Beneath the Surface: sub-lethal effects of parasites and pollutants on mammal energetics and implications for aquatic ecosystems.

By

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Abstract

Sub-lethal infections by helminth parasites are generally considered unimportant for wildlife fitness. However, data on the sub-lethal effects of parasites, combined with the effects of anthropogenic stressors, are often lacking. To fill this gap in knowledge, I carried out a meta-analysis of the literature to determine if sub-lethal infections of helminth parasites significantly influence mammal energetic condition. In parallel, I surveyed the parasites and metal contaminants of the North American river otter (*Lontra canadensis*) and mink (*Neogale vison*) in Alberta and British Columbia, modeling their effects on host energetic condition. Parasite communities and metal contaminants were also compared against previous reports on otter and mink across North America collected via a systemic search of the literature. Although river otter and mink are ecologically, economically, and charismatically important semi-aquatic mammals in Canada, little is known about their parasites and contaminants. As carnivores at the top of freshwater food chains, they are ideal sentinels of ecosystem health.

Results of the meta-analysis showed strong negative effects of infection on mammal energetic condition across major taxa. Effect size was influenced by methodological bias, suggesting that research limitations may have driven assumptions of insignificance. Surveys of the parasite populations in sampled mink and otter showed a diversity of parasites, including multiple species of zoonotic concern, and was characterized by four main species (*Alaria mustelae*, *Filaroides martis*, *Isthmiophora inermis*, and *Versteria rafei* n. spp.). Most prevalent were infections by mesocercariae of *A. mustelae* (Trematoda:Diplostomidae), a zoonotic species, related to subacute to chronic inflammation, not previously reported in otter and mink in North America. Due to their economic importance as furbearing species, especially for First Nation and Metís peoples, otter and mink are a potential pathway for the spread of zoonotic pathogens. Concentrations of metal contaminants were generally high compared to historical reports in these species in North America; Silver, Cadmium, Copper, Iron, Gallium, Rubidium, and Strontium were present at particularly high concentrations. Gallium and Rubidium, which have historically received little attention, were seen at much higher levels than previously reported and may represent emergent contaminants of concern. Anthropogenic sources of contaminants are significant drivers of this trace metal contamination, especially industrial, mining, pulp and paper, and oil and gas activities, across both local and sub-watershed scales. Areas of concern where populations may be more at risk include coastal populations along the Salish Sea, including Vancouver Island and the Sunshine Coast, the Columbia and Thompson River valleys and other environments impacted by coal mining, transport, and processing, and watersheds within the Alberta oil sands. Energetic condition was significantly influenced by mesocercariae of *A. mustelae* and elevated levels of essential metal contaminants, especially Copper and Zinc, and by Gallium, a rarely studied non-essential metal contaminant.

Overall, the findings of this study highlight serious gaps in knowledge concerning the importance of sub-lethal stressors such as parasites and metal contaminants in mammals. It synthesized the current state of the literature regarding sub-lethal effects of helminths on mammals and historical reports of metal contamination in otter in mink to create averages for easy comparison. As well as expanding our understanding of contaminant exposure and parasitic infection in aquatic mammals in Western Canada. This work suggests that parasites and contaminants are significant, interacting stressors on mink and otter populations. Future work on the potential effect of rising temperatures on parasite success, contaminant bioavailability, and their cumulative effects on mammal fitness is urgently needed.

Preface

This thesis is the product of collaborative work with other scientists, which is reflected by the authorship of published chapters and those in prep for submission. For all of these, I was responsible for study design, the organization of collaborations, the majority of data collection, data analysis, and writing. My collaborators gave invaluable assistance in the logistics around sample collection and processing, provided facilities and monetary support, as well as input in the design and writing phases.

Part of **Chapter 1** has been published as: Shanebeck, K.M. and C. Lagrue (2020). Acanthocephalan parasites in sea otters: Why we need to look beyond associated mortality... *Marine Mammal Science*, **36**, 676–689. KMS developed the review, conducted the literature search, and wrote the manuscript. CL helped develop the review and edited the manuscript.

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My path to academia has not been direct, made difficult by personal and economic struggles. I wish to acknowledge the hardships I have had to overcome as a queer and neurodivergent person to complete this achievement, including poverty, childhood abuse, workplace bias, harassment, and discrimination, mental health struggles, and much more. Though I have worked hard to overcome these things and have been successful, I know I could have just as easily failed despite my hard work and dedication. Because of that, I wish to also recognize the many queer, neurodivergent, racialized, and other marginalized people who did not make it, whose loss has weakened us as a scientific community.

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List of Abbreviations and Glossary of Terms

18S rRNA: gene associated with the 18S (small) subunit of the eukaryotic ribosome.

Absolute Fitness: Number of offspring produced by an individual over its life, therefore intrinsically including survival and lifespan.

Acanthocephalan: common name of members of the Phylum Acanthocephala, also known as thorny-headed worms.

Acute (pathology/toxicity): of a shorter duration, generally 4-6 days, but this is not absolute.

Body Condition: broad category of metrics used to measure the quality and overall health and functioning of an animal. Most often related to nutrition or body size.

Cestode: common name of members of the Class Cestoda, also known as tapeworms.

Chronic (pathology/toxicology): of a longer duration, generally over two weeks and often will be indicated by re-generation of tissue or repair.

Cohen's d: An effect size, the standardized difference of the means between two groups, adjusted by sample size.

CO1 or COX: gene associated with the coding of cytochrome c oxidase in the mitochondrial DNA.

Diffuse (pathology): describing the distribution of effect close up, lesions are spread throughout the affected area.

Diplostomid: members of the Family Diplostomidae

Disturbance: Negative effects on individual fitness due to behavioral or physiological changes in animals resulting from predator, parasite, or anthropogenic influence.

Downstream Effect (infection): The result of compounding or cascading effects due to the disruption of energetic condition. For example, infection by an intestinal nematode alters gut chemistry to kill competing bacterial fauna, leading to reduced digestive efficacy and diarrhea (*primary effect*), this digestive disruption leads to increased foraging behavior and consumption of suboptimal but easily accessible prey (*secondary effect*). Nutritionally deficient prey, malabsorption, and loss of bacterially synthesized vitamins lead to a Vitamin K deficiency which causes gastrointestinal and urinary bleeding, leading to anemia, and consequently to fatigue, weakness, etc. (*downstream effect*).

Echinostome: members of the Family Echinostomatidae, in the Class Trematoda, characterized by head collar spines surrounding their oral sucker.

Effect Size: A comparable measure of the strength of the relationship between factors of interest (in this case parasite infection and body condition), calculated from provided statistical information or raw data.

Energetic Condition: The energy available in an individual to be allocated to physiological and behavioral functions essential for survival (systemic functioning, resource acquisition, defense/stress response, nutritional status) and reproduction.

Fibrosis (pathology): repair of lesions or other damage, where connective tissue replaces parenchymal tissue to such a degree that it remodels tissue and leads to permanent scar tissue.

Focal or Multifocal (pathology): describing the distribution of effect close up, lesions are in discrete spots or nodules.

Foci (pathology): discrete lesions or nodules.

Generalized (pathology): describing effects from a distance, lesions are spread throughout the organ or area.

GI: Gastrointestinal

GLMM: Generalized Linear Mixed Model

Heterogeneity: In a meta-analysis, is the inter-study variation in the observed effect sizes beyond what would be expected by random chance; can be due to differences in study design, host species, response proxies, or environmental differences.

Ecchymotic (pathology): related to ecchymosis, the movement of blood from ruptured blood vessels into the surface of a tissue.

Hemorrhage (pathology): loss of blood from a ruptured blood vessel, external or internal, often referring to the presence of a bleed within tissue.

Hemorrhagic (pathology): related to bleeding.

Hyperemia (pathology): an increase in blood vessels in order to increase blood flow to a tissue.

Lesion (pathology): damage or abnormal changes to tissue, may be due to trauma or disease.

Localized (pathology): describing effects from a distance, lesions are limited to one portion of an organ or area.

Lontra canadensis**:** scientific name of the North American river otter.

Melena (pathology): dark tarry feces, indicative of gastrointestinal bleeding.

Mesentery: the organ that is formed by the two folds of the peritoneum and attaches the intestines to the posterior abdominal wall.

Mesocercaria: prolonged intermediate stage in trematode lifecycle using a second intermediate or paratenic host, and can transfer between multiple hosts.

Metacercaria: final larval stage in trematode lifecycle, encysted in the tissue of the final intermediate or paratenic host and is infective for the definitive host.

Multiplicative or compounding effects (infection): Concurrent factors that may interact to increase the severity of response. In the present context, these are the interactions between parasites and other stressors like secondary infection, temperature or food availability that may interact to increase the observed effect of parasite infection on energetic condition.

NAD1: gene associated with the coding of NADH-ubiquinone oxidoreductase in the mitochondrial DNA.

Nematode: common name of members of the Phylum Nematoda, also known as round worms.

Neogale vison**:** scientific name of the North American mink

NMDS: Non-Multimetric Dimensional Scaling

Omentum: the larger of the two folds of the peritoneum that encases the abdominal organs.

Ondatra zibethicus**:** scientific name of muskrat.

Trematode: common name of members of the Class Trematoda, also known as flatworms.

Peritoneum: abdominal membrane that encases the internal organs to prevent friction damage and has some immune function.

Primary Effect (infection): The presence or action of a parasite leads to a physiological or behavioral response. These effects often occur quickly in response to infection and are localized to the tissue or system that the parasite infects. For example, an intestinal trematode burrows into the intestinal wall, leading to a localized inflammatory response, or a respiratory nematode encysts in lung tissue leading to wheezing, reduced lung capacity, and tissue necrosis.

Peritonitis: inflammation of the peritoneum

Population Fitness: Mean fitness of all the members of a population.

Relative Fitness: Ratio of the absolute fitness of an individual to the average absolute fitness of the population.

Secondary Effect (infection): An effect not directly related to the presence or action of the parasite, but in response to the physiological or behavioral primary effects. For example, an intestinal acanthocephalan secretes chemicals to suppress the systemic immune response (*primary effect*) which leads to increased severity of an unrelated infection, such as by a bacterial agent (*secondary effect*).

Serosal (pathology): related to the serosa, the membranes that line the body cavity and organs.

Subacute (pathology/toxicology): a process, symptom, or illness that is not yet considered chronic, but has passed the acute period.

Table i. State and Provincial abbreviations. Postal abbreviations of all included US states and Canadian provinces.

AK	Alaska	ME	Maine	WI	Wisconsin
AL	Alabama	MI	Michigan	AB	Alberta
AR	Arkansas	MT	Montana	BC	British Columbia
CO	Colorado	NC	North Carolina	MB	Manitoba
CT	Connecticut	NE	Nebraska	NB	New Brunswick
FL	Florida	NY	New York	NL	Newfoundland
GA	Georgia	OH	Ohio	NT	Northwest Territories
ID	Indiana	OR	Oregon	NS	Nova Scotia
IL	Illinois	PA	Pennsylvania	NU	Nunavut
KS	Kansas	TN	Tennessee	ON	Ontario
LA	Louisiana	TX	Texas	PE	Prince Edward Island
MA	Massachusetts	UT	Utah	QC	Quebec
MD	Maryland	WA	Washington	YT	Yukon

Table ii. Metal abbreviations. List of the periodic abbreviations of all tested metals.

Al	Aluminum	Cu	Copper	Rb	Rubidium
Ag	Silver	Fe	Iron	Se	Selenium
As	Arsenic	Ga	Gallium	Sn	Tin
Ba	Barium	Mn	Manganese	Sr	Strontium
Bi	Bismuth	Mo	Molybdenum	Tl	Thallium
Cd	Cadmium	Ni	Nickel	V	Vanadium
Co	Cobalt	Pb	Lead	Zn	Zinc

Chapter 1: General Introduction

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1.1 Parasites and mammals, looking beyond associated mortality

Parasites are everywhere. Every mammal in the world likely has some parasite associated with it. Yet parasites are often only considered influential in population dynamics or for conservation research if they kill their host or cause serious disease (Pedersen et al. 2007; Pullan and Brooker 2008; Delahay, Smith, and Hutchings 2009; Han and Ostfeld 2019; Gatto and Casagrandi 2022). This lack of induced mortality of pathogenicity may be why helminths (cestodes, trematodes, nematodes, and acanthocephalans) have historically been considered seldom important in studies on mammals. As a result, they are underrepresented in the literature (Pedersen et al. 2007; Han and Ostfeld 2019).

In recent years, some studies have highlighted the importance of parasites' non-consumptive effects on food-web topology: strengthening trophic links, increasing biodiversity, altering connectedness, and influencing energy flow (biomass) (Hudson et al. 2006; Kurris et al. 2008; Lafferty et al. 2008). Outside the ecological sphere, agricultural research has long understood the relationship between helminths and the monetary costs they incur in their mammalian hosts. Farmers, the world over, are concerned with the effects of intestinal helminths on nutrition (Ezenwa 2004; Nieuwhof and Bishop 2005), immune health (Thumbi et al. 2014), milk production (Charlier et al. 2009), and reproductive success (van Dijk et al. 2009) of their stock. Yet, in wildlife health and conservation research, parasites are generally only considered to have significant effects on host populations if they cause direct mortality, severe pathogenicity, or damage host tissues (Pullan and Brooker 2008; Thompson et. al 2010).

The cumulative effects of various stressors are important considerations when modeling the stability of species at risk (Marcogliese and Pietrock, 2011; Hodgson and Halpern 2019), and it has become increasingly apparent to ecologists that sub-lethal interactions can have strong influence on organisms, populations, and food-web dynamics. For example, predators do not have to consume prey to influence behavior or populations as illustrated by the ecology of fear

(Zanette and Clinchy 2019). Sub-lethal pollutant exposure can significantly alter fecundity, through either body condition or reproductive loss (Rattner 2018). Anthropogenic disturbance is of great concern for conservation, and researchers have developed a variety of models to forecast its sub-lethal effects on populations (King et al. 2015). Similarly, pathogens of moderate virulence, like many helminths, are predicted to have stronger effects on host populations than lethal parasites (Irvine 2006; Riordan, Hudson, and Albon 2007; Tompkins et al. 2011). Sub-lethal parasites tend to infect more hosts in a population, and if they affect host resource availability or reproductive success, they are more likely to affect individual fitness and population dynamics than highly virulent pathogens that occur in low prevalence (Irvine 2006; Riordan et al. 2007; Tompkins et al. 2011). Here, we use one such relationship between a threatened host species and its sub-lethal parasite to illustrate the various ways such a parasite can influence host health.

1.2 Acanthocephalans and sea otters: a case study

The loss of apex predators such as sea otters (*Enhydra lutris*) is a serious concern for conservation due to their key roles in ecosystem functioning (Estes et al., 2011). Although the fur trade nearly drove sea otters to extinction in the early 1900's, they persist today as a keystone species in coastal Pacific kelp forest and eelgrass ecosystems (Watson and Estes, 2011; Hughes et al., 2013). This species exerts top-down cascading effects, with strong influences on many other organisms. These top-down effects are best seen in otters' capacity to control urchin populations in kelp forests and crab species in eelgrass ecosystems (Estes and Palmisano 1974; Watson and Estes, 2011; Hughes et al., 2013). Furthermore, near-extirpation of these coastal marine predators may have had other unrecognized effects, such as the extinction of the Steller's sea cow (*Hydrodamalis gigas*) through indirect effects on seaweed forage (Estes et al., 2016). Continued monitoring of sea otter populations is thus essential in the conservation and management of coastal ecosystems.

Sea otters have been extensively studied, focusing on their trophic level (Estes and Palmisano, 1974; Reisewitz, et al., 2006; Watson and Estes, 2011; Markel and Shurin, 2015; Estes et al., 2016), behavior, population dynamics (Estes et al., 2003; Tinker et al., 2009; Tinker et al., 2019), and diseases (Thomas and Cole, 1996; Kreuder et al., 2003; Miller et al., 2010; Miller et al., 2017). In disease research, particular emphasis has been placed on the significance of parasitism on sea otter mortality. The protozoan parasites *Toxoplasma gondii* (Kreuder et al., 2003; Burgess et al., 2018) and *Sarcocystis neurona* (Miller et al., 2010; Tinker et al., 2019), and acanthocephalans of the genus *Profilicollis* have been of particular interest for conservationists due to their lethal effects on sea otters (Thomas and Cole, 1996; Kreuder et al., 2003; Mayer et al., 2003; Tinker et al., 2019). Sea otters are a dead-end host for these parasites. *Toxoplasma gondii* and *S. neurona* are parasites of terrestrial mammals and infect marine mammals through transport of infective stages by freshwater run-off to the ocean. *Profilicollis* spp. commonly infect birds as their final hosts but can also infect otters eating sand crabs (*Emerita analoga*) (Mayer et al., 2003; Miller et al., 2008; Miller et al., 2010; Tinker et al., 2019). In contrast, the most prevalent and only parasite that utilizes sea otters as its only known definitive host, the acanthocephalan *Corynosoma enhydri* (Mayer et al., 2003), and its potential effects on otter health, have received much less attention. This species is often considered unimportant for otter fitness and population dynamics because of a lack of evident pathological effects on the host (see below).

Corynosoma enhydri is the most common intestinal parasite found in southern (*E. l. nereis*) and northern (*E. l. kenyoni*) sea otters (Margolis et al., 1997; Mayer et al., 2003). Though published data on prevalence are limited to older studies, data from 2018-2019 show that *C. enhydri* continues to be the most prevalent parasite of otters, both in Alaska and California (Shanebeck, 2018). The genus was described in 1904 by Lühe and now includes over thirty species (Amin, 2013). Members of this genus are obligate parasites in marine ecosystems, predominantly infecting marine mammals but also birds (Aznar et al., 2006). In sea otters, *C. enhydri* was first identified by Morosov (1940) in Russian sea otters, and then by Van Cleave (1953) in northern sea otters. This parasite was noted at first for its extremely large size compared to other *Corynosoma* species (Rausch, 1953). Since then, research into *C. enhydri* has been limited, and the intermediate host(s) and life cycle are still unknown (Mayer et al., 2003).

In 1953, Rausch published a study investigating mortality and cause of death in northern sea otters off the Aleutian Island of Amchitka in Alaska. Animals were examined for internal helminths and *Corynosoma* spp., likely *C. enhydri*, was identified from several individuals. Though limited in scope, and lacking data on prevalence or abundance, it was concluded that *Corynosoma* were “unimportant parasites insofar as the sea otter is concerned” (Rausch, 1953).

This conclusion was based upon the absence of a grossly apparent tissue response caused by the parasites, which was thus determined to be unlikely to affect the host “in any recognizable manner.” The author conceded that severe infections might be able to cause serious inflammation or fibrosis.

Since this early study, research on *C. enhydri* has been limited to assessing direct physical damage caused by the insertion of the parasite’s proboscis in the host gastrointestinal epithelium (Mayer et al., 2003). However, this research focused mainly on the pathophysiology and ecology of *Profilicollis* spp. and only reported *C. enhydri* as a secondary observation. No significant inflammation of the intestinal wall or host immune response were found, and *C. enhydri* was repeatedly suggested to have no significant effect on otter health (Mayer et al., 2003). Thomas and Cole (1996) performed gross necropsies and histopathology on 75 otters from California and concluded that, though *Corynosoma* was found at a high abundance, it “caused no detectable deleterious effects” based upon absence of significant tissue inflammation, immune response or peritonitis. Their assessment was based on the observation that *C. enhydri* has seemingly weaker penetrative effects and does not breach the intestinal wall in sea otters, in contrast with *Profilicollis* spp., which commonly cause enteritis, peritonitis, and death (Thomas and Cole, 1996; Mayer et al., 2003; Miller et al., 2017). For marine mammals, broader research on the potential sublethal effects of intestinal parasitism remains limited.

As resource availability and diseases significantly limit sea otter fitness and population dynamics, it is imperative that we investigate possible indirect effects of helminth infection. Conservation of sea otters and kelp forest or eelgrass ecosystems may depend on our ability to accurately model the viability of populations, particularly as researchers look towards possible relocation efforts (Davis et al., 2019). *Corynosoma enhydri* may affect both otters and intermediate host species, and thus have additive effects on the ecosystem. Until now, research on the effects of parasitism in sea otters has focused on their contribution to mortality (Thomas and Cole, 1996; Kreuder et al., 2003; Miller et al., 2010). However, analysis of the literature shows a diversity of significant sublethal effects of acanthocephalans and other helminths on individual host health and population success and suggests that *C. enhydri* is likely to have similar impacts.

1.3 Beyond mortality: potential significance for individuals and populations

1.3.1 Immunosuppression and polyparasitism

Many recent studies have focused on the effects of parasites on immune function in intermediate and definitive hosts. Gastrointestinal helminths can downregulate systemic allergic and autoimmune responses in humans and other vertebrates (Zaccone et al., 2006; Figueiredo et al., 2010). These effects are likely due to parasite adaptations to combat host inflammatory responses and force host toleration of parasite antigens, most commonly through suppression of the Th_1 immune response (Cox, 2001; Figueiredo et al., 2010). In fact, compared to other pathogenic groups, helminth parasites are considered “master manipulators” of the host immune response, often controlling the production of cytokines, immune cell activation, and tolerance (Zaccone et al., 2006; Barcante et al., 2012).

For example, Cornet et al. (2009) reported both host and parasite-specific immune suppression by three different acanthocephalans (*Pomphorhynchus laevis*, *Pomphorhynchus tereticollis* and *Polymorphus minutus*) in intermediate crustacean hosts. Immune suppression is beneficial for the parasites because it can enhance survival. However, impaired host immune function can also facilitate secondary bacterial infections (Cornet et al., 2009; Marcogliese and Pietrock, 2011). This effect is not limited to crustaceans. Interactions between helminths, definitive hosts, and bacterial infections have been recorded in vertebrate hosts as well. For example, Th_1 suppression by the trematode *Fasciola hepatica* in mice has been associated with exacerbated bacterial infections of *Bordetella pertussis* (Cox, 2001). Th_1 suppression by nematodes in cattle increases invasion success of bovine tuberculosis in Africa (Ezenwa et al., 2010). Helminth-induced immune modulation in ruminants can alter the efficacy of vaccinations and host immune response to concomitant infections (McNeilly and Nisbet, 2014). Immunodepression of the host by acanthocephalans may thus benefit concomitant infections during establishment in the parasitized host.

Traditionally, the severity of acanthocephalan infection in definitive hosts has been measured using two parameters: depth of proboscis penetration into the gastrointestinal wall, and infection intensity (i.e., number of parasites per infected host; Dezfuli et al., 2011). However, in humans and some domestic animals, there has been greater emphasis on characterizing indirect and sublethal health impacts, such as the influence of polyparasitism – co-infection of two or more parasite species in a host – and quantifying co-morbid conditions beyond the "one death, one disease approach" (Pullan and Brooker, 2008). Infection by nematodes can increase mortality in African cattle when there is co-infection by protozoan parasites (Thumbi et al., 2014). Pullan and Brooker (2008) highlighted the importance of hookworm-malaria co-infection in children, and the potential for multiplicative impacts on nutritional and organ health. The highest proportions of morbidity occur in populations with high rates of helminths and co-infection with *Plasmodium* spp. (Brooker et al., 2007).

Co-infections are often observed between helminth and protozoan parasites as well as between two species of helminths in vertebrates (Cox, 2001). Helminth-induced suppression of host immune response via cytokine IFN-γ has been linked to an increased density of microparasites in co-infected hosts (Graham, 2008). The greatest impacts on host health are likely to be exerted by helminth species that strongly suppress immune response, but do not reduce resource availability for other parasites (Graham, 2008). This could be particularly significant for intestinal helminths and neural microparasites that do not share niches within the host and may not compete for resources.

In sea otters, infection by the microparasites *T. gondii* and *S. neurona* can cause morbidity and death (Kreuder et al., 2003; Miller et al., 2010; Burgess et al., 2018). If enteric infection by *C. enhydri* induces immune suppression and promotes systemic *T. gondii* and *S. neurona* infection, this acanthocephalan may exert greater impacts on sea otter health and survival than was previously thought. If acanthocephalan infection enhances sea otter vulnerability to systemic protozoal infection and/or exacerbates the severity of infection, this would significantly change our collective understanding of the pathogenicity of *C. enhydri.* Potential impacts of infection on sea otter population recovery would need to be reassessed. Infectious diseases are an important cause of death in stranded southern sea otters, and immune suppression by *C. enhydri* could be an important contributing factor, which is currently ignored (Kreuder et al., 2003; Miller *et al*., 2017; Tinker et al., 2019).

1.3.2 Systemic effects and nutritional loss

In wild animals, it is very difficult to detect intestinal helminth infection without fecal analysis (which can be time-consuming, provide false negatives, and does not provide sensitive data on prevalence and abundance) or endoscopy. Consequently, there is a gap in the literature on the pathological effects of intestinal infection by acanthocephalans on live marine mammals, and *C. enhydri* is no exception. This is likely due to the difficulties in observing animals in the wild and the ethical issues with intentionally infecting vertebrates for observation, especially protected species such as sea otters. As a result, potential sublethal effects of *C. enhydri* infection are unknown. For comparison, there have been a few cases of accidental corynosomiasis in humans and symptoms included abdominal pain, ulceration, and melena, though these effects may be unique to infections in humans, an aberrant host for *Corynosoma* spp. (Fujita et al., 2016; Takahashi et al., 2016). In the bird-associated acanthocephalan *Plagiorhynchus cylindraceus*, early studies had considered the species to be of no pathological significance for its vertebrate host. However, upon further investigation, infection was found to have significant effects on the absorption of nutrients in the definitive host, lowering metabolic rate, increasing both nutrient intake and excretion, and reducing body weight in starlings (*Sturnus vulgaris*) (Connors and Nickol, 1991). The authors identified the limitations of research focusing only on the site of proboscis attachment as a benchmark for pathological significance. Host metabolism should be integrated into recorded metrics when assessing the pathology of intestinal infections (Connors and Nickol, 1991).

Indirect effects of parasites also include reduction of host energetics (Booth et al., 1993). Malnutrition has been linked with intestinal helminths in African bovid (Ezenwa, 2004), Soay sheep (*Ovis aries*) (Gulland, 1992), and bighorn sheep (*Ovis canadensis*) (Enk et al., 2001). In waterfowl, helminths can have strong negative effects on body condition and nutrient absorption during pivotal periods such as spring migration and cause morbidity in vulnerable individuals (England et al., 2018). Helminths can also affect host weight and behavior as infected hosts may modify their feeding habits to compensate for parasite burdens (Budischak et al., 2018). These effects may be of importance for sea otters, which live on a metabolic knife's edge, especially in California where otters are already resource limited (Mayer et al., 2003; Tinker et al., 2008; Tinker et al., 2019).

Though difficulties in analyzing wild populations have resulted in limited available data, the agricultural industry has long made the connection between intestinal helminths and reduction in productivity. Helminths are a widespread problem amongst livestock and have been shown to have a myriad of effects. In sheep, intestinal parasites reduce appetite and grazing, lowering metabolic efficiency and live weight (Vlassoff et al., 2001; Nieuwhof and Bishop, 2005; Louie et al., 2007). The dairy industry has long suffered from helminthiasis, as infected livestock show a reduction in milk production (Charlier et al., 2009; van Dijk et al., 2009), reduced calf weight gains (Stott, 2017), and reproductive loss, including abortions (van Dijk et al., 2009). Helminths can affect pigs, lowering feed to weight gain ratios (Dold and Holland, 2011). Helminthiasis in pigs also halts lactase activity in the intestine, and lowers food uptake efficiency (Crompton, 1986; Dold and Holland, 2011). In humans, helminths can weaken the intestinal barrier, alter microbiota, and reduce nutritional intake or appetite (Shea-Donohue et al., 2017). Malnutrition is common in children infected with helminths, and particularly concerning is the connection to a reduction in cognitive ability (Stephenson et al., 2000). Intestinal infection can affect nutrient uptake, reducing absorption of essential elements like sodium, potassium, and chloride (Stephenson et al., 2000).

This effect on nutrient uptake may be particularly relevant to sea otters as resource availability is a limiting factor for population growth in California (Tinker et al., 2008, Tinker et al., 2019). Sea otters have a high mass-specific metabolic rate, greater than any other marine mammal, and eat 20-25% of their body mass in food every day (Yeates et al., 2007; Thometz et al., 2014; 2016). Otters also invest a large amount of energy into pup rearing. The 6-month lactation period is the most energetically taxing time for female otters (Thometz et al., 2014; 2016). Nursing females must continually hunt for prey during lactation, which represents an extreme burden (Thometz et al., 2014; 2016). This is likely why post-nursing otters are at high risk of death from End Lactation Syndrome; lowered body condition resulting in mortality from concomitant infections or male mating aggression (Thometz et al., 2014; Chinn et al., 2016). If *C. enhydri* affects nutrient assimilation, it may also reduce milk production as other helminths do in cattle. It could thus be a contributing factor to pup loss/abandonment or post-lactation mortality in mothers. Southern sea otters are strongly affected by density-dependent mortality and rely on a consistent and high calorie diet (Tinker et al., 2019). Females tend to produce offspring each year although pup survival is dependent on female body condition, as females may abandon pups if they cannot support them (a “bet-hedging” strategy) (Monson et al., 2000; Tinker et al., 2019). There have been suggestions of regional differences in fecundity based upon resource availability, which may be one explanation for the slower recovery rate of the southern population compared to the northern sea otter (Tinker et al., 2019). The potential added effects of acanthocephalan infection on sea otter nutrient availability and body condition, which are an open question in sea otter-*Corynosoma* interactions, may thus be significant and must be investigated.

1.3.3 Behavioral changes

Helminth effects on host behavior are significant in a variety of species. They affect rodent spatial behavior, causing field mice to be more dispersed (Shaner et al., 2017). In Mangabey monkeys, helminth infection has been linked to increased testosterone in immigrant males, suggesting parasites may have some effects on dispersal behavior (Arlet et al., 2015). As mentioned before, helminths are of concern for livestock, and one of the side effects of intestinal helminthiasis includes changes in appetite and feeding habits (van Dijk et al., 2009; Dold and Holland, 2011; Stott, 2017).

A recent study in South America (Montalva et al., 2019) monitored wild fur seal pups (*Arctocephalus australis*) in a population that suffers near 100% infection rates with the hookworm *Uncinaria* spp. After treating some individuals with anti-helminthic medication, the researchers assessed behavior and weight gain in medicated and non-medicated (still infected) pups. They found that growth rate was stunted in severely infected individuals, also coinciding with lower blood hemoglobin levels (Montalva et al., 2019). This resulted in behavioral differences. Severely infected pups spent more time in the water and spent that time swimming rather than playing (Montalva et al., 2019). It should be noted that *Uncinaria* spp. have been shown to perforate the intestinal wall in a proportion of juvenile California sea lion (*Zalophus californianus*) cases (22.3%) (Spraker et al., 2004) as well as in in South American fur seals (*Arctocephalus australis*) (34.8%) (Seguel et al., 2017). The behavioral alterations observed in hosts may be a direct effect of that more invasive attachment, which *C. enhydri* has not been reported to display in sea otters. Seal pups that suffered from intestinal peritonitis had an average of 1,343.4 worms. It is thus possible that peritonitis is a result of extreme parasite loads (Seguel et al., 2017). It is unclear what the mechanisms of behavioral change in these infected animals were, but it should be noted that if they were due to direct damage, these effects may not be mirrored in otters. However, without more information, and a lack of data on sea otters, it is important to highlight the possible effects of infection on behavior.

Otters rely upon a high calorie diet, require extensive time foraging to sustain that diet, and moderate their core temperature through air trapped against their skin by a thick layer of hair (Estes et al., 2003; Yeates et al., 2007; Thometz et al., 2014). Parasites that alter their ability to forage or groom could significantly alter their health. Juvenile otters (weaned, 181-250 days old) have even higher metabolic requirements relative to other age groups, are at a disadvantage compared with adults in their foraging abilities (Thometz et al., 2014*)*, and have high intensities of *C. enhydri* infection (Mayer et al., 2003). As a result of these synergistic effects, there could be population-level implications including decreased growth rates, recruitment, and population recovery, due to burdens on immature individuals.

1.3.4 Intestinal microbiota

Attempts at understanding interactions between parasites and host gastrointestinal microbiota have increased and attracted interest in recent years. Multiple studies have shown the statistical significance of these relationships and their possible effects on host health. Helminth infections have been linked to significant changes in gut microbiome (Walk et al., 2010; Berrilli et al., 2012; Kreisinger et al., 2015). They are also a source of by-products (e.g. carbon dioxide, nitrogen, and enzymes) that can affect abiotic factors and bacterial populations (Vlcková et al., 2018). Specific variations in gut microbiota have been directly linked to the helminth species that inhabit a particular host (Kreisinger et al., 2015). Generally, gastrointestinal parasites may have implications for gut homeostasis in both vertebrate and invertebrate hosts (Kreisinger et al., 2015). This is significant as particular gut microbiota play critical roles in increasing nutrient yield and modulating host metabolism in vertebrates (Tremaroli and Bäckhed, 2012). Other microbiota in the intestine are essential in host immune defense, and a reduction in host commensal bacteria has been linked with an increase in pathogen infections (Kamada et al., 2013). While, with the exception of damage to the intestinal linings, there is no information currently available on sea otter parasites and intestinal health, such interaction could be a potential source of further strain on otter health and metabolism. As previously stated, sea otters have large mass-specific energy demands (Chinn et al., 2016; Thometz et al., 2016). Microorganisms, and potential helminth-induced changes, may thus influence nutrient assimilation in otters. If microbes are important assistants in sea otter's digestive process, their loss or alteration could be an additional strain, necessitating an increase in foraging to compensate.

1.4 Beyond mortality: possible sources of significance in the ecosystem

1.4.1 Behavioral changes in intermediate hosts

While *Corynosoma enhydri* is the only confirmed parasite to use otters as its preferred definitive host, its intermediate host(s) have not been identified and potential effects of the parasite on these intermediate hosts are unknown (Mayer et al., 2003). However, acanthocephalans commonly affect intermediate host behavior and phenotype. In crustacean intermediate hosts, larval acanthocephalan parasites can affect geotaxis, time-specific behavior, and reproduction (Bailly et al., 2017). For example, *Corynosoma constrictum* influences phototaxis, even preferentially directing infected amphipod hosts toward specific wavelengths of light (Benesh et al., 2005). *Pomphorhynchus laevis*-infected amphipods display increased phototaxis, are attracted to predator odors, and are found more frequently in moving waters, thus increasing their likelihood of being eaten by the parasite definitive host (Lagrue et al., 2007; Franceschi et al., 2010). Another species of acanthocephalan, *Acanthocephalus dirus*, which infects freshwater isopods, can reduce mating behavior, increase movement away from refuge, affect metabolism, and modify neurochemical levels (Kopp et al., 2016; Caddigan et al., 2017).

Parasite-induced host manipulation is a well-known and researched phenomenon. Many helminths affect intermediate host's physiology, phenotype, and behavior, in ways that increase the probability of transmission to the definitive host (Lefèvre et al., 2009; Poulin and Maure, 2015; Herbison, 2017). It is possible that *C. enhydri* similarly affects its intermediate host phenotype to increase predation, especially considering the high prevalence recorded in southern otters (Hennessey and Morejohn, 1977; Mayer et al., 2003). Since many sea otter prey species, such as abalone, urchin, and crab, also have broader ecological and commercial value, identifying *C. enhydri*'s intermediate host(s) and investigating effects of the parasite should be research priorities. The continued investigation of sea otter effects on in-shore communities and on commercially valuable species has been identified as an important future direction of research (Davis et al., 2019). Sea otters are often a point of contention for conservationists and commercial or local subsistence fishing groups (Davis et al., 2019). Efforts to correctly monitor the effects of re-introduction to previously extirpated areas will likely be inaccurate without an understanding of the concurrent effect of re-introduction of *C. enhydri* to invertebrate hosts.

1.4.2 *Corynosoma enhydri*: specialist or generalist?

Due to limited knowledge of *C. enhydri*'s intermediate host(s), it may be premature to rule out the possibility of alternative definitive host species. Host specificity needs to be documented to gain accurate knowledge of its actual biological niche, and before assessing the parasite's role in ecosystems (Poulin and Mouillot, 2003). The default classification of a species between generalist or specialist often assumes the latter and observed infections outside the preferred host are considered accidental or "dead end" infections (Johnson et al., 2009; Agosta et al., 2010). However, phenotypic plasticity has been shown to allow for ecological fitting, and quick parasite response to changing host environments (Little et al., 2006; Agosta et al., 2010). Sea otters were almost eradicated in California due to the fur industry; about 50 animals remained in Big Sur before they were eventually protected in 1911 (Riedman and Estes, 1990). However, only 60 years after this sharp population bottleneck, prevalence of *C. enhydri* was 91% in southern sea otters (Hennessey and Morejohn, 1977). While it is possible that *C. enhydri* persisted in the remnant population, there may be alternative host species that are still unknown and sustained the parasite populations in the absence of otters. Given the near extinction and isolation of otter subpopulations, it seems reasonable that *C. enhydri* may use alternative definitive host species, perhaps one most abundant in California, explaining the difference in observed prevalence between California (>90%) and Alaska (~50%) (Hennessey and Morejohn, 1977; Margolis et al., 1997; Mayer et al., 2003).

Though intermediate host(s) remain unidentified, invertebrates are most likely since fish are absent from otter diet in California (Estes et al., 2003). *Corynosoma enhydri* could be infecting any number of widespread prey items of otters such as urchins, crabs, snails, or bivalves, which are also consumed by many other predators (Estes et al., 2003). Elasmobranchs have been reported to harbor related members of the Genus *Corynosoma*. Knoff et al. (2001) reported infection by *Corynosoma australe*, a parasite of seals. However, infection was dismissed as accidental as *Corynosoma* "are known to use mammals and birds as definitive hosts". Unfortunately, they did not report whether the parasites were mature and gravid, which would have added more weight to the assertion that since mammals and birds are the traditional hosts, elasmobranchs are likely accidental hosts. Another species, *Corynosoma cetaceum* has been reported in hammerhead sharks (*Sphyrna mokarran*) off Australia. Infections were again considered accidental (Smales et al., 2018). *Corynosoma australe* has also been reported to switch from marine mammals to birds. Penguins in Argentina were infected with gravid females (Hernandez-Orts et al., 2017). The emphasis on the Red Queen hypothesis and Fahrenholtz's rule (strict co-speciation between host and parasite) leads to the assumption that parasites will tend toward specialization (Agosta et al., 2010; Goater et al., 2014), and any infections outside of identified "normal" hosts are accidental. While these events may indeed be dead-end infections, it must also be considered that due to the indiscriminate nature of exposure by the ingestion of infected prey, intestinal parasites have great opportunity for ecological fitting (Little et al., 2006; Agosta et al., 2010). *Corynosoma*, as a genus seems to have flexibility in marine mammal hosts (Aznar et al., 2006), and acanthocephalans as a whole seem to be specialists in their intermediate hosts, but generalists in their definitive hosts (Kennedy 2006).

While it is simpler to assume that parasites like *C. enhydri* are host specialists, if we are to understand their greater ecological significance, they must be put into an ecosystem context. Ecosystems, especially marine ones, tend to be very complex (Thompson et al., 2005; Brooks and Hoberg, 2007; Lafferty et al., 2008). It is conceivable that other kelp forest predators such as leopard sharks (*Triakis semifasciata*) or harbor seals (*Phoca vitulina*) could be secondary definitive hosts for *C. enhydri*. *Corynosoma* spp. have been seen in elasmobranchs previously and are common in seals (Knoff et al., 2001; Valtonen et al., 2004; Siebert et al., 2007; Smales et al., 2018). It is also possible elasmobranchs could act as a paratenic host to an unknown definitive host such as pinnipeds or cetaceans. Marine birds are also a common host of acanthocephalans, and if crabs are the intermediate host, it is possible gulls or other large species may be used as hosts (Nickol et al., 1999).

Parasites are essential parts of food webs, and understanding their interaction effects on the various species they use as hosts is essential (Lafferty et al., 2008). As previously stated, parasites can have wide ranging behavioral and metabolic effects on their hosts. Therefore, identifying the intermediate host(s) of *C. enhydri*, possible secondary definitive host species, and other predators of the discovered intermediate host(s) is imperative.

1.4.3 Climate change and *C. enhydri* infection dynamics

Looking into the future, if *Corynosoma enhydri* currently affects otter health and population dynamics, then potential further effects of climate change must be considered. Fluctuations in disease dynamics have been frequently associated with changes in temperature, particularly increases in infection rates with higher temperatures in marine species (Marcogliese, 2008). Possible effects of climate change on parasites and their ecosystems will involve a complex combination of variables including ocean acidification, UV radiation, eutrophication, weather changes, and fluctuations in ocean currents (Marcogliese, 2001). Overall, there is a strong possibility that increasing ocean temperatures will coincide with a change in parasite population dynamics and virulence (Lafferty et al., 2004; Marcogliese, 2008; Paull et al., 2015).

Helminth parasites, particularly those using invertebrate intermediate hosts, can be strongly affected by an increase in temperature. Larval development time can be dramatically reduced in some species, while others may have higher rates of reproduction and shortened generation time (Harvell et al., 2002; Lafferty et al., 2004). Thermal stress can increase host susceptibility by suppressing immune response, changing behavior, and lowering fitness due to reduced oxygen levels (Lafferty et al., 2004; Marcogliese, 2008). Theoretical models have linked the change of trematode parasite dynamics due to warming temperatures with strong cascading effects on intertidal communities (Poulin and Mouritsen, 2006). Success or failure of the host or parasite in response to global warming relies upon the difference in acclimation responses. If parasite tolerance to temperature increase is higher, it could lead to increased rates of parasitism due to speed of reproduction because of their metabolic rate and small size (Lafferty et al., 2004; Paull et al., 2015). In colder waters, rising temperatures may increase the rate of parasitic infection and induce range expansion (Davidson et al., 2011; Dudley et al., 2015). Changes will likely be parasite-specific, based on host-specificity, life cycle, intermediate host health, and the other effects of climate change beyond temperature like eutrophication, weather, or acidification (Goedknegt et al., 2015; Marcogliese 2016; Cizauskas et al., 2017).

If climate change increases *C. enhydri* prevalence, abundance or intensity, it may also have far reaching effects on ecosystem dynamics. Increased transmission and infection rates could have strong impacts on marine communities, altering intermediate host behavior, increasing rates of predation and resulting in indirect effects such as apparent competition, increasing parasite-induced host mortality or the frequency of harmful infections in novel hosts (Mouritsen and Poulin, 2002; Poulin and Mouritsen, 2006). An increase in parasite densities and a reduction in host fitness due to warming waters could exacerbate effects, such as metabolic disruption or immune suppression, through an increase in infection rates. This could be particularly important for sea otter populations in colder climates like Alaska, where parasite infection rates are historically lower (Margolis et al., 1997). Therefore, it is essential that more information be gathered about this prolific parasite of sea otters.

1.5 Conclusion

As sea otters are so important for coastal ecosystems, it is imperative we expand the current body of knowledge concerning otter health and explore the possible secondary, sublethal effects of parasitism, especially for a parasite like *Corynosoma enhydri,* which has such high prevalence (Mayer et al., 2003). Sublethal effects could be particularly strong in otters as resource availability is a limiting factor for otter population growth in California (Tinker et al., 2019). Since studies on live, wild sea otters present ethical, legal, and logistical challenges, observations are based on available carcasses that strand naturally. As a result, marine mammal health research focuses on cause of death as a significant indicator of population success. However, we believe this emphasis on direct mortality has led to an underestimation of the significance of the sublethal effects of parasitism, which may be more significant for populations as a whole.

This is not to say that direct mortality from other pathogens is unimportant for sea otters. However, an emphasis solely on direct mortality of any parasite or disease as the litmus test for population-wide significance is too limited. Judging the significance of all parasites by one standard when effects can be complex and difficult to accurately identify is problematic (Marcogliese and Pietrock, 2011). We thus strongly recommend that we expand the understanding of the interaction effects between parasitism, host behavior, and health. This is particularly true for southern sea otters, which are an important consumer in kelp forest and eelgrass ecosystems, but whose population is resource-limited and has been slow to expand (Lafferty and Tinker, 2014; Tinker et al., 2019).

Future research into the effects of parasites on mammals, especially species at risk, should expand on the broader perspective of morbidity, including an ecological perspective of the strong effects of secondary symptoms like metabolic disruption, behavioral modification, and intensification of concomitant infections. As parasitism is common and polyparasitism likely, investigations into the effects of intestinal parasites like *C. enhydri* and *Profilicollis* spp. on immune health are essential. Species of concern like the southern sea otter in California are often resource limited and any disruption of their nutrient assimilation could be extremely significant (Yeates et al., 2007; Tinker et al., 2008; Thometz et al., 2014; Tinker et al., 2019), and research into these vulnerable populations must include the potential effects of infection by sub-lethal parasites.

