; Lacy 1997).

In addition to direct consequences of human activities, the movement of coyotes into Cascade red fox habitat is another threat. The pre-colonial range of the coyote was limited to the Great Plains and since the 1800s they rapidly expanded their range to most of North America (Gompper 2002). Landscape alterations, warming climate, and decreasing snow pack may influence the range of coyotes, creating habitat more conducive to this wild canid species (Perrine 2005). Coyotes are known to be dominant competitors and predators of red fox (Sargeant and Allen 1989; Levi and Wilmers 2012), as well as host to a variety of canine parasites and pathogens (Radomski 1989; Foster et al. 2003; Gompper et al. 2003; Bridger et al. 2009; Liccioli et al. 2012; Redman et al. 2016). The possibility for future parasite spillover from coyotes into Cascade red fox populations is likely if these two wild canids share the same habitat.

My research aims to provide valuable insight into future potential threats to Cascade red fox populations through investigation and characterization of gastrointestinal parasites (helminths) within this species and the sympatric coyote. Information generated from this study will help inform wildlife managers and allow researchers to measure changes in parasite diversity and intensity to help inform conservation.

This study focused on parasite analysis of Cascade red fox scat and coyote scat that was collected during surveys conducted in the summer of 2016 and 2017 in MORA. Results showed that both Cascade red fox and coyotes carry common canine helminths that have pathogenic potential (Bowman and Nelson 2014; Nemzek et al. 2015; Taylor et

al. 2016; Seguel and Gottdenker 2017) as well as those with zoonotic importance (Overgaauw and van Knapen 2000; Lee et al. 2010; Ma et al. 2018). Overall prevalence of helminth infection in Cascade red fox for 2017 was 25% with a total of three helminth species identified: *Toxocara canis* (13%), *Toxascaris leonina* (13%), and *Ancylostoma caninum* in one individual. The prevalence of overall helminth infection was less in coyotes at 14% with two species identified: *Toxocara canis* (11%), and *Toxascaris leonina* (6%). Results from 2016 revealed an overall helminth prevalence of 45% with four types of helminths identified: *T. canis* (18%), *T. leonina* (23%), *A. caninum* (13%), and a Taeniid species recovered from one individual. The nematodes *T. canis* and *A. caninum* are among helminths with the potential to cause disease in their hosts, including the accidental human host. In canines, clinical symptoms are mainly seen in young pups and can include weight loss, diarrhea, vomiting, poor haircoat, and unthriftiness (Bowman and Nelson 2014; Taylor et al. 2016; Seguel and Gottdenker 2017). Pups infected with *A. caninum* may also suffer from anemia, which can be quite debilitating (Overgaauw and van Knapen 2000; Bowman and Nelson 2014). While parasites are ubiquitous, and alone do not always cause clinical disease, additional stressors such as coinfection with other parasites, and the added effects of climate change and habitat fragmentation can increase susceptibility to morbidity and mortality.

With a small genetic effective population size, Cascade red fox is facing additional loss of genetic diversity (Akins et al. 2018), and therefore may be less resilient in the future to undergo parasitism and disease. Going forward, it is important to continue including parasitological surveys with other research currently conducted on Cascade red fox. Knowledge on the parasite fauna of Cascade red fox and understanding of host-

parasite dynamics through a continually changing environment is a crucial component in the conservation of this species.

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