1.6 Thesis Scope

Mustelids, especially otters, have high metabolic rates in relation to other mammals (Stephensen et al. 1988; Pfeiffer and Cullik 1998; Chinn et al. 2016). As such, they require a high-calorie diet and can be strongly affected by fluctuations in nutrient availability (Pfeiffer and Cullik 1998; Chinn et al. 2016). Like sea otters, the North American river otter (*Lontra canadensis*) and mink (*Neogale vison*) play an important role in aquatic ecosystems, with top-down effects on fish and invertebrates, as well as in coastal communities as an importer of marine nutrients, which has been linked to increased habitat heterogeneity (Roemer et al. 2009). River otters are also particularly susceptible to anthropogenic pollution and are useful sentinel species in aquatic habitat conservation and management (Peterson and Schulte 2016). Pollution may also affect interactions between host and parasites. Exposure to pollution has been reported to be positively correlated with parasite intensity; parasites may increase pollutant exposure, or the pollutant reduces host defense to parasitic infection (Sures 2004). Sub-lethal or sub-clinical exposure to contaminants may also be an energetic burden on the animal due to the costs of homeostatic regulation (Rajotte and Couture 2002, Deb and Fukushima 2007). Since otters and mink are key predators in aquatic ecosystems and are at high risk of pollutant exposure; they are an ideal sentinel species (Peterson and Schulte 2016).

The study of pathogens with complex life cycles such as helminth parasites necessitates interdisciplinary research (Moore 2008). Research can be further complicated if the host is a threatened, charismatic species like otters, due to sampling limitations of protected species, and since stakeholders can include not only ecologists, but non-profit conservation groups, wildlife veterinarians, government agencies, and commercial enterprises. There are three main spheres of application in disease ecology: agriculture, wildlife health, and natural resource conservation, and these disciplines tend to be insulated from one another (Thompson et al. 2010). This work is an important intersection between wildlife health research, which until now has been predominantly concerned with individual morbidity, and conservation efforts by utilizing the wealth of experimental knowledge from the agricultural sphere, which has been specifically concerned with the energetic burden of parasites on the individual. We focus on describing the effect of sub-lethal helminth infection in river otter and mink in Western Canada in association with contaminant exposure to investigate the cumulative effect of these stressors on their energetic condition.

Do sub-lethal (or sub-clinical) infections of helminths negatively affect host energetic condition and therefore fitness in mammals? In Chapter 2, we present a meta-analysis of the literature with the goal of investigating the sub-lethal effects of helminth infections on mammal energetic condition (the balance of costs for maintenance, repair, and food energy acquisition), classically called "body condition" as a broad term including metrics related to host health and energy availability. The goal of this study was to address the question: do sub-lethal helminth parasites influence mammal energetic condition? We conducted a systematic review of the literature and extracted effect sizes from relevant studies to answer this question, including analysis of both biological and methodological factors to determine the average strength of the effect of helminth infection on mammal energetic condition.

What is the diversity and prevalence of helminth parasites in aquatic mammals in western Canada? In Chapter 3, we report on the diversity and prevalence of helminths in river otter, mink, and muskrat in British Columbia and Alberta, including a summary of the literature concerning parasite diversity and distribution throughout North America. We use both morphological and molecular tools to identify parasite species, and phylogenetic taxonomic analysis to establish the place of unknown species. This data fills a gap in knowledge concerning the parasite communities of Western Canada and may have implications for wildlife populations, ecological function, and human health.

What is the type and prevalence of contaminant exposure in river otter and mink in western Canada, and how do anthropogenic factors explain observed results? In Chapter 4, we summarize all previous reports of metal contaminant exposure in river otter and mink in North America and calculate weighted averages for twenty-two metals including pooled standard deviations as a tool for comparison. We then report on the type, concentration, prevalence, and distribution of contaminants of concern in British Columbia and Alberta, and compare them against historical averages. We use geospatial analysis to determine the effect of various environmental and anthropogenic factors on the concentration of contaminants in otter and mink livers. The results fill a gap in knowledge concerning modern contaminants in Western Canada for aquatic carnivores, as well as for human communities, and highlight the importance of ongoing monitoring of sentinel species as an early warning for environmental threats.

Does parasitic infection and contaminant exposure negatively affect the energetic condition of mink and otter? In Chapter 5, we use multivariate and generalized linear mixed models to investigate the effect of parasitic infection and contaminant exposure on the energetic condition of mink and otter via morphological proxies. These results fill a gap in knowledge concerning the energetic burden of highly prevalent parasitic infections and metal contaminant exposure on aquatic carnivores in Alberta and British Columbia and may have dramatic implications for freshwater ecosystems, especially in a warming climate.

Chapter 2: The energetic costs of sub-lethal helminth parasites in mammals, a meta-analysis

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This chapter also benefitted greatly from the help of external contacts who gave of their time or knowledge in support of this project. I am grateful to the authors of publications who included open-source data, R codes, summaries of the literature and clear instructions for best practice in meta-analysis, which greatly benefited this chapter (Nakagawa and Santos, 2012; Sanchez *et al*., 2018; Sánchez-Tójar *et al*., 2020; O'Dea *et al*., 2021). Thank you to the authors of papers who provided data when there was not sufficient information in their publication to extract effect sizes. Many thanks as well to Dr. Daniel Noble at the Australian National University, one of the authors of *orchaRd*, for their willingness to answer questions about the package. I am also very grateful to Dr. Nicole Thometz, University of San Francisco, for taking time to give invaluable feedback concerning the binning of various health measurements into their respective energetic condition categories and hypotheses for a timeline of observed responses.

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2.1 Introduction

2.1.1 Helminths and mammals

Parasites are ‘bad’, meaning that, by definition, they must have net negative effects on their hosts, although these effects can be subtle (Poulin, 2007). In wild mammal research and conservation, emphasis has been placed on ‘infectious diseases’ or ‘pathogens’ and their relation to observed morbidity and mortality at a local scale (Han and Ostfelt, 2019). As a result, the literature has been strongly biased towards studies of microparasites such as bacteria, viruses, and protozoan parasites with clear pathogenic effects on their hosts (Geraci and St. Aubin, 1987; McCallum and Dobson, 1995; Woodroffe, 1999; Lafferty and Gerber, 2002; Altizer *et al*., 2003; De Castro and Bolker, 2004; Smith, Sax and Lafferty, 2006; Pedersen *et al*., 2007; Han and Ostfeld, 2019). Historically, macroparasites (helminths: nematodes, trematodes, cestodes, and acanthocephalans), were often assumed to be benign (Irvine, 2006; Pedersen *et al*., 2007; see Supporting Information, Appendix A.1) and still receive less attention (Han and Ostfeld, 2019). Mammals were frequently reported to be unaffected by helminths due to the lack of ‘observable’ damage directly related to infection (Aznar *et al*., 2001; Samuel, Pybus and Kocan, 2001; Delahay, Smith and Hutchings, 2009; Raga *et al*., 2009), even when found in their thousands in a single host (Geraci and St. Aubin, 1987; Daily, 2005). For example, cestodes and trematodes were commonly assumed to have no ‘significant’ effects on wildlife populations (Samuel *et al*., 2001; Botzier and Brown, 2014). However, helminths are ubiquitous and can have many complex effects on host behavior, body condition, fecundity, population dynamics, systemic health, and concomitant infections (Hutchings *et al.*, 2006; Degen, 2008; Beldomenico *et al*., 2008*a*; Bordes and Morand, 2011; Buck and Ripple, 2017; Sanchez *et al*., 2018; Defolie, Merkling and Fichtel, 2020). Recent studies in disease ecology have emphasized the importance of these diverse effects on host health and fitness. However, measuring such effects remains a challenge and research on sub-lethal infections and their links to physiological or behavioral changes in mammalian hosts remain underrepresented (Han and Ostfelt, 2019).

Past assumptions of insignificance may thus have been due to the difficulties in identifying links between endoparasites and host health. Inherent methodological limitations associated with wild mammal research also restricts empirical assessment of these links (Irvine, 2006; Lachish and Murrary, 2018). This may explain why wildlife health disciplines often focus on identified diseases or pathogens, and how they influence observable clinical signs. Such signs are easier to analyze from carcasses, but also limit our ability to assess the potential significance of endoparasite effects that are not obviously related to morbidity (Samuel *et al*., 2001). Ethical concerns around sampling of and experimentation on wild mammals further restrict studies to opportunistic collection and *post-hoc* analysis of carcasses. Proxies for endoparasite effects are thus *de facto* biased towards direct pathology or mortality, with confounding factors on host health not always quantifiable. Conversely, field observations of live animals mostly restrict parasite identification to faecal egg counts (FECs) and non-invasive metrics of host health, behavior, or population dynamics (Sanchez *et al*., 2018; Shanebeck and Lagrue, 2020). Controlled, experimental infection and observation of wild mammals is rare, which limits our knowledge of sub-lethal effects like stress, reproductive behavior/output, food intake, physiochemical disruption, or even common, non-specific clinical indicators like diarrhea, lethargy, pain, or fever (Samuel *et al*., 2001; Sanchez *et al*., 2018).

2.1.2 Shifting the relevance paradigm: fitness rather than mortality

Host health, in a broad sense, and the relevance of various parasites or pathogens are often defined by the presence or absence of 'disease' (pathological signs and mortality) (Tompkins *et al*., 2011; Stephen, 2014; Sanchez *et al*., 2018), not fitness. Yet, while highly virulent parasites are often assumed to be the greatest threat for hosts, those of moderate virulence are predicted to affect host population health more strongly (Riordan, Hudson and Albon, 2007; Ryser-Degiorgis, 2013). Sub-lethal parasites tend to infect larger proportions of host populations and have a deeper and longer lasting influence on host energetics, reproductive success and population growth compared to lethal parasites that infect and kill a small percentage of the host population (Irvine, 2006; Riordan *et al*., 2007; Tompkins *et al*., 2011).

Individual and population fitness result from a precarious balance between fecundity and survival (maintenance, repair, growth), the currency of which is energy availability (Schoener, 1971; Araújo, Bolnick and Layman, 2011; Berta, Sumich and Kovacs, 2015; Costa-Pereira *et al*., 2019). Mammals (along with birds), and especially marine mammals, have the highest energetic demands of modern fauna due to metabolic temperature regulation, expensive mechanisms of movement, and the high costs of pregnancy and lactation (McNab, 2012; Berta *et al*., 2015; Thometz *et al*., 2016; Heldstab, Van Schaik and Isler, 2017). As a result, sub-lethal trophic, environmental or health factors may significantly influence host energetics and thus individual and population fitness (Clinchy, Sheriff and Zanette, 2013; King *et al*., 2015; May *et al*., 2019).

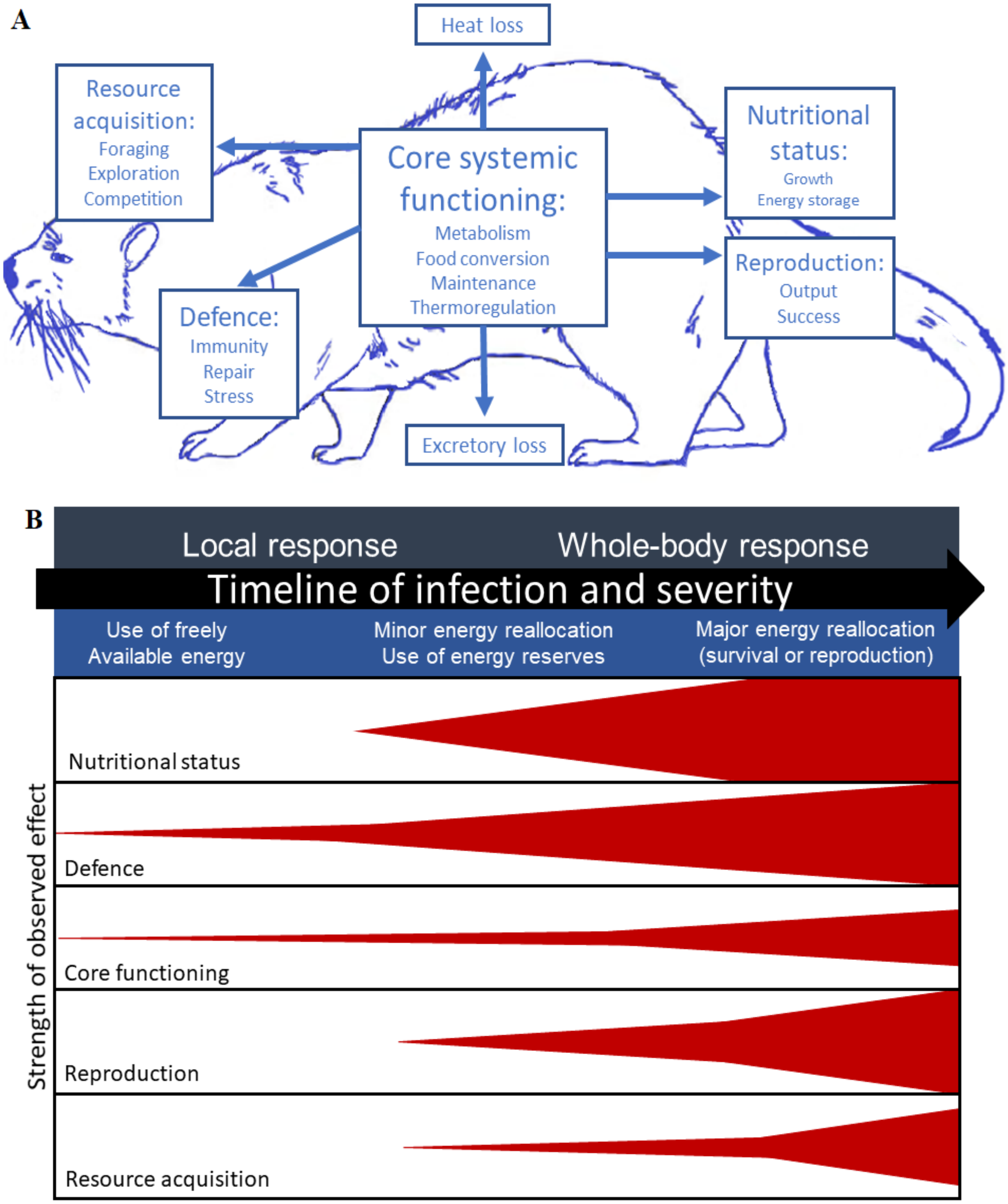

Figure 2.1 Suggested framework for assessing mammal energetics. Including (A) pathways of energy expenditure *via* energetic functions and loss (adapted from Berta *et al*., 2015) and (B) a hypothesized timeline for observed response by energetic function. During local responses using freely available energy, changes will be small or non-apparent, while lasting infections or those of higher severity may induce more strongly negative changes in host energetic condition.

Mammals budget the energetic costs of survival and reproduction through a cascading system of responses, somewhat like an energy grid. Limited resources/energy induce prioritization (redirection of freely available energy), rationing (redirection from energy reserves, reduction of allocation for systemic function to minimum requirements) and rolling blackouts (redirection from essential systems or between survival and reproduction) (Beldomenico *et al*., 2008*a*; McNab, 2012; Berta *et al*., 2015; Collins *et al*., 2016; Heldstab *et al*., 2017). Energy allocation is centralized around core systemic functioning, which will tend to have a small range of tolerable change (Fig. 2.1). However, some resources/energy can be reallocated quickly to help mount a localized response to infection, such as increasing blood flow and creating metabolic heat (Berta *et al*., 2015; Maceda-Veiga *et al*., 2015; Levesque, Nowack and Stawski, 2016). Energy supply is dependent upon resource acquisition [e.g. exploration, foraging, or competition (McNab, 2012; Collins *et al*., 2016)]. An individual's ability to acquire energy partly determines its nutritional status (growth, fat storage, muscle quality, 'body condition'), which is related to energy abundance and, especially in mammals, to absolute fitness and the individual's ability to store energy (Parker, Barboza and Gillingham, 2009; Malerba, White and Marshall, 2018; Sanchez *et al*., 2018). Stored energy can then be allocated to defense: mounting an immune response, repairing damaged tissue, or activating hormonal stress responses (Dias *et al*., 2017; Thompson, 2017), and ultimately absolute fitness is determined by the energy available for allocation to reproduction, both in terms of output (quantity of offspring) and success (survival to adulthood or the 'quality' of offspring) (Tompkins *et al*., 2011; McNab, 2012; Costa-Pereira *et al*., 2019).

Resource rationing and allocation to specific energetic demands will depend on intra- and interspecific, as well as environmental factors. However, we suggest a general framework to predict the timing and magnitude of parasite effects on host energetic condition arranged by the five main areas of expenditure (Fig. 2.1B). Any energetic challenge should increase the overall burden on an animal's energetic reserves (McNab, 1989, 2012; Nagy, Girard and Brown, 1999; Berta *et al*., 2015). This may be especially important to consider in research methodology. A failure to consider the period/severity of infection and which system is affected energetically by infection, at that time, may lead to type-II errors in our conclusions of a parasite's effects on host condition. We thus suggest that the influence of parasitic helminths on individual and population fitness, measured by energetic condition as the intersection of physiological, behavioral, and environmental influencers (Ryser-Degiorgis, 2013; Stephen, 2014), should be accounted for, not just pathological signs or mortality.

2.1.3 Measuring energetic condition

The most common proxy used to quantify energetics, or the general health of an animal, is ‘body condition’, classically measured by fat stores, muscle tone or body size [also defined subjectively as ‘robustness’, ‘vigor’, ‘quality’, ‘overall health’, ‘competitive ability’, or ‘energetic reserves’ (Milner *et al*., 2003; Beldomenico *et al*., 2008*a*; Peig and Green, 2010; Sanchez *et al*., 2018)]. Body condition is linked to individual fitness in the context of the animal’s ability to gather excess energy and convert it into reproductive success, although this can vary as not all mammals store substantial amounts of fat in their body (Tompkins *et al*., 2011; Stephen, 2014; Sanchez *et al*., 2018).

The complexity of mammal physiological and behavioral responses to direct and indirect energetic (classically ‘nutritional’) challenges has led to some inconsistencies in the selection of response variables in studies of the effects of parasites on host energetics (Brown, Marquet and Taper, 1993; Milner *et al*., 2003; Peig and Green, 2009; Levesque *et al*., 2016; Sanchez *et al*., 2018). Primary or direct effects are often easier to quantify, while secondary or downstream effects are more subtle and difficult to attribute conclusively to a parasite. For example, some intestinal parasites cause ulcerative colitis or inflammation of the intestinal wall, an obvious burden on defense as they require both an inflammatory response and tissue repair. However, another intestinal parasite may simply reduce digestive efficacy, suppress appetite, or alter the gut microbiome, producing no obvious, direct pathological signs. Nevertheless, infection will lead to changes in the host’s resource acquisition behavior and the loss of fat reserves (Charlier *et al*., 2009; Dorny *et al*., 2011; Suzuki, 2017), or perhaps show lowered hematocrit and packed cell volume (PCV) due to nutritional deficiencies (Budischak, Jolles and Ezenwa, 2012). Another parasite may suppress the host’s immune response, increasing the severity of secondary infections or causing normally neutral symbionts to become pathogenic (Shanebeck and Lagrue, 2020). This explains why many studies have tried to quantify the effect of parasites in relation to ‘body condition’ as a broad indicator for subtle or hard-to-measure effects on energy availability (Samuel *et al*., 2001).

Effect sizes included in this study may reflect direct, secondary, or downstream effects of infection, and are broadly defined as the strength of disruption of host energetic condition in relation to parasitic infection (Peig and Green, 2010; Levesque *et al*., 2016; Sanchez *et al*., 2018). It should also be noted that causality between parasite and host body condition is not unidirectional; pre-existing energetic challenges may predispose a host to infection, but the parasite may also increase the severity of these existing energetic challenges (Beldomenico *et al*., 2008*a*). Furthermore, in wildlife studies, infection duration is often unknown, data collection being limited in time, and focused on broad indices that only report downstream effects (Budischak *et al*., 2012). All of this makes interpreting the results of studies assessing the effects of infection on energetics and animal fitness difficult as they are subject to confounding (especially temporal) variables.

2.1.4 Meta-analysis goals and hypotheses

Through a meta-analysis of the literature, we asked: (1) do sub-lethal (or 'sub-clinical') infections by helminth parasites reduce mammal energetic condition, and therefore influence fitness? (2) If so, how do observed effects change according to biological and methodological factors? Historically, helminth parasites were assumed to have limited effects on host energetics (Appendix A.1). However, a cursory survey of the literature suggests otherwise. A wealth of research from the agricultural sphere, which has long been concerned with the exact energetic (and therefore financial) burden helminths have on their mammalian hosts, seems to indicate that negative effects on host energetic condition are common (Crompton, 1986; Vlassoff, Leathwick and Heath, 2001; Nieuwhof and Bishop, 2005; Charlier *et al*., 2009). Evidence from wildlife research is more variable and sometimes conflicting; species, environmental factors, methodological constraints, and other unknown factors may be influencing the ultimate observed magnitude of the effects of infection on wild mammal fitness (Coulson *et al*., 2018; Han and Ostfelt, 2019).

A previous review of the literature on the effects of anti-parasitic medications on wild vertebrate fitness reported that only 62% of studies showed a significant positive effect of antiparasitic treatment (Pedersen and Fenton, 2015). A review and meta-analysis by Sanchez *et al*. (2018) noted an overall negative effect of infection by helminths on body condition in wild vertebrates. Specific to mammals, a recent meta-analysis by Defolie *et al*. (2020) showed a strong association between parasitic infection and stress hormone response (glucocorticoids) in mammal hosts. How these effects are quantified and qualified, then reported in the literature, vary by context, host, and discipline (Schulte-Hostedde, Millar and Hickling, 2001; Peig and Green, 2010; Labocha, Schutz and Hayes, 2014; Sanchez *et al*., 2018). Consequently, results and conclusions on the magnitude and significance of these effects may largely depend upon study designs. What is increasingly clear from parasitological research is that helminths have complex, significant effects on both hosts and ecosystems. Yet, they have been largely neglected in evaluations of ecological functioning beyond disease and mortality (Irvine, 2006; Lafferty *et al*., 2008; Buck and Ripple, 2017).

To build upon these previous reviews, excluding microparasites which are well reported to cause mortality or disease (pathological signs and symptoms), and to try to explain the observed variations in the strength of effects, we focused on studies investigating sub-lethal infections by helmith parasites in mammals (both domestic and wild). We tested the common assumption that mammals are less likely to be affected energetically by sub-lethal parasitic infection. Domesticated animals were included to examine the wealth of experimental reports on the specific energetic consequences of helminth infections, as the agricultural industry is concerned with the exact financial costs of reduced nutrition, growth and reproductive success on meat and dairy mammals (Charlier *et al*., 2009). To address the heterogeneity in wildlife studies and conflicting reports of helminth effects on mammalian hosts, we emphasized careful analysis of various moderators like project design, sampling procedures, energetic condition proxies, identification and quantification of parasite infection, and phylogenetic variation. We hypothesized that, overall, helminths would have significant negative effects on host energetic condition, moderated by both methodological and biological factors which may induce variance and heterogeneity in the literature (Table 2.1).

Table 2.1 Hypothesized effects of included moderators. Effects of helminth infection on energetic condition in mammals, according to methodological and biological categories.

	Moderator	predicted relationship	suggested reasoning	references
Methodology	**Experimental design** *Cross-sectional* *Experimental* *Field experiment*	Cross-sectional designs will generally lead to lower resolution (weaker effect, greater variance).	Time of infection is an important factor in disruption of energetic condition; cross-sectional studies have difficulties controlling this factor.	Beldomenico *et al*. (2008*a*); Sanchez *et al*. (2018)
	Host sampling origin *Wild live sampled* *Wild opportunistic* *Domestic bred* *Laboratory bred*	Live sampling of wild animals will increase effect strength and reduce variance compared to opportunistic sampling. Sampling of domestic or laboratory-bred animals will increase the strength of the interaction.	Opportunistic sampling is species and system specific, and especially for mammals can be limited. Animals found dead in the wild are more likely already to be in poor health/condition and sample quality may be reduced by decomposition. Human-raised animals can be controlled for both infection and general health, allowing an equal baseline for comparison of infected/uninfected groups.	Budischak *et al*. (2012); Ryser-Degiorgis (2013); Kołodziej-Sobocińska (2019); Shanebeck and Lagrue (2020)
	Infection confirmation *Visual confirmation* *Faecal egg count* *Infection assumed*	Faecal egg count will produce weaker effect sizes with more variance compared to visual observation. Assumed infections will produce non-significant effects due to high variance.	Faecal egg count can result in false negatives and limit resolution of infection intensity. Lack of confirmation may lead to inclusion of false positives, adding variance.	Nielsen *et al*. (2010); Byrne *et al*. (2018)
	Parasite quantification *Intensity* *Presence/absence* *Treated/untreated* *Infection over time* *Species richness*	Correlation between parasite intensity and host energetic condition will show stronger effects with less variance compared to presence/absence. Infection over time and species richness will also show strong effects on energetic condition.	Presence/absence measurements reduce resolution *via* variance due to inclusion of low-intensity infections which may have limited host effects. Including infection timeline in analysis is essential, although many wild studies cannot measure this. Intensity may be a good substitute as parasites tend to accumulate over time.	Bush *et al*. (1997); Fenton *et al*. (2014); Sanchez *et al*. (2018); Defolie *et al*. (2020)

			Concomitant infections of parasites can have compounding effects on host health.	
Biology	**Host Taxa** *Ungulates* *Rodents* *Primates* *Marsupials* *Lagomorphs* *Cingulates* *Carnivores*	Effects will generally be consistent across groups, with significant variance, although ungulates will be associated with the strongest effect.	Variation will be more due to species- or system-specific factors, as all mammals have high metabolic requirements, and will have unique physiological and behavioral adaptations to energetic stressors. However, ungulates are the best studied, and may be easier to monitor for changes due to their size, accessibility, and propensity to store fat.	Parker *et al*. (2009); McNab (2012); Berta *et al*. (2015); Flores-Saavedra *et al*. (2018)
	Energetic proxy *Systemic function* *Nutrition* *Defense/stress* *Reproduction*	Mammal condition will similarly be strongly affected across all moderators. Systemic functioning will show slightly stronger effect.	Due to strong differences in study design, variations are more likely to be explained by methodology than by features of the response system. Systemic function response metrics often have quick response times, i.e. are more likely to show changes rapidly, reducing variation due to timeline of infection.	Table S2.1; Brock *et al*. (2013); Maceda-Veiga *et al*. (2015)
	Energetic scale *Physiological* *Morphological* *Observational*	Effect will depend on interaction with proxy, although physiological and observational measurements will tend to show stronger effects.	Physiological proxies tend to show changes rapidly, although with less range of difference. Similarly, observational proxies like behavior can be easily measured and change rapidly, although differences in measurement can increase variance.	Brock *et al*. (2013); Maceda-Veiga *et al*. (2015); Collins *et al*. (2016); Levesque *et al* (2016)
	Parasite group *Cestode* *Trematode* *Nematode* *Mixed*	Mixed infections with multiple groups will have the strongest effects; all other groups will show similar strength of effects.	Co-infections are reported to increase host susceptibility due to parasite's ability to suppress host immune responses, as well as the compounding effects of infection in multiple tissues/systems.	Bordes and Morand (2011); Budischak *et al*. (2012); Serrano and Millán (2014)
	Sex and age *Female/male* *Juvenile/adult*	Female and juvenile condition will be more strongly affected by helminth infection.	Female energetic burdens are greater due to the high cost of reproduction and lactation. Juvenile energetic burdens are greater due to the cost of growth.	Degen (2008); McNab (2012); Heldstab *et al*. (2017)

2.2 Methods

2.2.1 Literature search

For this analysis, we ran a systematic literature search, according to the standards outlined in the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA; Shamseer *et al*., 2009). The initial search was performed on February 13th, 2020, based on titles, abstracts, and key words from the *Web of Science* and *Scopus* databases. We ran the following search parameters: (Helminth* Or Cestod* OR Trematod* OR digenea* OR Acanthoceph* OR Nematod* OR ascari* OR worm* OR fluke* OR flatworm OR tapeworm OR roundworm* OR hookworm* OR "intestinal parasite" OR "gastrointestinal parasite*") AND (fitness OR "reproductive success" OR "milk production" OR "lactation" OR reproduction OR longevity OR fecundity OR sub-lethal OR sublethal OR "cumulative effect*" OR stress* OR "reduce tolerance" OR "antagonistic interaction*" OR "negative interaction*" OR "host health" OR "host behavior*" OR "host behavior*" OR "body condition*" OR Pathology OR "host physiology" OR performance OR "body mass index" OR "host susceptibility") AND (mammal* OR cetacea* OR primate* OR rodent* OR marsupial* OR carnivor* OR livestock OR cattle OR bovid* OR sheep OR swine OR ruminant* OR buffalo OR ungulat* OR pinniped* OR seal OR mustelid*) AND (host*) AND NOT (tick* OR elegans OR schistosom* OR treatment OR antihelminth* OR bird* OR vaccine*). Adding the specific exclusion categories after initial searches avoided these unrelated categories overwhelming the search results.

After combining the search results between *Scopus* and *Web of Science* and deleting duplicates, the search yielded 1115 potential articles. These articles were screened independently through blind review of abstracts by one of the authors (K.M.S.) and an independent reader, mediated by another author (C.L.), using Rayyan, an online software for meta-analysis (Ouzzani *et al*., 2016). When it was unclear from the abstract if the study was relevant, it was included by default for full-text review. A total of 265 articles were identified for full-text assessment. Review papers were included during this blind screening and used to conduct a backward search of the literature, assessing their cited references for inclusion. This identified an additional 78 papers for full-text assessment. Full-text reviews were conducted to confirm relevance according to inclusion/exclusion criteria and to determine whether usable data were provided. If articles did not report sufficient information, the corresponding author was contacted to request the relevant data. After full-text review, 85 of the original and 58 of the additional backward search references were used for data extraction. Inclusion and exclusion decisions followed a predetermined criterion (Appendix A.2).

2.2.2 Data extraction

From the 143 studies included, we recorded the host species, taxonomic reference number from the Open Tree of Life (McTavish *et al*., 2015), sex, standardized age, parasite group (cestode, nematode, trematode, mixed), species (if available), location in the host body, how infection severity was quantified, how infection was confirmed, and study design. In addition, for each effect size calculation, we recorded the energetic condition proxy measured and what general function it related to. The scale of the proxy used (physiological, morphological, observational; Table S2.1), proxy data type (direct numerical, scaled numerical, proportional, ordinal), sample size, test statistics, how the effect size was calculated, and the direction of the effect (positive or negative relationship between infection and condition) were also recorded.

We used Cohen's *d* as our metric of effect size between parasite infection and host condition (Cohen, 1988). Cohen's *d* was calculated using open-source effect size calculators from the Campbell Collaboration (Lipsey and Wilson, 2001; Wilson, 2001) and Psychometrica (Lenhard and Lenhard, 2016). We converted reported effect sizes, test statistics, data, graphical data, etc. into Cohen's *d* following a predetermined hierarchy of conversion methods (Fig. S2.1), recording how the effect size was calculated in the metadata. We determined the direction of effect based upon the correlation between infection and host energetic condition. When changes in an energetic proxy were related to a reduction or challenge to energetic condition, the effect was considered negative. If both increasing and decreasing values in a specific condition proxy are known to be associated with reduced host health (for example liver mass, where both increases and decreases are related to poor health) (Penzo-Méndez and Stanger, 2015), any effect was considered negative. When experimental studies included repeated measures over time, we compared the means of the change over time between control and experimental groups when possible. However, if data were not available, we extracted the mean and standard deviation from the last measurement of the experiment. From the 143 studies, we calculated a total of 602 effect sizes. However, after investigation for negative bias, three strongly negative outliers were excluded ($d = -11.86, -8.30, -7.96$). All were from laboratory experiments on rodents. One of these was the only effect size calculated from a particular article, bringing our total number of included studies down to 142 and included effect sizes to 599.

2.2.3 Defining moderators: methodology and energetic condition

Metrics of ‘body condition’ can vary strongly across ecological studies. They may include qualitative and quantitative (proportional, direct numerical, adjusted numerical) measurements. Methodology also differs widely in terms of sampling period, sampling source, parasite identification methods, etc. (Brown *et al*., 1993; Beldomenico *et al*., 2008*a*; Peig and Green, 2010; Sanchez *et al*., 2018). As these differences may influence assessment of host condition–parasite correlations (Sanchez *et al*., 2018), we grouped moderators as described below.

Energetic condition assembled response metrics by the specific response system or effect they indicate changes in (Table S2.1). These included metrics related to (1) reproduction, (2) core systemic functioning (excretory, digestive, cardio-pulmonary systems and metabolism), (3) nutritional status (fat storage, growth, muscle tone, body size, mass, and other qualitative assessments) and (4) defense (physiological and behavioral stress responses, immunity). Very few studies investigated resource acquisition as a response metric; two measured time spent foraging and some recorded host activity ($k = 9$) such as time spent travelling. To reduce the bias of zero-inflation, these effects were nested within stress/defense as changes in these behaviors are also often linked to systemic stress response.

Energetic scale grouped response metrics by physiological, morphological, and observational scales (Table S2.1). Physiological metrics were defined as small-scale, physiochemical proxies linked to specific systemic processes such as hormone concentrations, waste products, proteins in blood or tissue, blood parameters (such as hematocrit or hemoglobin), concentrations of specific nutrients and others (Table S2.1). Physiochemical processes may vary strongly over time and be directly or indirectly affected by parasite infection (Beldomenico *et al*., 2008*b*; Defolie *et al*., 2020). For example, infection by intestinal helminths may be related directly to reduced hemoglobin levels *via* blood loss (Seguel *et al*., 2018). However, hemoglobin level also is affected by nutrition and therefore could be indirectly related to helminth infection due to reduction in digestive efficacy (Beldomenico *et al*., 2008*b*; Budischak *et al*., 2012). Reduced hemoglobin also has implications for cardio-pulmonary and metabolic health and can lead to fatigue, behavioral changes, and reproductive effects (Budischak *et al*., 2012). Similarly, cortisol is used as a proxy for systemic stress response and may be related directly to immune challenge by parasitic infection (Seguel *et al*., 2019). However, systemic stress may also be a cumulative response to compounding factors indirectly linked to nutritional challenge due to infection (Pawluski *et al*., 2017; Defolie *et al*., 2020).

Morphological metrics were defined as proxies that can be easily observed, measured, and calculated, such as organ mass, body mass, body/scaled mass indices, muscle depth, fat levels, milk production, and others (Table S2.1). For example, splenic mass is linked to immune response and increases in animals with significant immune challenges (Corbin *et al*., 2008). Liver mass tends to be regulated strictly in the bodies of mammals and restricted to a specific percentage of total body mass. Changes in size are often related to disease or nutritional challenge (Penzo-Méndez and Stanger, 2015).

Observational metrics were defined as any proxies that did not directly measure physical changes in the host's body, but instead recorded external changes directly linked to energetic condition, such as behavior, reproductive success (e.g. number of offspring produced or mass of offspring post weaning), physical endurance, elective food intake, and others (Table S2.1). This category of metrics is closely related to trade-off theory where responses to energetic challenges induce adjustment in energy allocation and behavior in mammals (Hutchings *et al.*, 2006; Brock *et al*., 2013; Berta *et al*., 2015). For example, milk production or offspring body mass gain in controlled settings are good indicators of the mother's nutritional condition and ability to allocate resources to milk production and quality, which may be challenged by parasitic infection (Landete-Castillejos *et al*., 2005; Moore and Hopkins, 2009).

Research methodologies were grouped according to sampling and parasite identification methods. *Research method* identified if the study was (1) cross-sectional (observational studies that investigated a sub-set of a population), (2) experimental (studies that investigated effects in a controlled setting), or (3) field experimental (semi-controlled studies conducted in the field). *Host origin* recorded the sampling source of the host. Sources were (1) laboratory bred (animals reared in a laboratory setting for experimentation), (2) domestic (domesticated animals, raised for human industry), (3) live-caught wild animals (hosts sampled directly for the study following set sampling protocols; may have been killed but were sampled live) and (4) opportunistically collected wild animals (carcasses collected opportunistically from roadkill, hunters, stranding, etc.). *Parasite identification* recorded how parasite infection was confirmed. Identification could be *via* (1) direct observation (visual confirmation of parasites in the host), (2) faecal egg count (FEC, confirmation of eggs in the host's feces), (3) assumed infection (study included a group treated with anti-helminthic and untreated groups assumed to be infected). *Parasite quantification* relates to how parasite infection severity was accounted for in study analyzes. Infection classically could be reported as (1) intensity/abundance (number of parasites per individual host), (2) presence/absence (including studies that measured by prevalence) or (3) richness (diversity of parasite species). Alternatively, some studies reported infection as (4) treated/untreated hosts (i.e. compared animals treated with anti-helminthics *versus* untreated hosts) or (5) infection over time (quantified infection severity by period of infection, artificially infecting animals with an equal number of parasites).

2.2.4 Statistical analysis

2.2.4.1 Addressing non-independence

The effects of phylogenetic and methodological non-independence are important considerations to include in the synthesis of meta-analytical data (Noble *et al*., 2017). To control for non-independence, we included phylogenetic relationships using the packages *rotl* (Michonneau, Brown and Winter 2016) and *ape* (Paradis and Schliep, 2019) to extract a phylogeny from the Open Tree of Life (Rees and Cranston, 2017), construct a phylogenetic tree, randomized to deal with polytomies, and create a correlation matrix (Sanchez *et al*., 2018; Sánchez-Tójar *et al*., 2020).

For laboratory or other controlled studies that included multiple groups with a shared control, non-independence of experimental groups is a possible source of type-I error in the analysis due to artificial inflation of sample size (Noble *et al*., 2017; Fernández-Castilla *et al*., 2020). To avoid this, we included the sub-group variance in the pooled SD or used multigroup effect size calculations to provide a single effect (Lipsey and Wilson, 2001).

Non-independence can also affect analysis of overall effect size when studies provide multiple measurements for the same group. Our final data set had an average of 4 effect sizes per study. Studies may have reported multiple condition proxies from the same population, effects on different age groups or sexes, differences between parasite groups from the same sampled population, or included multiple independent experimental designs. For example, conducting an experiment for males and females or food-supplemented *versus* non-supplemented, each with their own control group, would yield multiple effect sizes. During data extraction, to monitor this, effect size metadata included not only the study the measurement was taken from, but also a unique observation group number to identify effect sizes measured from the same experimental group. For example, Arias-Hernández *et al*. (2019) investigated the effect of parasite infection on rabbit energetics and included two different experimental protocols. In one, rabbits were fed a normal commercial diet and in the other, rabbits were fed to obesity. This produced effect sizes for each metric of energetic condition, which were identified by a group ID number between the normal and obese rabbit experiments. Both observation ID and study were included as random effects to structure inter- and intra-study variance in the model.

2.2.4.2 Meta-regression modelling

Effect size analysis and graphical representation were carried out in R Studio (RstudioTeam, 2020), with the package *metafor* (Viechtbauer 2010). Within our multiple working hypothesis framework, we used a random effect multivariate regression model ('rma.mv' function, *metafor* package), fitted with a restricted maximum likelihood (REML) estimator to reduce underestimation of heterogeneity (Viechtbauer, 2005). We then calculated heterogeneity as I^2, a better measure of true variance (Nakagawa and Santos, 2012). We used a crossed design explicitly to model residual variance, including the random effects: observation group (intra-study variance), study, and host species (phylogenetic correlation). We investigated the effects of our various moderators *via* multimodel inference, through variance explained (R^2), heterogeneity (I^2 and Q), and the Akaike Information Criterion (AIC) to summarize the relative importance of our various predictors hypothesized *a priori* to be of concern (Elliott and Brook, 2007). To address publication bias, we analyzed meta-regression models *via* contour-enhanced funnel plots (Peters *et al*., 2008).

2.2.4.3 Graphical representation

Visual representation was conducted in R studio (RstudioTeam, 2020) with the new package *orchaRd*, which expands the classic forest plot including trunk (point estimate) and branches (confidence intervals) only to an 'orchard plot', adding 'twigs' (prediction interval) and 'fruit' (individual effect sizes scaled by the inverse of their standard error in semi-random clustering (Nakagawa *et al*., 2021). Point estimates (PIs) give a perspective of the range of possible or 'plausible' effect sizes for hypothetical new studies and give a perspective of the heterogeneity of the meta-regression. Heterogeneity is to be expected in meta-analysis of ecological research and is therefore important to represent (Senior *et al*., 2016; Nakagawa *et al*., 2021).

2.3 Results and Discussion

Our final data set (see Datafile S2.1) included 599 effect sizes from 142 studies, across 78 species of mammals grouped into seven clades: cingulates ($k = 3$), marsupials ($k = 12$), primates ($k = 35$), lagomorphs ($k = 49$), carnivores ($k = 81$), rodents ($k = 165$), and ungulates ($k = 254$) (Fig. S2.2). Recorded effect sizes came from domesticated species (21.5%), laboratory-bred species (10.4%), live-sampled wild species (46.2%), and opportunistically sampled carcasses of wild species (21.9%). Effect sizes by energetic condition proxy skewed toward nutritional status (50.1%), then core systemic functioning (20.0%), defense/stress (17.4%), and reproduction (12.5%). Most effect sizes were measured on the morphological (60.4%), then split between the physiological (22.0%) and observational/external (17.5%) measurement scales (see Section II.3). Effect sizes also came from studies across many years and systems, using various methods (Appendix A.3, Fig. S2.3). Average effect size (Cohen's d) for the intercept-only multivariate meta-analytic model of the correlation between helminth infection and mammal energetic condition was strongly negative ($d = –0.59$ [95% CI = –0.78 to –0.39], se = 0.10, $p < 0.0001$, $k = 599$), with moderate heterogeneity ($I^2 = 56.89\%$) (Fig. 2.2), supporting our hypothesis that sub-lethal helminth infections are important influencers of mammal energetic condition.

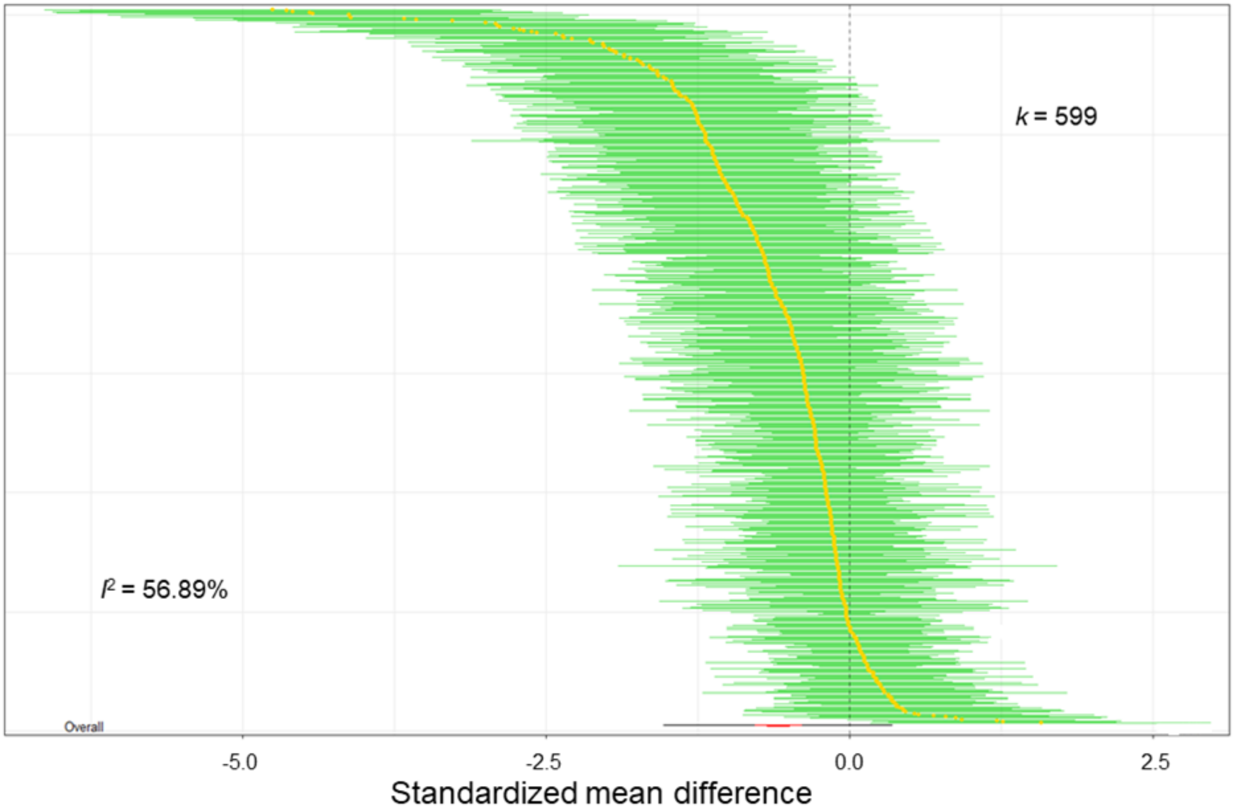

Figure 2.2 Overall effect on energetic condition. Mean effect sizes (yellow dots), 95% confidence intervals (dark green lines), and precision interval (pale green lines) across effects from all studies (*k* = 599), including the heterogeneity (I^2), average effect (red box) with 95% confidence interval (red line) and precision interval (black line) of the intercept-only model

2.3.1 Effects of methodological factors

Methodological differences among studies strongly influenced effect size. As predicted, cross-sectional studies showed weaker negative effects of infection on host energetics (*d* = –0.47 [95% CI = –0.71 to –0.24], se = 0.12, p < 0.001, *k* = 320) when compared to experimental studies (*d* = –0.82 [95% CI = –1.09 to –0.55], p < 0.001, *k* = 227), though not significantly so as their confidence intervals overlapped (Fig. 2.3A). They also made up the largest proportion of included effect sizes. Semi-controlled field experiments yielded intermediate effect sizes and showed the most variance (Fig. 2.3A). This may be because infection period (i.e. time since infection) is an essential variable (Sanchez *et al*., 2018), which cross-sectional studies can rarely include in their analysis. However, such trends may also be influenced by a variety of environmental or physiological variables. This could similarly explain the increased variance observed in semi-controlled field experiments, as study design and duration were highly variable.

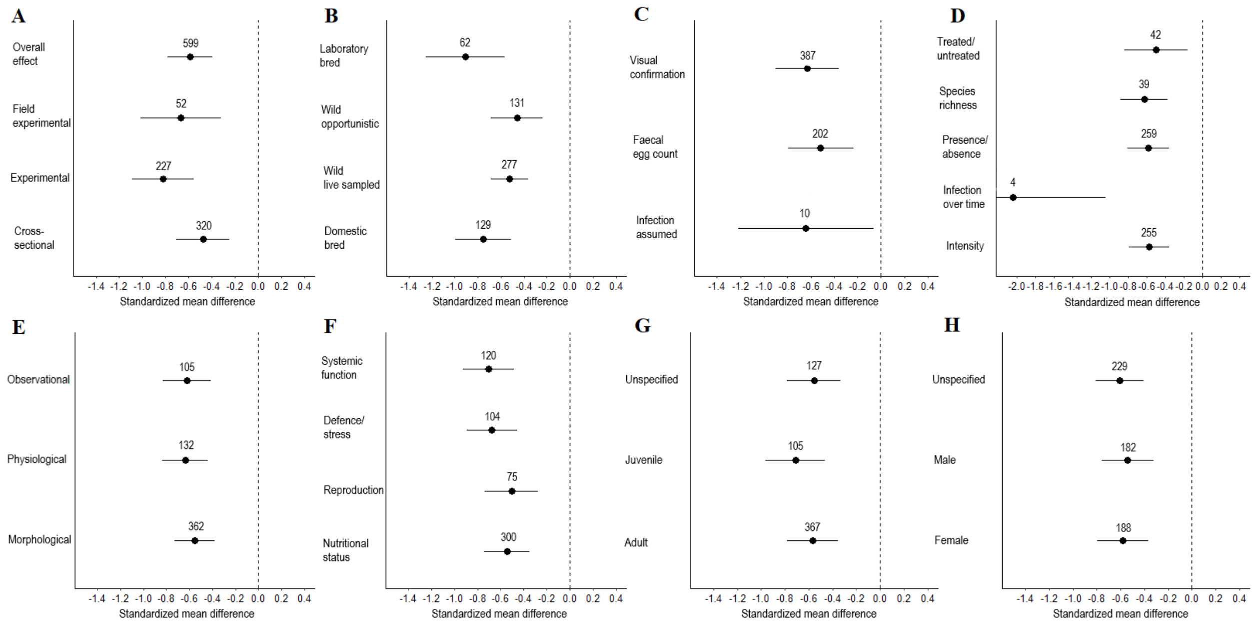

Figure 2.3 Effect of moderators on energetic condition. Average effect sizes of the hypothesized moderators, number of included effect sizes and 95% confidence intervals according to methodological factors. (A) Overall effect across all effect sizes and by project design, (B) host sampling origin, (C) method of infection confirmation, (D) parasite quantification as well as the biological factors, (E) energetic proxy scale, (F) energetic proxy, (G) age, and (H) sex.

In studies where wild animals were treated against parasites, it could not always be confirmed that animals were not re-infected after treatment, or that the 'control' animals were infected (Newey and Thirgood, 2004; Pederson and Greives, 2008; Gobush, Baker and Gulland, 2011; Ezenwa and Snider, 2016). Furthermore, anthelminthics can have a range of efficacy in the per cent reduction of parasite abundance and among parasite species (Murray, Keith and Cary, 1998; Pederson and Fenton, 2015), all of which may increase variance. Infection period may also explain the stronger negative effects seen in mammals sourced from domestic or laboratory-bred animals, as they almost always were used in controlled experiments and artificially infected, allowing for exact measurement of infection period and inclusion of this factor into the model (Fig. 2.3B). Wild hosts were most often used in cross-sectional studies, which may explain the generally weaker effect size detected. The effect size was, as predicted, weakest for opportunistic sampling. This may be because mammals found deceased in the wild are more likely to be in poor condition already due to disease processes or physical harm (Shanebeck and Lagrue, 2020). Cross-sectional studies often cannot quantify infection period, limiting them to snapshot comparisons between infected and uninfected hosts. Observed effects of parasitic infection may thus be weakened by confounding factors influencing host condition such as unrelated diseases, injuries, or anthropogenic effects.

Methods used to identify and quantify parasitic infection did not have a strong effect on the average effect size. Our prediction that visual confirmation should show a stronger negative effect compared to FEC was not confirmed (Fig. 2.3C). This suggests that, while FEC has its limitations when trying to quantify infection intensity, these did not significantly obscure the effects of infection. Again, infection period may be more important in determining the strength of effect (Nielsen *et al*., 2010; Byrne *et al*., 2018). The few studies that used infection over time as their metric produced a very strong negative effect, albeit with strong variance, likely due to low sample size of effects (Fig. 2.3D). Studies that assumed infection without confirmation predictably showed high variance, suggesting that infection status of hosts should always be confirmed, rather than assumed (Fig. 2.3C). Similarly, defining individual host infection status as ‘treated’ or ‘untreated’ increased variance and weakened the strength of the negative effect, likely because these studies did not always guarantee that all ‘treated’ individuals received treatment or were parasite free (Fig. 2.3D). Contrary to our prediction, there was little difference in effect size among studies where parasite infections were quantified by intensity, presence/absence or species richness.

2.3.2 Effect of biological factors

Biological factors were also significant determinants of the strength of the negative effect of infection on host energetic condition. As predicted (Table 2.1), this effect was similar across energetic proxy and proxy scale but slightly stronger for proxies and scales more likely to respond quickly to infection (physiological and observational scales, and systemic function and defense/stress), although this difference was not significant (Fig. 2.3E, F). Age and sex did not lead to strong variation in effect sizes among groups; juveniles and females showed slightly stronger negative effects of infection on host energetic condition (Fig. 2.3G, H), although this was not significant. This variance may have come from a variety of sources, and many studies did not differentiate between ages or sexes, which may have influenced these results.

The increased energetic requirements for growth in juveniles and pregnancy and lactation in females can be extreme in some groups like mustelids (Thometz *et al*., 2016; Heldstab *et al*., 2017; Felska-Blaszczyk and Seremak, 2020). Furthermore, some energetic burdens such as pregnancy and lactation are time specific, and cross-sectional studies may not have observed females during periods of higher energetic burden. Additionally, very few studies differentiated adults to include aged adults or adults past their reproductive prime or considered to be of advanced age (Milner *et al*., 2003). This is an important consideration when analyzing energetic condition as this group is also likely to be in poorer condition, regardless of parasitic infection, due to the increased rates of age-related health problems that may affect their body condition and ability to acquire sufficient resources (Milner *et al*., 2003; Beldomenico *et al*., 2008*a*; McNab, 2012). Many studies did not report or differentiate the age or sex of the host animals, which reduced the overall sample size to quantify this difference. Future studies would benefit from collecting and explicitly modelling the effect of such information when investigating energetic effects related to helminth infections. Species-specific host life-history traits such as age and sex can affect energetic requirements as well as average base energetic condition (McNab, 2012).

Interestingly, there was little difference among parasite taxonomic groups but, contrary to previous assumptions that cestodes are generally of little significance for mammals (Appendix A.1), cestodes showed a strong negative effect ($d = -0.61$ [95% CI = –0.91 to –0.30], se = 0.15, $p < 0.0001$, $k = 49$) (Fig. S2.4). When location of the parasite was considered, all were significantly negative moderate or strong effects except for those located in muscle tissue, likely due to low sample size ($k = 2$) (Fig. S2.5). Our survey of the literature found no study investigating the effects of sub-lethal acanthocephalan infections on energetic condition, although acanthocephalans are ubiquitous parasites of marine mammals (Shanebeck and Lagrue, 2020). Most effect sizes came from gastrointestinal infections ($k = 485$). It should be noted that for both parasite tissue location and parasite clade, mixed infections where more than one parasite group infect the same host or infections were in multiple tissues (which was most often also mixed-clade infections) showed the strongest negative effects on the host. This matches our prediction (Table 2.1), and the available literature showing that concomitant infections and the compounding effects of multiple nutritional, immune, and systemic burdens have stronger effects on overall host condition (Bordes and Morand, 2011; Budischak *et al*., 2012; Serrano and Millán, 2014).

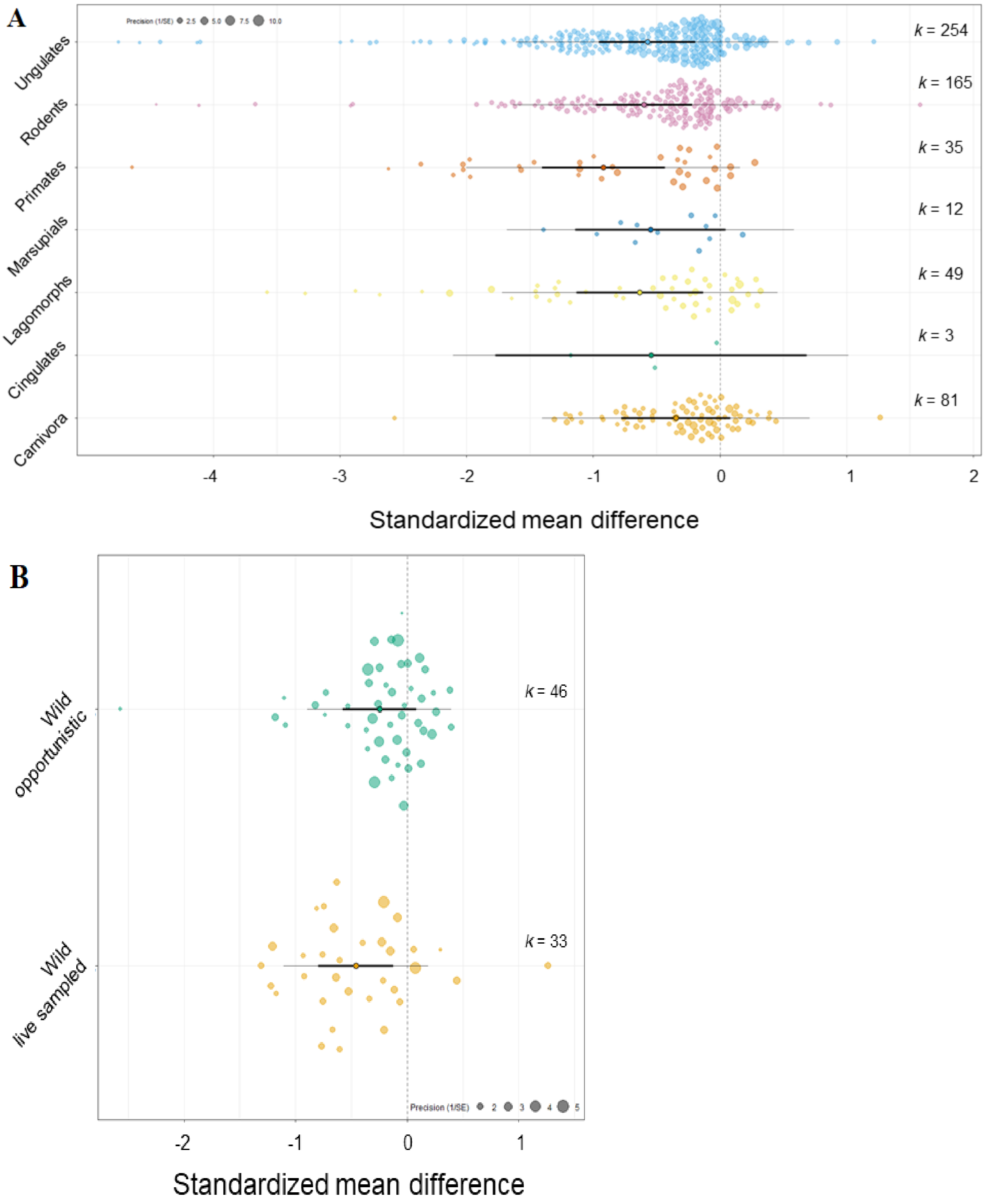

Figure 2.4 Effect of infection by taxonomic group. (A) Orchard plot showing average effect sizes (point, 'trunk') including 95% confidence intervals (bold line, 'branch') and precision intervals (small line, 'twig'), individual effect sizes scaled by precision ('fruit') spread on the *y*-axis based on quasi-random noise (Nakagawa *et al*., 2021), and number of included effect sizes (*k*) according to taxonomic group. (B) Effect sizes for carnivores only according to sampling origin of the host: opportunistically collected as carcasses or sampled live according to specific experimental protocols (included studies were predominantly cross-sectional in design).

There were strong variations among host taxonomic group (Carnivora, Ungulata, Rodenta, etc.), although interpretation of differences may not be fully due to taxonomic differences (Fig. 2.4A). For example, of the five clades with the largest number of reported effect sizes, primates showed the strongest effect. However, studies of primates were focused predominantly on defense (stress response), *via* analysis of cortisol levels in feces or behavioral responses. Effects based on metrics that can change rapidly, like physiological measurements of defense/stress proxies (e.g. cortisol), are likely to produce stronger negative effects when time period is not included, compared to proxies like nutritional condition that may take more time to show significant changes (Stevenson and Woods, 2006; St. Juliana *et al*., 2014; Sanchez *et al*., 2018). Contrary to our prediction (Table 2.1), there was no significant difference between members of Ungulata (the clade with the largest average body size), and Rodenta or Lagomorpha (the clades with the smallest average sizes). Of the five clades with the most recorded effect sizes, when considered alone, only Carnivora did not show a significantly negative effect (Fig. 2.4A). While this seemed to confirm an assumption that carnivores (like pinnipeds) are less likely to be affected by sub-lethal infections like gastrointestinal helminths; all these studies were cross-sectional, and variance may be explained by methodological bias. When the interaction of host sampling origin and taxonomic group was modelled (Fig. 2.4B), carnivores sampled live in the wild *via* proactive experimental protocols showed a significant negative effect of infection on energetic condition ($d = –0.48$ [95% CI = –0.80 to –0.17], se = 0.16, $p = 0.003$, $k = 33$). Contrastingly, limitations inherent to opportunistic sampling led to a weaker, non-significant effect ($d = –0.24$ [95% CI = –0.54 to 0.05], se = 0.15, $p = 0.106$, $k = 46$).

2.3.3 Best fit models: two-factor interactions

Host sampling origin largely explained variations in the data and was a contributing factor in the two best models based on AIC, heterogeneity and variance explained (Table 2.2). This underscores the importance of explicitly modelling the effect of methodology in meta-analysis of complex, difficult-to-measure effects. When separated by host sampling origin and energetic proxy, differences in methodology revealed some interesting trends. Studies investigating wild mammals showed the same trend but slightly stronger effects in live-sampled hosts compared to opportunistically sampled hosts. There was however little variation among energetic proxies (Fig. 2.5).

Table 2.2 Intercept-only model compared against the top five moderator models. The top five models are shown based on the lowest Akaike Information Criterion score (AIC). Heterogeneity (I^2) is also shown, Cochran's Q [residual heterogeneity (Q_E), and the heterogeneity explained by moderators (Q_M)], the per cent variance explained by the model, [R^2 conditional (R^2_C)], and the variance explained by moderators [R^2 marginal (R^2_M)].

Model	I^2	Q_E	Q_M	R^2_C	R^2_M	*AIC*
Intercept only	56.89	1174	–	89.64	–	1134
Host sampling: energetic proxy	53.96	1062	226	99.99	25.36	1090
Host taxa: energetic proxy	55.88	1052	75	87.76	21.08	1098
Parasite quantification: energetic proxy	56.06	1101	73	89.11	21.21	1108
Experimental design: parasite quantification	56.38	1031	46	77.84	18.75	1112

For laboratory-bred and domestic hosts (which are most often tested in controlled experiments), a few strong differences were apparent. For laboratory animals, the effects of infection on stress/defense were much larger than for any other group, which may be an artefact of selective breeding making laboratory rodents more tolerant of large changes to systemic function. This may artificially induce larger differences between normal and highly stressed systemic functioning or indicate that stress/defense responses require strictly controlled settings when investigating the breadth of change. The weakest average effects of infection were seen in domestic host reproduction. However, this does not necessarily mean that parasites do not have significant effects on reproduction. First, most research on farm animals investigating the effects of helminth infections offered consistent *ad libitum* feed for all individuals, potentially allowing hosts to compensate for some of the sub-lethal effects of parasites *via* higher food intake. Second, these experiments often use controlled infections over short periods (weeks/months not years). As reproduction should be most strongly affected by long-term infections and additional energetic burdens (secondary effects like lethargy, behavioral stress response, and reduced ability to secure food resources), short-term infections and subsidized energy intake may have masked long-term, cumulative effects of parasite-induced nutritional deficiency on reproduction. Laboratory-bred hosts did not show a similar weak effect on reproduction, but this may be because those studies often used observational proxies like reproductive behavior (e.g. number of mounting attempts) which can be rapidly altered but may not be the best proxy for reproductive success. This may explain why the next best model (host taxa: energetic proxy; Table 2.2) showed similar patterns, again likely due to methodological trends nested within the various groups, like primate studies which are often limited to observational metrics and commonly focus on stress response.

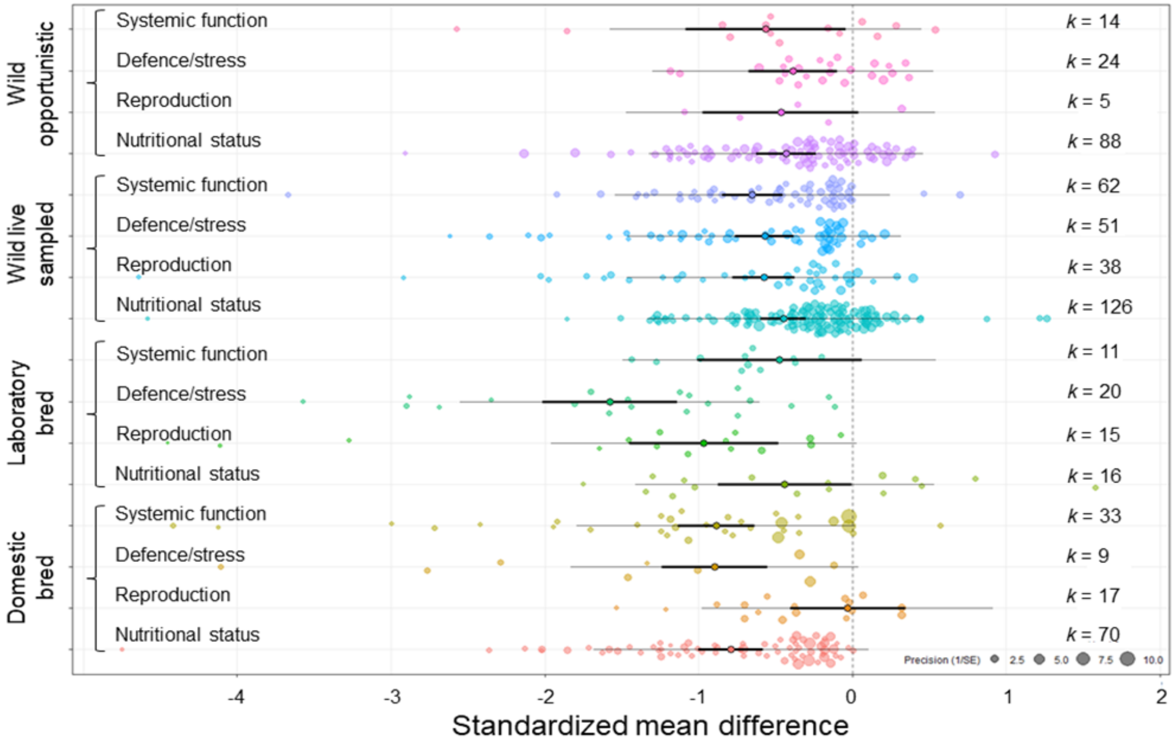

Figure 2.5 Effect of infection by host origin and energetic proxy. Orchard plot showing average effect size (point) of energetic proxy by host sampling origin and energetic category (core systemic function, defense/stress response, reproduction and nutritional status), with 95% confidence interval (bold line), precision interval (thin line), and individual effect sizes scaled by precision and clustered quasi-randomly. Domestic and laboratory-bred species tend to have a higher starting energetic condition due to food supplementation and being bred to put on muscle and fat easily. Wild species in comparison will have a lower average starting condition due to differences in resource availability, environmental stressors, or other negative influences of condition. The 'Wild live sampled' group sampled by an experimental protocol tends to represent a more accurate cross-section of the population, although is still limited by the researcher's ability to sample randomly. 'Wild opportunistic' sampling tends toward lower average body condition, as animals found deceased in the wild are more likely to be in poor condition due to disease, injury, or other processes that led to their death.

For laboratory-bred and domestic hosts (which are most often tested in controlled experiments), a few strong differences were apparent. For laboratory animals, the effects of infection on stress/defense were much larger than for any other group, which may be an artefact of selective breeding making laboratory rodents more tolerant of large changes to systemic function. This may artificially induce larger differences between normal and highly stressed systemic functioning or indicate that stress/defense responses require strictly controlled settings when investigating the breadth of change. The weakest average effects of infection were seen in domestic host reproduction. However, this does not necessarily mean that parasites do not have significant effects on reproduction. First, most research on farm animals investigating the effects of helminth infections offered consistent *ad libitum* feed for all individuals, potentially allowing hosts to compensate for some of the sub-lethal effects of parasites *via* higher food intake. Second, these experiments often use controlled infections over short periods (weeks/months not years). As reproduction should be most strongly affected by long-term infections and additional energetic burdens (secondary effects like lethargy, behavioral stress response, and reduced ability to secure food resources), short-term infections and subsidized energy intake may have masked long-term, cumulative effects of parasite-induced nutritional deficiency on reproduction. Laboratory-bred hosts did not show a similar weak effect on reproduction, but this may be because those studies often used observational proxies like reproductive behavior (e.g. number of mounting attempts) which can be rapidly altered but may not be the best proxy for reproductive success. This may explain why the next best model (host taxa: energetic proxy; Table 2.2) showed similar patterns, again likely due to methodological trends nested within the various groups, like primate studies which are often limited to observational metrics and commonly focus on stress response.

Another model where variance was well explained (parasite quantification: energetic proxy), showed consistent results. Categories with the strongest or weakest results were those with the lowest sample effect size (Fig. S2.6). However, as seen in the parasite-quantification only model (Fig. 2.3D), for the categories with the largest sample sizes, defense: presence/absence ($k = 42$), defense: intensity ($k = 46$), nutrition: presence/absence ($k = 104$), nutrition: intensity ($k = 155$), there was no strong difference between intensity or presence/absence. Similarly, in the last model (study design: parasite quantification), effect sizes were consistent between intensity and presence/absence (Fig. S2.7), with similar differences based on experimental, field experimental, or cross-sectional studies as seen in the study-design only model (Fig. 2.3A). This contradicted our hypothesis that measuring parasite infection by intensity would detect stronger effects than simply presence/absence, suggesting that even low intensity infections can have a significant influence on host energetic condition. If studies are limited in their ability to measure intensity, presence/absence is still a valuable metric.

2.3.4 Effect of gastrointestinal parasites on standard body condition metrics

When the data set was reduced to effect sizes calculated only from infections by gastrointestinal helminths using classic metrics of 'body condition' (nutritional status; Table S2.1), the effect was still significant and moderately strong ($d = –0.43$ [95% CI = –0.62 to –0.24], se = 0.1, $p =$ <.0001, $k = 239$; Table S2.2; $I^2 = 52.06\%$, $R^2_c = 88.90$). The meta-regression model by specific groupings of condition proxies ($I^2 = 47.93\%$, $R^2_c = 85.71$, $R^2_M = 16.27$) showed strong differences in effect according to how nutrition was measured (Table S2.2); again indicating that methodological considerations are essential when reviewing experimental findings. Observations based on a subjective body condition score (often a categorical qualitative assessment) and body size/length (which is too unspecific) showed the weakest effects; body size/length leading to a non-significant effect (Fig. 2.6, Table S2.2). This is consistent with previous reports that caution the use of these indices because of the loss of resolution inherent to qualitative scores, and overly general metrics like length that vary strongly by species, age, sex, and mitigating circumstances (Stevenson and Woods, 2006; Peig and Green, 2010; Sanchez *et al*., 2018). Growth rate, which inherently accounts for time of infection, showed the strongest negative effect. The most consistent and significant metrics, all with an effect size around –0.4, were body mass index, body mass, and measurements of fat (kidney fat index, fat mass, fat depth), which are better indicators of the availability of energy in the animal for storage in fat or for growth (Brown *et al*., 1993; Peig and Green, 2010). While historically gastrointestinal parasites have been seldom considered influencers of mammal health (Appendix A.1), it is clear that sub-lethal infections of gastrointestinal helminths are negatively affecting mammal energetic condition and should clearly be included into the analysis of both mammal health and population dynamics.

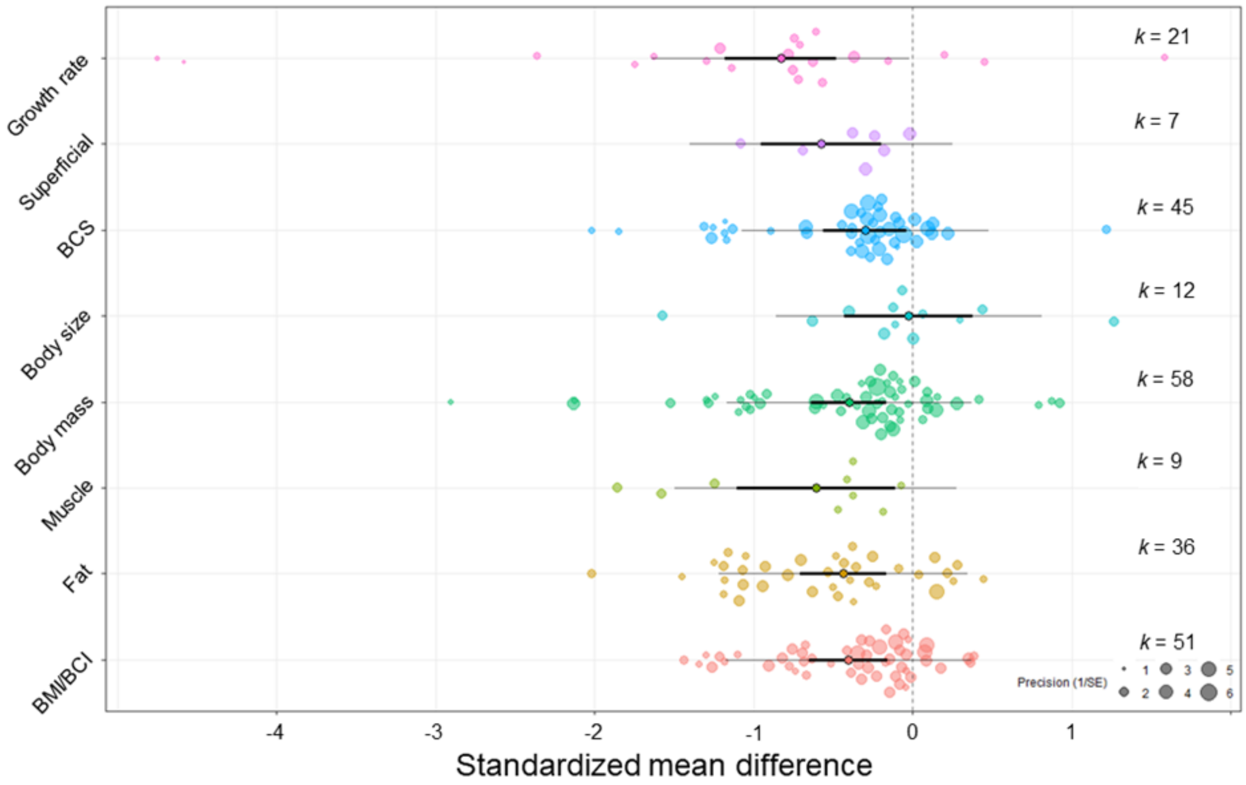

Figure 2.6 Effect of gastrointestinal infection by body condition proxy. Orchard plot showing average effect size (point) of gastrointestinal parasite infection on 'Body condition' (morphological energetic proxy measures), with 95% confidence interval (bold line), precision interval (thin line), and individual effect sizes scaled by precision and clustered quasi-randomly. Sub-sets of included effect sizes of studies that investigated gastrointestinal helminths and measured energetic condition by nutritional status, i.e. classic metrics of body condition. These include growth rate, superficial features (like antler size or fur quality), body condition score (BCS), body size, body mass, muscle (often muscle depth at specific locations), fat (such as fat mass or kidney fat index), and body mass index (BMI) or body condition index (BCI).

2.3.5 Heterogeneity and bias

Heterogeneity is expected to be high in ecological meta-analysis, generally I^2 = 83–92% (Senior *et al*., 2016). This is due likely to limitations in ecological sampling which create diverse methodological practices. Surprisingly, even though most of our included effect sizes came from studies of wildlife (*k* = 408), overall heterogeneity was moderate (I^2 = 56.89%), and closer to what would be expected in meta-analyzes of human medicine and controlled experiments (Senior *et al*., 2016). Analysis of publication bias showed a left skew in the funnel plot of the intercept-only model. However, when repeated for the best-fit meta-regression model, this was reduced (Fig. S2.8). Some level of skewed distribution should be expected due to the nature of the effect size measurement, a comparison of means, most often between infected and uninfected animals.

This means that we are less likely to get strongly positive effect sizes as that would require infected animals independent of the control group to increase their energetic condition drastically. It is more likely they would have similar averages, leading to a weakly positive or weakly negative effect.

Publication bias tends to lead to type-1 errors as studies producing significant results are more likely to be published. This is why ecological cross-sectional studies generally have higher heterogeneity and stronger effect sizes than controlled experimental studies which tend to reduce negative bias and moderate the observed effect (Peters *et al*., 2008). However, heterogeneity may not always be due to publication bias. Any factor that can influence effect size may increase heterogeneity, such as sampling bias and poor study design (Peters *et al*., 2008). Our results showed the opposite trend; methodological limitations of cross-sectional studies reduced the strength of the negative effect, while experimental, controlled studies increased the strength of the negative effect (Fig. 2.3A). This suggests that methodological bias, not publication bias, may be increasing heterogeneity, feeding the incorrect assumption that large wildlife, like marine mammals, may be unaffected by helminth parasites.

Similar trends are obvious within cross-sectional studies. In the meta-regression where only host taxonomic group was considered, the negative effect of infection was not significant for carnivores (Fig. 2.4A). If sampling methodology had not been considered in our analysis, this distinction would have been missed. In addition to publication bias, we encourage the consideration of methodological bias in meta-analyzes, especially in ecological research, as it might also induce type-I or -II errors.

2.3.6 Guidelines for studying sub-lethal effects in wildlife

Our results highlight how methodological variations may influence our perceptions of the influence of sub-lethal parasites on wildlife populations. Going forward, researchers should explicitly state and model the context and importance of energetic condition (‘health’) metrics as they relate to individual and population fitness for the species. We suggest some general guidelines below.

For *semi-controlled field experiments*, it is preferable that individuals with known infection status (best practice to treat uninfected 'control' animals with anthelmintics paired with faecal analysis to confirm they are parasite free) be observed for differences in behavior, reproductive output, or hormonal proxies (which can be measured *via* feces), and if possible, re-captured for serological and morphometric energetic proxies. Since infection period is unlikely to be known, and faecal analysis often limits parasite quantification to presence/absence, it is best that analysis of the effect of anthelmintics or artificial infection be measured as change over time. Thus, re-capture of individuals is preferred over random sampling of a population where individual hosts cannot be confirmed as treated or not. Depending on the duration of the study, energetic proxies that respond quickly to energy availability should be used, such as time spent in an elective behavior, metabolic parameters, concentrations of specific vitamins, etc. (Table S2.1).

For *cross-sectional studies*, animals should be sampled according to controlled experimental protocols whenever possible to ensure adequate sampling of the population depending on study goals. Opportunistic methods often introduce bias into the sample group and should be interpreted with caution. During data collection, considerations of both age class and sex should be included, along with consideration of the time of year and species-specific life-history traits. If this is logistically impossible, for opportunistically collected carcasses, researchers must explicitly model cause of death in the analysis to differentiate individuals that died of accidental (i.e. unnatural events such as roadkill or fishing bycatch) causes and those that died of natural causes (e.g. disease, age). Hosts that die of natural processes are more likely to be in poor condition regardless of parasitic infection, which reduces the sensitivity of the comparison between infected and uninfected individuals. Researchers should also comment on any possible compounding effects of parasite and natural cause of death. For example, in marine mammals opportunistically sampled from carcasses washed up on shore, it would be beneficial to include cause of death in the model to account for the variance in condition for the uninfected 'control' hosts between 'trauma', 'disease', and 'age'. Those that died from boat strike or other traumatic events, but in otherwise good condition, would constitute a better uninfected control group than those that died of old age or starvation and may obfuscate more subtle effects of infection.

Relying upon a single energetic proxy may lead to type-II errors, which can be avoided by combining different proxies. For example, when infection period is unknown, using a mix of proxies with some that respond quickly and others that take longer to respond to energetic challenge (for example cortisol stress response and a nutritional status proxy such as muscle depth) will provide a more complete and less-biased picture of the effects of infection. If using a proxy like fat (nutritional status), use multiple body locations such as subcutaneous and organ fat, as different host species may deplete their fat stores at different points during the infection period. Explicitly modelling the effect of infection on each would document infection effects better and help avoid type-II errors if a species, for example, depletes their organ fat first when energetically challenged. Proxies should also be chosen according to species-specific host life history. For example, for a host that does not tend to store large quantities of fat, even when healthy, and instead pours all excess energy into reproduction or metabolism, condition would be best measured by reproductive output, core systemic functioning, or 'optional' activities such as time spent patrolling or exploration.

Overall, it is difficult to quantify unequivocally the effects of sub-lethal infection on host fitness in any single study. Due to methodological inconsistencies and other constraints/limitations, data is often limited, and our conclusions biased by incomplete information. Going forward, studies aiming to assess parasite influence on mammal hosts should aim to use multiple condition proxies, clear methodology, and account for the potential limitations of host and parasite sampling.

2.3.7 Helminths matter

Our meta-analysis shows that contrary to some assumptions, mammals are significantly affected by sub-lethal infections of helminth parasites. Generally, infection lowers host energetic condition, which may be directly related to fitness. Methodological factors strongly influence our ability to detect and quantify infection effects and the significance of helminth parasites on host health. Cross-sectional studies relying on opportunistic sampling may have contributed to a perception that these parasites do not influence host health or fitness. However, our results show that this is not the case. Similarly, differences such as host species, age, sex, and the condition proxy assessed also influence the significance of the negative effects of helminth infection. The strength of effect is also likely linked to duration of infection, as there were no strong differences between intensity and presence/absence. This is consistent with Sanchez *et al*. (2018), who noted a similar lack of differentiation between presence/absence and intensity data. Energetic proxies more likely to change quickly, or those that explicitly included time (such as growth rate), showed slightly stronger negative effects of infection on host energetics.

Historical assumptions that helminths do not significantly influence mammal health, unless they occur in overwhelming numbers (Appendix A.1), may derive from both sampling limitations and common emphasis on disease in the literature (Fig. S2.9). However, we should not draw conclusions on complex host–parasite interactions based solely on pathogenicity or intensity of infection (Samuel *et al*., 2001). Indicators of wildlife health need to go beyond death and disease and consider host individual and population fitness. Similar to non-consumptive predator effects and the ecology of fear (Clinchy *et al*., 2013), or the stress of anthropogenic disruptors on energy budgeting (Bennett *et al*., 2009), sub-lethal helminth infections should be considered influencers of host fitness. Helminths can disrupt digestive functions, suppress the immune system, activate a stress response, and alter other physiochemical processes (Irvine, 2006; Beldomenico *et al*., 2008*a*; Parker *et al*., 2009; Tompkins *et al*., 2011; Stephen, 2014; Shanebeck and Lagrue, 2020). Concomitant infections, lower competitiveness, and stress on already resource-limited systems may also be linked to sub-lethal helminth effects on mammal health (Samuel *et al*., 2001; Stevenson and Woods, 2006; Beldomenico *et al*., 2008*a*). As such, they clearly have the potential to be significant influencers of host fitness and ecological functioning. While the significance of these effects may depend on specific systems, host and parasite species, and situations, helminths should be considered guilty until proven innocent.

2.4 Conclusions

(1) Mammal energetic condition is significantly negatively affected by sub-lethal helminth parasites, across all major groups (including ungulates, carnivores, primates, and rodents).

(2) Methodological limitations and disparities in wildlife research can reduce the resolution of these effects and may have driven previous assumptions of insignificance. Cross-sectional observational studies produced weaker effect sizes compared to experimental or semi-controlled studies. Sampling protocols also explained inter-study variance in wildlife research; opportunistic sampling of carcasses led to reduced effect sizes and increased variance compared to studies using active sampling protocols of live animals.

(3) There was no difference in parasite effects on host condition between infections measured by intensity or presence/absence, suggesting that duration of infection may be more important when analyzing sub-lethal infections. This contradicts assumptions that only high-intensity infections are relevant, highlighting an important limitation of observational studies where infection period is unknown.

(4) We hope this review and meta-analysis will instruct ongoing research and provide a comprehensive context for the inclusion of sub-lethal parasites into ecological and management models. We strongly recommend that sub-lethal parasites be considered a significant potential threat to wildlife populations and ecosystem structure and functioning.

Chapter 3: Parasite populations and pathobiology of larval trematode infections in aquatic mammals of Western Canada.

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3.1 Introduction

3.1.1 Natural history of river otter and mink

River otter (*Lontra canadensis*) and North American mink (*Neogale vison*) are semi-aquatic mesocarnivores that occur across a broad swath of North America (Larivière and Walton 1998, Larivière 1999). Once reduced by the fur trade, river otters have returned to a large portion of their historical range, though are still absent from the southern parts of the Canadian prairie provinces, as well as parts of the central and southwest United States (Larivière and Walton 1998, Roberts et al. 2020). Multiple successful reintroduction efforts have taken place across the United States. However, populations are not regularly monitored and the status of many *L. canadensis* populations in North America is uncertain (Ellington et al. 2018). Though not listed as a threatened species in Canada, they are internationally listed in Appendix II of the Convention on International Trade of Endangered Species (CITES), and as furbearing species, they are potentially still at risk if trapping and other anthropogenic stressors are not regulated (Engelstoft and Mogensen 2005). In contrast, *Neogale vison* occupy most of their historic range, and are not a species of concern in North America. They are also invasive around the world (Larivière 1999).

Important constituents of both riparian and coastal ecosystems, river otters and mink are mesocarnivores with top-down influence on fish and invertebrate species (Roemer et al. 2009). River otters feed on a broad range of riparian organisms including fish, frogs, turtles, waterfowl crustaceans, and other invertebrates, though fish make up most of their diet (Searing 1979, Larivière and Walton 1998). Similarly, mink feed on fish, frogs, waterfowl, and crustaceans, but may add muskrats and small terrestrial mammals to their diets depending on the season and environment (Searing 1979, Larivière 1999). Opportunistic predators, both of their diets are broad and tend to be determined by habitat, availability of food, and vulnerability of prey and change by region and season (Searing 1979). For example, in lake-dominated regions, both otter and mink increase the frequency of waterfowl and their eggs in diets likely due to easy availability of molting and nesting birds (Gilbert and Nancekivell 1982). In marine habitats, both mink and otters will feed on fish and intertidal invertebrates like crab, though distinct niche separation has been reported. Otters favor habitats with high exposure and stronger wave action while mink prefer low or moderate exposure (Ben-David et al. 1996). Coastal populations may feed almost entirely in marine habitats and are importers of marine nutrients, which has been associated with increases in coastal habitat heterogeneity (Roemer et al. 2009). In riparian habitats, otters and mink have greater habitat overlap but show resource partitioning when co-occurring in high densities, mink shifting their diet to include more terrestrial prey such as lagomorphs and small rodents (Larivière 1999, Bonesi et al. 2004).

Another important furbearer of North America is the muskrat (*Ondatra zibethicus*). Primary as well as secondary consumers in freshwater ecosystems, significant harvest declines have been seen in recent years though population data is limited, and the cause is unknown. Infectious and non-infectious pathogens have been suggested as potential causes (Ahlers and Heske 2017, Ganoe et al. 2020). As both primary and secondary consumers, muskrats are exposed to some similar parasites as river otter and mink but will also be exposed to species unique to them via grazing of aquatic grasses (Ganoe et al. 2020). Historically, muskrats have been used to test many ecological hypotheses related to trophic-level interactions and population dynamics because of their unique position in riparian ecosystems (Ahlers and Heske 2017). They were included in this study to compare their parasites against those of otter and mink to infer pathways of trophic transmission and compare their parasite populations against those reported in the literature.

3.1.2 Parasites and pathobiology

Despite the key role of otters in the functioning of aquatic ecosystems, their parasites are seldom investigated in North America. Reports are limited to the eastern/southeastern United States during the 20th century (Shoop and Corkum 1981, Flemming et al. 1997, Hoberg et al. 1997, Kollars 1997, Kimber and Kollias 2000). Parasites of mink have been more frequently recorded, perhaps due to their greater numbers, frequency of trapping, and commercial farming for the fur trade. However, studies include farmed and invasive animals and may not represent wild parasite populations. Recent studies have focused on microparasites that cause diseases of concern such as *Cryptosporidium* spp., *Giardia* spp. (Gaydos et al. 2007), *Leptospira* spp., parvovirus, and *Toxoplasma gondii* (Sanders et al. 2020, Cotey et al. 2022). Current monitoring is also focused on zoonotic diseases such as COVID-19 and avian flu.

To summarize past knowledge on the populations and prevalence of parasitic helminths in river otter and mink, we conducted a systemic search for studies that surveyed parasite communities of otter and mink in North America, including backwards search via citations, producing 27 peer-reviewed reports. We present here the most frequently reported species and some other species of note (Table 3.1 and 3.2). For muskrats, we used published data from a comprehensive review of their parasites by Ganoe et al. (2020) (Table 3.3). Due to the large number of reports in muskrats, we shortened the list by combining members of the same genus and removing any species with two or less records. Reports that looked at only one animal were also omitted. Helminth parasites included echinostomes (Trematoda:Digenea), diplostomids (Trematoda:Digenea), nematodes, cestodes, and acanthocephalans. Species causing serious pathology are usually of special interest to researchers. These include the guinea worm *Dracunulus* spp. (Crichton and Beverley-Burton 1973, Elsasser et al. 2009, Tumlison and Surf 2018), nasal worm *Skrjabingylus* spp. (Sealander 1943, Dorney and Lauerman 1969, Scherr and Bowman 2009), heart worm *Dirofilaria immitis* (Snyder et al. 1959, Zabiega 1996), and kidney worm *Dioctophyme renale* (Sealander 1943, Miller and Harkema 1964, Hoberg et al. 1997).

Table 3.1 Review of parasite prevalence in *L. canadensis* in North America. The table presents the species or genus within each class of parasites, state or province, (P_w) weighted average of the prevalence of infection (%) across all studies, and (SD_w) the weighted standard deviation. Note that when prevalence values came from a single study, no SD_w can be calculated.

SPECIES	LOCATION	P_w	SD_w	REFERENCES
Trematoda				
Baschkirovitrema canadense	AL, AR, NC, TN	47.4%	14.3	(Miller and Harkema 1964, Forrester 1992, Flemming et al. 1997, Kollars 1997, McAllister 2016)
Isthmiophora inermis	OR/WA	13%	-	(Hoberg et al. 1997)
***Clinostomum* spp.**	AR	3%	-	(McAllister 2016)
Enhydrodiplostomum alaroides	NC, AL, FL	12.7%	18.0	(Miller and Harkema 1964, Forrester 1992, Flemming et al. 1997)
Cestoda				
Schistocephalus solidus	OR/WA, NL	6.2%	5.4	(Smith and Threlfall 1973, Hoberg et al. 1997)
Nematoda				
Strongylids	OR/WA, TN, AL, FL	32.8%	27.4	(Forrester 1992, Flemming et al. 1997, Hoberg et al. 1997, Kollars 1997)

Capillarids	NC, FL	52.9%	16.1	(Miller and Harkema 1964, Forrester 1992)
Anisaskids	OR/WA	24.1%	10.9	(Hoberg et al. 1997)
***Dracunculus* spp.**	AR, ON	51.6%	41.0	(Crichton and Beverley-Burton 1973, Elsasser et al. 2009, Tumlison and Surf 2018)
Skrjabingylus lutrae	AR, ON	31.7%	17.7	(Scherr and Bowman 2009, Tumlison and Tumlison 2019)
Acanthocephala				
***Acanthocephalus* spp.**	AL, TN	6.8%	1.9	(Flemming et al. 1997, Kollars 1997)
***Corynosoma* spp.**	OR	4%	-	(Hoberg et al. 1997)

Table 3.2 Review of parasite prevalence in *N. vison* in North America. The table presents the species or genus within each class of parasites, state or province, (P_w) weighted average of the prevalence of infection (%) across all studies, and (SD_w) the weighted standard deviation. Note that when prevalence values came from a single study, no SD_w can be calculated.

SPECIES	LOCATION	P_w	SD_W	REFERENCES
Trematoda				
Baschkirovitrema canadense	NC, FL	8.3%	6.5	(Miller and Harkema 1964, Foster et al. 2007)
***Isthmiophora* spp.**	NC, WI, MN	13.1%	10.8	(Erickson 1946, Miller and Harkema 1964, Dorney and Lauerman 1969)
Alaria mustelae	NC, FL, MN, WI	28.1%	29.7	(McAllister 2016)
Enhydrodiplostomum alaroides	NC	5%	-	(Miller and Harkema 1964)
***Paragonimus* spp.**	MI, IL, MN	8.1%	4.5	(Sealander 1943, Erickson 1946, Zabiega 1996)
Cestoda				
***Versteria* spp.**	MT, NL, NC	8.3%	6.8	(Miller and Harkema 1964, Jennings et al. 1982)
Nematoda				
Capillarids	NC, FL, MN, ON, WI, NL, IL	19.5%	24.5	(Erickson 1946, Miller and Harkema 1964, Dorney and Lauerman 1969, Butterworth and Beverley-Burton 1981, Jennings et al. 1982, Zabiega 1996, Foster et al. 2007)
Strongylids	FL, MT	32.4%	19.9	(Barber 1973, Foster et al. 2007)
Filaroides martis	NC, MN, WI, MI, IL	41.9%	14.6	(Sealander 1943, Erickson 1946, Anderson 1962, Miller and Harkema 1964, Dorney and Lauerman 1969, Zabiega 1996)
***Molineus* spp.**	NC, FL, MN, WI, IL	13.8%	15.2	(Erickson 1946, Miller and Harkema 1964, Dorney and Lauerman 1969, Zabiega 1996, Foster et al. 2007)

***Dracunculus* spp.**	ON, AR	11.9%	13.8	(Elsasser et al. 2009, Schulte-Hostedde and Elsasser 2011, Tumlison and Surf 2018)
Dioctophyme renale	NC, MI, ON, QC, NS	11.3%	12.8	(Sealander 1943, Miller and Harkema 1964, Klenavic et al. 2008)
Skrjabingulus nasicola	ON, MI, WI	92%	3.6	(Sealander 1943, Dorney and Lauerman 1969, Santi et al. 2006)
Acanthocephala				
Pseduocorynosoma constrictum	WI	6%	-	(Dorney and Lauerman 1968)
Centrorhynchus conspectus	NC	14%	-	

Table 3.3 Review of parasite prevalence in *O. zibethicus* in North America. The table presents the species or genus within each class of parasites, state or province, (P_w) weighted average of the prevalence of infection (%) across all studies, and (SD_w) the weighted standard deviation, and n number of studies that reported that parasite. All data was collected from Ganoe et al. (2020) who collated data from reports from the last 100 years.

SPECIES	LOCATION	P_w	SD_W	N
Trematoda				
Alaria mustelae	AK, MB, ON	19.7%	15.3	3
***Echinoparyphium* spp.**	BC, IL, KS, ME, MI, N, NE, NL, OH, ON	14.9%	11.4	11
Echinostoma revolutum	BC, IL, KS, ME, MN, MD, MI, NB, NL, NY, OH, ON, OR, PA, UT	65.6%	40.8	24
***Echinostomum* spp.**	AR, CO, NE, ON, VA	7.2%	16.0	5
Fibricola cratera	IL, OH, OR, VA, UT	4.4%	4.6	6
***Notocotylus* spp.**	AR, BC, IL, NE, NB, NY, ME, MB, MI, OH, ON, OR, UT	43.7%	27.5	14
Nudacotyle novicia	IL, ME, MD, MI, NB, OH, TX	16.5%	12.1	7
Plagiorchis proximus	AR, BC, CO, IL, ME, NB, NL, NY, OH, ON, PA, UT	55.3%	80.8	15
Quinqueserialis quinqueserialis	AR, BC, CO, IL, ME, MB, MD, MI, NB, NL, NY, OH, ON, OR, PA, UT	99.5%	91.2	24
Wardius zibethicus	IL, KS, ME, MB, MD, MI, NB, NY, OH, PA, VA	26.6%	9.2	14
Cestoda				
Hydatigera taeniaeformis	BC, IL, LA, MB, MD, MI, NY, OH, ON, OR, PA, PE, TX, VA	28.8%	26.3	19
***Rodentolepis* spp.**	AR, BC, CO, IL, KS, LA, ME, MB, MD, MI, NB, NL, NY, OH, ON, OR, UT	50.8%	52.5	26
***Versteria* spp.**	AR, IL, NB, UT	2.1%	0.6	4
Nematoda				

Ascaris lumbricoides	IL, PA	3.4%	1.9	3
Capillarids	BC, CT, ME, MB, MI, NL, NY, OH, PA, UT	57.4%	83.7	12
***Trichostrongylus* spp.**	NL, OH, OR	14.6%	5.7	3
Trichuris opaca	AR, BC, CO, IL, MD, MI, NY, OH, ON, OR, PA, UT	12.2%	13.6	14
Acanthocephala				
Polymorphus paradoxus	AK, BC, MB	2.9%	1.4	4

3.1.2.1 Nematodes and Acanthocephalans

A common parasite of mink, *Filaroides martis* has been reported across Canada and the United States (Table 3.2). Found in the lobes, parenchyma, or bronchioles of the lungs, *F. martis* larvae migrate through the stomach mucosa into the abdomen, using the adventitia (outermost layer) of various blood vessels to guide themselves to the lungs, taking up to 41 days. They can be found in the adipose or connective tissue of various organs during their journey and may sometimes migrate to various aberrant tissues such as the abdominal fat, kidneys, or aorta (Stockdale and Anderson 1970). Upon arrival in the lungs, males and females coil tightly together forming nodules (Stockdale and Anderson 1970, Ko and Anderson 1972). Reports indicate that *F. martis* causes little pathological effect once encysted in the lungs beyond mechanistic collapse of adjacent alveoli, localized fibroplasia (development of fibrous tissue often due to mechanical damage), and infiltration by white blood cells in the nodule, which sometimes lead to hemorrhage. Migration of the larvae through tissue is associated with lesions, inflammation, and fibroplasia (Stockdale 1970, Stockdale and Anderson 1970).

Prevalence of *F. martis* can be high. One study in ON, Canada reported 45% of mink (n=976) infected (Anderson 1962). Infection is passed from egg to a freshwater gastropod intermediate host, then a rodent transport host (Anderson 1962). *Filaroides martis* can infect other mustelids such as marten (Seville and Addison 1995), but are not reported in otter, likely because they infrequently include terrestrial rodents in their diet. Anderson (1963) identified *Filaroides* spp. in a single male river otter from ON, which the author asserted were distinct from *F. martis*, naming them *F. canadensis*. No further reports have seen this parasite in otters or verified this identification.

Capillarids (*Capillaria* spp. and *Pseudocapillaria* spp.) are ubiquitous nematodes of mammals that have been reported in both river otter and mink (Table 3.1, 3.2). Various species may infect the urinary tract, lungs, liver, and intestine, but have not generally been associated with clinical disease (Kimber and Kollias 2000). The exception to this is *Capillaria hepatica*, a zoonotic parasite of concern which is generally rare in carnivores but can lead to hepatitis and death in sea otters (*Enhydra lutris*) (Miller et al. 2020). Muskrats have also been reported to be host to a variety of capillarids. Up to 19 species of nematode have been reported, though the most prevalent are *Trichuris opaca* (Trichuridae) and *Calodium hepatica* (syn. *Capillaria hepatica*; Capillariidae). Prevalence of *Capillaria* spp. has been reported in some studies as higher than 50% (Ganoe et al. 2020).

Reports have also identified acanthocephalans in river otter and mink, though none document potential pathology. Many species are assumed to be incidental infections and therefore unimportant (Kimber and Kollias 2000). However, acanthocephalan-induced peritonitis and death is known to occur in sea otters infected with *Profilicollis* spp. and in farmed mink infected by *Corynosoma* spp. from marine fish (Nuorteva 1966, Mayer et al. 2003). *Corynosoma* spp. also cause localized tissue damage in seals, their normal definitive host, due to the insertion of their proboscis. *Corynosoma* infection is associated with granulomatous and eosinophilic enteritis and thickening of the intestinal wall which may influence nutrient absorption (Lakemeyer et al. 2020). Reports of acanthocephalans in muskrat are limited and often record low prevalence, mainly of *Polymorphus* spp. (Ganoe et al. 2020). *Polymorphus proximus* (Polymorphidae) was identified as a new species from muskrats sampled in Alberta (Connell and Corner 1957).

3.1.2.2 Cestodes and Trematodes

Other helminth parasites have received limited attention beyond reporting their presence. Cestodes are generally considered unimportant due to the lack of obvious clinical disease, and unlikely to affect their host unless they are immunocompromised (Kimber and Kollias 2000). Trematodes similarly have not been associated with clinical disease in river otters and mink. Exceptions include *Paragonimus* spp. and mesocercariae of *Alaria canis* which may be associated with respiratory illness (Sealander 1943, Kimber and Kollias 2000). Only one report has identified mesocercariae of *Alaria canis* in the abdominal fat of a single otter from Ontario. In other species, mesocercariae may migrate to the lungs to develop into metacercariae to await transport to another definitive host or migrate up the trachea to be swallowed and develop into adults (Pearson 1956). Adults of *Alaria mustelae* (Diplostomidae) have frequently been reported in mink (Table 3.2), though no reports of mesocercarial infection could be found. No reports have been published documenting the helminth parasites of river otter and mink in western Canada, and there is only one report from the western United states (Hoberg et al. 1997). Muskrats have also been reported to be infected by *A. mustelae* and may play host to a variety of other digeneans. The most reported include *Echinostoma revolutum* (Echinostomatidae), *Quinqueserialis quinqueserialis* (Notocotylidae), *Notocotyle urbanensis* (Notocotylidae), *Plagiorchis proximus* (Plagiorchidae), and *Wardius zibethicus* (Paramphistomoidae). *Quinqueserialis quinqueserialis*, one of the largest digeneans infecting muskrat, is reported to occur at the highest prevalence and intensities (over 4,000 worms) (Ganoe et al. 2020).

A limited number of reports have also identified *Versteria* spp. (syn. *Versteria mustelae, T. mustelae, T. tenuicollis*) (Taeniidae) in North American mink (Skinker 1935, Miller and Harkema 1964, Jennings et al. 1982). Formerly in the Genus *Taenia*, and formerly the sole confirmed member of *Versteria*, *V. mustelae* was historically identified based on its small hooks and mustelid hosts (Freeman 1956, Nakao et al. 2013), though measurements provided by the literature vary widely (Table S3.2). Recent molecular analysis identified new genotypes in North America from larval cestodes infecting humans and an orangutan in a zoo (Lee et al. 2016, Deplazes et al. 2019, Lehman et al. 2019), and a new species described in South America, *Versteria cuja* (Bagnato et al. 2022). According to molecular data, at least two, if not more, unidentified species of *Versteria* exist in North America (Lee et al. 2016, Niedringhaus et al. 2022). Because of this, and due to the wide variation in reported morphological characteristics between North American and European samples, previous reports of *V. mustelae* in North America may have been *Versteria* spp., and descriptions should be interpreted carefully. Adults of an unidentified species were recently reported from a mink in Oregon and ermine from Colorado, and another unique lineage in an Ermine from Wisconsin, though the samples were without scolices and could not be fully described (Lee et al. 2016). Molecular analysis confirmed them to be the same lineage as those identified from human cystercercosis in North America (Lee et al. 2016).

Muskrat are one of the confirmed intermediate hosts for undescribed lineages of *Versteria* spp. in North America, along with other species of rodents (Freeman 1956, Niedringhaus et al. 2022). Infections have even been reported as so intense in the liver and lungs as to be the cause of death in some muskrat (Niedringhaus et al. 2022). Muskrats also act as both intermediate and definitive host for a variety of other cestodes. Most commonly as definitive host for species of Hymenolepididae, and as intermediate host for *Hydatigera* spp. (Taeniidae).

3.1.2.3 Parasites of zoonotic concern: *Toxoplasma gondii*

While, historically, domestic animals have been the focus of research into the sources of zoonotic parasites, in the last couple of decades, it has become increasingly clear that wildlife are a major source of emergent human diseases (Polley 2005, Jenkins et al. 2013). However, there is a significant gap in research concerning the prevalence and distribution of zoonotic parasites, especially in North America, and a lack of knowledge of how widespread various zoonoses are in humans, especially in rural populations (Jenkins et al. 2013). Perhaps due to the disconnect between human populations and wildlife in rich urban centers, there is a general belief in ‘developed’ countries that parasites are less of a concern, even though urban wildlife and pets are a large transmission risk for a variety of pathogens (Mackenstedt et al. 2015, Luong et al. 2018). Yet, rural populations, especially First Nation communities and others that rely on wild sources of meat or frequently handle carcasses for traditional uses, are exponentially more exposed to zoonotic parasites in North America and at high risk for zoonosis (Jenkins et al. 2013). Zoonotic infections may also have more serious implications for rural and First Nation communities due to a historical lack of access to sufficient medical care and resulting higher rates of comorbid conditions which may increase the severity of otherwise generally subclinical parasitic infections (Elmore et al. 2012, Jenkins et al. 2013). Infections are also less likely to be reported in these communities due to limited access to testing and the non-specificity of symptoms in mild to moderately pathogenic infections (Sampasa-Kanyinga et al. 2012).

One widespread pathogen of concern for wildlife, especially aquatic mammals, as well as for humans, especially First Nation communities, which can have three times the infection rate of the broader population, is *Toxoplasma gondii* (Jenkins et al. 2013, Ahlers et al. 2015). *Toxoplasma gondii* uses felids as their definitive host but can use almost any endothermic vertebrate as intermediate or paratenic host (Dubey et al. 2021). Wild or domestic cats shed oocysts in their feces, which become sporulated cysts. Infection of vertebrate hosts occurs via ingestion (Jones and Dubey 2010). Inside the intermediate host, *T. gondii* become tachyzoites, the motile stage, which travel to various tissues such as muscle or brain. This stage can also asexually reproduce in the intermediate host. In host tissues, tachyzoites encyst to become bradyzoites. These are infective to both secondary intermediate and felid definitive hosts. Vertebrate intermediate hosts can be infected through ingestion of sporulated oocysts from water or by accidental ingestion from contact with feces, through ingestion of other bird or mammal with encysted bradyzoites in their tissue, or via the migration of tachyzoites across the placental barrier to a fetus (Jones and Dubey 2010, Ahlers et al. 2015).

Toxoplasma gondii infection hotspots have often been associated with anthropogenic centers and domestic cats (Barros et al. 2018), though there is debate about the strength of the effect domestic felid species have on the prevalence of *T. gondii* in wild populations (Conrad et al. 2005, Elmore et al. 2012). Various surveys have identified *T. gondii* infections in carnivores across North America, including pinnipeds, ursids, canids, and mustelids (Elmore et al. 2012, Sharma et al. 2019). Infections have been reported in both river otter and mink, causing blindness, encephalitis, and even death. Infections can also lead to neonatal mortality (Dubey et al. 2021). Seroprevalence in *L. canadensis* can be high, including 28% in MI (Cotey et al. 2022) and 24% in NC (Sanders et al. 2020). Mink are particularly susceptible to fatal toxoplasmosis, which is of concern due to their widespread farming and economic importance (Dubey et al. 2021). Abortion rates can be as high as 26% in infected females (Frank 2001). Both mink and muskrat have been reported to have high seroprevalence of *T. gondii* in IL in high agriculture areas, much higher than their terrestrial counterparts (Ahlers et al. 2015). However, in contrast to the effects in other mammals, no clinical disease has been reported in muskrat (Ganoe et al. 2020). This may be because rodents are the normal intermediate host for *T. gondii*, which therefore may be less likely to cause observable pathology beyond its documented effects on rodent behavior in order to increase transmission (Vyas 2015). High seroprevalence has also been seen in sea otters (*Enhydra lutris*) and other marine mammals in coastal California (Conrad et al. 2005), which have highlighted the threat of water-borne transmission of *T. gondii* to wildlife and potentially humans (Jones and Dubey 2010).

3.1.3 Scope of study

The aim of this study is to fill a gap in our knowledge of the diversity, distribution, and prevalence of helminth parasites of *L. canadensis*, *N. vison*, and *O. zibethicus* in Western Canada, with special emphasis on parasites of zoonotic concern. Necropsies of river otter, mink, and muskrat are used to document parasite infection host tissues, including description of any associated pathology. Parasites collected are identified using both morphological and molecular techniques, including phylogenetic analysis and description of a previously unnamed species. Results show a diverse community of helminths dominated by larval trematodes of *Alaria mustelae*, a species of zoonotic concern, frequent infection by the lung worm *Filaroides martis* in mink, and high prevalence of other species of zoonotic concern including *Versteria* spp. and *T. gondii*. This data is the first of its kind in Western Canada and has implications not only for wildlife populations and ecological functioning, but for human health as well.

3.2 Materials and Methods

3.2.1 Carcass Collection

Carcasses of river otter and mink were obtained from licensed fur trappers in Alberta and British Columbia (BC), Canada, in association with the Alberta Trappers Association and the BC Ministry of Forests during the 2020-21 and 2021-22 trapping seasons. In total, 155 river otters and 106 mink were included in this study. One river otter from Alberta was excluded due to lack of information about its capture location, and three river otters from BC were excluded due to an advanced state of decomposition. In addition, two Alberta trappers submitted 41 muskrat during the 2020-21 trapping season. By year and province, this included: 74 river otters and 22 mink from Alberta, and 26 river otters and 35 mink from BC in the 2020-21 trapping season. For the 2021-22 trapping season, we obtained 17 river otters and 15 mink from Alberta, 36 river otters and 34 mink from BC, from various regions in the provinces (Figure 3.1). The fur trappers who submitted carcasses provided information about the location and month of capture. Unfortunately, reporting was not always consistent for the Alberta trappers. If no exact date of capture was provided the middle point of the trapping period was used for all animals. For example, if the trapper trapped from December to February, all the animals they provided were recorded as trapped in January. If specific GPS coordinates were not provided, but a general area was, then GPS coordinates were selected from the general area indicated via Google Maps. If no general location was provided, then the location of capture was recorded as the geographic center of the trapline they were collected from.

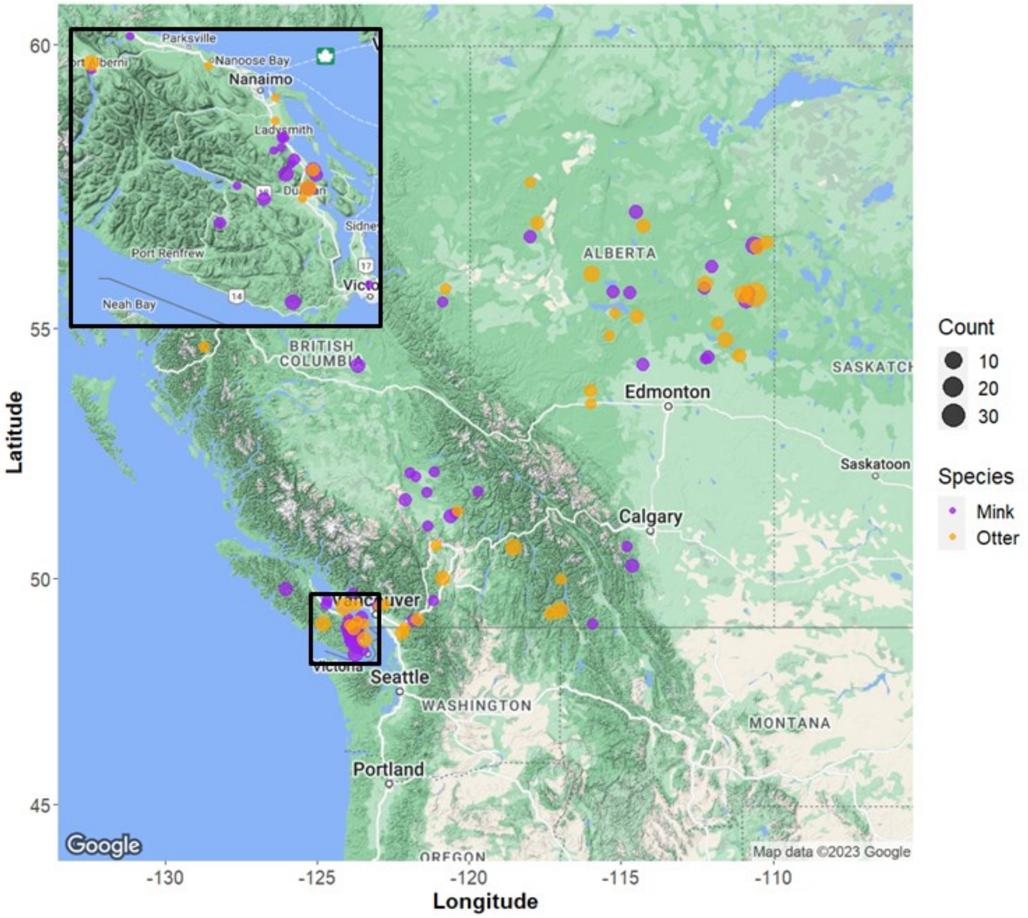

Figure 3.1 Distribution of sampled animals. Locations of trapped animals by GPS coordinate of exact location of capture, general area, or geographic center of trapline. Points are scaled by the number of animals sampled from that location. Map was created in R studio with the packages: *ggplot2*, *maps*, *mapdata*, and *ggmap*, points spread by jitter (w=3, h=3).

3.2.2 Carcass condition evaluation and tissue sample collection

Carcasses were frozen after skinning and kept at -20°C, except during shipping or transport, until necropsy. Gross necropsies were performed on all animals according to protocols outlined by the Coast Region Research Section (BC Ministry of Forests) and Environment and Climate Change Canada. Morphometric parameters (body length, tail length, chest girth, and weight) were taken, and age was estimated according to observed wear on the teeth, size of the animal, and development of the temporalis muscle. Sex was determined by visual confirmation. Actual age was later determined by Matson's Laboratory (Manhattan, MT, USA) by cementum annuli aging

(Stephenson 1977). General health was assessed by visual observation of both external and internal conditions; any abnormalities were recorded, including the specific tissues affected.

Liver, spleen and testes were also weighed for later analysis. Samples of heart, lung, liver, kidney, stomach, reproductive organs, baculum, muscle, hind legs, fur, brain, heart blood were collected, and nasal/rectal swabs were performed for COVID-19 testing. Various tissue samples were sent to the Beaty Museum, University of British Columbia or the specimen bank at the National Wildlife Research Center, Environment and Climate Change Canada for long-term storage and analysis.

3.2.3 Parasite identification

During necropsy, all animals were macroscopically examined for parasitic infection by dissection of their internal organs and visual assessment of various tissues. Organs were examined by cutting open in the case of trachea, bronchioles, heart, GI tract, kidney, and bladder, by transverse cuts at regular intervals in the case of the lungs and liver, or by careful observation and palpation of the tissue in the case of the pancreas, musculature, and omentum. Any parasites observed were removed by forceps, relaxed in tap water, and then transferred to 70% ethanol for later identification. In the case of animals with larval infections detectable due to extensive diffuse small white foci, tissue samples were preserved in 70% ethanol, and later digested with pepsin. The filtrate was examined for embedded parasites according to standardized protocols for parasite identification (Comission 2005). In six cases, representative samples of gross lesions from various tissues were collected for histological examination, fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned, mounted and analyzed by the BC Ministry of Agriculture, Food, and Fisheries' Animal Health Center (Abbotsford, Canada).

Collected parasites were identified morphologically and/or molecularly. For light microscopy, helminths were stained with acetic acid carmine, cleared in clove oil and mounted permanently in Canada balsam. Molecular identification was carried out using suggested primers for various subregions of both ribosomal and mitochondrial DNA, following the cycling instructions provided by the authors (Table 3.4). DNA was extracted from whole worms or from infected tissues using commercially available kits (DNEasy Blood and Tissue, QIAGEN). Amplification was conducted in an ABI 2720 Thermal Cycler in a total volume of 25μl using PCR Master Mix

(Invitrogen) or Taq PCR Kit (New England BioLabs Inc) according to the manufacturer's instructions. Sequencing was done via the Sanger method by the University of Alberta's Molecular Biology Service Unit (Edmonton, Canada). The produced sequences were trimmed using MEGA11 (Kumar et al. 2018) and compared against those available in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 3.4 Primers used for molecular identification of parasites. Organized by taxonomic group the primer was used to identify and the sub-region either ribosomal (18s, 28s) or mitochondrial (CO1, NAD1) the provided primer targeted.

Taxa	**Region**	**Primer (5'-3')**	**Reference**
Trematoda	CO1	F 5'-TGTAAAACGACGGCCAGTTTWCITTRGATCATAAG R 5'-CAGGAAACAGCTATGACTGAAAYAAYAIIGGATCICCACC	(Moszczynska et al. 2009)
Cestoda	NAD1	F 5'-AGATTCGTAAGGGGCCTAATA R 5'-ACCACTAACTAATTCACTTTC	(Trachsel et al. 2007)
	18s	F 5'-CAGCTATGGTTCCTTAGATCRTA R 5'-TATTTTTCGTCACTACCTCCCCGT	(Schols et al. 2019)
	CO1	F 5'-TGTAAAACGACGGCCAGTTTWCITTRGATCATAAG R 5'-CAGGAAACAGCTATGACTGAAAYAAYAIIGGATCICCACC	(Moszczynska et al. 2009)
Nematoda	28s	F 5'-AGCGGAGGAAAAGAAACTAA R 5'-TCGGAAGGAACCAGCTACTA	(Carreno and Nadler 2003)
Acanthocephala	CO1	F 5'-AGTTCTAATCATAA(R)GATAT(Y)GG R 5'-TAAACTTCAGGGTGACCAAAAAATCA	(Garcia-Varela et al. 2009)
	18s	F 5'-AGATTAAGCCATGCATGCGTAAG R 5'-TGATCCTTCTGCAGGTTCACCTAC	(Verweyen et al. 2011)

Parasites identified as *Isthmiophora inermis* and *Versteria* spp. were analyzed for phylogenetic relatedness to similar sequences available in GenBank. Samples were aligned using ClustalW as implemented in MEGA11 software against sequences from related parasites (Kumar et al 2018). The resulting alignments were used to create phylogenetic trees. Evolutionary divergences were estimated by calculating pairwise distance and bootstrapped at 1000 replicates.

3.2.4 Identification of *Toxoplasma gondii*

Brains from Albertan otters were also removed by opening the skull with a transverse cut through the frontal bone behind the orbital sockets with a hacksaw, then two lateral cuts from the zygomatic processes of the frontal bone to the foramen magnum. The now separated sections of frontal and parietal bone were carefully removed. The brain was finally extracted with dissecting scissors to cut away nerves and pull the dura mater from the rest of the skull and stored in sterile whirl-pak® bags (VWR, Mississauga, ON, Canada). Tools were washed and bleached between each skull dissection. Brains were frozen at -20 ºC and sent to the University of Saskatchewan's Zoonotic Parasite Research Unit (Saskatoon, Canada) where they were tested for infection by *Toxoplasma gondii* via magnetic capture qPCR using standardized protocols (Opsteegh et al. 2010). The lowest detectable tachyzoite limit has been previously described as 445 tachyzoites per 100g of tissue (Bachand et al. 2018). The number of tachyzoites in the otter brains were estimated by comparison against 5 standardized samples of 100g of beef tissue spiked with tachyzoites ranging in regular intervals from 530 to 5,300,000. These standards were fitted in a generalized linear model in R statistical software 4.2.2 (Team 2018), and the slope and y-intercept used to predict the Log_{10}(concentration) of tachyzoite-equivalents in the river otter samples (Bachand et al. 2019).

3.3 Results

Of the 302 animals investigated for parasites, 90% of river otters (n_{otter}=155), 88% of mink (n_{mink}=106), and 100% of muskrat ($n_{muskrat}$=41) were infected by helminth parasites. Average species richness (*R*; number of species per individual) was low for otter and mink ($R_{otterAB}$= 1.11±0.62; $R_{otterBC}$= 1.18±0.61; R_{minkAB}= 1.18±0.69; R_{minkBC}= 1.40±0.90) compared to muskrat ($R_{muskrat}$= 3.29±1.21). Helminths identified included at least 22 separate species: 8 species of trematodes: *Alaria mustelae* (Diplostomidae), *Isthmiophora inermis* (Echinostomatidae), *Plagiorchis proximus* (Plagiorchiidae), *Quinqueserialis quinqueserialis* (Notocotylidae), *Wardius zibethicus* (Paramphistomatidae), *Baschkirovitrema canadensis* (Echinostomatidae), *Echinostoma revolutum* (Echinostomatidae), and *Clinostomum poteae* (Clinostomidae), 6 species of nematodes: *Filaroides martis* (Filaroididae), *Contracaecum* spp. (Anisakidae), *Anisakis simplex* (Anisakidae), *Porrocaecum depressum* (Ascarididae), *Baylisascaris devosi* (Ascarididae), and *Molineus* spp. (Molineoidea). Multiple *Capillaria* and *Pseudocapillaria* (Capillariidae) nematodes were also seen, but often too damaged for species level identification. We were unable to successfully sequence capillarid nematode parasites, except for one specimen that blasted to *Pseudocapillaria* spp.. Capillarid nematodes were thus nested together as "capillarids." We also identified four species of cestodes: *Mesocestoides* spp. (Mesocestoididae), *Rodentolepis evaginata* (Hymenolepididae), *Hydatigera taeniaeformis* (Taeniidae), and *Versteria* spp. (Taeniidae), and four species of acanthocephalans: *Corynosoma strumosum* (Polymorphidae), *Polymorphus paradoxus* (Polymorphidae), *Centrorhynchus conspectus*

(Centrorhynchidae), and *Pseudocorynosoma anatarium* (Polymorphidae). Other common parasites of mink and otter such as *Strongyloides* spp.*,* *Paragonimus* spp., *Enhydridiplostomum alaroides, Dracunculus* spp., and *Dioctophyma renale* (Table 3.1, Table 3.2) were not found in our samples.

3.3.1 Parasites of the gastrointestinal tract and liver

3.3.1.1 Trematodes

The majority of parasites were recovered from the gastrointestinal tract (GI) of study animals. Adults of the trematode *A. mustelae*, were found in the intestine of all three species (n_{mink}=15 [14.0%]; n_{otter}=4 [2.6%]; $n_{muskrat}$= 12 [29.2%]). River otters were infected with *I. inermis* (n_{otter}=15 [9.7%]), found in the esophagus, stomach, and intestine, specimens of *B. canadensis*, a similar species, were also found in the stomach though in very low prevalence and only in Alberta (n_{otter}=2 [1.3%]). Unique from otters and mink, muskrats were infected with *P. proximus* ($n_{muskrat}$= 15 [36.6%]), *W. zibethicus* ($n_{muskrat}$= 3 [7.3%]), and *E. revolutum* ($n_{muskrat}$= 4 [9.8%]) in the intestine or stomach, and *Q. quinqueserialis* ($n_{muskrat}$= 32 [78.0%]) always found in the caecum.

3.3.1.2 Cestodes

Adult cestodes identified as *Versteria* spp. were found in all three species (n_{mink}= 8 mink [7.5%]; n_{otter}= 5 [3.2%]; $n_{muskrat}$= 2 [4.9%]), an undescribed North American species of the genus (Lee et al. 2016). Larval infections of *Versteria* spp. were also identified in the livers of mink and muskrat in Alberta (n_{mink}=4 [3.7%]; $n_{muskrat}$= 18 [43.9%]), at intensity ranging from 3 cysts to over 50 (Plate 3.1). The mink infected with over 50 cysts in the liver was also notable for concurrently being infected with 21 adults of the species. Muskrat livers were also infected with strobilocerci of *H. taeniaformis* ($n_{muskrat}$= 4 [9.6%]). Less common adult cestodes in the intestine included *Mesocestoides* spp. (n_{otter}= 2 [1.3%]) and *R. evaginata* ($n_{muskrat}$= 5 [12.2%]) in Alberta.

3.3.1.3 Nematodes

Species of nematodes included capillarids (n_{mink}=13 [12.2%]; n_{otter}= 10 [6.5%]; $n_{muskrat}$= 13 [31.7%]) found mostly in the intestine, though one case of liver infection was found in an otter in Alberta, possibly *C. hepatica*, two cases in the kidney of an otter and a mink, and two cases in the lungs of otters. Low incidences of infection by other nematodes from Anisaskidae and

Ascarididae were seen including: *A. simplex* from 1 otter and 1 mink in BC as well as 2 Alberta muskrat, *Contracaecum* spp. in 3 Alberta otters, *B. devosi* in 1 Alberta mink, *P. depressum* in 1 Alberta mink, and *Molineus* spp. in 1 BC mink.

3.3.1.4 Acanthocephalans

In British Columbia, coastal populations were infected with the marine parasite *C. strumosum* (n_{mink}= 4 [3.8%]; n_{otter}= 1 [1.1%]), *P. anatarium* (n_{otter}= 1 [1.1%]) on Vancouver Island and *C. conspectus* (n_{mink}= 7 [6.5%]) five of which came from Vancouver Island. Both *C. strumosum* and *C. conspectus* caused a single case of acanthocephalan induced peritonitis in juvenile mink from Vancouver Island, immature worms burrowed through the intestinal wall and were found in the abdominal cavity or captured by the omentum which was actively gathering parasites (Plate S3.1) (Shanebeck et al. 2022). Muskrats were also infected with *P. paradoxus* ($n_{muskrat}$= 5 [12.2%]), found from the stomach to the caecum, though when present in large numbers, found clustered just anterior to the caecum and large intestine.

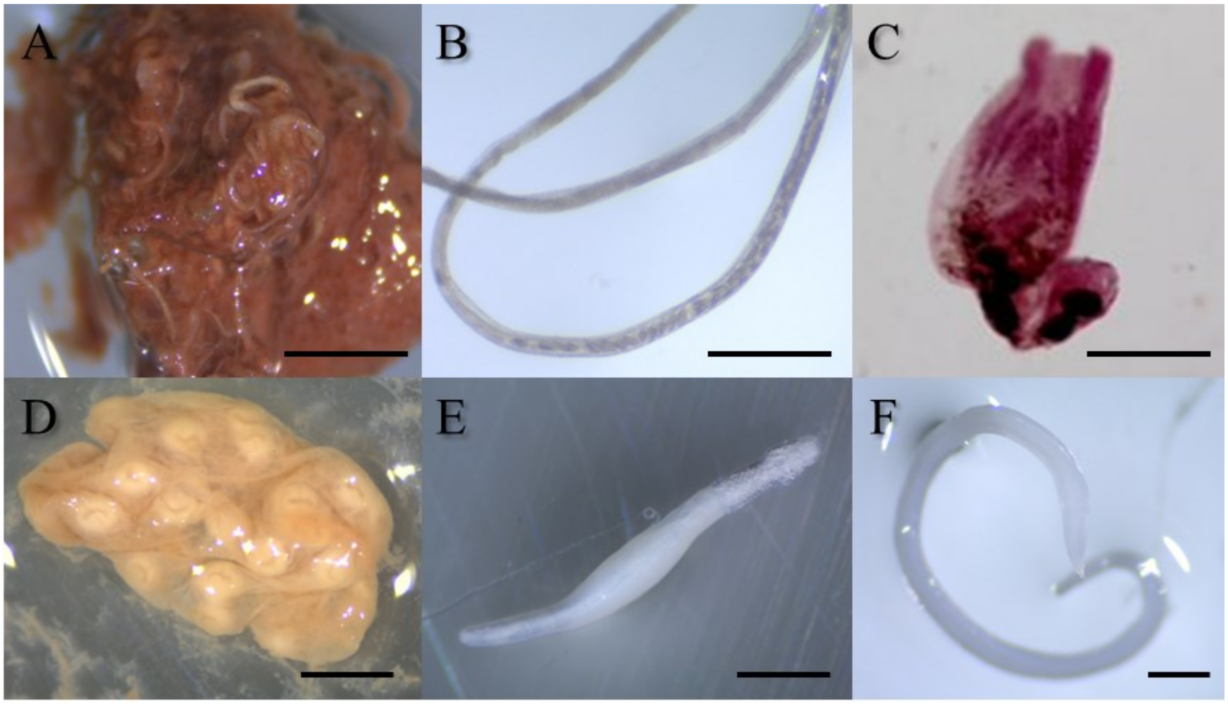

Plate 3.1 Examples of collected parasites. Specimens photographed via light microscopy A) lung cysts of tightly coiled *Filaroides martis*, scale bar=2mm, B) gravid adult of *Capillaria* spp. from the intestine, scale bar=2mm, C) gravid adult specimen of *Alaria mustelae*, stained with acetic acid carmine, cleared in clove oil and mounted permanently in Canada balsam, scale bar=125µm, D) liver cysts containing cystacanths of *Versteria* spp., scale bar=4mm, E) immature *Centrorhynchus conspectus* recovered from the abdominal cavity of a mink, scale bar=1mm, and F) *Contracaecum* spp., scale bar=1mm.

Table 3.5 Parasites of river otter and mink. Parasites identified from animals according to province. Details include prevalence (*Pr*) of infection (number of hosts infected) and range of intensity (*I*) (total number of parasites in host, or cysts for larval) by parasite species and taxonomic group, for total animals sampled and for just males or females (samples sizes provided). A checklist of tissues that species were found in is also included, S=stomach, I=intestine, L=liver, A=abdominal tissues (omentum, adipose, musculature), K=kidney, and R=respiratory.

	River Otter						**Mink**											
Alberta	**Total (n=92)**		**Males (n=47)**		**Females (n=45)**		**Total (n=36)**		**Males (n=25)**		**Females (n=11)**		**Tissues affected**					
	Pr	***I***	***Pr***	***I***	***Pr***	***I***	***Pr***	***I***	***Pr***	***I***	***Pr***	***I***	**S**	**I**	**L**	**A**	**K**	**R**
Trematoda																		
Alaria mustelae (adult)	1	83	-	-	1	83	6	33-349	2	87-349	4	33-126		X				
Alaria mustelae (larval)	81	-	41	-	40	-	28	-	21	-	7	-	X	X	X	X	X	X
Isthmiophora inermis	9	15-326	4	15-27	5	19-326	-	-	-	-	-	-	X	X				
Bashkirovitrema canadense	2	3-21	-	-	2	3-21	-	-	-	-	-	-	X					
Cestoda																		
Versteria rafei n. spp. (adult)	3		2	3-4	1	23	4	5-54	4	5-54	-	-		X				
Versteria rafei n. spp. (larval)	-	-	-	-	-	-	4	3-50+	4	3-50+	-	-			X			
Mesocestoides spp.	2	1-2	2	1-2	-	-	-	-	-	-	-	-		X				
Nematoda																		
Capillarid	5	-	3	-	2	-	1	-	1	-	-	-		X	X		X	X
Filaroides martis	1	3	1	3	-	-	11	1-6	16	1-6	-	-						X
Contracaecum spp.	3	3-12	2	8-12	1	3	-	-	-	-	-	-	X					
Porrocaecum depressum	-	-	-	-	-	-	1	4	1	4	-	-	X					
Baylisascaris devosi	-	-	-	-	-	-	1	1	1	4	-	-		X				
British Columbia	**Total (n=63)**		**Males (n=40)**		**Females (n=23)**		**Total (n=70)**		**Males (n=57)**		**Females (n=13)**		**S**	**I**	**L**	**A**	**K**	**R**
Trematoda																		
Alaria mustelae (adult)	3	31-74	2	44-74	1	31	9	19-171	6	19-171	3	14-43		X				
Alaria mustelae (larval)	55	-	35	-	20	-	41	-	34	-	7	-	X	X	X	X	X	X
Isthmiophora inermis	6	12-62	3	12-51	3	21-62	-	-	-	-	-	-	X	X				
Clinostomum poteae	1	3	-	-	1	3	-	-	-	-	-	-		X				
Cestoda																		
Versteria rafei n. spp. (adult)	2	3	2	3	-	-	4	7-23	4	7-23	-	-	X	X				
Nematoda																		
Filaroides martis	-	-	-	-	-	-	23	1-17	20	1-17	3	3-6				X	X	X
Capillarid	5	-	2	-	3	-	12	-	10	-	2	-		X	X		X	X
Anisakis simplex	1	1	1	1	-	-	1	1	1	1	-	-		X				
Molineus spp.	-	-	-	-	-	-	1	38	1	38	-	-		X				
Acanthocephala																		
Pseudocorynosoma anatarium	1	4	-	-	1	4	-	-	-	-	-	-		X				
Corynosoma Strumosum	1	4	1	4	-	-	4	1-34	3	1-3	1	34		X		X		
Centrorhynchus conspectus	7	1-61	6	1-41	1	61	-	-	-	-	-	-	X	X		X		

Table 3.6 Parasites of muskrat in Alberta. Details include the prevalence of infection (number of hosts infected) and range of intensity (*I*) (total number of parasites in host, or cysts for larval) by parasite species and taxonomic group for total animals sampled and for just males or females (samples sizes provided). A checklist of tissues that species were found in is also included, S=stomach, I=intestine, C=caecum, L=liver, and K=kidney

	Total (n=41)		**Male (n=24)**		**Female (n=17)**		**Tissues affected**				
	Pr	***I***	***Pr***	***I***	***Pr***	***I***	**S**	**I**	**C**	**L**	**K**
Trematoda											
Alaria mustelae (adult)	13	3-82	8	3-82	5	13-62		X			
Alaria mustelae (larval)	2	-	2	-	-	-	X	X			
Echinostoma revolutum	4	6-86	2	6-86	2	1-6	X	X			
Plagiorchis proximus	30	11-231	20	11-231	10	16-85	X	X	X		
Quinqueserialis quinqueserialis	32	8-187	18	8-187	14	11-121			X		
Wardius zibethicus	3	13-57	2	13-14	1	57		X			
Cestoda											
Versteria rafei n. spp. (adult)	3	1-4	1	4	2	1-2	X	X			
Versteria rafei n. spp. *(larval)*	18	1-9	10	1-8	8	1-9				X	
Hydatigera taeniaeformis	4	1	1	1	3	1				X	
Rodentolepis evaginata	5	1-4	3	1-4	2	3-4	X	X			
Nematoda											
Capillarid	16	-	11	-	5	-		X	X	X	X
Anisakis simplex	2	2-3	1	2	1	3	X				
Acanthocephala											
Polymorphus paradoxus	5	1-46	2	16-46	3	1-24	X	X	X		

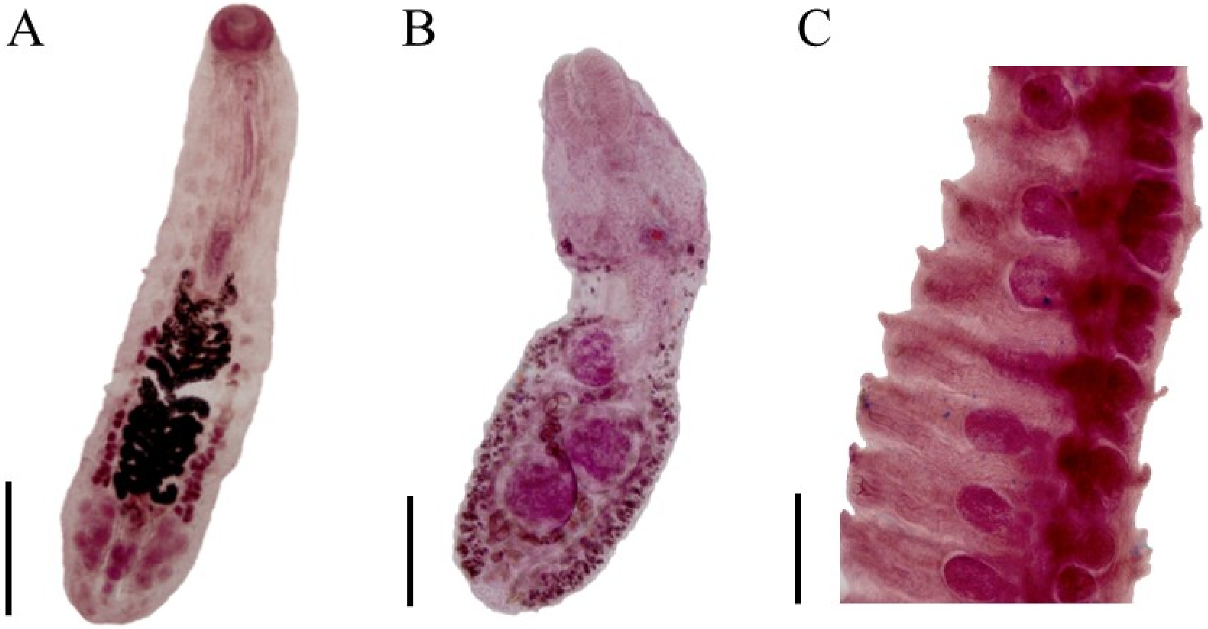

Plate 3.2 Parasites of muskrats. Common parasites of muskrats from Alberta, including A) *Quinqueserialis quinqueserialis* always found in the caecum, scale bar=1mm, B) *Plagiorchis proximus* scale bar=0.5mm, C) *Rodentolepis evaginata* scale bar=4mm.

3.3.2 Parasites of the respiratory tract

The most common infection in the lungs of mink was *F. martis* (n_{mink}= 27 [25.2%]), with a greater percentage infected in British Columbia than Alberta (n_{minkBC}=23 [32.9%]; n_{minkAB}= 4 [10.8%]). Infection was also seen in a single river otter from Alberta, trapped near Winefred Lake in the center east of the province. *Filaroides martis* formed large nodules in the lung tissue of the hosts and were surrounded by a tight fibrous capsule, ranging from 3-10mm in diameter, containing tight bundles of adult worms (Plate 3.1a). Immature worms were also seen in connective tissues around the trachea or bronchi (Plate 3.2). In three cases, immature worms were found in the renal capsule, and in one case throughout the thoracic and abdominal cavity, including the thymus, bronchi, liver, pancreas, omentum, and internal back fat. Intensity of infection ranged from 1 to 17 cysts, with an average of 5±4 cysts. Besides *F. martis*, two otter lungs were infected with *Capillaria* spp., though the worms did not sequence and were too degraded for species level identification (possibly *C. aerophila*) (Kimber and Kollias 2000).

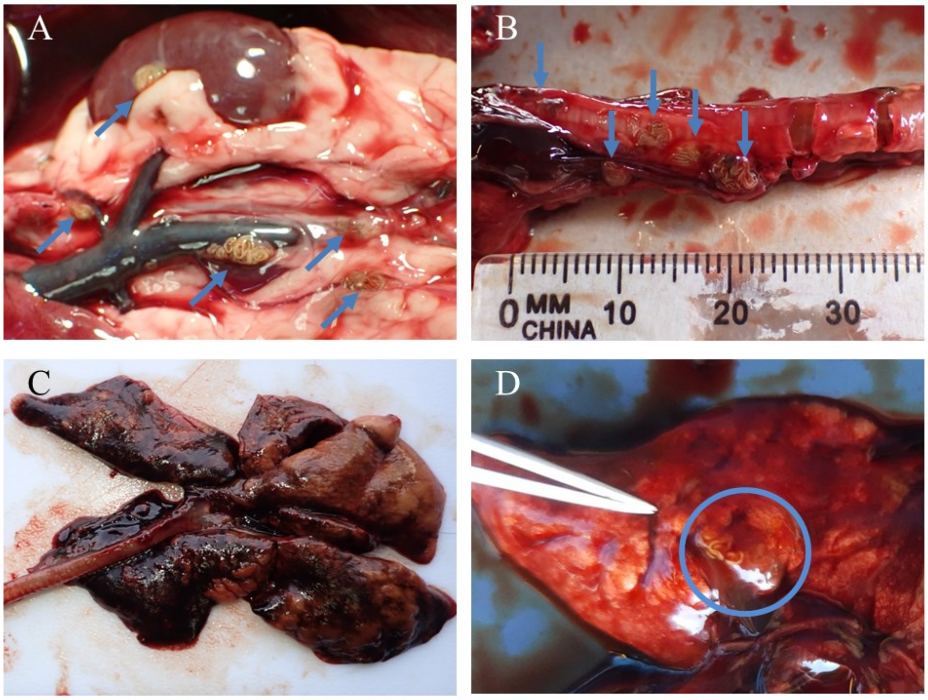

Plate 3.3 Infections of *Filaroides martis*. Pictures of mink infected in various tissues including A) immature worms in on the kidney, hepatic portal vein, and adipose tissue (arrows), B) immature worms in the connective tissue of the trachea (arrows), C) the lungs of a heavily infected individual, showing hemorrhaging, tan-yellow discoloration, and emphysema, and D) a cyst containing mature worms in a lobe of the lungs (circled).

3.3.3 Infection of multiple tissue types by mesocercariae of *Alaria mustelae*

During necropsy, numerous small white nodules were observed in various tissues in the abdominal cavity of many of the animals (n_{otter}= 106 [68.4%]; n_{mink}= 69 [64.5%]; $n_{muskrat}$= 2 [4.9%]). These foci were localized most often in the intestinal and gastric serosa, and pancreas, though at times also found in the liver, spleen, kidney, mesentery, bladder, retroperitoneum, and musculature of the abdominal wall. Artificial digestion via pepsin produced mesocercaria with morphologic features consistent with descriptions of *Alaria* (Bosma 1934, Johnson 1970), though not in large amounts as was expected considering the prevalence of white nodules throughout the viscera and mesentery. Digestion with pepsin also degraded the parasite tegument and made staining difficult, which other reports have noted and recommend mechanical digestion of fresh tissue to make use of *Alaria*'s mobility out of the tissue, and inability to swim against gravity (Riehn et al. 2010). Unfortunately, our samples were taken during necropsy and fixed in 70% ethanol for later analysis, and it was too late to identify motile mesocercariae. The collected tissues were digested, and DNA extracted for molecular analysis, and sequencing with primers specific to digenean parasites. Of 96 tissue samples extracted, 13 successfully sequenced (11 intestine, 1 kidney, 1 liver) and all matched with available GenBank sequences of *A. mustelae* with over 99% identity (Appendix B, Table S3.1).

The pattern of the lesions suggested migration of the parasites out of the intestine into the abdominal cavity and then to various tissues, at times spreading to tissues in direct communication or apposed to a loop of the intestine, implying non-vascular migration. This was consistent with the general gross pathology, which showed common signs of peritonitis in the moderate to severe infections, including thickening and hyperemia (increase in blood vessels) of the mesentery, enlarged mesenteric lymph nodes, and ecchymotic (paintbrush) serosal hemorrhaging of the intestine. Severe cases were also associated with melena (bleeding in the GI tract), multifocal punctate red-black lesions in affected organs, tan-yellow discoloration of the lungs, enlarged thymus, and in the case of infected kidneys, hematuria (bloody urine). In one case of a young juvenile otter, severe infection by mesocercaria was associated with heavy bleeding in the intestine and signs of generalized anemia (hemorrhagic anemia), including pallor of the mucus membranes of the mouth and internal organs.

Histopathology of a subset of infected river otter and mink showed subacute to chronic lymphocytic and eosinophilic inflammation, though post-mortem decomposition and possible freeze artefact limited our histopathological assessment of tissues. Inflammation was accompanied by nodular to diffuse fibrosis (the replacement of normal parenchymal tissue with fibrous connective tissue due to damage), predominantly seen in abdominal adipose tissue. For example, thin to moderately thick laminae of dense mature fibrous connective tissue were observed along the serosal surface and occasionally within interlobular (between sections or lobes of organs) planes of abdominal adipose tissue. These fibrous tissues occasionally intercalated with adjoining loops of bowel, which may also be indicative of the migration of parasites through the intestinal wall into the abdomen and adjoining tissues. Except for two cases, no trematodes were detected in the sectioned tissues. In one case, close evaluation of the observed foci of fibrosis in the adipose tissue showed a small fragment of presumptive parasite stroma. However, due to the chronicity of the lesions and lack of discernable pathogens, a specific etiology could not be proposed histologically.

In one severe case of a river otter from British Columbia, the infection presented with lymphoplasmacytic and eosinophilic enteritis and fibrosis of the abdominal fat and pancreas. Interstitial renal fibrosis and membranous glomerulopathy were also present and would have contributed to at least moderately impaired renal function. These symptoms are consistent with chronic nonspecific inflammation. There was reactive mesothelia with micropapillary proliferations in the splenic capsule, indicative of chronic, nonspecific localized peritonitis. Cholangiohepatitis (inflammation of the bile ducts and liver) may be secondary to disruption of the intestinal mucosa due to helminth infection and secondary bacterial invasion, which extended retrograde via the bile duct to the liver. Another severe case, a mink from British Columbia, also presented with adipose, renal (interstitial, perivascular), pancreatic (perivascular, interlobular), and gastric (foveolar) fibrosis, cholangiohepatitis and glomerulopathy. The spleen was nodular with multi-focal red-black lesions. Histology showed lymphoid depletion and hemosiderosis. Iron pigment accumulation in the liver may be secondary to malnutrition, chronic inflammation, peracute sepsis, a maladaptive type process, or other disease entity.

Overall, chronic peritonitis was likely due to the mesocercariae identified as *Alaria mustelae* and may represent an ectopic migration, aberrant host parasite relationship, potentiated pathology secondary to some other pre-existing or underlying disorder, or some other process. Moderate to severe multisystemic inflammation and fibrosis would likely have contributed to some degree of morbidity and contributed significantly to the suboptimal nutritional condition of affected animals (negative energetic burden on the hosts).

3.3.4 Phylogenetic placement of key parasites

3.3.4.1 Isthmiophora inermis

A frequent infection observed in river otters was a long thin echinostome with 27 spines (Plate 3.3). Previous reports of echinostome infection in otters included *Isthmiophora inermis*, *I. beaveri* (Detwiler et al. 2012), *Bashkirovitrema canadense*, and *B. incrassatum*. Samples were confirmed to not be *I. beaveri,* which have a short post-testicular region (Kostadinova and Gibson 2002). They were also confirmed not to be *B. canadensis* or *B. incrassatum* according to available measurements in the literature, and a clear difference in size (Dronen 2009). Two otters in Alberta were confirmed to concurrently be infected with a small number of *B. canadensis* that were discernable mainly by their much longer filiform body, and the position and shape of the testes. All other echinostomes collected in otter and mink matched measurements provided by Hoberg et al. (1997) for *I. inermis* except for length, the specimens from this study slightly longer. They were therefore identified morphologically as likely to be *Isthmiophora inermis*.

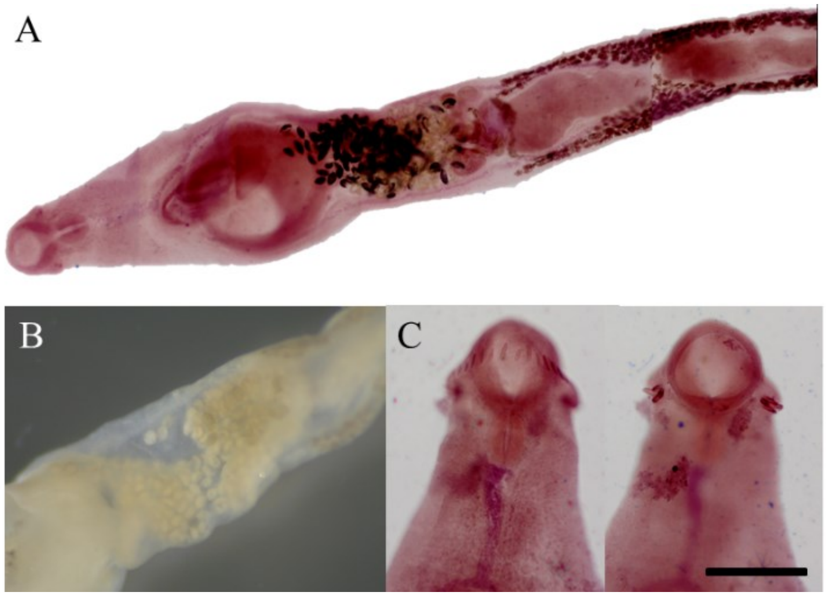

Plate 3.4 Specimens of *Isthmiophora inermis*. Parasites stained with acetic acid carmine, cleared in clove oil and mounted permanently in Canada balsam, including A) composite photo of whole mounted worm, B) unstained photo under dissecting microscope showing eggs in the body cavity, and C) dorsal and ventral view of the head showing oral sucker, mouth, and collar spines, scale bar=400μm.

Sequences of the 28S ribosomal subunit region blasted most closely to *Isthmiophora* spp. from a study by Tkach et al. (2023) from North American mink, the authors did not comment on whether their specimens could be *I. inermis*. Sequences of the CO1 region did not closely match to any available sequences, including *I. hortensis, I. melis,* or *Isthmiophora* spp. from Europe and Asia. There are no available sequences in GenBank for *I. inermis*.

3.3.4.2 Versteria spp.

Adult cestodes were found from 5 river otter and 8 mink. They matched descriptions for the genus *Versteria* (Freeman 1956) and could be the same as a previously undescribed species in North American mustelids (Lee et al. 2016). Molecular analysis of the adult worms and larval cysts from mink and muskrat liver, produced sequences for the NAD1 mitochondrial region. Those sequences matched those from larval infections identified in humans and an orangutan from North America (Table S3.1). Lee et al. (2016) and Niedringhaus et al. (2022) noted these were likely a separate species from *V. mustelae*. A sample from a river otter from Alberta was also sequenced for the CO1 mitochondrial region, again matching sequences from larval infections in North America. Phylogenetic analysis of available NAD1 sequences for taeniids confirmed this study's specimens and larval infections to be a related lineage, separate from *V. mustelae*, and likely represent a separate species (Figure 3.2). One worm from a river otter in British Columbia produced a sequence unique from the other 30. With pairwise distance of 0.046, which is more than the distance reported between the newly described *Versteria cuja* and *Versteria* spp. in North America, it likely represents another species. Unfortunately, only one fragmented specimen was found so description was not possible. Morphological analysis of diagnostic regions also showed differences between previous reports of *V. mustelae* and the cestodes retrieved in this study. Our samples are most likely a new species which we name and describe as *Versteria rafei* n. spp. (Appendix B.1, Figure S3.1, Plate S3.3).

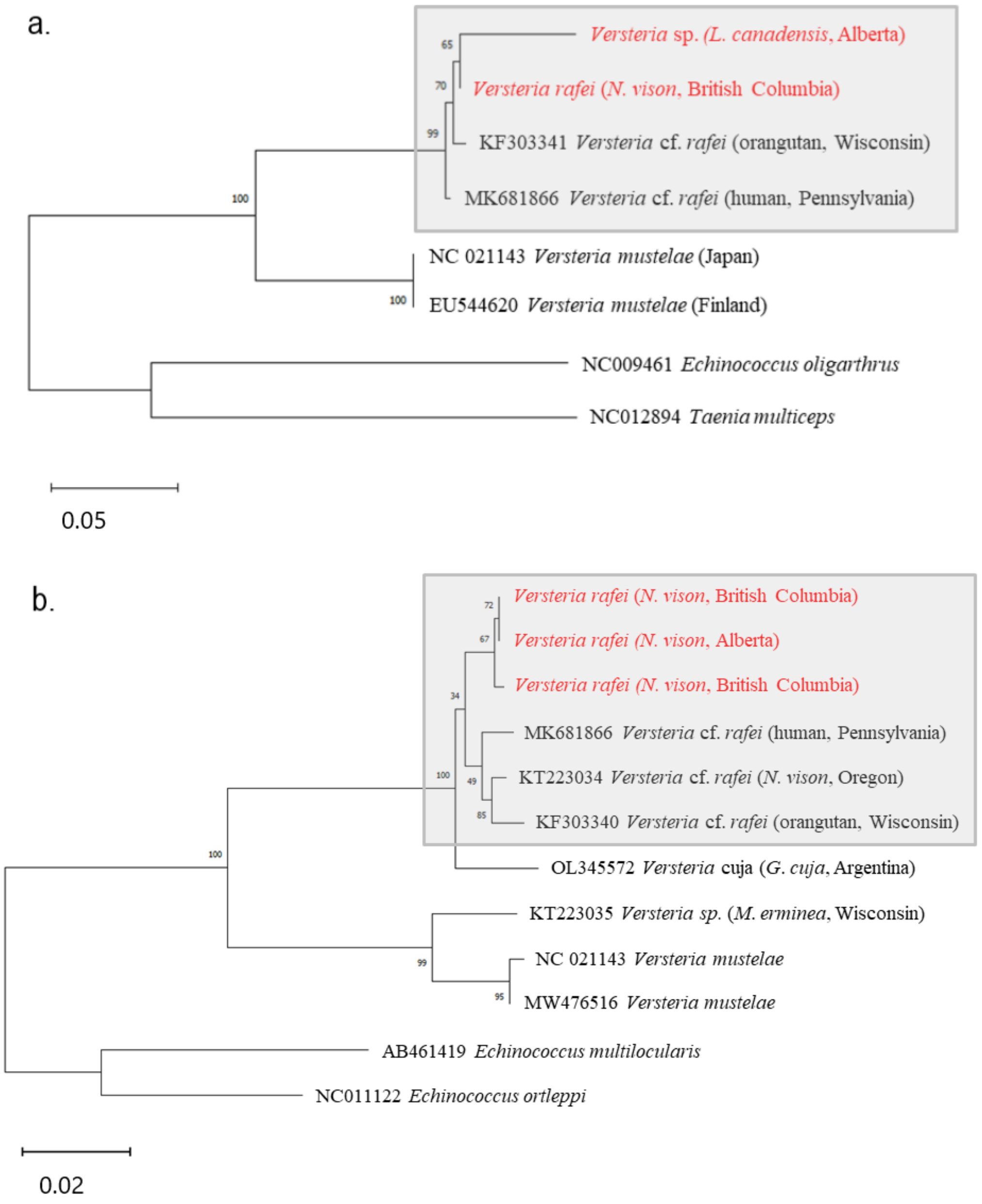

Figure 3.2 Phylogenetic trees of parasites described as *Versteria rafei n. spp.* Mitochondrial sequences from *Versteria rafei n. spp.* sampled in this study identified in red, including host species and location of capture in brackets, compared against sequences from GenBank, identified by ascension number before their name, specimens identified as *Versteria* spp. or *Versteria mustelae* but are likely examples of *V. rafei* are identified as *V. cf. rafei* and in a grey box, for the A) *nad1* and B) *cox1* mitochondrial regions. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1993). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

3.3.7 Toxoplasma gondii in river otters from Alberta

A generalized linear model was fitted to calculate the number of tachyzoite-equivalents in the tested tissue, using the Ct-values from the five standardized samples of beef muscle spiked with known concentrations against the log starting quantity of the standardized sample. The best fitted model was described as Ct = 39.3–3.2*t*, where the y-intercept of the regression line was 39.3 and the slope -3.2, and *t* the log quantity of tachyzoites. The equation was simplified as *t* = (39.3–Ct)/3.2) and used to calculate the estimated number of tachyzoite-equivalents in the tissue against the standard, which was equal to $5.3x10^{t}$ per 100g of tissue. Of the 89 river otter brains tested, 30 (34%) (20 males (44%, n=45) and 10 females (23%, n=44)) tested positive for *T. gondii* with an average estimate of 48,639 (SD=820,818) and range of 586 to 4,061,167 tachyzoite-equivalents per 100g of brain tissue. Four animals had over 1 million tachyzoites-equivalents per 100g of tissue.

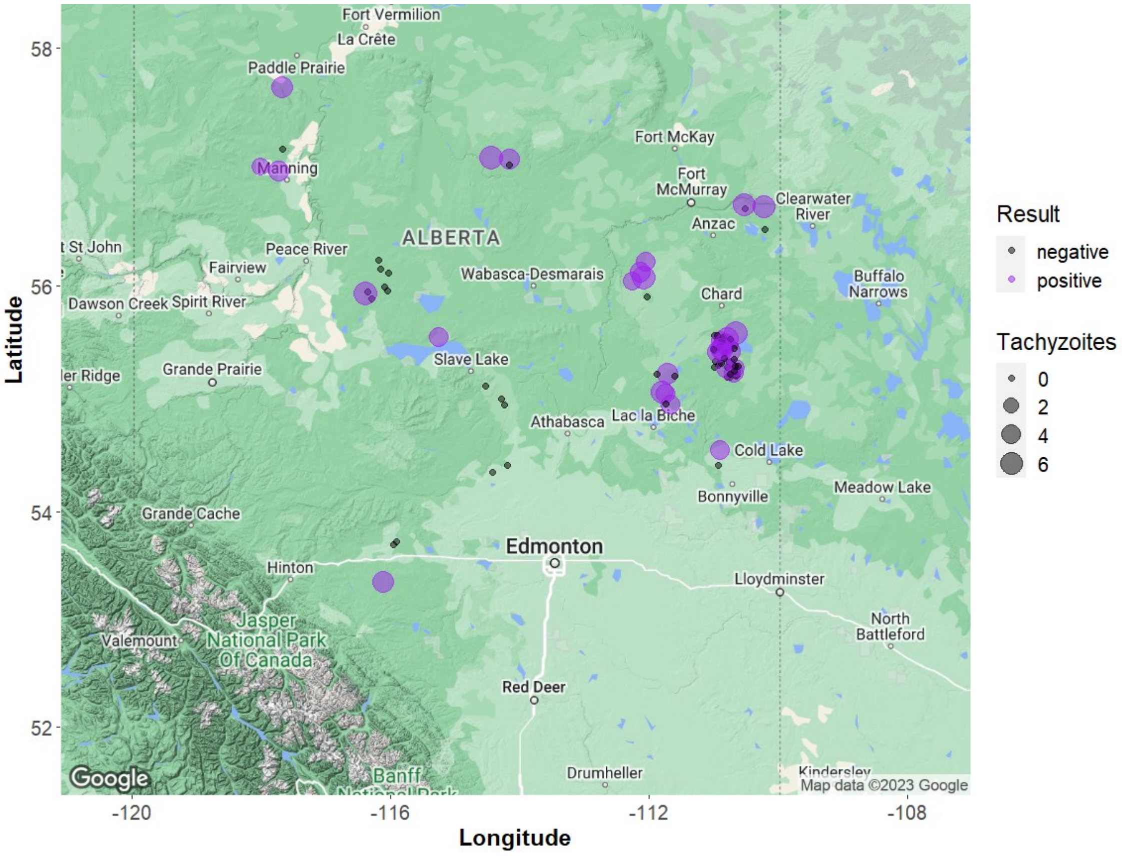

Figure 3.3 Distribution of *Toxoplasma gondii* in Alberta. Results of magnetic capture real time qPCR for the detection of *T. gondii* in river otter brains from Alberta. Dots show all sampled otter brains by result of test, whether negative or positive for *T. gondii* infection, and scaled by the log-transformed number of tachyzoite-equivalents per 100g of tissue as a measure of parasite intensity of in the sample. Overlapping points are spread by jitter (h=0.2, w=0.2).

Toxoplasma gondii was identified from all regions (Figure 3.4), except in an area of central Alberta west of Athabasca where all five otters tested were negative. Similarly, prevalence was low in a cluster north of Slave Lake where 8 otters were tested and only one was positive. Highest densities of *T. gondii* were identified in the Lakeland Region and Athabasca River basin south of Fort McMurray in the east of the province (Figure 3.3). The largest sampling group came from this region, near Winefred Lake, where 11 of 47 otters sampled (23%) were positive.

3.4 Discussion

This study investigated the helminth communities of *L. canadensis* and *N. vison* in Western Canada where there is little knowledge of the presence or prevalence of helminth parasites in these important aquatic mesocarnivores, as well as *O. zibethicus*, an aquatic sentinel species that is reported to be in decline (Roberts et al. 2020). As otter return to their historic range (Figure 3.4), and with the possible decline of muskrat populations in North America, it is essential to understand the parasites and pathogens of these important aquatic mammals (Ganoe et al. 2020, Roberts et al. 2020).

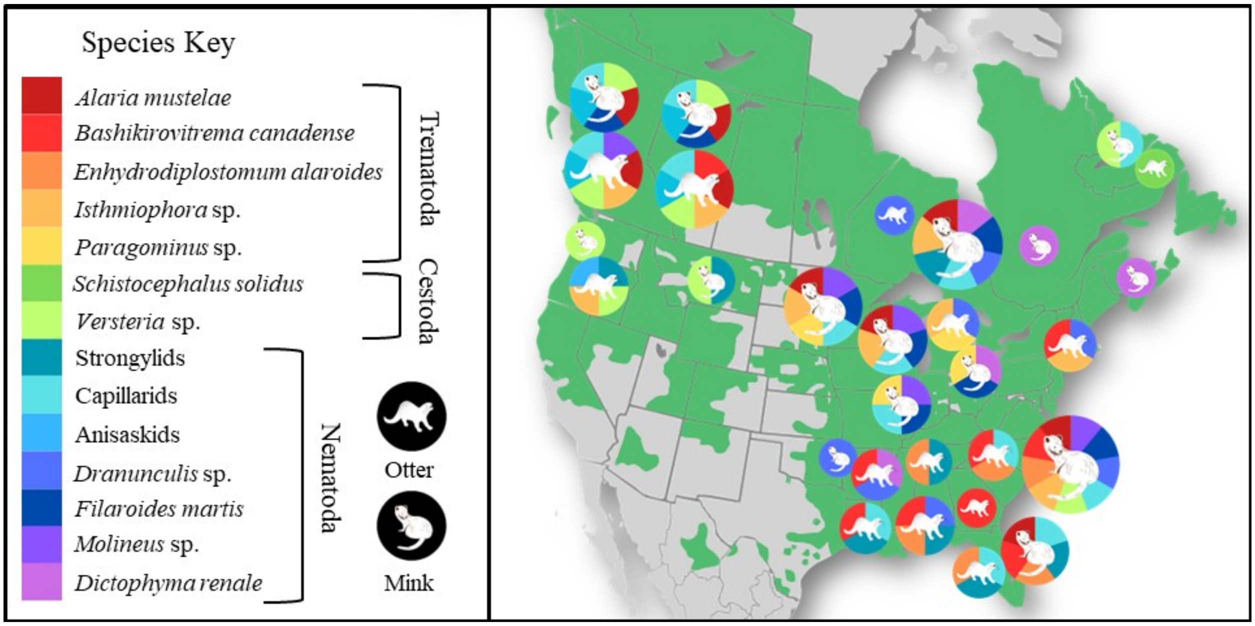

Figure 3.4 Known distribution of some common parasites of otter and mink. A non-exhaustive distribution of common parasites of otter and mink in the states or provinces in which they were reported. Parasite species represented by color, host species by illustration, circles scaled by number of parasite species identified in that region. Background color (green) marks the current known distribution of North American river otter based on reports synthesized by Roberts et al. (2020). Empty spaces include areas where otter are confirmed to be absent or where there is no information. For parasite data see Table 3.1 and 3.2. Additional reports of presence of some parasite species by studies that did not include prevalence and were therefore not included in our systemic search were included from reviews of parasites in mink

and otter (Swales 1933, Kimber and Kollias 2000). The figure was made via the open-source software canva.com and their open-source illustrations.

Our data revealed a diverse community of trematodes, nematodes, cestodes, and acanthocephalans, with trematodes being the largest taxonomic group by prevalence, richness, and intensity. This is consistent with previous reports on muskrat, whose parasites communities are dominated by gastrointestinal trematodes (Table 3.3), though differs from reports of otter and mink that saw a greater richness and prevalence of nematodes in past studies (Table 3.1; 3.2). The most prevalent species was *A. mustelae* found both in its mesocercarial and adult stages. Adult stages in mink occurred at a lower prevalence than the weighted average of previous reports (14% vs. 24%) but fell well within one standard deviation, though higher in muskrat (29% vs. 20%). *Alaria mustelae* has not previously been reported in river otter. *Filaroides martis* infected 33% of mink in British Columbia, which is lower than the 42% weighted average, but again within one standard deviation. Mink in Alberta had much lower prevalence at 11%.

Infections of *Isthmiophora inermis* occurred at similar prevalence to previous studies, however, we only found members of this genus in river otter. *Isthmiophora inermis* has only been reported in otter from the Pacific Northwest (Hoberg et al. 1997), though *I. melis* has been reported in otters of an unknown prevalence in Massachusetts (Kimber and Kollias 2000) and a single animal in Michigan (Beaver 1941). Three studies reported *Isthmiophora* spp. in mink from North Carolina, Wisconsin, and Maine. We saw none in mink from this study. Hoberg et al. (1997) noted differences in size for specimens identified as *I. inermis* between North American and Eurasia, though stated there was insufficient data to identify them as a unique species. Our samples were even larger than those identified by Hoberg et al. (1997), and it should be considered that they are a unique species in North America. Future molecular and morphological comparison is needed.

There have been very few reports of acanthocephalan infection in river otter and mink, with limited records of species such as: *Corynosoma strumosum* (OR; Hoberg et al. 1997), and *Acanthocephalus* spp. (AL, TN; Fleming et al. 1977; Kollars et al. 1997) in otter, and *C. conspectus* (Miller and Harkema 1964) and *P. constrictum* (Dorney and Lauerman 1968) in mink. We observed all of these except *Acanthocephalus* spp. in British Columbia, mostly on Vancouver Island. It is unclear why they were not found elsewhere. It is possible that high rates of urbanization and deforestation in the southern parts of the Island have induced reduction of habitats resulting in interspecific overlap and therefore competition. Otter and mink may include more amphibians, terrestrial mammals, or marine fish in their diets leading to a greater richness of acanthocephalan parasites using those species as intermediate host.

All parasites reported previously in muskrat at a weighted prevalence of 20% or higher (Table 3.3) were seen in this study except for one species, *Notocotylus* spp., which was previously reported in British Columbia, MB, and ON (Ganoe et al. 2020). Most of the species had similar prevalence or within one standard deviation of the weighted mean of previous studies except *E. revolutum*, which occurred at low prevalence comparatively (10% vs. 66%) and *W. zibethicus* (7% vs. 27%). Most notably, we saw infection by *Versteria rafei* n. spp. in muskrat at much higher prevalence than previously reported (44% vs 2%) and may present a serious increase in infections in Alberta.

Our results suggest distinct parasite communities between the Pacific Northwest and Eastern populations of mink and otter (Figure 3.4). While some species are spread across North America, such as *F. martis* and *A. mustelae*, many frequently reported species were not found in this study or by Hoberg et al. (1997) in OR, such as *Enhydrodiplostomum alaroides*, *Dioctophyme renale*, *Dracunculus* spp., *Paragonimus* spp., and *Skrjabingylus* spp. While we did find *B. canadense* in Alberta, prevalence was 1%, which is much less than previous reports from the Southeastern US where the weighted average of prevalence between five studies was 47% (Table 3.1). We could also not find any reports of *I. inermis* in otter outside of the Northwest, and it may be unique to otters of this region. Otter and mink in the Northwest may also be more exposed to anisakids, which have only been reported there, though these infections are likely accidental. These differences may be explained by a variety of factors, but we believe the most likely is the slow recovery of otter after extirpation from much of North America, which left the central prairies free of otter until recent reintroduction efforts begun to close the gap (Roberts et al. 2020). Due to this isolation, especially for the coastal regions of British Columbia, WA, and OR, and differences in climate and ecosystem, it is reasonable that northwestern populations would have unique parasite communities.

3.4.1 Implications of mesocercarial infection by *Alaria mustelae*

Alaria mustelae is a generalist parasite with a complex life cycle that can include up to five hosts. Sporocysts develop in the first intermediate host and release cercariae into the water. Cercariae infect the second intermediate host where they transform into mesocercariae. At this point mesocercariae can infect a paratenic host (possibly multiple species of vertebrate hosts, including amphibians, birds, and mammals). When the second intermediate or paratenic host are consumed by a competent third intermediate host, they transform into metacercaria in the lung parenchyma or muscles such as the diaphragm (Bosma 1934). When the third intermediate host is eaten by the definitive host, the parasite develops into an adult in the intestines (Mohl et al. 2009). Definitive hosts can include a variety of mustelids, canids, and other carnivorous mammals. Experimental studies have reported metacercariae fed to dogs, cats, and ferrets all developed into adults (Bosma 1931).

3.4.1.1 Exposure and intermediate hosts

Some reports identify amphibians as the only second intermediate host of *A. mustelae* but there has been debate about the possibility of fish acting as second intermediate hosts (Mohl et al. 2009). Considering the high prevalence in otter and mink across multiple infrapopulations, and the low percentage (0-5%) of amphibians reported in their diet in Alberta and British Columbia (Gilbert and Nancekivell 1982, Melquist et al. 2003), it is unlikely frogs are the only vector. Older reports claim fish act as intermediate hosts, and other Diplostomidae are known to encyst in the eyes of fish (Erickson 1946). As fish make up the majority of otter and mink diets in Western Canada, they are much more likely to explain the high prevalence of infection by mesocercariae (Gilbert and Nancekivell 1982, Melquist et al. 2003). A human case of infection by *Alaria* spp. mesocercariae was also reported to be caused by the ingestion of undercooked wild goose in MB, which may act as a paratenic host (Kramer et al. 1996) and is a possible pathway of infection in otter and mink as they both consume waterfowl (Gilbert and Nancekivell 1982).

The mesocercarial stage of *A. mustelae* is very unspecific and can use almost all types of vertebrates as hosts (Mohl et al. 2009). Previous reports identified raccoons (*Procyon lotor*) infected with *A. mustelae* both as adults and as metacercariae, noting that they may be competent definitive as well as paratenic hosts (Shoop and Corkum 1981). *Alaria* spp. mesocercariae have also been identified in wild pigs and an opossum (*Didelphis virginiana*) in Oklahoma (Johnson et al. 2017), as well as from red-sided garter snakes (*Thamnophis sitalis*) in MB (Uhrig et al. 2015). In Europe, infections of *A. alata* have been on the rise in domestic pigs and are of concern for public health (Portier et al. 2014). Mesocercarial infections in humans are associated with serious pathology, and at least in one case, death (Freeman et al. 1976, Mohl et al. 2009). *Alaria* spp. can be transmitted via lactation in rodents infected with mesocercariae where parasites migrated to the mammary glands and were passed on to their young (Shoop and Corkum 1983). This may be a serious concern for heavily infected female otters and mink and could explain the high infection rates detected if mesocercarial infections are being passed from mother to pup. Future studies should investigate these potential routes of exposure.

This study observed both gravid adults and mesocercariae in river otter and mink, which suggests that they may be paratenic as well as definitive hosts. This is the first time that infections of *A. mustelae* mesocercariae have been reported in river otter in North America, Bosman (1934) noted that mink could be infected with metacercariae, but no recent reports could be found. The closely related *A. alata* can infect European mink (*Mustela lutreola*) as adults and mesocercariae (Tabaran et al. 2013). Both otter and mink are preyed upon or scavenged upon by wolves (*Canis lupis*), coyotes (*Canis latrans*), and wolverines (*Gulo gulo*) (Melquist et al. 2003). Mink are also eaten by red foxes (*Vulpes vulpes*), bobcats (*Lynx rufus*), and even river otters (Larivière 1999), all of which are suitable definitive hosts for *A. mustelae* (Mohl et al. 2009). *Alaria canis* can be transmitted by auto-infection from metacercariae traveling up the trachea then swallowed (Pearson 1956). If *A. mustelae* exhibits a similar phenomenon, this may also explain the dual infections of mesocercarial and adult parasites.

3.4.1.2 Pathology of infection

Pathology of infection was similar to that observed in European mink infected by *A. alata.* Multiple, well-defined small white nodules were found in skeletal muscle and subcutaneous tissues. The authors noted that granulomas and fibroplasia were likely due to the continuous movement of mesocercariae through tissue, but when encysted in muscle tissue, induced no immune response (Tabaran et al. 2013). Upon entering a paratenic host, mesocercariae can travel to muscle, diaphragm, adipose tissue, or the lungs to encyst and potentially develop into metacercariae (Bosma 1934, Johnson 1979), which may explain our difficulty in finding mesocercariae or to successfully sequence samples from intestinal, liver, pancreatic, and renal tissues. Muscle, diaphragm, adipose, or lung tissues were not collected for artificial digestion, as they did not present white nodules like the abdominal organs, though nodules were observed occasionally in the musculature of the abdominal wall. If they had been collected, it is likely encysted mesocercariae would have been found much more frequently.

Both the observed pattern of lesions and histopathology were consistent with the migration of parasites through the abdominal or gastrointestinal walls and other tissues en route to muscle or adipose tissue to mature. The observed white foci likely represent encapsulated degenerate parasites or past and resolving migration tracks, and thus is not indicative of the parasite's current presence in those tissues. Artificial digestion of tissues in all "serious" cases produced mesocercariae consistent with *Alaria*. However, definitive identification was not possible due to degradation of the tegument by the pepsin. Because of this and our inability to definitively identify trematodes as the etiological agent of the observed fibrosis and inflammation, it is possible not all cases were due to *A. mustelae*. Some may have been caused by another species such as *A. canis* or other Diplostomid. However, 13 separate individual infections were confirmed to be *A. mustelae* based on molecular characterization of the CO1 mitochondrial region. Due to the similarity in presentation and common infection by adults of *A. mustelae* in both river otter and mink, it is likely the cases were all the same species. Future research is needed to expand upon this discovery to investigate the lung, diaphragm, adipose tissue, and musculature of animals presenting with the diffuse multifocal white nodules observed to confirm the identification of the mesocercaria and determine if they are maturing into metacercaria in otter and mink.

3.4.1.2 Implications for human communities and aquatic ecosystems

This is the first report of *A. mustelae* infection in river otter in North America and is of concern for both wildlife management and human health. With moderate to severe respiratory or ocular symptoms, and anaphylaxis, alariasis can be a serious condition in humans and has been associated with mortality even in the case of healthy young individuals (Mohl et al. 2009, Portier et al. 2014). While previous cases were assumed to be caused by the consumption of undercooked frog legs as they were the only known intermediate host (Mohl et al. 2009, Portier et al. 2014), the widespread infection in otter and mink which infrequently consume amphibians, likely confirms previous reports suggesting that fish may also be suitable second intermediate or paratenic hosts. Other members of the family Diplostomidae commonly use various species of fish as intermediate hosts, encysting inside the eye, muscles, or organs (Kohn et al. 1995, Ribeiro et al. 2019). As fish are more frequently consumed by First Nation Community members than the general population, risk of infection should be of concern for rural populations in Western Canada. We also observed mesocercaria of *A. mustelae* in two muskrats, which are a traditional food source for indigenous peoples (Wein and Freeman 1995) and may be a further source of infection for at risk groups. Further research is needed, and rural medical providers should consider alariasis as a potential differential diagnosis for respiratory and ocular disease.

Infections of diplostomid mesocercariae can affect a range of fish species and affect host health and population fitness. Mesocercarial infections are the cause of 'black-spot' disease in freshwater and marine fish (Harrison and Hadley 1982, Duflot et al. 2023), which refers to the melanization of the encysted parasite, a defense mechanism by the host (Steedman 1991). Black-spot disease can be caused by a large variety of trematodes and can stunt growth, cause deformities, lower thermal tolerance, and reduce survival and reproductive success (Hockett and Mundahl 1989, Steedman 1991, Hagmayer et al. 2020, Duflot et al. 2023). Future research is needed to determine if *A. mustelae* causes similar disease in fish species in Alberta and British Columbia, especially considering the high prevalence and range of the species observed.

3.4.2 *Corynosoma strumosum* infection and acanthocephalan-associated peritonitis

Acanthocephalan infections were common in British Columbia, mainly in mink, and included the marine parasite *Corynosoma strumosum* and a parasite of birds, *Centrorhynchus conspectus.* In two cases, infection led to acanthocephalan-associated peritonitis due to migration of the parasites into the abdominal cavity; septic peritonitis likely the cause of death for a juvenile female mink infected with *C. strumosum*. Infection by *C. strumosum* in North American mink farmed in Europe was reported to cause serious pathology in the gastrointestinal tract, including mortality (Nuorteva 1966). However, this may be the first confirmed report of *C. strumosum* infection in wild North American mink (Van Cleave 1953). We also observed a case of acanthocephalan induced peritonitis in a juvenile mink by immature *C. conspectus*, as well as non-perforating infections in six adult mink. Infection by immature *C. conspectus* has previously been reported in mink, foxes, opossums, and raccoons (Richardson and Nickol 1995), though this is the first report of peritonitis linked to the infection. *Centrorhynchus conspectus* is a parasite of birds of prey, and little is known about its intermediate hosts. Potential groups include fish, amphibians, and rodents (Richardson 2014). We only observed infection in mink, which may be due to differences in diet between otter and mink. The latter includes terrestrial vertebrates in their diet more frequently, especially when sharing habitat with otters (Larivière 1999). The majority of infections were seen from mink on Vancouver Island where high levels of anthropogenic change may increase otter-mink overlap and lead to mink altering their diet leading to increased infection.

Acanthocephalan-associated peritonitis often occurs in unsuitable hosts. It is a major cause of mortality in southern sea otter (*Enhydra lutris*) infected by the acanthocephalan *Profilicollis* spp., which normally infect shore birds (Mayer et al. 2003). Infection by *Corynosoma* spp. in young hosts is also not unique. A previous report found the related species *Corynosoma enhydri* in a sea otter pup only four weeks of age. The pup was infected with only one parasite and infection was not associated with migration into the abdominal cavity and peritonitis (Shanebeck et al. 2020). Mink begin to ingest solid food as soon as their deciduous teeth erupt at around four weeks. They join their mothers hunting after around eight weeks (Larivière 1999) and could thus be exposed to infection at a very young age. As infections by *C. strumosum* have been reported in the past to cause serious disease in farmed mink (Nuorteva 1966), it may also be an unknown natural cause of juvenile mink mortality in coastal areas. Infections in adults do not seem to lead to peritonitis, indicating that young animals are more susceptible. However, it is possible that infection intensity influences the occurrence of intestinal perforation. Both individuals where infection led to peritonitis were also the most heavily infected.

Though pathologically significant infections have not been reported in river otters, juveniles may be similarly vulnerable to intestinal infections by these acanthocephalans. Unfortunately, monitoring of populations is limited, let alone consistent identification of intestinal helminths. We recommend further investigation into determining the prevalence of marine parasite infections in coastal foraging mink and river otter, and in the suitability of these species as definitive hosts. If we are to manage and conserve furbearers in coastal regions, we must include consideration of the influence of exposure to novel marine parasites on individual and population health, as parasites can have strong influence on populations and food webs and can be important bio-indicators (Lafferty et al. 2008).

3.4.3 Implications of *Versteria rafei* n. spp. infections

Human alveolar and cystic echinococcosis as well as taeniid cysticercosis and coenurosis have historically been diseases of concern for both humans and wildlife. These pathologies may be caused by a variety of parasite species (Deplazes et al. 2019). Notoriously difficult to identify in their larval form, many cases may simply be recorded as 'echinococcosis' or 'cysticercosis' without species level identification. The genus *Versteria* was erected in 2013 based on molecular characterization of *Taenia* which clustered with *Versteria mustelae* as a closer relative of *Echinococcus* than *Taenia* (Nakao et al. 2013). Using molecular approaches, recent reports have identified diseases similar to alveolar and cystic echinococcosis caused by an unknown species of *Versteria* in humans as well as an orangutan in a zoo in North America, suggesting it should be considered as a differential diagnosis for cases of 'echinococcosis' (Barkati et al. 2019, Deplazes et al. 2019). In one such case, a patient even tested positive for two different ELISA tests for *Echinococcus granulosus* antigens, though negative for a confirmatory western blot (Barkati et al. 2019), highlighting the potential for misidentification. However, this has only been recently discovered and it is impossible to determine how many historical cases of 'echinococcosis' or 'cysticercosis' are due to *Versteria*, or how significant a threat it is to human communities.

We documented infections of adult *Versteria rafei* n. spp. in both river otter and mink from Alberta and British Columbia, as well as larval infections in the livers of mink and muskrat from Alberta. A single adult of this then-unnamed species was previously identified from both a mink and an ermine, though the samples were too degraded for extensive description (Lee et al. 2016). Our report is the first to document infection on a broad scale for river otter and mink, with infection intensities as high as 54 worms in a single individual. The prevalence and intensity of infection as well as the presence of mature and gravid proglottids confirm both *L. canadensis* and *N. vison* as competent definitive hosts for the unnamed species. Due to historical differences in nomenclature, it is hard to definitively identify the intermediate host of *Versteria rafei* n. spp., but previous reports of '*Taenia mustelae*' identified the intermediate host in North America as rodents, including muskrat (Freeman 1956). We found larval cysts of taeniid parasites in the livers of muskrats, which were confirmed as *Versteria rafei* n. spp. based on molecular characterization. Muskrats are prey items for both river otter and mink, though mink more often include terrestrial rodents in their diet (Larivière and Walton 1998, Larivière 1999). Infection prevalence and intensity were slightly higher in mink, which may be explained by this dietary difference.

One mink from Alberta had over 50 cysts of *Versteria rafei* n. spp. in its liver and was concurrently infected with 21 adults of the species. Because of the high number of cysts and adults present in this individual, it indicates that hosts may be auto-infected by cysticerci; autoinfection via reverse peristalsis of gravid proglottids into the stomach or release of eggs in the intestine have been documented in other species. *Versteria*'s closest relatives, *Echinococcus* and *Taenia*, have both been shown to do this in canids and humans (Ito 1997, Peregrine 2009). Infections may thus be of concern for trappers handling wild mustelids, which could lead to contact with eggs and therefore infection by cysticerci. Indigenous groups who hunt and eat intermediate host species may be infected with adults and potentially autoinfected by cysticerci, muskrat are a traditional food source for First Nation groups in Canada (Wein and Freeman 1995, McLachlan 2014).

Investigating the life history and prevalence of zoonotic pathogens in the wild is essential to prevent future infections. Infections of larval *Versteria rafei* n. spp. can be fatal in various hosts, including humans, captive animals, and wildlife (Lee et al. 2016, Barkati et al. 2019, Lehman et al. 2019, Niedringhaus et al. 2022). Considering infection presents the same as *Echinococcus* spp., it is possible infections by *Versteria rafei* n. spp. are more prevalent than reported, and currently diagnosed as Echinococcosis. *Versteria rafei* n. spp. has also been reported to encyst in patient's brains (Lehman et al. 2019). Coenurus type cysts of cestodes were identified in the cerebral spinal fluid of patients but could not be identified. However, the author suggested they may be *Versteria rafei* n. spp. due to similarities in reported dimensions of cysts, hooklets, and strobilates (MacDonald 2021). Since histology is not an accurate guide to the identification of larval cestode infections, molecular characterization is essential to understand infection pathways and factors that may predispose people to infection by *Versteria* and other taeniid species (Niedringhaus et al. 2022).

3.4.4 Implications of *Toxoplasma gondii* infections

Infection by *T. gondii* was confirmed in 34% of Albertan river otters. Such prevalence is slightly higher than previous studies of *L. canadensis*, which ranged between 24-28% (Sanders et al. 2020, Cotey et al. 2022). This is lower than most terrestrial carnivores, such as red fox (*Vulpes vulpes*) (Bachand et al. 2018). We did not have the resources to test both otter and mink brains, but considering mink and otter share similar habitats and diets, infection rates may be similar in mink. Mink may even present higher infection rates due to the importance of rodents in their diet (Larivière 1999). Little is known about the effects of toxoplasmosis in river otters. However, infection in Californian sea otters can be associated with meningitis and myocarditis. Toxoplasmosis was identified as the cause of death in 72% of cases (Shapiro et al. 2019). While *T. gondii* can encyst in various tissues, location in the brain is associated with clinical toxoplasmosis and likely represent more serious cases of infection (Hill et al. 2005, Sanders et al. 2020). Our results indicate that toxoplasmosis may represent a serious threat for Canadian river otters, especially since *T. gondii* is known to cause fatal encephalitis in their closest relative, the sea otter (Shapiro et al. 2019). Clinical infections can also be related to concurrent infections of morbilliviruses like Canine Distemper, which occurs in most species of mustelids including *L. canadensis* and *N. vison* (Kimber and Kollias 2000). These viruses can have an immunosuppressive effect leading to more severe diseases (Dubey et al. 2021).

In this study, males were twice as likely to be infected as females, a pattern documented before (Cotey et al. 2022). This may be due to male’s propensity to roam further than females, target different prey, which could increase their exposure to the disease. Females tend to range less, especially when caring for young (Larivière and Walton 1998, Blundell et al. 2000). There was no discernable connection between infection rate and proximity to urban areas. It is thus likely that wild felids are the source of *T. gondii* infection in wild river otters. Connections between agricultural land and *T. gondii* infection are possible, due to increased runoff from changes in soil structure and landscape, or from a higher likelihood of feral cats on farms (Cotey et al. 2022). However, most of our infected animals came from the Lakeland and Fort McMurray areas where agriculture is marginal. Little is known about the effects of *T. gondii* infection in river otters and the prevalence of infection in definitive felid hosts in Canada, and more research is needed to address this gap in knowledge.

Human infections with *T. gondii* can be serious, both for immunocompromised or pregnant people, and children. Recent reports have confirmed significant correlation between *T, gondii* infection and spontaneous abortions (Kalantari et al. 2021) and reduced cognitive functions in children (Mendy et al. 2015). However, toxoplasmosis is not considered a pathogen of concern in Canada and information on the prevalence of human infection is limited (Elmore et al. 2012). First Nation communities may be particularly at risk for infection, as previous reports from across North America have identified high seroprevalence in First Nation people, even in the absence of domestic or wild felids (Elmore et al. 2012). Felids are still the only confirmed definitive host, though parasites can reproduce asexually in intermediate hosts and be passed on through multiple hosts, which may explain high prevalence in areas without large felid populations (Dubey 2009). The observed infections in river otters, a sentinel species in riparian ecosystems, should be of concern for both First Nation and settler populations that frequently draw from rivers and lakes for water or as a regular part of their diets. Considering the lack of medical resources for First Nation and rural communities who may be at higher risk of infection, this is an important issue of equity. Future research needs to collaborate with indigenous groups and health professionals that serve rural areas to investigate the potential threat of *T. gondii* infection for Albertan, especially First Nation communities.

3.4.5 Conclusion

Helminth populations of *L. canadensis, N. vison*, and *O. zibethicus* identified in Alberta and British Columbia included many of the characteristic species previously reported. However, notable species of concern such as *Dracunculus* spp. and *Dioctophyme renale* (Table 3.1, 3.2) were not found. Like previous reports, overall species richness was low (Sealander 1943, Forrester 1992, Hoberg et al. 1997, Foster et al. 2007), and was characterized by four main species (*Alaria mustelae*, *Filaroides martis*, *Isthmiophora inermis*, and *Versteria rafei* n. spp.). Helminth communities in river otter and mink were likely influenced by both habitat variation, including vast home ranges in the case of otters, and their eclectic diets. Diet can vary broadly to include rodents, waterfowl, and amphibians, though it is usually dominated by fish (Larivière and Walton 1998, Larivière 1999). As mesocarnivores that dominate aquatic ecosystems, they are definitive hosts to a range of parasite species, but also potential transport hosts, sometimes for the same species like in *Alaria mustelae* and *Versteria rafei* n. spp. infections. Sentinels in our aquatic ecosystems, investigation of their helminth communities revealed multiple species of concern for zoonotic transmission. Our data highlights a serious gap in knowledge around helminth communities in Western Canada and the threat related to vulnerable communities. Ongoing research is needed to assess the risk of infection by *Versteria* spp., *Toxoplasma gondii*, and *Alaria mustelae* for First Nation communities and fur trappers who handle these organisms. Furthermore, we need research into the distribution of these parasites in other intermediate and definitive hosts to understand the range of this threat to wildlife and human communities.

Chapter 4: River otter and mink as sentinels for anthropogenic metal contamination in aquatic ecosystems of Western Canada.

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4.1 Introduction

The major watersheds of Western Canada include multiple large, complex river basins that flow into the Pacific and Arctic Oceans. In Alberta, two of the largest are the Athabasca and Peace River basins, which occupy most of the northern part of the province, originating in the Rocky Mountains. They meet at the Peace-Athabasca Delta and Athabasca Lake, combining to form the Slave River, which drains into Great Slave Lake, becoming the Mackenzie River. These waters eventually drain into the Arctic Ocean, making them part of the largest river basin in Canada (D'Souza and Parlee 2019, Lima and Wrona 2019). In British Columbia, the province is dominated by the Fraser River basin, which originates in the Rockies and drains into the Pacific Ocean at the Strait of Georgia (Marshall et al. 2017). These large watersheds are integral parts of Canada's ecosystems, as well as essential for human communities, especially First Nation groups like the Salish, Secwepemc, Dance-zaa, Cree, Sekani, and many others who have used these rivers since time immemorial (Marshall et al. 2017, D'Souza and Parlee 2019). Due to environmental contamination and other anthropogenic threats these ecosystems are at risk (Dietz et al. 2019).

While there are natural sources of metals in the environment, human activity is a major driver of environmental contamination (Fisk et al. 2005, Gautam et al. 2016). The environmental fate and effects of contaminants are largely dependent on the chemical class, exposure duration, physiochemical properties of the environment and organism physiology. Major pathways include atmospheric transport which can lead to settlement in soil and water, leaching into groundwater and surface water from landfills, agriculture, and industry, or direct deposition into waterways (AMAP 1998, Gautam et al. 2016). In water, metal contaminants can be found in solution (dissolved) or in suspension (solid particles) and can travel long distances to be deposited in sediment or taken up by flora or fauna. Contaminants can also travel as droplets, particles, gas, bound to organics, or hitched onto other particles, though droplets tend to not travel as far (Briffa et al. 2020). They are not biodegradable and persist for long periods, they may also interact chemically with other elements to break down or become even more toxic (Briffa et al. 2020). Some trace metals also tend to bioaccumulate up trophic chains in aquatic ecosystems, meaning top predators, including humans, are at higher risk of toxic exposure (Gautam et al. 2016).

4.1.1 Historical health of the Alberta and British Columbia Watersheds

Due to a variety of anthropogenic stressors including agriculture, forestry, mining, oil sands developments, industrial waste, and urban development, significant environmental changes have occurred in central and northern Alberta over the past 100 years (Lima and Wrona 2019). The upper portion of the Athabasca River is subject to extensive forestry, coal mining, and pulp and paper mills, while oil sands developments are the largest influencer in the lower portion (Lima and Wrona 2019). Similarly, the Peace River has pulp mills and some oil production, while municipal sewage is potentially also a source of contamination (Wrona et al. 2000). Indigenous knowledge has highlighted many anthropogenic effects, especially of hydroelectric development, in flood patterns, water levels, small creek and tributary dry-ups, and shift in creek beds and flows; these are implicated in a decline in the water quality of the Peace River over the last 50 years (D’Souza and Parlee 2019). Previous reports have identified seven priority pollutants that have exceeded both federal and provincial water quality guidelines meant to protect aquatic life. Cadmium (Cd), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni), Silver (Ag), and Zinc (Zn), have been detected above safe levels in the Athabasca River downstream of oil sands developments, which are likely the main source of metal contamination (Kelly et al. 2010).

In British Columbia, the Fraser River basin supports a population of over 2.5 million people. The watershed's lower portion is dominated by high levels of anthropogenic landscape alteration through agriculture and urban development, while its upper portions are used for forestry, pulp mills, and fisheries (Marshall et al. 2017). In the last 100 years, a wide variety of pollutants from pulp and sawmills, mining, sewage, industrial waste, and agricultural as well as urban run-off, have been reported as serious threats to ecosystem functioning. However, the largest threat may be metal mining, as the basin currently supports 60% of all metal mines in the province (Kristensen et al. 2013, Marshall et al. 2017). Pollutants of concern include Cu, Ni, Zn, Cobalt (Co), Pb and Hg (Gallagher et al. 2004). Like the Fraser River, the Columbia River basin has historically suffered from contamination related to mining and smelting, including what used to be the largest zinc-lead smelter in the world (Johnson et al. 2005), and more recently, increased risk of contamination due to mountain top removal coal mining in the Elk Valley. Pollutants of concern include Cd, Co, Cu, Pb, Antimony (Sb), Selenium (Se), and Zn (Sofield and Bollinger 2009, Child et al. 2018, Cooke and Drevnick 2022).

Vancouver Island is the largest island on the west coast of North America, and has a long history of anthropogenic activity, predominantly forestry. Recent information on metal concentrations and outflows on Vancouver Island is limited; however, some sources include extensive forestry and wood processing (saw, pulp, and paper mills), mines both active and decommissioned, agriculture, and urbanization. Pollutants of concern on the island including As, Cd, Cu, Mn, Pb, and Zn (Laurinolli and Bendell-Young 1996, Bailey et al. 1999, Barroso and Melnechenko 2019). Mining is a serious driver of contamination in aquatic ecosystems, especially due to acid drainage from coal mining and discharge from abandoned metal mines. Even long abandoned mines can disproportionately contribute to metal contamination in watersheds and remain bioavailable to wildlife (Byrne et al. 2012, Camizuli et al. 2018). British Columbia has a long history of heavy mining activity throughout the province, which is likely a major cause of widespread metal contamination (EMLI 2023).

4.1.2 River otters, mink, and pollutants

River otters and mink are ideal species for monitoring the bioaccumulation of various contaminants in our riparian ecosystems due to their position as the top predators in North American boreal aquatic food webs (Ben-David et al. 2001, Basu et al. 2007, Crowley et al.

2018). Otters are also useful indicator species due to their sensitivity to disturbance (Ben-David et al. 2001), and mink have been suggested as an excellent complement to otters due to their shared diets, controlled laboratory data concerning exposure, and more limited home ranges, which allow for better resolution to specific water bodies (Crowley and Hodder 2019). Additionally, piscivorous mammals like mink and otter are ideal sentinels to monitor the risk of contaminant exposure for humans, such as methylmercury which is neurologically toxic in both wildlife and humans (Basu 2012). Even so, there is limited research in Canada on the effects of pollutants on river otters. In recent years projects have focused on exposure to Hg (Ben-David et al. 2001, Klenavic et al. 2008, Crowley et al. 2018, Eccles et al. 2020), persistent organic pollutants (POPs) (Huang et al. 2018, Wainstein et al. 2022), and polycyclic aromatic compounds (PACs) (Harding et al. 1999, Xia et al. 2019). In British Columbia, river otter and mink have been reported to have site specific exposure to Cd, Cu, Hg, Pb, Magnesium (Mg), and Manganese (Mn) (Harding et al. 1998). In Albertan otters, metals of concern may include Cd, Hg, Strontium (Sr), and Thallium (Tl) along with PACs (Eccles et al. 2020, Thomas et al. 2021).

While metal contaminants are broadly studied, there is limited information available concerning specific toxic thresholds in liver, kidney, or other tissues for most mammalian vertebrates, including river otter and mink. Studies are often limited to experiments investigating the effect of metals in feed to determine dose-effect or dose-response relationships. Research often focuses on acute toxicity to support the development of legislation and policy on environmental concentration limits. Since environmental levels are generally consistent, except in the case of large-scale spills or disasters, exposure is often chronic and cumulative (AMAP 1998). Subtle community changes or behavioral and physiological effects are also much harder to study and are underrepresented in the literature. It is also often unclear at what point accumulation in liver tissues is related to adverse outcomes (AMAP 1998, Byrne et al. 2012). Abiotic and biotic factors that alter the bioavailability of the metal, including temperature, salinity, pH, dissolved oxygen, and water hardness, may also mediate its toxicity in vertebrates, further complicating analysis (AMAP 1998, Rattner and Heath 2002, Fisk et al. 2005). Essential metals such as Cu, Fe, Mn, Molybdenum (Mo), Se, and Zn are required for normal homeostatic control and healthy functioning. A deficiency of these metals can cause detrimental effects, such as the anemia, appetite loss and organ damage resulting from Cu deficiency in mammals (Łanocha-Arendarczyk and Kosik-Bogacka 2019). However, in excess, these metals also have adverse effects, and they tend to have more variable ranges of concentrations than non-essential metals. For some such as Cu and Se, the difference between toxic and beneficial is narrow which further complicates the forecasting of negative effects (Tchounwou et al. 2012, Briffa et al. 2020). Toxic effects also vary between metals, and present in a variety of ways, such as developmental defects, spontaneous abortion, kidney and liver damage, oxidative stress, carcinogenesis, disruption of cell processes and apoptosis, and neurological effects. Other effects are difficult to measure, and there are some that have not yet been described in mammals (Domingo 1994, Tchounwou et al. 2012, Briffa et al. 2020, Teschke 2022).

Due to these limitations, some reported toxic thresholds may be very conservative values and represent only the upper end of toxic concentrations while lower concentrations may still negatively influence individuals and overall population health (Sample et al. 1996, AMAP 1998, Byrne et al. 2012, Briffa et al. 2020). Observed metal concentrations in wild carnivores are predominantly due to accumulation through repeated exposure via prey items such as fish, which may carry high levels of metal contaminants. However, exposure via contaminated water, inhalation, and in some instances absorption through the skin may also contribute to accumulation. Many studies assume that these secondary pathways are negligible; however, little research is available on the rates of uptake in wild mammals and this assumption may be false.

4.1.3 Goals and Hypothesis

The objective of this study was to quantify metal toxicants in river otter and mink in Alberta and British Columbia and compare detected levels against historical reports to determine if animals in Western Canada are at higher current risk of metal contamination. Furthermore, we sought to determine if observed concentrations were due to anthropogenic sources of contamination, and if so, what sources were contributing to elevated levels and at what spatial scale those sources were detectable. We hypothesized that metal contaminants would be correlated with the presence of anthropogenic sources of contamination in Alberta and British Columbia reported to be major sources of those metals (Table 4.1), mediated by the interaction effect of age in sampled animals due to bioaccumulation.

Table 4.1 Review of pollutant sources and associated hypotheses. Information presented includes the metal pollutant tested, anthropogenic sources hypothesized as drivers of that metal in our animals and source references. Interaction effects are denoted by joining two drivers with a colon.

Metal	Anthropogenic Sources	Hypothesized Drivers	References
Ag	Mining, including as byproduct in Cu, Au, Ni, and Pb-Zn; industrial uses, electronics, and sterilizing medical equipment; oil and gas industry	Mines Industrial Intensity Oil and Gas count	(Huang et al. 2016, Strużyńska 2019)
Cd	Smelting; industrial uses, including metallurgic and electronic; burning of fossil fuels; agriculture, via fertilizers; municipal waste, and pulp paper production; oil and gas industry	Industrial Intensity Road Density Urban Cover Oil and Gas count	(Singh and Chandra 2019, Tomza-Marciniak et al. 2019)
Co	Mining; Industrial uses, including metallurgic and ceramic; power plants, coal burning; road transport	Mines Industrial Intensity Road Density	(Kosiorek 2019)
Cu	Mining and smelting; industrial uses, including polymers, lubricants, plastics, gas sensors, and antimicrobials; urban development, including piping, roofing, coolers, heating; agriculture, as feeding additive and fungicide; pulp and paper production; oil and gas industry	Mines Industrial Intensity Urban Cover Crop Cover Pulp and Paper Mill Oil and Gas count Mines:Industrial Crop:Wildfire	(Singh and Chandra 2019, Łanocha-Arendarczyk and Kosik-Bogacka 2019, Raoelison et al. 2023)
Fe	Mining and smelting; industrial uses, mainly steel production and products; pulp and paper production	Mines Industrial Intensity Pulp and Paper Oil and Gas count Mines:Industrial	(Kosik-Bogacka et al. 2019, Singh and Chandra 2019)
Hg	Mining, especially un-remediated mines; fossil fuel combustion; Industrial uses, including metallurgy, cement, coal, and electrical; Oil and Gas, pulp and paper industry	Mines Industrial intensity Oil and Gas count Road Density Pulp and Paper Mill	(Wiener et al. 2002, Kalisińska et al. 2019)
Mn	Industrial uses, including metallurgic, chemical, ceramic, textile, and electrochemical; agriculture, including fertilizers and plant protectants; pulp and paper production; burning of fossil fuels, especially modern unleaded	Industrial Intensity Urban cover Crop Cover Pulp and Paper Mill Road Density	(Singh and Chandra 2019)

Pb	Mining; burning of fossil fuels; ammunition; industrial uses, including metallurgic; urban waste, including paint, pipes, and landfills; pulp and paper production; oil and gas industry; wildfires increase air and aquatic concentrations	Mines Industrial Intensity Urban Cover Pulp and Paper Mill Oil and Gas sites Wildfire Cover	(Baranowska-Bosiacka et al. 2019, Singh and Chandra 2019, Raoelison et al. 2023)
Rb	Mining; Industrial uses, including pharmaceuticals, photoelectric, spectrophotometers, infrared lamps, semiconductors, vacuum tubes; Coal burning	Mines Industrial Intensity Oil and Gas Count Mines:Industrial	(Johnson et al. 1975, Campbell et al. 2005a)
Se	Mining, including the processing of copper ore, and especially coal; burning of fossil fuels; industrial uses; pulp and paper production; municipal waste; agriculture, via phosphate fertilizers	Mines Industrial Intensity Urban Cover Crop Cover	(Ohlendorf 2002, Pilarczyk et al. 2019)
Zn	Mining, especially coal; industrial uses, including production of alloys, galvanizing steel, rubber, cosmetics and pharmaceuticals, flooring, plastics, textiles, electrical, etc; agriculture, including nutrient supplementation and fungicide; energy production via the burning of coal; wildfires increase air and aquatic concentrations	Mines Industrial Intensity Crop Cover Oil and Gas Count Wildfire Cover Crop:Wildfire	(Kosik-Bogacka and Łanocha-Arendarczyk 2019, Raoelison et al. 2023)

4.1.4 Scope of study

Liver tissues from previously described necropsies (see section 3.2) were used to determine pollutant exposure of river otter and mink in Alberta and British Columbia and evaluate whether geospatial factors or parasitic infection influenced pollutant concentrations. The results indicate elevated levels of multiple metal contaminants including Ag, Al, Bi, Cd, Ga, Rb, and Sr in British Columbia and Ba, Fe, Ga, Rb, and Sr in Alberta. Metal contamination strongly correlated with anthropogenic sources, both at the watershed and home range buffer scales. The results fill a knowledge gap regarding modern pollutants of concern in Western Canada for aquatic carnivores, as well as for human communities, and highlight the importance of monitoring sentinel species as early warning indicators for environmental threats.

4.2 Materials and Methods

4.2.1 Literature review

To summarize past reports of 22 target metals, Aluminum (Al), Ag, Arsenic (As), Barium (Ba), Bismuth (Bi), Cd, Co, Cu, Iron (Fe), Gallium (Ga), Mn, Mo, Ni, Pb, Rubidium (Rb), Sb, Se, Tin (Sn), Sr, Tl, Vanadium (V), and Zn, in the liver of mink and otter, we conducted a systemic literature review. We searched a combination of keywords including mink, otter, mustelid, pollutant, metal, and contaminant, including variations of those words as well as other specific contaminants in Google Scholar and Web of Science (Appendix D.1). We also conducted a backwards search via references in those publications included in the original search, to add additional publications within our scope. We included studies that reported concentrations of metal in the liver of *L. canadensis* or *N. vison*, either wild or farmed (in the case of mink) in North America and provided both a mean and measure of variation to calculate standard deviation. If a study did not include standard deviation or error, it was excluded unless it was the only report of a given metal, in which case a standard deviation was estimated based on provided minimum, maximum, and median values. Only values measured from liver tissue were included.

For each study we recorded the species, location (state or province), time of sampling, sample size, the metal reported, whether the metal was reported in wet weight or dry weight, and average concentration and standard deviation (if standard error or confidence interval was provided, we calculated standard deviation from this information). Values provided in wet weight were then converted to dry weight using an assumed percent moisture of 70% if no percent moisture was provided, which has been previously reported as a general average for mustelids (Blus et al. 1987). We excluded any studies that did not provide a measure of variance or report sample size, we excluded any reported concentrations from a sample size of less than 8 animals. We excluded reported values of Cu from Stejskal et al. (1989) in farmed mink, as the authors reported the feed was Cu supplemented. If our search did not provide at least two reports for a given metal, we expanded our parameters to include publications of other species of otter or mink in other parts of the world.

4.2.2 Calculation of historical trace metal concentrations

To create a standard for comparison of the trace metals measured by this study in Alberta and British Columbia, we calculated the weighted mean and pooled standard deviation of each metal from the data collected by the literature search, using the following formulas:

$$\bar{x}_w = \frac{\sum n_k \, x_k}{\sum n_k}$$

Where $\bar{x}_w$ is the weighted mean of a given metal, n the sample size and x the average for "k" number of averages.

$$SD_p = \sqrt{\frac{\sum(n_k - 1)SD_k^2}{\sum n_k - k}}$$

Where SD_p is the pooled standard deviation of a given metal, n_k the sample size and SD_k the standard deviation for "k" number of averages.

The weighted mean was then used as a threshold to determine if concentrations observed in otter and mink in this study were "elevated" or not. Elevated was defined as any concentration above the weighted mean for that metal. Since information about the toxicity of essential metals is scarce, and some may vary widely, "elevated" for the essential metals that may be tolerated at higher levels, Fe, Mn, Rb, and Zn, was defined as any concentration above the weighted mean plus one pooled standard deviation as a more conservative threshold. These values were also used to determine a standardized mean difference to quantify the magnitude of the difference between our results and previous reports:

$$d = \frac{x_m - \bar{x}_w}{SD_p}$$

Where d is the standardized mean difference, x_m the mean concentration of a given metal in our animals, $\bar{x}_w$ the weighted mean and SD_p the pooled standard deviation from the literature.

4.2.3 Sample Collection

River otter and mink were collected from licensed fur trappers and necropsied according to the methods outlined in Section 3.2. During necropsy, livers were removed for pollutant analysis, stored in chemically cleaned (triple rinsed with acetone and hexane, Optima Grade, Fisher Scientific, Ottawa, ON) amber glass jars and then frozen at -20ºC and shipped to the National Wildlife Research Center (NWRC; Ottawa, ON) for analysis. Livers were then thawed and homogenized without homogenization buffer according to protocols of the NWRC (#SOP-TO-PROC-07F). For trace element analysis, an aliquot of ~3.0g (wet weight; ww) was tested at the NWRC.

4.2.4 Trace metal analysis

For trace element analysis, samples were stored in pre-weighed, acid-washed, polypropylene vials, and their exact wet weight recorded. They were then freeze-dried for at least 48h at -50 ºC, their dry weight recorded immediately after, and the difference used as percent moisture. After drying, samples were stored in a desiccator at room temperature. Around 100g of the dried sample was put in digestion tubes (15ml DigiTUBEs, SCP Science) and mixed with nitric acid (1.5mL- trace metal grade, Fisher Scientific, Ottawa, Canada), along with a blank and quality control sample. After being left at room temperature overnight, samples were heated to 70 ºC for 100 minutes in a DigiPrep MS Graphite Digestion system (SCP Science) then at 100 ºC for 330 minutes, shaken periodically by hand. Samples were left to cool overnight at room temperature. The volume was then adjusted to 5mL with ultrapure water and stored at room temperature until analysis.

After digestion, samples were tested for trace elements at the National Wildlife Research Center, (Ottawa, ON) using a PerkinElmer NexION 300d inductively-coupled plasma-mass spectrometer (ICP MS) (SOP# MTH-MET-TE-01C), against certified atomic absorption standards (Sigma-Aldrich Chemical Company, Oakville, ON, Canada). Quality assurance and control also included correction for percent recovery of each internal standard, calibration blanks in each set of 9 samples, and duplicate/triplicate extractions for a random subset of liver samples, cross-contamination prevented by an injection of 1% HNO_3 blanks between samples and calibration standards (see Appendix C for reference standards and detection limits; S4.1 Final results were reported in µg/g (dry weight; dw)).

4.2.5 Spatial analysis

There were no a priori assumptions made about the spatial scale at which metal contamination would interact with our focal species, therefore all were calculated at 2 spatial scales: within the *subwatershed* and species home range *buffer*. For otters, the home range was defined as a 150 sq. km circular buffer around the sample point (*i.e.*, a circle with radius 6.910 km). The *buffer* was calculated as 1.5 times the typical 80 sq. km home range for male otters, plus 30 sq. km to account for uncertainty in the location along a trapline the sample may have been taken. For mink, a similar approach was taken based on a 6 sq. km home range (6 sq. km * 1.5 + 30 sq. km) resulting in a *buffer* of 39 sq. km (*i.e.*, a circle with radius 3.523 km). Watershed and subwatershed boundaries were obtained from the National Hydro Network (NHN), which was published by Natural Resources Canada (NRCan; Government of Canada, 2017a). NHN data were created using the best available data in partnership with various federal, provincial, and territorial partners. Subwatersheds are also called NHN Work Units or Sub-Sub-Drainage Areas, while watersheds are referred to as Sub-Drainage Areas. Spatial analyzes were conducted using R Programming Language version 4.1.0 (R Core Team, 2021), ArcPro version 3.1.1 (ESRI Inc., 2023), and QGIS version 3.22.7 (QGIS Development Team, 2021). Watersheds were on average ~50,000 km^2 and subwatersheds ~6,000 km^2 in total area. It should be noted that watersheds and subwatersheds on the Alberta-Saskatchewan border only included the portions located in Alberta as data was only available for anthropogenic drivers there. A small portion of the Lower Central Athabasca watershed, specifically the Christina and Clearwater subwatersheds, are located in Saskatchewan.

We calculated spatial explanatory variables representing potential anthropogenic sources of trace metals in the environment including *crop cover*, *wildfire cover, road density*, *oil and gas sites*, *urban cover*, *industrial intensity*, *mine count, mine distance*, and *pulp and paper mills*.

Crop cover. The 2021 Annual Crop Inventory was used to identify land cover classified as crops (Agriculture and Agri-Food Canada, 2021). The *crop cover* variable was created using the crop raster data: percent crop cover within the subwatershed and a binary variable indicating presence or absence of crops within the species' home range. A binary variable at the home range scale was calculated as there was little variability in percent cover, resulting in 2 variables.

Wildfire cover. The *wildfire cover* variable was calculated using the 2020 Canadian National Fire Database's (CNFDB) National Burned Area Composite (NBAC) polygon dataset for land classified as burned within the last 10 years (2011 to 2020) (Hall et. Al 2020). Percent area burned by forest fire was calculated within the subwatershed and a binary variable indicating presence or absence of forest fire within the home range, resulting in 2 variables.

Road density. Linear road data were obtained from the transport features of the 2023 CanVec series (Government of Canada, 2023a), which is derived from the National Road Network (NRN). *Road density* was calculated as kilometer of road per total area of the spatial scale, resulting in 2 variables.

Oil and gas sites. Point data representing locations of oil and gas wells and facilities were obtained from the resource management features of the 2023 CanVec series (Government of Canada, 2023b). *Oil and gas sites* was calculated as total number of sites in the subwatershed and home range buffer, resulting in 2 variables.

Industrial intensity. Point source pollutant releases were calculated using the 2021 National Pollutant Release Inventory (NPRI), which is Canada's public inventory of releases, disposals, and transfers of over 320 pollutants to air, water, and land (Government of Canada, 2021). Pollutant releases are submitted by factories, mines, oil and gas operations, power plants, and sewage treatment plants that meet one of three criteria: 1) employees of the facility work a combined total of more than 20,000 person hours annually, 2) specific activities take place at the facility (e.g., fuel combustion, wastewater treatment, wood preservation), or 3) NPRI substances are used, processed, or manufactured above reporting thresholds.

For the purposes of this analysis, we focused on eight trace elements of concern: Pb, As, Cd, Se, Co, Hg, Zn, and Mn. Due to the expected pathway of exposure for mink and otter, only releases to air and water were considered. For each of the eight substances and two exposure pathways (i.e., air and water) industrial releases were measured as the intensity of the substance released (amount per sq. km) per year. Each metric was calculated based on facilities located in the subwatershed (home range buffers were too small to contain any reporting facilities), resulting in a total of 16 variables. Pollutant release metrics were calculated as the 5-year average of releases for each substance (2016-2020). All were measured in kg, except Zn and Mn, which were measured in tonnes. Air and water releases were also used to calculate total *industrial intensity* which was calculated as the sum of releases for all 8 metals in both air and water transformed into a ranged scale, resulting in 1 additional variable.

Mine count and *mine distance.* Mine-related variables were calculated using two datasets: 1) 2023 CanVec mine locations from the resource management features series (Government of Canada, 2023b), and 2) 2021 producing mine locations from Natural Resources Canada (Government of Canada, 2022). The CanVec mine data contains the locations of ore extraction sites as points, and polygons for sites with a larger footprint, then converted to points by calculating the centroid of each polygon. The producing mines data were compiled by NRCan's Lands and Minerals Sector and the Canada Energy Regulator, in collaboration with provinces and territories, and represent mines in production as of 2021. *Mine count* was calculated as the total number of mines for the two spatial scales resulting in 2 variables. Last, the distance from each sample point to the nearest mine (km) was calculated, which produced two separate values from the CanVec and producing mine datasets, *mine distance* was determined as the minimum distance to a mine between the CanVec and producing mine datasets resulting in 1 variable.

Pulp and paper mills. Presence absence data of pulp and paper mills was determined from previous reports (Lima and Wrona 2019, Branch 2020). This binary moderator was determined only at the subwatershed level due to the limited number of mills.

All quantitative continuous variables were transformed to a ranged scale keeping the relative distance between values constant.

$$y_{new} = \frac{(y_{old} - y_{min})}{(y_{max} - y_{min})}$$

4.2.6 Statistical analysis

Statistical analysis was carried out in R version 4.2.1 (R Core Team, 2022), using R studio (Rstudio Team 2020) using the packages *car* (Fox and Weisberg 2019)*, lme4* (Bates et al. 2015)*, MASS* (Venables and Ripley 2002), *MuMIn* (Barton 2023), and *ggplot2* for graphical representation (Wickham 2016). Working within a multiple working hypothesis framework, we compared the effect of spatial explanatory variables on the concentration of 11 metals (Ag, Cd, Co, Cu, Fe, Hg, Mn, Pb, Rb, Se, Zn) hypothesized to be influenced by anthropogenic sources (Table 4.1). We used Generalized Linear Mixed Models (GLMM) to account for non-independence of repeated sampling by individual trappers, with the trapper included as a random effect along with subwatershed to account for spatial correlation on a larger scale. Early analysis of the differences in metal concentrations and sex showed minimal non-significant differences between males and females, except in the case of metals detected in a much smaller percentage of animals like Al or Sr (Figure S4.2) Due to a lack of hypotheses about the role of sex in acquiring contaminants, we excluded sex from further analyzes. For some metals, there was no obvious correlation with species, while for others there were differences between species. We retained species as a random effect given known behavioral and dietary differences between otter and mink, which may predispose otters to higher concentrations due to the higher proportion of fish in their diet (Larivière and Walton 1998, Larivière 1999).

We sought to model the effects of anthropogenic sources on the extent to which liver metal concentrations were elevated above average values from previous reports rather than overall concentration of metals, because levels above the average are more likely to be indicative of contamination leading to detrimental health effects. We thus transformed our response variable (metal concentration) to a standardized mean difference from the calculated weighted mean from animals in previous reports. Explanatory variables included anthropogenic sources hypothesized to contribute to each metal concentration (Table 4.1) and animal age in years, as age is often reported to be a significant factor due to trophic transfer and bioaccumulation over time. We also included an interaction between age and all anthropogenic sources to determine if the observed interactions were due to high environmental levels and short term exposure (if not correlated with age) or due to long term accumulation if older animals were more likely to have higher concentrations (Harding et al. 1998, Dehn et al. 2006, Dietz et al. 2019), hypothesizing that *age* would have a strong effect on the observed liver concentration of most metals.

For all eleven metals, we ran two models: one with the explanatory variables calculated at the subwatershed scale and the other with explanatory variables calculated at the home range buffer scale. This was the case for all variables except *mine distance* which was calculated at the individual level and used only for the home range buffer models, and *mine count* which was used at the subwatershed level, and *pulp and paper mills* and *industrial intensity* which were measured at the subwatershed level and used for both the subwatershed and home range buffer models. The GLMMs were run using the glmer function from the lme4 package and included the hypothesized explanatory variables, including any hypothesized interaction effects (Table 4.1), and the interaction effect between *age* in years and all anthropogenic moderators, family Gaussian with the "identity" link function. We investigated the effect of our moderators and interaction effects via multimodel inference by a stepwise removal of non-significant effects ('ANOVA' function, *car* package) selecting the best fit model via the Akaike Information Criterion ('AICc' function, *MuMIn* package), all models checked for multicollinearity between variables via Variance Inflation Factor ('vif' function, *car* package). A histogram of all best fit model residuals was checked for normalcy, and variance explained examined via R^2 ('r.squaredGLMM' function, *MuMIn* package).

4.3 Results

4.3.1 Historical reports and calculated averages

Our literature search identified 29 publications reporting 299 average trace metal liver concentrations. While most target metals had at least one record, the most frequently tested and reported were Cd (n_{Cd}=33), Cu (n_{Cd}=31), Hg (n_{Hg}=47), Pb (n_{Pb}=33), and Zn (n_{Zn}=27). Six metals, Sb, Ga, Rb, Ag, Tl, and V had only one record, and most of these came from a study investigating river otters from contaminated sites around oil sands in Alberta (Guo et al. 2020). There were no reports of Bi. Expanding the search to include other species of otter and mink globally yielded an additional four studies. Two studies provided information about Ag in *N. vison* in Sweden, where that species is invasive (Ljungvall et al. 2017), and in Eurasian river otter (*Lutra lutra*) in the United Kingdom (Brand et al. 2020). Only one report of Rb was found in otters (*L. lutra*) in Italy (Esposito et al. 2020). No other reports of Bi, Ga, Sb, or V from liver tissue that fit our reporting standards could be found. Since we had no reports of Bi, we used the average from a report on sea otters (*Enhydra lutris*) in California that had been excluded as they did not include a measure of variance, estimating a standard deviation based on their reported minimum, maximum, and median (Kannan et al. 2006).

Table 4.2 Weighted means of trace metals in otter and mink of North America. The calculated weighted mean (X_w) and pooled standard deviation (SD_p) for historical reports of trace metals of concern, including the number of reported averages (*N*) used to calculate the weighted mean, the location the animals were sampled from, and the range of years the reported concentrations were collected in. Note that for Ga and V where only 1 report was available, we provide the average and standard deviation reported by the study, instead of a weighted mean and pooled standard deviation.

Metal	***N***	X_w	SD_p	**Location**	**Period**	**Reference**
Ag	3	0.113	0.177	AB, Sweden[1], UK[2]	2004-2017	(Ljungvall et al. 2017, Brand et al. 2020, Guo et al. 2020)
Al	8	6.232	5.452	BC, NT, ON	1984-1996	(Wren 1984, Poole et al. 1995, Harding et al. 1998)
As	4	0.456	0.271	AB, MT, NC	1992-2017	(Szumski 1998, Guo et al. 2020, Sanders et al. 2020)
Ba	4	0.061	0.103	AB, BC	1990-2017	(Harding et al. 1998; Guo et al. 2020)
Bi	1	0.01	0.001*	CA[3]	1992-2002	(Kannan et al. 2006)
Cd	33	0.312	0.485	AB, BC, ID, MT, NC, NT, NY, ON, VA, WA, Farmed	1981-2016	(Anderson-Bledsoe and Scanlon 1983, Ogle et al. 1985, Blus et al. 1987, Stejskal et al. 1989, Blus and Henny 1990, Poole et al. 1995, Harding et al. 1998, Szumski 1998, Martin et al. 2011, Mayack 2012, Sanders et al. 2020)
Co	5	0.114	0.070	AB, BC, NC	1990-2016	(Harding et al. 1998, Guo et al. 2020, Sanders et al. 2020)
Cu	31	21.59	18.60	AB, BC, ID, IL, MT, NC, NT, ON, VA, WA, Farmed	1981-2016	(Anderson-Bledsoe and Scanlon 1983, Ogle 1984, Wren 1984, Blus et al. 1987, Stejskal et al. 1989, Poole et al. 1995, Halbrook et al. 1996, Harding et al. 1998, Szumski 1998, Brzezinski et al. 2014, Guo et al. 2020, Sanders et al. 2020)
Ga	1	0.01	0.002	AB	2017	(Guo et al. 2020)
Hg	47	5.643	6.183	AB, BC, ID, ON, NY, YK, NS, IL, QC, NC, WI, MT, Farmed	1981-2017	(Wren 1984, Wren and Stokes 1986, Blus et al. 1987, Foley et al. 1988, Halbrook et al. 1996, Harding et al. 1998, Szumski 1998, Evans et al. 2000, Mierle et al. 2000, Fortin et al. 2001, Gamberg et al. 2005, Klenavic et al. 2008, Strom 2008, Haines et al. 2010, Martin et al. 2011, Mayack 2012, Brzezinski et al. 2014, Guo et al. 2020, Sanders et al. 2020)
Fe	21	1039.1	324.2	AB, BC, IL, NC, NT, ON, Farmed	1984-2016	(Wren 1984, Stejskal et al. 1989, Poole et al. 1995, Halbrook et al. 1996, Harding et al. 1998, Guo et al. 2020, Sanders et al. 2020)
Mn	22	8.299	3.338	AB, BC, IL, NC, NT, ON, Farmed	1984-2017	(Wren 1984, Stejskal et al. 1989, Poole et al. 1995, Halbrook et al. 1996, Harding et al. 1998, Brzezinski et al. 2014, Guo et al. 2020, Sanders et al. 2020)

Mo	8	2.381	0.711	AB, BC, IL, NC, Farmed	1984-2017	(Halbrook et al. 1996, Harding et al. 1998, Brzezinski et al. 2014, Guo et al. 2020, Sanders et al. 2020)
Ni	7	1.103	0.711	AB, ON, NT, Farmed	1984-2017	(Wren 1984, Poole et al. 1995, Brzezinski et al. 2014, Guo et al. 2020)
Pb	33	0.886	4.549	BC, ID, MT, NC, NT, NY, ON, VA, WA	1981-2011	(Anderson-Bledsoe and Scanlon 1983, Ogle et al. 1985, Blus et al. 1987, Blus and Henny 1990, Poole et al. 1995, Harding et al. 1998, Szumski 1998, Martin et al. 2011, Mayack 2012, Brzezinski et al. 2014, Sanders et al. 2020)
Rb	3	13.44	3.342	AB, Italy[2]	1998-2017	(Esposito et al. 2020, Guo et al. 2020)
Se	19	4.150	2.115	AB, BC, NC, ON, YT, Farmed	1984-2017	(Wren 1984, Harding et al. 1998, Gamberg et al. 2005, Martin et al. 2011, Brzezinski et al. 2014, Guo et al. 2020, Sanders et al. 2020)
Sb	1	0.650	0.06*	IL	1984-2002	(Halbrook et al. 1996, Kannan et al. 2006)
Sn	3	2.697	1.213	BC	1990-1996	(Harding et al. 1998)
Sr	5	0.253	0.226	AB, BC	1990-2017	(Harding et al. 1998; Guo et al. 2020)
Tl	1	0.04	0.01	NC	2009-2016	(Sanders et al. 2020)
V	1	0.09	0.03	AB	2017	(Guo et al. 2020)
Zn	27	81.72	23.38	AB, BC, ID, IL, MT, NC, NT, ON, VA, WA, Farmed	1981-2017	(Anderson-Bledsoe and Scanlon 1983, Wren 1984, Blus et al. 1987, Poole et al. 1995, Halbrook et al. 1996, Harding et al. 1998, Szumski 1998, Guo et al. 2020, Sanders et al. 2020)

[1]Invasive North American mink, *Neogale vison*, [2]Eurasian otter, *Lutra lutra*, [3]California sea otter, *Enhydra lutris*, *SD was estimated using provided median, minimum, and maximum values against the mean.

4.3.2 Tissue trace metal concentrations

Average metal concentrations in the livers of river otter and mink sampled in British Columbia and Alberta were broadly similar to previous reports from across North America, with some exceptions, and are summarized in Table 4.3. Metal concentrations did not vary strongly between males and females or between otter and mink. However, for some metals, especially the non-essential metals like Ag, Al, and Sr, elevated levels were found mostly in adults. Concentrations were similar in otter and mink, except in the case of rarely detected metals like Ag, Al, and As that were seen mostly in otters in British Columbia. Strontium was rarely detected, found mostly in mink in Alberta (Figure 4.1). In general animals in British Columbia had higher concentrations of Ag, Al, As, Cd, Co, and Se while other trace metal concentrations were generally similar between the provinces.

Table 4.3 Trace metals in Alberta and British Columbia. Mean and standard deviation of detected trace metals in both river otter and mink livers (µg/g dw), including the number of animals (*k*) in which the metal was detected and the range of concentrations (lowest detected to highest), the standardized mean difference (*d*; positive values indicate elevated levels), given by the province in which they were trapped out of the total sample size (n) of animals tested.

	Alberta (n=89)				**British Columbia (n=63)**			
Metal	***k***	**Mean (SD)**	**Range**	***d***	***k***	**Mean (SD)**	**Range**	***d***
Ag	82	0.067 ± 0.055	0.014-0.343	-0.260	52	0.257 ± 0.724	0.02-3.96	0.814
Al	0	ND	-	-	7	29.34 ± 25.14	6.44-69.2	4.238
As	12	0.061 ± 0.013	0.043-0.085	-1.463	27	0.487 ± 0.608	0.04-2.08	0.114
Ba	10	0.137 ± 0.112	0.045-0.455	0.738	39	0.064 ± 0.019	0.041-0.116	0.029
Bi	47	0.007 ± 0.005	0.003-0.029	-3.000	27	0.021 ± 0.033	0.012-0.172	11.00
Cd	89	0.096 ± 0.162	0.006-1.22	-0.445	63	0.969 ± 3.340	0.01-25.8	1.355
Co	25	0.095 ± 0.027	0.069-0.181	-0.271	25	0.144 ± 0.085	0.07-0.41	0.429
Cu	89	26.02 ± 13.53	10.1-73.2	0.238	63	30.73 ± 21.96	8.48-137	0.491
Fe	89	1416.7 ± 569.9	623-5680	1.165	63	1126.3 ± 641.2	442-5700	0.269
Ga	89	0.064 ± 0.008	0.046-0.093	27.00	63	0.072 ± 0.008	0.055-0.102	31.00
Hg	89	3.546 ± 3.011	0.256-23.5	-0.339	63	3.583 ± 3.500	0.157-20.1	-0.333
Mn	89	8.932 ± 2.635	3.96-26.2	0.190	63	8.212 ± 2.178	2.80-13.0	-0.053
Mo	89	1.980 ± 0.215	0.534-4.11	-0.564	63	1.737 ± 0.441	1.60-3.79	-0.906
Ni	44	0.548 ± 1.172	0.113-7.65	-0.781	47	0.304-0.259	0.113-1.69	-1.124
Pb	43	0.095 ± 0.277	0.011-1.79	-0.174	62	0.302 ± 0.648	0.015-3.43	-0.128
Rb	89	17.80 ± 15.79	3.76-154	1.305	63	15.64 ± 17.76	3.07-141	0.658
Sb	2	0.031 ± 0.011	0.02-0.042	-10.32	6	0.204 ± 0.266	0.021-0.731	-7.433
Se	89	2.327 ± 0.755	0.858-5.35	-0.862	63	4.496 ± 3.298	1.27-16.9	0.164
Sn	1	0.622	-	-1.711	6	0.214 ± 0.055	0.129-0.293	-2.047
Sr	3	0.608 ± 0.215	0.428-0.911	1.571	13	6.362 ± 9.046	0.350-26.9	27.03
Tl	33	0.007 ± 0.004	0.004-0.018	-3.300	29	0.013 ± 0.013	0.004-0.057	-2.700
V	89	0.019 ± 0.010	0.006-0.060	-2.367	63	0.056 ± 0.043	0.014-0.311	-1.133
Zn	89	81.32±26.38	48.6-284.0	-0.017	63	84.21 ± 18.59	56.8-115	0.107

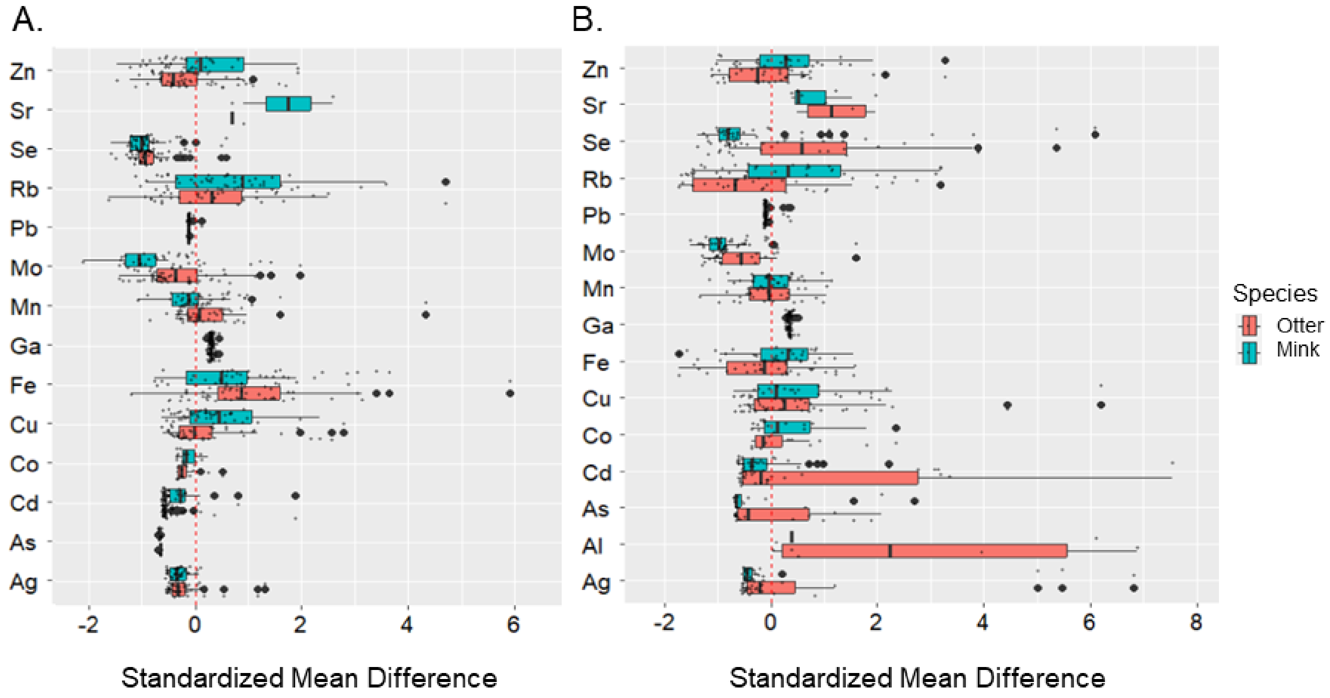

Figure 4.1 Standardized mean difference of trace elements. Box and jitter plot of observed metal concentrations presented as the standardized mean difference against the weighted mean of previously reported values, values of zero represent no difference, highlighted as a dashed red line, negative values lesser than and positive values greater than the weighted mean, values greater than 10 excluded. Including various metals (y-axis) presented by species (red=otter, blue=mink) and by province, A) Alberta and B) British Columbia.

4.3.3 Effect of anthropogenic sources

Several anthropogenic sources of trace metal contaminants were significantly correlated with elevated trace metal levels in mink and otter liver tissue at both the subwatershed and home range buffer spatial scales. We found significant relationships for most metals at both spatial scales, except for Rb in either spatial scale and Ag and Zn in the home range buffer models (Table 4.4). Variance explained by the included moderators, both fixed and random (R^2 conditional), varied between metals, the best models included those for Ag ($R^2_{sw} = 0.507$; $R^2_{buffer} = 0.524$), Cd ($R^2_{sw} = 0.991$; $R^2_{buffer} = 0.992$), Co ($R^2_{sw} = 0.358$; $R^2_{buffer} = 0.394$), Cu ($R^2_{sw} = 0.439$; $R^2_{buffer} = 0.480$), and Se ($R^2_{sw} = 0.616$; $R^2_{buffer} = 0.613$) (Table S4.2).

Table 4.4 Summary of significant explanatory variables. Table of variables that best explained increases in hepatic metal concentrations for each best fit model by spatial scale (subwatershed or home range buffer), including the significant variables from the model (significant *p*-values denoted by asterisk; ***<0.001, **<0.01, *<0.05, near significant values denoted by $^{-}$), interaction effects denoted by a colon between variables, and the variable's estimate, and standard error (*se*).

	Subwatershed			Home Range Buffer		
Metal	Significant Variables	Estimate	*se*	Significant Variables	Estimate	*se*
Ag	(Intercept)	0.05667	0.33547	No significance	-	-
	Age	0.03185	0.07761			
	Industrial Intensity*	-1.08783	1.19075			
	Mine Count:Age***	6.10625	1.18017			
	Industrial:Age***	-4.85353	1.41792			
Cd	(Intercept)	1.46733	1.55595	(Intercept)	2.07255	1.66704
	Age***	-1.43662	1.31786	Industrial Intensity	-1.61529	3.86999
	Road Density***	0.06581	0.04521	Urban Cover***	-31.79289	8.36616
	Road Density:Age***	0.41511	0.10713	Age***	0.08914	0.04307
				Industrial Intensity:Age*	1.95779	0.76647
Co	(Intercept)	-0.73246	0.07121	(Intercept)	-0.67154	0.05723
	Industrial Intensity***	1.92572	0.23690	Industrial Intensity***	1.83591	0.23680
	Age	0.05898	0.02494	Age	0.03172	0.01965
	Oil and Gas	0.35045	0.25748	Industrial Intensity:Age***	-1.13497	0.22050
	Industrial Intensity:Age***	-1.19437	0.22553			
	Oil and Gas:Age^{-}	-0.16511	0.08863			
Cu	(Intercept)	-0.80661	0.14311	(Intercept)	-0.83267	0.17706
	Age	-0.06757	0.02858	Mine Distance	0.65173	0.45142
	Pulp and Paper	-0.02576	0.22593	Crop Cover*	-0.37879	0.18949
	Pulp and Paper:Age*	0.13406	0.05778	Mine Distance:Age*	-0.18478	0.07354
Fe	(Intercept)	0.31401	0.29421	(Intercept)	0.48468	0.36529
	Age	0.10003	0.10828	Age	-0.02879	0.07257
	Oil and Gas***	4.22087	0.98937	Oil and Gas**	4.56211	1.07234
	Oil and Gas:Age*	-0.77800	0.35978	Oil and Gas:Age**	-1.17613	0.42132
Hg	(Intercept)	-0.38728	0.08280	(Intercept)	-0.54193	0.11183
	Age*	0.02665	0.02087	Mine Distance*	0.58759	0.23457
	Industrial Intensity	-0.51929	0.27511	Age*	0.02301	0.02293
	Oil and Gas*	0.50603	0.22080	Road Density	0.12952	0.41494
	Industrial Intensity:Age*	0.55449	0.24401	Road Density:Age*	0.60485	0.26321
Mn	(Intercept)	0.07749	0.11738	(Intercept)	0.23261	0.10265
	Age	-0.06200	0.02950	Age	-0.04251	0.02567
	Urban Cover	-0.46400	0.34232	Urban Cover	-0.17882	1.39602
	Crop Cover*	0.59280	0.23427	Pulp and Paper*	-0.35152	0.17486
	Urban Cover:Age*	0.23626	0.10134	Urban Cover:Age*	1.62134	0.74867
Pb	(Intercept)	-0.119199	0.007530	(Intercept)	-0.119199	0.007530
	Age	0.003738	0.002763	Age	0.003738	0.002763
	Pulp and Paper***	0.0773614	0.013713	Pulp and Paper***	0.0773614	0.013713
	Pulp and Paper:Age**	-0.017699	0.005497	Pulp and Paper:Age**	-0.017699	0.005497
Rb	No significance	-	-	No significance	-	-
Se	(Intercept)	-0.54274	0.21921	(Intercept)	-0.58504	0.21312
	Mine Count***	2.90390	0.70770	Age	0.02160	0.03654
	Age	-0.01612	0.04095	Urban Cover	0.35049	2.24388
	Urban Cover*	-0.25343	0.54207	Industrial Intensity*	1.98116	0.64720
	Urban Cover:Age***	0.57630	0.15145	Urban Cover:Age***	3.76960	1.06812
	Industrial Intensity:Age**	-1.51668	0.52362	Industrial Intensity:Age*	-1.09177	0.52096
Zn	(Intercept)	-0.17038	0.19152	(Intercept)	-0.1470	0.2005
	Age	0.03252	0.04765	Mine Distance	0.8705	0.5492
	Wildfire Cover	-0.00465	0.51094	Oil and Gas^{-}	1.0946	0.5846
	Crop Cover	-0.95052	0.59972			
	Oil and Gas*	2.92188	0.79391			
	Oil and Gas:Age**	-0.59944	0.18719			
	Wildfire:Crop Cover**	24.61433	9.13441			

At the subwatershed scale, the best predictor of elevated Ag was *mine count* as an interaction effect with *age*, followed by *industrial intensity* which was negatively associated with Ag levels including as an interaction effect with *age*, there were no significant effects at the home range buffer scale. This suggests Ag contamination is caused predominantly by mining activity and that levels in otter and mink are likely due to long-term bioaccumulation, hence the significance of age in the model (Figure 4.2A), while high levels of Ag contamination from industrial activity may also in part explain exposure in younger animals especially in areas where mining was not occurring.

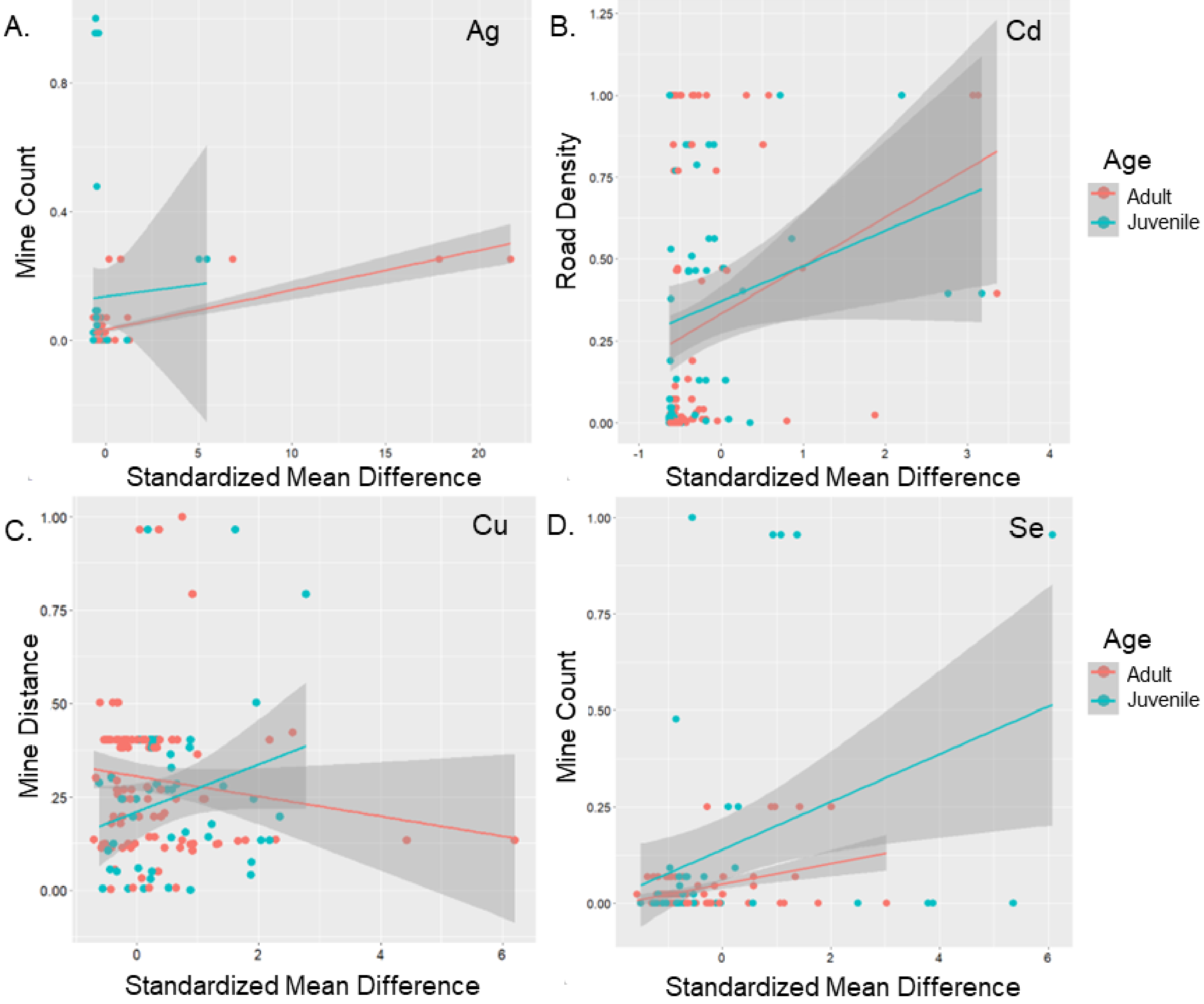

Figure 4.2 Effects of anthropogenic moderators on elevated metal concentrations. Scatterplot of the standardized mean difference of liver metals against significant anthropogenic sources by age class (juvenile < 1 year, adult > 1 year), A) silver by number of mines in the subwatershed, B) cadmium by road density in the subwatershed, values greater than 4 excluded C) cobalt by industrial intensity in the subwatershed, D) selenium by mine count in the subwatershed.

Elevated levels of Cd were best explained in the subwatershed model by the interaction effect of *road density* and *age* as well as by *age* and *road density* alone (Table 4.4; Figure 4.2B). At the home range buffer scale, Cd levels were best explained by *age* and the interaction effect of *age* and *industrial intensity* which had a positive association with Cd levels. The model also showed a very strong negative association with *urban cover* (Figure S4.2). The results suggest that long term bioaccumulation from fossil fuel emissions is the main cause of Cd exposure, and not direct water run-off from urban centers, while localized waste-water output from industrial activity may be an important source of localized high intensity exposure.

Elevated levels of Co were best explained in the subwatershed model by *industrial intensity* and likely due to high environmental exposure as opposed to long term bioaccumulation as the significant interaction effect of *age* and both *industrial intensity* altered the directionality of the effect to be negative (Table 4.4). The interaction effect of *oil and gas sites* and *age* was also nearly significant (p=0.06), also altering the directionality of the effect from positive to negative, suggesting that industrial intensity is the main driver exposure, but oil and gas activity may be a contributing factor at a smaller scale. In the home range buffer model, Co concentrations were best explained by *industrial intensity*, its interaction effect with *age* also significant. The strong negative interaction effect of *age* and *industrial intensity* suggests that observed concentrations are due to high levels in the environment as opposed to long term bioaccumulation.

Copper concentrations were best explained in the subwatershed model by the interaction effect of *pulp and paper mills* and *age*, which was positively associated with elevated Cu levels. In the home range buffer model, Cu concentrations were best explained by the interaction effect of *mine distance* and *age*, which showed a negative effect while *mine distance* on its own showed a positive effect, meaning Cu concentrations increased with a decrease in distance to the closest mine but only in relation to the animals age, *crop cover* was also negatively correlated with Cu concentrations. These results suggest that long-term accumulation is likely the main driver of elevated Cu levels in mink and otter and are highest in adults in areas close to mining activity (Figure 4.2C), while contamination from the pulp and paper mills may contribute on a broadscale to fish exposure and ultimately bioaccumulation in otters and mink.

Mercury levels were best explained in the subwatershed model by *oil and gas count* and the interaction effect of *industrial intensity* and *age*, both positively associated with Hg levels. In the home range buffer model, *mine distance* and the interaction effect of *road density* and *age* were significantly positively associated with Hg concentrations (Table 4.4). This suggests that Hg levels are driven broadly by high environmental concentrations due to oil and gas and mining activities not related to long term accumulation, while outputs from industry and fossil fuel burning may be at lower levels and require accumulation to explain the levels observed, which is why they were only significant as an interaction effect with age.

Observed levels of Pb were explained both in the subwatershed and home range buffer models by *pulp and paper mills* and their interaction effect with *age*. The presence of pulp and paper processing plants had a very significant positive association with increasing levels of Pb, while the interaction effect with age in years led to a negative association, suggesting that high environmental levels are the cause of the observed concentrations as opposed to long term accumulation. It should be noted that the three animals with the highest observed levels of Pb were trapped near Crofton, BC on Vancouver Island, next to the Crofton pulp and paper mill.

Concentrations of Se were best explained in the subwatershed model by *mine count* and the interaction effect of *age* and both *urban cover* and *industrial intensity* (Table 4.4). The strongest effect was due to the number of mines, which were positively associated with increases in selenium concentrations (Figure 4.2D). Urbanization was negatively correlated with Se until its interaction effect with *age* was considered. The interaction effect of *industrial activity* and *age* was negatively associated with Se levels. At the home range buffer scale, the interaction effect of *urban cover* and *age* best explained Se levels, at this scale *industrial intensity* was positively associated with Se though became negatively associated when the interaction effect with *age* was considered. Our results suggest that selenium contamination is largely due to the presence of mining activity and urbanization with high concentrations in the environment due to mining, while long-term accumulation may be due to urbanization at a local scale as *urban cover* showed the strongest association at the buffer scale as an interaction effect with *age*. Industrial activity is also an important driver of high concentrations in the environment at smaller geographic scales.

4.3.4 Geographical areas of significance

Spatial analysis of trace element concentrations revealed four regions of concern in Alberta and British Columbia due to elevated levels of various metals, including the Central Athabasca, Vancouver Island, Southern Coast, and Columbia River/Thompson watersheds. The Central Athabasca showed elevated levels of Fe, Mn, Rb, and Zn, especially in the Clearwater and Christina subwatersheds (Figure 4.3), which include parts of the Athabasca oil sands and high levels of oil and gas activity (Lima and Wrona 2019). This included 6 animals with Fe concentrations ranging from 2030-3070 µg/g dw, 4 with elevated Co (0.111-0.181 µg/g dw), 3 with elevated Hg including the highest recorded value (23.5 µg/g dw), 2 with toxic levels of Ni (2.56-7.65 µg/g dw) (Outridge and Scheuhammer 1993), 56 with elevated Rb, 19 of which were potentially toxic (20.2-154 µg/g dw) (Yamaguchi et al. 2007), 6 with elevated levels of Ag, and 14 with elevated levels of Mo. Adjacent to the Athabasca, the smaller Beaver watershed, which adjoins the Cold Lake oil sands also showed elevated levels of Fe, Hg, Mn, Ni, Rb, Zn, including 4 animals with potentially toxic levels of Hg (>6.67 µg/g dw) (Wren et al. 1987), the highest recorded values of Mn (26.2 µg/g dw) and Zn (284 µg/g dw), some individuals with elevated levels of Ag, Cd, Co, and Sr as well.

In the Columbia and Thompson River valleys, elevated levels of Se were observed, including the two highest concentrations measured, six animals (60%) in the Thompson River valley with elevated levels (max. 15.4 µg/g dw) and 4 (66%) in the Columbia (max. 16.9 µg/g dw). Four animals in the Thompson also had elevated levels of Hg, 3 of which were potentially toxic (6.06-20.1 µg/g dw). Highly elevated levels Rb were also seen in the Thompson River valley, as well as Co in the Columbia (Figure 4.3).

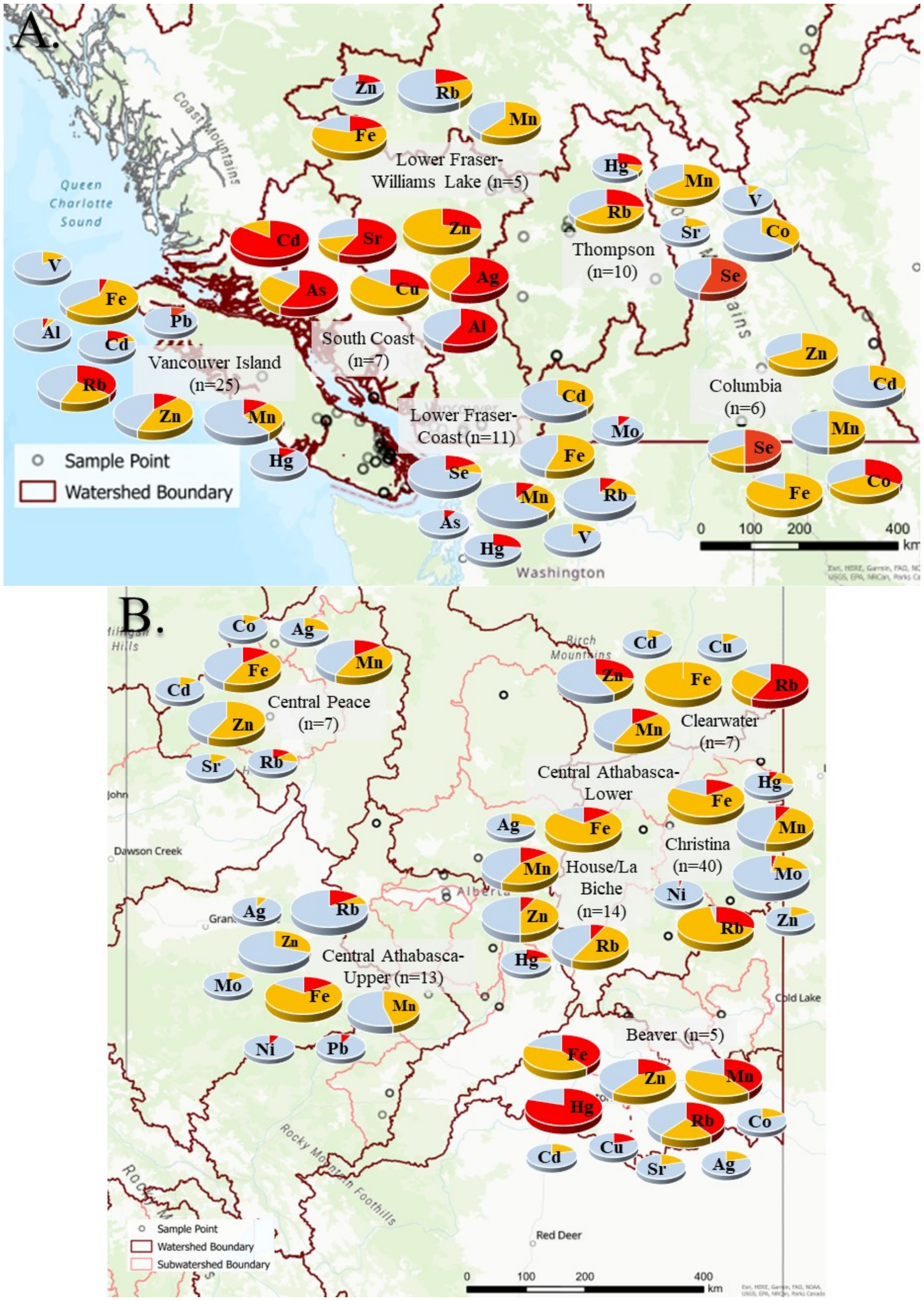

Figure 4.3 Distribution of metals in mink and otter by watershed. Map of sample sites and various metal pollutants detected in mink and otter livers in A) British Columbia and B) Alberta. Watersheds are delineated by dark red lines, subwatershed by light red lines (Alberta only), with the watershed or subwatershed name provided along with the sample size of animals tested. Metal presented as pie charts, represented the proportion of total animals sampled in which any given metal was elevated (above the weighted mean from the literature), presented in yellow, or at potentially toxic levels (either two pooled standard deviations above the weighted mean, or above a known toxic threshold), presented in red. Maps were created in QGIS via information about watershed and subwatershed boundaries obtained from the National Hydro Network published by Natural Resources Canada (NRCan; Government of Canada, 2017a).

In the Southern Coast watershed, animals showed toxic levels of multiple metal contaminants (Al, Ag, As, Cd, Cu, Se, Sr, and Zn) including the highest recorded values for Al (68.2 µg/g dw), Ag (3.96 µg/g dw), Cd (25.8 µg/g dw), Cu (137 µg/g dw), and Sr (26.9 µg/g dw). On Vancouver island otter and mink had elevated levels of (Al, Cd, Ga, Fe, Mn, Pb, Rb, V, Zn) including the highest recorded values of Fe (5700 µg/g dw), Ga (0.102 µg/g dw), Pb (3.43 µg/g dw), and V (0.311 µg/g dw), mainly near Crofton on the coast (Figure 4.3). Three animals were trapped in Crofton had potentially toxic levels of Pb (2.49-3.43 µg/g dw) and Hg (7.49-11.3 µg/g dw), and strongly elevated levels of Mn (5.92-11.8 µg/g dw), Rb (17.7-29.8 µg/g dw), and Zn (83.5-155 µg/g dw), one with the highest recorded level of Pb and the highest recorded level of V, another with the highest recorded level of Fe.

4.4 Discussion

4.4.1 Metal contaminants in Western Canada compared to historical reports

In British Columbia Ag, Al, Bi, Cd, Ga, Rb, Sr and in Alberta Ba, Fe, Ga, Rb, and Sr all had strongly elevated averages compared to historical reports. Metal concentrations were transformed into a standardized mean difference, which can be understood as the proportion of the pooled standard deviation from the literature that separates concentrations measured in this study from the historical average, a difference of at least 60% of the pooled standard deviation (d=0.6) was considered a “strong” difference.

4.4.1.1 Non-essential trace metals

Our results highlighted multiple pollutants of concern for aquatic life in British Columbia and Alberta. Most concerning were the high levels of harmful non-essential metals. Animals from both BC and Alberta also showed elevated levels of Mercury; 18 at potentially toxic levels (>6.67 µg/g dw) (Wren et al. 1987) from the Central Athabasca and Beaver watersheds in Alberta and multiple locations in BC. Two individuals, one from BC and one from Alberta, had extremely high levels (over 20 µg/g dw). Both the Central Athabasca and Beaver watersheds have large oil sands deposits and extensive human activity related to the extraction and processing of oil and gas, which produces metal contaminants in both air and water (Huang et al. 2016) and are likely the main source of Hg in the province (Kelly et al. 2010, Lima and Wrona 2019). Oil and gas sites were a significant driver of Hg only in the subwatershed model and not related to age. Elevated levels of Hg observed in younger animals may thus be due to high environmental concentrations from oil and gas activity, for which atmospheric deposition may be a significant driver. However, on average mercury concentrations were lower than the historic weighted average from otter and mink across North America, though consistent with reports from British Columbia and Alberta (Harding et al. 1998, Guo et al. 2020).

Strontium was elevated in Alberta (0.608 µg/g dw) and severely elevated in British Columbia (6.362 µg/g dw), though detected in a limited number of animals (3% in Alberta, 31% in British Columbia). Previous studies in otter and mink are limited to Western Canada, reporting averages of 0.21 µg/g dw in Alberta (Guo et al. 2020) and 0.45 µg/g dw in British Columbia (Harding et al. 1998). These averages are similar those reported from ringed seals (*Phoca hispida*) in the arctic, with an average of ~0.33 µg/g dw (Campbell et al. 2005b). Five animals from the Sunshine Coast in BC had extremely high, potentially toxic levels (range 4.31-26.9 µg/g dw). No information about specific toxic thresholds in mammals could be found. However, Sr has been reported to have toxic effects on calcium metabolism, and therefore development, due to high calcium demand during growth. It is related to bone disease and has toxic effects on liver function in humans (Morohashi et al. 1995, Chowdhury and Blust 2011, Feng et al. 2023).

Cadmium is one of the most commonly measured metals, leading to a large sample size of reports to calculate the weighted mean from (n=33), which was 0.312 µg/g dw. Reported averages ranged from 0.07 µg/g dw in otter from BC (Harding et al. 1998) to 0.932 µg/g dw in mink from Ontario (Martin et al. 2011). Average concentration of Cd in animals in this study from British Columbia was higher, with an average concentration of 0.969 (range 0.01-25.8) µg/g dw, much higher than the weighted mean. This is also almost 9 times the average for animals that were measured by Harding et al. (1988). The major drivers in the model for cadmium concentrations were road density as it interacts with age at the subwatershed scale. This pattern suggests that the burning of fossil fuels and constant atmospheric deposition of Cd leading to long-term accumulation is the main diver at the broadest scale. In the home range buffer model, the effect of industrial activity as it interacts with age was the main driver, suggesting that the burning of fossil fuels for industrial uses and industrial wastewater may be significant local drivers.

Vanadium, though a widespread and potentially highly mobile and toxic metal, is also infrequently tested in mammals. However, it has recently been identified as an emerging global contaminant of concern due to anthropogenic enrichment via the burning of fossil fuels that are Vanadium-rich (Chetelat et al. 2021, Ścibior et al. 2021). Again, the only study was by Guo et al. (2020), reporting an average of 0.09 (range 0.036-0.15) µg/g dw; though Kannan et al. (2006) reported an average of 0.181 µg/g dw in sea otters, excluded from the weighted average because of a lack of standard error. The authors did not provide the number of animals that had detectable levels. We detected V in every animal tested. Average concentration was lower than previously reported, though with a higher maximum in British Columbia, 0.311 µg/g dw. Vanadium is released in the environment via fossil fuel production, mining, industrial processes, and tanker disasters (Chetelat et al. 2021, Ścibior et al. 2021). The highest values of V were found in animals from coastal regions of Vancouver Island. Since coastal dwelling otter and mink forage in marine habitats, observed high levels of V may be due to shipping related contamination, the Salish Sea one of the busiest shipping lanes in the world (Bendell et al. 2019, Waugh et al. 2022). Future research is needed to analyze V concentrations in the Salish Sea.

We could only find one report for Ga in a study investigating otters (n=18) from contaminated areas around oil sands, with an average of 0.01 µg/g dw and max concentration also of 0.01 µg/g dw. Again, the number of animals with detectable levels were not indicated (Guo et al. 2020). This limitation was not unexpected as Ga is infrequently tested or not detected and therefore not reported (Ciesielski et al. 2006). However, in this study, Ga was detected in every animal investigated, all above the maximum reported by Guo et al. (2020). In Alberta, mean Ga concentration was 0.064 (range 0.046-0.093) µg/g dw (Table 4.3). Little data about Ga toxicity is available, but it can have similar effects to As. Chronic exposure in mice is associated with growth reduction and lower female survival (Venugopal and Luckey 1978). This is a concerning increase and future research is urgently needed.

4.4.1.2 Essential trace metals

For the essential trace metals, values in the literature can vary more widely, and toxic thresholds are not always clear. In this study, Cu was elevated above historical averages in British Columbia (*d*=0.238) and more strongly so in Alberta (*d*=0.491). Elevated concentrations of Cu were distributed throughout all watersheds in both provinces. The diffuse nature of these high concentrations, and the significance of age in the model suggests that the elevated values seen in our animals is due to long term and widespread bioaccumulation of Cu, driven by wood processing and mining activity.

Selenium was slightly elevated in BC (*d*=0.164) and lower in Alberta (*d*=-0.862). However, the historical average was driven by a single report from British Columbia which reported multiple averages from populations around the province. The highest average was 7.41 µg/g dw from the Columbia River watershed (Harding et al. 1998), which has a long history of coal mining and is also where we saw some of the highest values of Se (Johnson et al. 2005). When values from BC were excluded, the adjusted weighted average was 2.88 (±1.98) µg/g dw, against which the standardized mean difference for our animals would be 0.82, a strong difference between the two. This suggests that British Columbia has higher levels of Se contamination on average than other parts of North America such as Alberta, Ontario, North Carolina, and the Yukon (Gamberg et al. 2005, Martin et al. 2011).

Iron was also seen at elevated levels compared to previous reports. Our literature search produced 21 studies reporting liver concentrations with an average of 1029.1 µg/g dw (Table 4.2). We observed an average concentration of 1416.7 (range 623-5680) µg/g dw in our animals, 9 (10%) with levels over 2020 µg/g dw. However, we could not find reference to the threshold at which Fe begins to have detrimental effects. Iron can vary widely in mammals, and therefore is rarely studied due to the difficulties in analysis (Kosik-Bogacka et al. 2019). However, previous reports of river otter and mink in North America have shown generally consistent levels between 980-1330 µg/g dw in otter and 1030-1310 µg/g dw in mink (the same for farmed mink) (Wren et al. 1988, Stejskal et al. 1989, Harding et al. 1998). In our animals, 21% had liver concentrations of Fe greater than one standard deviation above the highest previous reported average (>1500 µg/g dw) (Harding et al. 1998), 10% with over 2000 µg/g dw, to a maximum of 5700 µg/g dw. Mammals cannot excrete Fe and excess amounts in tissue can lead to damage such as cirrhosis, cardiomyopathy, and endocrine failure (Coffey and Ganz 2017).

Rubidium was high in both provinces, 17.80 µg/g dw in Alberta and 15.64 µg/g dw in British Columbia, and higher than the 13.44 µg/g dw weighted average produced by the literature. Twelve animals had more than double the weighted mean of Rb; 2 mink, one from each province with levels over 140 µg/g dw. The high value of the weighted mean was due to the report by Guo et al. (2020) from contaminated areas of Alberta, where the average was 16.82 µg/g dw. The only other report included was from Eurasian otters in Italy, which had an average of 2.88 µg/g dw (Esposito et al. 2020). Other reports have shown liver concentrations ranging from ~3 to 9 µg/g dw in marine mammals (Mackey et al. 1995), an average of ~5.7 µg/g dw in ringed seals (Campbell et al. 2005a), and an average of 2.88 (range 0.91-6.3) µg/g dw in sea otters, which was excluded in the calculation of the weighted mean due to a lack of reported standard error (Kannan et al. 2000). Because of this it is likely that previous reports from Alberta are not a good benchmark, and the differences seen due to more widespread Rb contamination in the province. In the model, there were no significant anthropogenic drivers of Rb out of the data available. While Rb is a byproduct of mining, industry, and oil and gas activity, it is also abundant in the earth's crust and can be released due to land clearing and erosion and is a major byproduct of coal burning (Johnson et al. 1975, Campbell et al. 2005a, Elias et al. 2020). As both coal burning and land clearing are ubiquitous in both provinces via multiple industries, it was likely difficult for our models to detect trends. Rubidium contamination is also driven by atmospheric deposition and long-term biomagnification in the ecosystem which may further obfuscate the effect of specific sources on the observed concentrations (Campbell et al. 2005a).

Little information is available about Rb toxicity in wild mammals, let alone toxic thresholds. Rubidium can be toxic both to liver and kidney health, may inhibit weight gain, impair reproduction, reduce longevity, and it may be more toxic when it is equal to around 10% of dietary potassium in mammals (Venugopal and Luckey 1978, Chatterjee et al. 1979, Yamaguchi et al. 2007). Reports from feral dogs in Europe showed significant correlation between Rb liver tissue concentrations over ~15 µg/g dw and neoplastic disease (Skibniewska et al. 2012). The strongly elevated levels of Rb in otter and mink in Western Canada compared to historical averages should be of concern due to limited knowledge about toxicity thresholds and the potential for fitness effects on individuals. As Rb is rarely tested and generally considered unimportant for wildlife, this may represent a serious gap in knowledge, and future research should investigate the potential threat of Rb contamination in the heavily altered landscapes of British Columbia and Alberta.

4.4.1.3 Limitations of historical averages

While some metals were well below the weighted mean calculated from the literature (Figure 4.1), it should be noted that these studies were predominantly of wild animals of a variety of ages and locations, often from polluted areas like the Alberta Oil Sands, and may not represent a “normal” concentration from a healthy animal. This is valuable as it creates a more conservative metric for comparison, especially considering the potential variability of essential elements in animals, but also requires caution in interpretation. Some studies have investigated the concentration of metals of concern in farmed mink as a baseline to compare wild studies too, as farmed animals with a controlled diet may offer a better “standard” level for comparison (Brzezinski et al. 2014). Though the studies do not indicate if farmed animals may be exposed to an increase in any metals due to chemicals for cleaning or tanning, cramped spacing, or processed feed. The mean concentration of four metals measured in our animals, which were either below or evenly distributed around the weighted mean (Mn, Mo, Se, and Zn), when compared against values in farmed animals from Brzninski et al. (2014), do show a much stronger difference, especially for Mn and Mo overall and Se in BC animals (Figure 4.2).

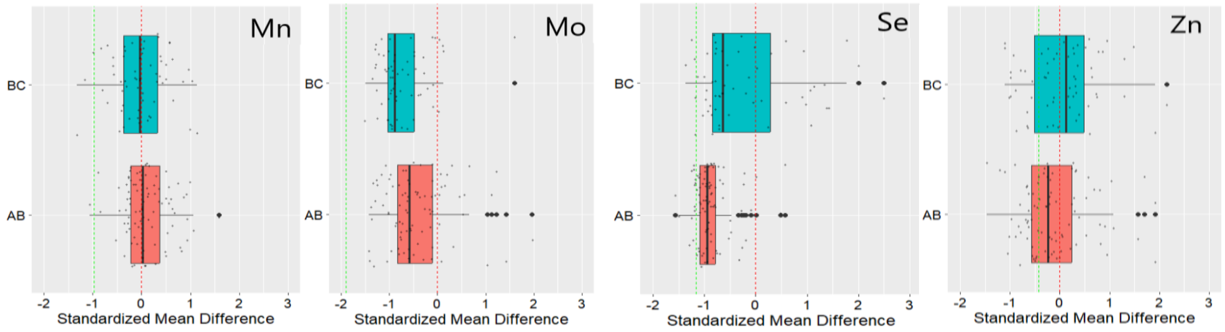

Figure 4.4 Standard mean difference of metals compared against values in farmed mink. Box and jitter plot of metal concentrations measured in this study presented as the standardized mean difference against the weighted mean of previously reported values (values greater than 3 excluded), values of zero represent no difference, highlighted as a dashed red line, the standardized mean difference of reported values from farmed mink (Brzezinski et al. 2014) represented as a dashed green line.

4.4.2 Effect of anthropogenic sources

As hypothesized, anthropogenic sources of metal contaminants were significantly correlated with elevated levels of trace metal pollutants in river otter and mink in Western Canada, though not all drivers hypothesized to influence specific trace metals were significant. However, it should be noted that a lack of significance in our modeling does not mean that other anthropogenic drivers are not influencing metal contaminants in British Columbia or Alberta. Due to the limitations in sample size in specific areas and the large geographic scale, the sensitivity of our models is limited, and observed elevated levels of contaminants may be due to multiple overlapping sources. The results of our modeling likely represent the anthropogenic drivers that are disproportionally influencing specific trace metal concentrations. Also contrary to our hypothesis, age was not a significant driver of metal concentrations for all trace metals. We saw increased concentrations of trace metals in both young and old animals. Some juvenile animals (below 1 year of age) had the highest concentrations of some metals, including Al, Fe, Mn, and Rb (Figure 4.1). While animals may still be more prone to increasing concentrations as they get older due to bioaccumulation over their lifetimes, the elevated values found in such young animals suggest that high environmental concentrations may also be an important factor in observed liver concentrations.

Major drivers included industrial and mining activities, which influenced metal concentrations at all spatial scales and both in long-term accumulation and high environmental concentrations. Oil and gas activity, and pulp and paper production may be more strongly associated with high environmental concentrations. Road density and urban cover were related with age and therefore likely related to long-term accumulation (Figure 4.5). The liver metal concentrations related to age and therefore most likely to be due to long term accumulation included Ag, Cd, Cu, and Hg, which agrees with the literature as both Cd and Hg have been reported to have age dependent associations in mink and otter (Mayack 2012, Kang et al. 2015), though this is not always the case (Evans et al. 2000, Klenavic et al. 2008). Conversely, high levels of environmental exposure may better explain elevated levels of Co, Fe, Pb, and Zn observed in younger animals (Figure 4.5). Of these metals, the models that explained the most variance were for Ag, Cd, Co, Cu, and Se. Mining activity significantly influenced Ag, Cu, and Se; industrial intensity Cd, Co, and Se; road density and urbanization Cd and Se.

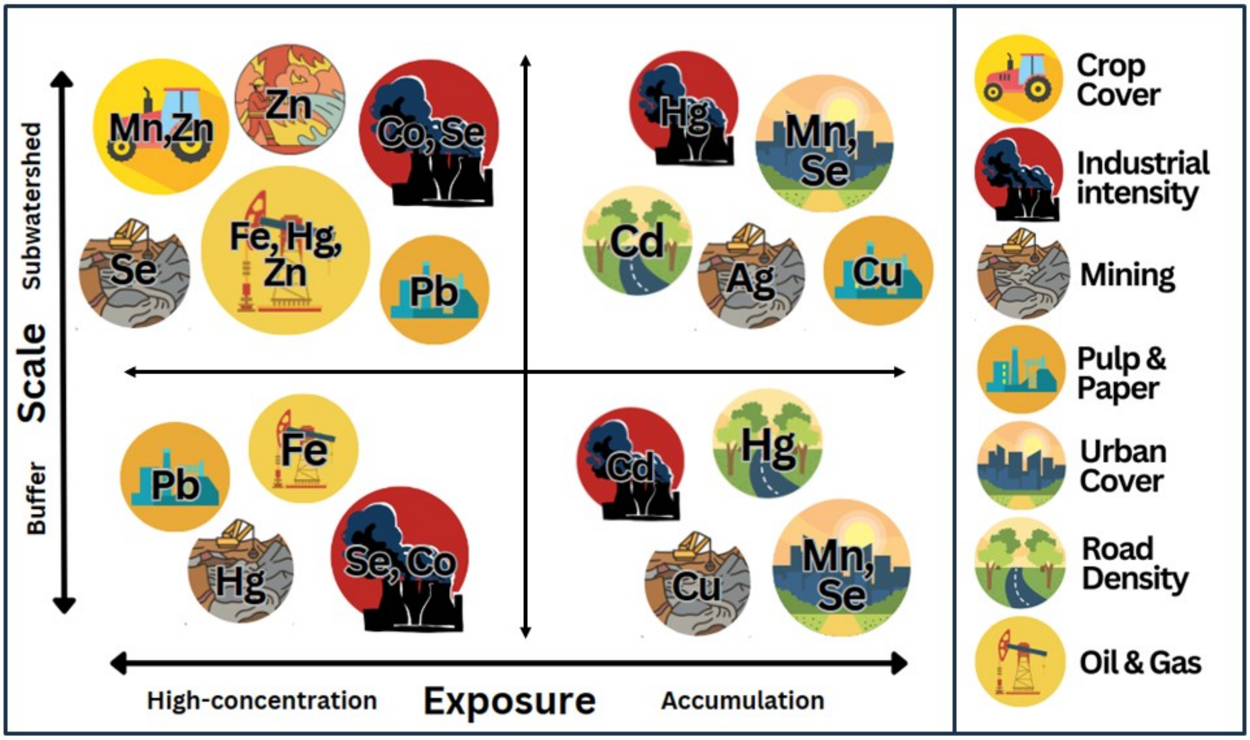

Figure 4.5 Significant drivers of metals in aquatic mammal tissues. Visual representation of the anthropogenic sources of metal contaminants that were significantly related to metal concentrations in river otter and mink tissues. Trace metals for which that driver was significantly correlated presented within the circles (size scaled by number of metals associated with that driver), clustered by geographic scale (whether that variable was significant in the subwatershed or home range buffer model) and by likely driver of exposure: "High concentrations" indicates that age did not significantly interact with that anthropogenic driver, and elevated levels were likely due to high environmental concentrations, while "Accumulation" indicates that age did significantly interact with that driver and long term accumulation most likely explains elevated levels.

Other models explained less variance. Notably many of the essential metals Fe, Mn, Rb, and Zn (Table 4.3), which exist naturally in higher concentrations and may vary more broadly, leading to a smaller standardized mean difference due to their larger pooled standard deviation. While anthropogenic sources may explain a small number of animals with extremely high concentrations of essential metals as well as a good portion of the variation, much of the variation observed may also be due to natural differences in diet or other biological moderators. There are also interactions between certain essential metals that can further complicate analysis. For example, Zn can reduce uptake of Cu and Fe, and Cu deficiencies may also be associated with high levels of Fe and Mo (Venugopal and Luckey 1978, Kalisińska and Budis 2019, Kosik-Bogacka and Łanocha-Arendarczyk 2019, Łanocha-Arendarczyk and Kosik-Bogacka 2019). Though age was significant in a few models, it was not always due to an increase in age as we would expect for long-term accumulation, but sometimes due to highly elevated levels in juvenile animals (Figure S4.2). Most metals we tested for were occasionally found at the highest concentrations in juveniles, even if there was a significant relationship between age in years and higher concentrations. Overall, anthropogenic sources were influential at all spatial scales, though contrary to our hypotheses, age did not significantly interact with all metals.

It should be noted that some studies have reported maternal transfer of trace metals either cross-placentally or via milk, which could explain elevated levels in juvenile animals. While it is impossible to definitively attribute the high levels of many metals seen in juveniles to either maternal transfer via milk or exposure to high environmental levels, we suspect that environmental exposure is more likely to explain these levels in our young animals due to the low rates of maternal transfer reported in other aquatic carnivores and the early age at which both otter and mink begin to consume prey items. Some metals are reported to transfer cross-placentally or via milk such as Hg (Sanchez-Chardi et al. 2007), though this is not the case for all metals and the proportion of transfer is often species specific. Cadmium for example does not transfer in high proportions (Grawé and Oskarsson 2000). We could find no information about transfer of metals from mother to pup in mink or otter. However, a study of the American fur seal (*Arctocephalus australis*) did not detect a significant transfer of metals (Cd, Cu, Hg, Zn) from mother to pup, metal concentrations spiking instead post weaning when the pups switched to solid food (Gerpe et al. 2009). A review of various species of seal also reported significantly lower concentrations of metals (Cd, Hg, Pb) in pups. Concentrations then increased in sub adults and peaked in adults, suggesting a generally low rates of transfer (Sepulveda et al. 1997). River otters begin ingesting solid food at 9-10 weeks (Larivière and Walton 1998), and mink are weaned at 5 weeks (Larivière 1999), therefore exposure to environmental pollutants via prey items can begin very early in life. Higher concentrations in young animals may be better explained by higher digestive absorption rates, higher energetic needs due to growth, or a reduced capacity to process and excrete trace metals compared to adults, and therefore high levels of environmental contamination may lead to higher levels in young animals (Sanchez-Chardi and Nadal 2007, Reijnders et al. 2009, Blagojević et al. 2012)

4.4.3 Geographic areas of concern

4.4.3.1 Alberta

Around the Athabasca and Cold Lake oil sands, animals were observed with concentrations of metal contaminants above historical averages, including Ag, Ba, Cd, Co, Fe, Hg, Mn, Mo, Ni, Rb, Sr, and Zn. A significant driver of Fe, Hg, and Zn, and nearly significant for Co, was *oil and gas sites*, which likely best explains some of these elevated levels (Table 4.3). The data on oil and gas sites include multiple methods of extraction which does limit the sensitivity of the model and may not fully capture all metals linked to oil and gas extraction. Hydraulic fracturing for natural gas would have been captured within our count of oil and gas sites but may be related to different types of contaminant outputs, such as Fe, Mn, Sr, and Zn (Folkerts et al. 2021), then the extraction and processing of bitumen (crude oil) which is the predominant oil and gas industry in Alberta (Huang et al. 2016, Lima and Wrona 2019). Trace metals above water quality guidelines for the protection of aquatic life associated with bitumen activity in Alberta have included atmospheric deposition of Ag, Cd, Cu, Hg, Ni, Pb, and Zn and direct water deposition of Cd, Cu, Hg, Ni, Pb, and Zn (Huang et al. 2016). This may be why *oil and gas sites* were not a significant driver in the models of Mn as this may be mainly associated with natural gas extraction. The models may be further complicated by the presence of inactive well sites that are still a source of pollutants but may not be included in our count of *oil and gas sites* in the model (Alboiu and Walker 2019).

Due to the limitations in sampling of our study and the broad geographic scale, we can only catch a small glimpse of affected animals in a variety of locations that may or may not be physically associated with a specific anthropogenic driver of trace metal contamination. The elevated levels we observed for other contaminants may be a complex mix of sources including industrial activity, the burning of fossil fuels, urban and agricultural runoff, and others. But it is clear that the oil and gas industry is a major driver of Fe, Hg, and Zn contamination in Albertan aquatic mammals. Because *age* was only a significant effect when related to *oil and gas sites* in our models for Co, Fe, and Zn where more young animals were affected than old, it is likely the observed high levels of these contaminants is not due to long term accumulation but high environmental levels. This is supported by previous studies that have reported levels of Co, Hg, and Zn associated with oil and gas activity that exceeded provincial or federal safety limits (Huang et al. 2016).

4.4.3.2 British Columbia

Potentially toxic levels of Se were detected in animals from the Columbia and Thompson Watersheds along with Co, Hg, and Rb. Extensive mining in the Columbia River watershed, especially for coal, is likely the main source of excess environmental Se as coal mining activity and transport is a major source of Se contamination (Lemly 2014, Cooke and Drevnick 2022). Mining and burning of coal are also associated with Co contamination, which further points to coal industry as the cause of these elevated levels in the Columbia River watershed (Momoh et al. 2017, Raj et al. 2017, Kosiorek 2019). While there are no current coal mines in the Thompson watershed, there are coal fields and historical coal mines near Kamloops, and mining operations for other elements including Au, Ag, Cu, Mn, Mo, Pb, and Zn (EMLI 2023). Cobalt and Se can both be byproducts of acid drainage mining, which is used in the Thompson River valley (Sheoran and Sheoran 2006, Sofield and Bollinger 2009, Momoh et al. 2017).

Coastal dwelling otter and mink on Vancouver Island also showed high levels of many metals (Al, Cd, Ga, Fe, Mn, Pb, Rb, Sr, V, Zn). Three animals caught within 2 km of the Crofton pulp mill, had some of the highest concentrations of Fe, Pb and V observed, which are most likely due to their proximity to the mill as Fe and Pb are reported outputs of sawmills and pulp and paper production (Bailey 1999; Singh and Chandra 2019). In the GLMM models analyzing the effects of anthropogenic drivers of contamination on Pb, pulp and paper mills were the only significant driver, the interaction effect of age and pulp and paper mills negatively associated with Pb levels as younger animals tended to have higher levels of Pb. Of the 10 animals with the highest levels of Pb, 70% were under one year of age, two were 1 year of age, and the last 4 years old. Toxic thresholds for Pb may vary widely across species (Baranowska-Bosiacka et al. 2019). Chronic exposure and liver concentrations as low as 1.47 µg/g dw in wild rodents (Powolny et al. 2023) and increasing liver concentrations between 1-3.71 µg/g dw in mink (Capodagli and Parker 2007) have been associated with decreased energetic condition. Three adult minks near the Crofton mill had concentrations within this range (2.49-3.43 µg/g dw), and Pb contaminants from pulp and paper industry on the island may represent a stressor for adjacent aquatic populations on Vancouver Island.

In the Southern Coast watershed, near Secret Cove, BC, only 80 km away to the next grouping of animals in the coastal Lower Fraser, 6 otters and 1 mink were submitted, trapped as nuisance animals at a local marine fish farm. These animals all showed toxic levels of at least one metal contaminant, and included the highest recorded values for Al, Ag, Cd, Cu, and Sr. Coastal dwelling otter and mink may feed almost entirely in marine habitats on intertidal fish and invertebrates (Ben-David et al. 1996, Roemer et al. 2009), and as they were trapped as nuisance animals at a marine fish farm, we can confirm they were foraging at least in part on marine fish. Information about Al toxicity is limited, however concentrations above 25.5 µg/g dw in human liver have been associated with renal failure, liver disease, and encephalopathy (Alfrey et al. 1980). Three female otters had levels over this threshold (41.8-68.2 µg/g dw). There can be wide ranges in reported toxicity thresholds for Cd exposure in wildlife causing reproductive effects and cardiac, hepatic, and renal toxicity. Average environmental exposure is reported to generally be below 4 µg/g dw in birds and mammals and known toxic effects occur around 20 µg/g dw (Tomza-Marciniak et al. 2019). We observed one adult male mink with 25.8 µg/g dw in its liver.

Toxic effects of Ag contamination include oxidative stress and inflammatory, endocrine, and developmental effects. Silver is also very toxic to microorganisms, which may have effects on gut microbiota, though information about liver concentration thresholds is limited (Du et al. 2018, Strużyńska 2019). Five otters had Ag levels at least 10 times the historic average of 0.113 µg/g dw, ranging from 1.00-3.96 µg/g dw. Normal liver concentrations of Cu in farmed mink have been seen around 5.60-41.2 µg/g dw, and mustelids may be particular susceptible to the effects of excess Cu (Łanocha-Arendarczyk and Kosik-Bogacka 2019). Concentrations of Cu around 65 µg/g dw in the liver tissue of marine mammals has been associated with toxic effects (Puls 1994), and we measured two adult females with over 100 µg/g dw. Concentrations of Ag and Cu were both driven by mining activity in our models, and BC has a long history of extensive Ag and Cu mining throughout the province (Laurinolli and Bendell-Young 1996, Johnson et al. 2005, Northcote 2019).

Data on toxic thresholds of Sr is limited, but may include behavioral disruption developmental defects, and reduced fitness has been associated with exposure (Chowdhury and Blust 2011). Previous averages have been around 0.253 µg/g dw, and five animals on the Sunshine Coast had the highest concentrations well above this ranging from 4.31-26.9 µg/g dw. Elevated levels were also seen in the Lower Fraser, Thompson, and Vancouver Island watersheds ranging from 0.350-0.754 µg/g dw. Due to low detection levels we were not able to model the effect of anthropogenic drivers on observed concentrations, however sources may include metal refineries, ceramic industries, lumber processing, and pulp and paper production (Chowdhury and Blust 2011). Pulp and paper mills are located in the Thompson, Lower Fraser, Southern Coast, and Vancouver Island watersheds, and are likely the main source of Pb contamination (Branch 2020).

The extremely elevated levels of metal contaminants, especially non-essential metals like Al, Ag, As, and Sr, is of great concern as it may indicate pervasive concentrations of these elements in marine fish in the Strait of Georgia. Local anthropogenic sources of metal contaminants include a pulp and paper mill ~45 km down coast in the Howe Sound and another ~40 km directly across the Strait of Georgia in Nanaimo, sand and gravel mining ~12 km away in Sechelt, and multiple industrial mineral mines ~30 km away on Hardy Island, ~50 km on Texada (limestone) and Valdes Islands (Northcote 2019). Texada Island was also historically mined for a variety of metals including Au, Ag, Cu, Fe, Mo, and Zn (EMLI 2023). The strait of Georgia is also strongly impacted by the Fraser River and municipal waste from Vancouver and is one of the world's busiest shipping lanes (Bendell et al. 2019, Waugh et al. 2022). As toxic effects for these metal contaminants may include a physiological, behavioral, or energetic burden on these animals, it is possible this disturbance is to blame for the human interactions that led to their deaths, the stress of metal contamination leading to a prioritization of access to easy prey over avoidance of humans. Further research should investigate the potential effect of sub-lethal contaminants as drivers of human-wildlife conflict, which may be significant in British Columbia, which has high levels of metal contamination and important fishery industries.

4.4.4 Implications for ecosystem health

While the sub-lethal effects of metal contaminants and the concentrations at which mammals experience negative effects from exposure are often unclear, there is a wealth of understanding concerning toxic potential in fish and aquatic species.

Copper has been reported to have widespread effects on aquatic ecosystems from individuals to communities (O'Brien and Keough 2014) and can influence fish and amphibian health (Brix et al. 2022). Cadmium is a gonadotoxin, which reduces fertility, acts as a carcinogen, and inhibits DNA expression and repair (Tomza-Marciniak et al. 2019). Selenium can reduce growth and reproduction in aquatic invertebrates, and affect the health, physiology, and survival of fish, especially larvae (Ohlendorf 2002). Zinc exposure can affect cardiovascular and renal health, damage the gills of fish (Jaiswal et al. 2018), and inhibit the growth and survival of plankton and algae (Sinouvassane et al. 2016). Though rarely considered as a contaminant of concern, Fe was greatly elevated in Albertan animals, as high as 5 times the historical average. Iron contamination can have severe effects on fish health, negatively affecting development, tissue growth, physiological processes, and metabolism (Ranjitha and Sharath 2020). In British Columbia Cu, Cd, Se, and Zn contamination have been reported as a threat to commercially important species like sturgeon, rainbow trout, and salmon, which are among the most sensitive of freshwater fish species (Bailey et al. 1999, Johnson et al. 2005, Vardy et al. 2014).

Ecotoxicology literature is biased toward certain contaminants that have been historically of concern, while others such as Ga, Rb, Sr, and V have received less attention. Reports have identified Ga and V as emergent contaminants of concern (Yang and Chen 2003, Chetelat et al. 2021, Ścibior et al. 2021, Akarsu et al. 2023), or the ability of Rb to biomagnify in aquatic systems (Campbell et al. 2005), but information on toxicity and prevalence are still limited. Rubidium can replace potassium and disrupt osmoregulatory and other processes (Peters et al. 1999, Tipsmark and Madsen 2001), which can lead to toxic effects in bivalves (Salaun and Truchet 1996) and reduced reproductive capacity in fish (Yamaguchi et al. 2007). Strontium is also rarely monitored and poorly understood, but it is known to affect egg hatchability and larval survival in freshwater fish (Chowdhury and Blust 2011). Gallium poses high ecological risk for ecosystems (Akarsu et al. 2023), it is a hepatotoxin, causes renal damage, and reduces the survival of larvae in fish (Yang and Chen 2003, Yang 2014). Considering the elevated levels of contaminants observed and the potential toxic effects of these contaminants in aquatic ecosystems, it is imperative future research investigates the exposure and burden of these contaminants on fish and invertebrates, especially in threatened species such as salmon and sturgeon.

4.4.5 Conclusions

Concentrations of trace metals are generally high in Western Canada compared to historical reports in otter and mink in North America; Ag, Al, Bi, Cd, Cu, Ga, Rb, Sr were strongly elevated in British Columbia and Ba, Fe, Ga, Rb, and Sr in Alberta. Ga and Rb, which have historically received little attention, were seen at much higher levels than previously reported and may represent emergent contaminants of concern. Anthropogenic sources of contaminants are significant drivers of this trace metal contamination, especially industrial, mining, pulp and paper, and oil and gas activities, across both local and sub-watershed scales. Areas of concern where populations may be more at risk include coastal populations along the Salish Sea, including Vancouver Island and the Sunshine Coast, the Columbia and Thompson River valleys and other environments impacted by coal mining, transport, and processing, and watersheds within the Alberta oil sands. Future research is needed to assess the impact of these elevated levels of trace metal contaminants and determine the reason for ineffectual policy around industrial waste management and water quality standards for the protection of aquatic species.

Chapter 5: Interactive and cumulative effects of parasites and contaminants on energetic condition in important aquatic mesocarnivores.

This manuscript is a collaboration with multiple research partners. See Chapters 3 and 4 for an outline of their contributions.

Chapter 5 will be submitted as: Shanebeck, K.M., P. Thomas, M. Todd, C. Lagrue, and S.J. Green (*in prep*). Interactive and cumulative effects of parasitic infection and pollutants on aquatic mesocarnivore energetic condition. Target Journal: *Frontiers in Ecology and the Environment*.

5.1 Introduction

Meta-analysis of experimental and observational research shows that helminth parasites, even if non-lethal or sub-clinical, can significantly influence energy availability in their hosts through a variety of mediators including behavioral alteration, gastrointestinal disruption, changes in host microbiome, and immune challenge or suppression (Shanebeck et al. 2022). This relationship may be complicated by an infection-condition positive feedback loop, where poor energetic condition may also predispose an animal to infection. Poor health, nutritional challenge, and behavioral disruption may alter feeding strategies, potentially increasing representation in the diet of prey items more likely to be infected or exposing the host to toxicants and other infections such as in urban areas. This can increase the severity of infections or lead to concomitant infections which then further reduce host condition (Beldomenico et al. 2008, Murray et al. 2019). It is difficult to easily separate out the complicated variation in parasitic infection and host condition as they are often interrelated. Poor health may be the cause or consequence of infectious disease or parasitism, which can be further complicated by external stressors such as climate change or contaminants which may interact with parasitic infection and further stress homeostasis and immune health (Beldomenico et al. 2008, Blanar et al. 2009, Dietz et al. 2019).

5.1.1 Metal contaminants and host condition

The toxic effects of metal contaminants are well known in a few non-essential metals that can have dramatic effects on animal health, including the three most often reported: Cd, Hg, Pb (Baranowska-Bosiacka et al. 2019, Kalisińska et al. 2019, Tomza-Marciniak et al. 2019). However, like previous trends in the literature concerning parasitic infection which have been biased toward parasite species that cause mortality or easily observable pathology (Shanebeck et al. 2022), essential metals or others that are only known to be toxic at high concentrations are often underreported. It is also generally unclear at what point they may begin to have deleterious effects on their host, and "normal" ranges are poorly understood (Dietz et al. 2019). Most studies of wild animals have focused on allowable limits for environmental exposure and acute toxicity, while studies of chronic exposure and the effects of long-term accumulation are limited. Toxicity is further complicated by interactions with the pathway of exposure (e.g. inhalation or ingestion), rate of exposure, distribution in the tissues, excretory capacity of the host, and resting concentration (Briffa et al. 2020).

Metal contaminants can be toxic through a variety of pathways, such as through tissue and cellular damage, carcinogenesis as seen in Cd and Ni, free radical formation and resulting DNA damage which has been shown in Cd, Cu, Fe, Ni, Hg, and V. Toxicity is also dependent on the chemical properties of the metal, environmental factors, and the susceptibility of the organism, including factors like life history, age (young or very old animals may be more susceptible), and females may suffer stronger effects than males (Rattner and Heath 2002, Burger 2007, Tchounwou et al. 2012, Briffa et al. 2020). Though essential trace metals are necessary for physiological processes, they can have a small window between essentiality and toxicity, such as with Ni, Mn, Fe, Cu, and Zn, of which Cu and Zn are the most toxic in the environment (Ali and Khan 2018). Metal toxicity can occur through a variety of pathways and physiological systems, many of which have not been fully described for all metals (Tchounwou et al. 2012). However, recent reports have correlated increases in metal contaminants with decreased energetic condition in birds (Espejo et al. 2020), snakes (Lettoof et al. 2022), and small mammals (Tete et al. 2013, Rodriguez-Estival and Smits 2016, Camizuli et al. 2018). Camizuli et al. (2018) reporting that Cd, Cu, Pb, Zn, and the interaction of Cu and Zn had significant negative effects on the body condition of wood mice (*Apodemus sylvaticus*) near historical mining sites.

Vertebrates also have the capacity to regulate levels of many essential metals in their bodies, which has led some researchers to conclude they are only deleterious at levels above the body's ability to regulate (Kosik-Bogacka et al. 2019, Kosik-Bogacka and Łanocha-Arendarczyk 2019, Łanocha-Arendarczyk and Kosik-Bogacka 2019). However, this disregards the energetic burden of active metal regulation and excretion, which may also stress the liver and renal system

(Rajotte and Couture 2002, Deb and Fukushima 2007). Metal contaminants can also have behavioral effects on sensory based decision making, predator avoidance, and risk taking (Atchison et al. 1987, Scott and Sloman 2004, Sievers et al. 2019). While not lethal or always obviously pathogenic, energetic stress may have serious implications for individual and population fitness related to increasing prevalence and intensity of stressors. It may be thought of like a machine. As more processing and excretion is required, the main effect on the system is the increasing energy requirements and the stress due to the extra work. When the amount of work surpasses the machine's ability, parts begin to break due to stress and the pileup of material affects the machine and the building around it. Up until now, the focus has been on the point of toxic effects, when the work overwhelms the system, ignoring the period of increasing regulatory costs as the body fights to maintain homeostatic control of the system, mounts immune response, and repairs localized damage (Rajotte and Couture 2002).

5.1.2 The interaction of parasites and metal contaminants

Parasite infection can influence contaminant availability and effect in their hosts, though this is dependent on the parasite and contaminant. Parasites may be associated with reduced levels of contaminants in their host but may also increase the severity of toxic effects or be associated with an increase of some contaminants (Sures 2008). Many gastrointestinal parasites readily accumulate metals, predominantly cestodes and acanthocephalans which are absorptive feeders through their tegument and may more readily accumulate contaminants than nematodes and digeneans which have a digestive tract (Sures et al. 2017). Much of the research in preferential accumulation is in fish, where cestodes may accumulate metal levels as much as 1.175 times higher than in host tissues (Malek et al. 2007). In a review by Sures et al. (2017) the authors summarized studies that investigated this, including in mammal hosts, though not all studies reported higher concentrations in parasites. The following metals were investigated in mammals, including the number of studies that saw higher concentrations in their parasites out of the total number of studies that investigated that metal: As (1/1), Cd (3/8), Cu (2/4), Cr (2/5), Lead (8/8), Mn (2/2), Ni (2/2), and Zn (2/4) (Sures et al. 2017). Most studies note these differences to indicate parasites as useful sentinels of exposure and did not investigate if infected hosts had reduced exposure compared to uninfected hosts.

Beyond showing higher concentrations of metal contaminants than their hosts, helminths may also significantly reduce contaminant concentrations in host tissue (Sures 2008). This is particularly true in acanthocephalans which rely on bile salt from their host. Many vertebrates move metal contaminants through the hepatic-intestinal cycle by creating organometallic complexes that they transport via the bile duct to the intestine for excretion or re-absorption, which may allow acanthocephalans to actively remove metals from this cycle (Sures 2008, Sures et al. 2017). Rodents infected with cestodes showed significantly lower levels of lead in their tissues (Jankovska et al. 2009), the same in sheep infected with cestodes (Jankovska et al. 2010b). However, this is not the case for all parasite-host systems. In the same study, infected sheep exposed to cadmium instead of lead had higher concentrations of the metal in their tissues (Jankovska et al. 2010a). Buffalos parasitized by digeneans had significantly higher levels of cadmium, chromium, copper, and lead in their livers or muscle tissue compared to those uninfected (Lotfy et al. 2013). Foxes parasitized by cestodes had significantly lower concentrations of lead in their kidneys, but significantly higher levels of cadmium, copper and manganese in their liver and kidneys than the uninfected (Jankovská et al. 2010). Mink parasitized by the giant kidney worm (*Dioctophyme renale)* had significantly higher concentrations of mercury than uninfected mink (Klenavic et al. 2008).

Other studies have also reported infection by helminths can lead to more serious side effects of metals in the host which may moderate benefits of reduced exposure (Sures 2008). Metal contaminants and parasites may also act as cumulative stressors, reducing host resistance or tolerance of secondary infection (Marcogliese and Pietrock 2011). Due to limited research and conflicting reports on the interactions of parasites, contaminants, and mammals, it is difficult to predict precisely how a given system may react.

5.1.3 Goals and hypotheses

We sought to determine if the severity of mesocercarial infections with *Alaria mustelae* and the overall richness of helminth species were correlated with metal contaminant concentrations. Because of the difficulties in conducting controlled laboratory experiments to analyze the effects of infection in wild species of concern, we are often limited to cross-sectional studies across broad populations with high levels of variance in the data, which makes traditional cause-effect hypotheses useless. In light of this, multivariate methods may be powerful tools to reduce complex datasets into two-dimensional scales in order to infer relationships between infection, poor condition, and contaminants (Dexter et al. 2018). We asked the following:

1. Is otter and mink energetic condition, measured as fat index score (FIS, amount of fat reserves), girth length index (GLI, size as a proxy for growth), and liver somatic index (LSI, deviation from expected ratio as a proxy for systemic stress), influenced by parasitic infection and metal contaminants?
2. Are parasite assemblages in otter and mink hosts, specifically the intensity of *A. mustelae* infections and total helminth species richness, influenced by metal contaminants?
3. If so, what is the directionality of these relationships, and what are the potential ecological and physiological explanations for the observed interactions?

We predicted that the cumulative effects of contaminant exposure and parasitic infection would negatively influence energetic condition. We further hypothesized that contaminant exposure would also be related to an increase in parasite infection intensity. We predicted that increasing severity of infection by mesocercariae of *A. mustelae,* higher helminth species richness, and their interaction effect would be associated with reduced energetic condition in our animals due to the energetic burden of immune response and systemic stress. We also predicted that elevated levels of metal contaminants would be correlated with poorer energetic condition due to energetic costs of homeostatic regulation. This was predicted the essential metals to be related most strongly, especially Cu and Zn which are reported to be the most toxic of the essential metals (Ali and Khan 2018). Lastly, we predicted that metal contaminant exposure would be associated with higher intensity infections of *A. mustelae* and greater overall helminth species richness, with the stress of exposure further stressing the immune response-energetic condition feedback loop.

5.1.4 Scope of study

Data from Chapters 3 and 4 concerning parasitic infection and metal contaminant concentrations were used to determine their effect on the energetic condition of river otter and mink in Alberta and British Columbia. The results showed that infections by mesocercaria of *Alaria mustelae* were significantly correlated with poor condition measured by fat content, and elevated levels of essential metals, specifically Cu and Zn, were correlated with reduced fat content as well as a lower girth to body ratio. Gallium concentrations were associated with changes in liver size, which may indicate toxic effects. The results address a gap in the literature concerning the sub-lethal effects of compounding stressors on aquatic mammal energetic fitness and emphasize the importance of the costs of homeostatic regulation in the face of persistent and highly prevalent stressors.

5.2 Methods

See Chapters 3 and 4 for an outline of the methods related to the collection of hosts, parasites, and pollution data for this study. Due to the limited prevalence of helminths beside larval infections of *Alaria mustelae*, parasitic infection was modeled as *Alaria intensity* (the intensity of infection of *A. mustelae*: absent, mild, moderate, severe) and *richness* (number of parasite species infecting an individual). Liver concentrations of the included trace metals were transformed for normality into a ranged scale (See Chapter 4.2.4) instead of standardized mean difference to avoid negative values which cannot be used in canonical correspondence analyzes.

5.2.1 Quantifying energetic condition

Energetic condition of each animal was determined by both external and internal metrics. Fat stores were scored qualitatively using a four-class descriptive scale as poor (none or very little), moderate (small amount), good (moderate), and excellent (large amount), except for the overall coverage which was measured as none/scant, poor, moderate, good, and abundant (Hatler et al. 2003). This included external fats: overall coverage, the coverage and thickness of fat on the whole body; intercostal, amount of fat surrounding the intercostal muscles; inguinal, amount of fat in large fat deposits present in the inguinal (groin) region; and tail, amount of fat at the base of the tail (only for otters, as mink generally do not store fat here), and internal fats: heart, omentum, kidney, and internal back (Plate 5.1). Ratings were numerically transformed, 0 (poor), 1 (moderate), 2 (good), 3 (excellent), then added together to produce a fat index score (*FIS*) or added separately to produce external (*EFIS*) and internal (*IFIS*) scores.

Plate 5.1 Examples fat condition. Examples of fat from otter A) excellent inguinal and good tail fat, B) poor inguinal and tail fat, and none/scant overall; and mink C) excellent back and good kidney fat, D) poor kidney and back fat.

Chest girth was measured and used to calculate a girth length index (*GLI*), dividing it by the body length of the animal, as a proxy for the general size of the animal (Labocha et al. 2014). The liver was also weighed and divided by total mass to determine the liver somatic index (*LSI*). Liver mass is strictly regulated in the mammal body, at around 5% in most species, though around 3% in mink (Piórkowska 2020), and an increase or decrease may be indicative of poor health (Konarzewski and Diamond 1995, Zhu et al. 2013, Penzo-Méndez and Stanger 2015).

5.2.3 Statistical Analysis

Statistical analysis was carried out in R version 4.2.1 (R Core Team, 2022), using R studio (Rstudio Team 2020) using the packages *vegan* (Oksanen et al. 2022), *car* (Fox and Weisberg 2019), *lme4* (Bates et al. 2015), *MASS* (Venables and Ripley 2002), *MuMIn* (Barton 2023), and *ggplot2* (Wickham 2006) for graphical representation.

5.2.3.1 Multivariate analysis

To investigate general patterns of both parasitic infection (*Alaria intensity, richness*) and metal concentrations (*Cu, Cd, Fe, Ga, Hg, Mn, Mo, Rb, Se, V, Zn*) in relation to the energetic condition indices (*IFIS, EFIS, LSI, GLI)* we conducted nonmetric multidimensional scaling (NMDS;

“metaMDS” function, distance= “bray”, 3 dimensions, *vegan* package), which is a flexible technique to analyze highly dimensional data with non-normal distributions, excluding metal contaminants that had low detection rates as the NMDS does not do well with zero inflation (Dexter et al. 2018). Goodness of fit for the NMDS checked by stress plot and R^2 (“stressplot” function, *vegan* package), significance of variables by permutation test (“adonis2” function, Bray-Curtis method, *vegan* package).

To investigate the major gradients in the combinations of the predictor variables (*Alaria intensity, richness, species, age,* and *sex*) as they relate to the concentrations of metal contaminants (*Ag*, *As, Cd, Co, Cu, Fe, Ga, Hg, Mn, Mo, Ni, Pb, Rb, Se, Sr, V, Zn*) and energetic condition indices in individuals (I*FIS, EFIS, GLI, LSI*), we conducted a canonical correspondence analysis (CCA; “cca” function, *vegan* package). The CCA allows for the comparison of two or more sets of variables, including predictor (“environmental”) variables and criterion (“response”) variables, which can be interchanged without influencing the results (Graffelman 2007). Goodness of fit was checked via inertia explained and permutation test (“anova.cca” function, *vegan* package).

5.2.3.2 Generalized Linear Mixed Models

To test the significance and directionality of correlative relationships, we conducted post hoc analysis of the correlations between energetic condition indices and stressors (parasites and contaminants) observed in the NMDS and CCA, via Generalized Linear Multivariate Models (GLMMs). We constructed five GLMMs that modeled the effects on energetic condition (*FIS, GLI, LSI*) and parasitic infection (*Alaria intensity*, *richness*), with trapper, species, and month of capture included as random effects to account for non-independence of animals trapped in the same area by the same trapper, species specific behavior, and potential fluctuations in fat content due to time of year. Explanatory variables were based on the resulting correlations from the multivariate analysis and included the interaction effect of *Alaria* intensity and parasite richness (for the energetic condition models) to account for the confounding effects of concomitant infections which can increase severity of the effects of infection, and the interaction effect of Cu and Zn, as Zn can decrease the availability of Cu for the body.

To test the overall effects of elevated levels of metal contaminants on energetic condition we created two indices: an essential contaminant index (ECI; Cu, Fe, Mn, Mo, Se, Zn) and non-essential contaminant index (NCI; Ag, Al, As, Cd, Co, Ga, Hg, Ni, Pb, Sr, V) that were equal to the total number of metals in each animal that were elevated above the historical average (see Chapter 4.3, Table 4.2). While Co and Ni are considered ultra-trace essential metals, they were included in with the non-essential metals as they are only necessary in very small amounts and are more likely to be toxic at lower concentrations than the other essential metals (Tchounwou et al. 2012, Briffa et al. 2020). Three additional GLMMs were constructed to model the relationship between the energetic condition indices (*FIS, GLI, LSI*; response variables) and the new metal indices (*ECI, NCI*), *Alaria intensity*, *richness*, and their interaction effect, *sex*, and *age* (explanatory variables).

Final models were selected via multimodel inference by a stepwise removal of non-significant effects (‘ANOVA’ function, *car* package) and the Akaike Information Criterion (‘AICc’ function, *MuMIn* package), models with the lowest AIC score selected as the final model. All models were checked for multicollinearity between variables via Variance Inflation Factor (‘vif’ function, *car* package). A histogram of all best fit model residuals was checked for normalcy, and variance explained examined via R^2 (‘r.squaredGLMM’ function, *MuMIn* package).

5.3 Results

5.3.1 Multivariate relationships between parasites, trace elements, and energetic condition

We observed significant clustering in the energetic condition of animals related to parasitic infection and metal contaminant composition. The NMDS model explained a high amount of variance ($R^2_{\text{linear-fit}}$= 0.925, Figure S5.1), with the significant variables explaining the most variance including *Alaria intensity* (R^2=0.279, p=0.001), *age* (R^2=0.386, p=0.001), *EFIS* (R^2=0.397, p=0.001), and *IFIS* (R^2=0.386, p=0.001). Other variables explained less variance but were still significant: *richness* (R^2=0.080, p=0.001), *LSI* (R^2=0.023, p=0.029), *GLI* (R^2=0.07, p=0.001), *Zn* (R^2=0.079, p=0.001), *Cu* (R^2=0.038, p=0.004), *Rb* (R^2=0.021, p=0.033), and *Fe* (R^2=0.029, p=0.006).

The ordination plot showed an opposing relationship between infection by *A. mustelae* mesocercaria and fat scores, *IFIS* and *EFIS* clustering with absent or mild *A. mustelae* infection, the separation stronger for increasing internal fat and animals not infected with *A. mustelae* (Figure 5.1). Similarly, there was an opposing relationship between fat index scores and essential element concentrations of *Cu*, *Fe*, *Rb*, *Se,* and *Zn* and helminth species *richness*, though not as strong. Other energetic condition metrices, *GLI* and *LSI*, did not seem to be related strongly to *A. mustelae* infection, clustering at the center of the ordination, though these indices may be related to trace metal concentrations and helminth richness (Figure 5.1). There was some weak clustering related to secondary biological factors including: province, BC animals more likely to be related with secondary infections; sex, males more likely to be related with secondary infection; age class, which showed juveniles slightly more likely to have poorer fat scores; and species, which showed mink more likely to have secondary infections (likely due to the high prevalence of infection by the lung parasite *Filaroides martis*, see Chapter 3) (Figure S5.2).

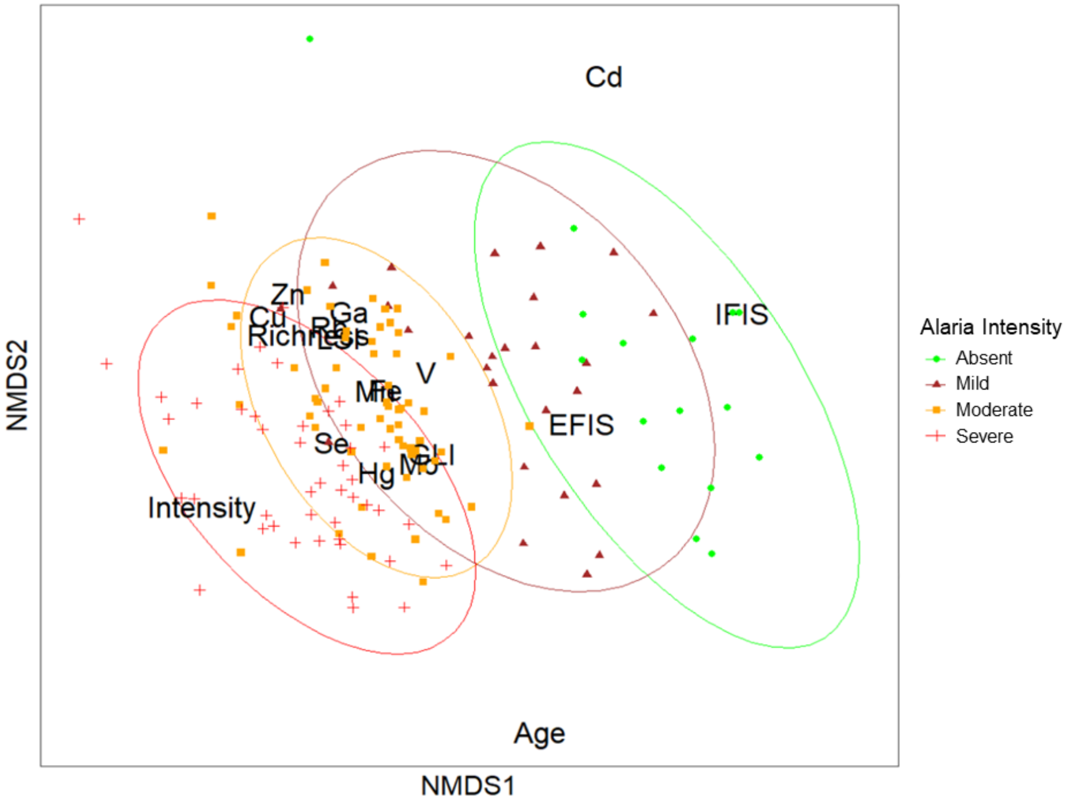

Figure 5.1 NMDS ordination diagram. Differences between animals related to their parasitic infection, liver concentrations of essential elements, and energetic condition (NMDS of non-scaled data, Bray-Curtis similarity). *Alaria mustelae* mesocercariae infection intensity shown by color and clustering identified by ellipse ("stat_ellipse" function, *ggplot2*).

In the CCA, the explanatory effect of parasite infection related to energetic condition and metal concentrations showed significant relationships between the first two axis' (p_{CCA1}= 0.001,

λ_{CCA1}=5.1; p_{CCA2}= 0.002, λ_{CCA2}=2.0, where λ=eigenvalue), and significant effect of predictor variables on the composition of metal concentrations and energetic condition in host animals including host *species*, *sex*, *age*, and the *Alaria intensity* ($p_{species}$=0.001, p_{sex}=0.002, p_{age}=0.002, p_{alaria}=0.001), total helminth *richness* was not significant (Figure 5.2). Overall variance in metal concentrations and energetic condition explained by the predictor variables (parasite infection, sex, age, and species) was low at 11% of the inertia. This may in part be due to the significant effect of anthropogenic drivers on metal contaminant exposure, which likely explains significant variance related to contaminant concentrations (see Chapter 4).

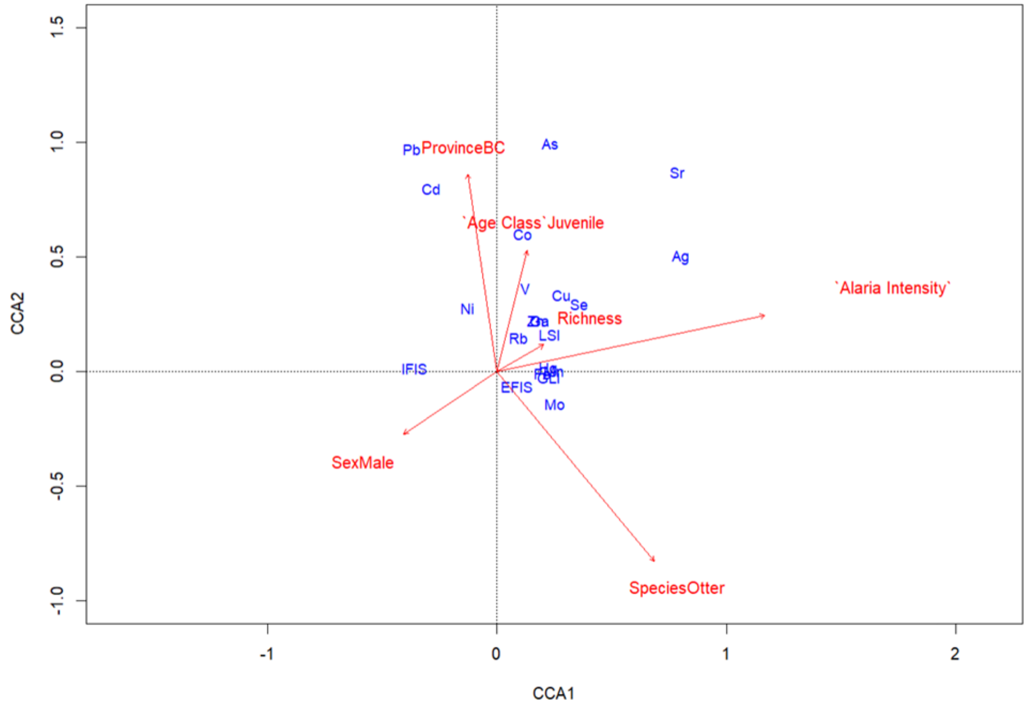

Figure 5.2 CCA ordination diagram. Results of the Canonical Correspondence Analysis (CCA) response variables (*FIS*=fat index score, *GLI*=girth-length index, *LSI*=liver somatic index) clustered in relation to the influence of predictor variables which included biological moderators (*age, sex, species*) and parasitic infection (*Alaria intensity*, *secondary infection* besides *Alaria*, and total helminth species *richness*).

The significance of *species* was likely driven by the correlation between mink and *Pb* (Figure 5.2), which was found at its highest value in mink (Figure S5.3). *Sex* and *age* were also significant predictor variables, both related with *FIS*, females tending to have poorer condition and *age* related to both condition indices and metal contaminant concentrations (Figure S5.3). *Age*, as expected, was related to *GLI* which is a proxy for growth (Figure S5.3), as well as weakly related to *Hg* concentrations (Figure S5.3d). Like in the NMDS model, fat index score and *A. mustelae* infection intensity were inversely related, in addition *A. mustelae* was associated with increasing concentrations of Ag, Cu, and Se (Figure 5.3).

5.3.2 GLMMs: moderators of energetic condition and parasitic infection

5.3.2.1 Energetic condition

Fat index score decreased in relation to increasing intensity of infection by *A. mustelae* mesocercariae as well as elevated concentrations of *Cu* and *Zn* (Table 5.1), moderated as well by the interaction effect of Cu and Zn. While the combined FIS was modeled, there was a noticeable difference between internal and external fat scores when graphed, the decrease in overall fat content likely being driven by the IFIS (Figure 5.3). Animal fat decreased in relation to higher Cu and Zn concentrations (Figure 5.4A), the combined concentrations of Cu and Zn also showing a strong negative effect on FIS, though when Zn was at higher concentrations compared to Cu they were weakly related to higher FIS (Figure S5.4).

Table 5.1 Significant variables from GLMM models. Table of variables that best explained energetic condition and parasitic infection for each best fit model and the variance explained by the model (R^2_c), including the significant variables from the model (significant *p*-values denoted by asterisk; ***<0.001, **<0.01, *<0.05, near significant values denoted by $^-$), interaction effects denoted by a colon between variables, and the variable's estimate, and standard error (*se*).

Response Variable	Explanatory Variables	*p*-value	estimate	Standard error
Fat Index Score	(Intercept)		-2.8280	61.8792
$R^2_c=0.671$	*Alaria* Intensity***	$2.2e^{-16}$	-2.8084	0.2873
	Cu	0.486	-53.9808	26.4490
	Zn**	0.004	15.4178	31.2576
	Fe*	0.027	35.2816	24.4629
	Cu:Zn*	0.045	27.5202	13.7299
Girth Length Index	(Intercept)		0.50070	0.08929
$R^2_c=0.582$	Cu	0.941	-0.08816	0.04818
	Zn***	0.0001	-0.39637	0.03023
	Mo***	0.001	0.11720	0.03044
	Cu:Zn*	0.011	0.59487	0.23376
Liver Somatic Index Difference	(Intercept)		0.01383	0.00239
	Age*	0.022	-0.00086	0.00038
$R^2_c=0.179$	Ga*	0.047	0.00989	0.00498
	Mn**	0.002	-0.02264	0.00724
Alaria Intensity	(Intercept)		1.6844	0.1474
$R^2_c=0.210$	Cu**	0.009	1.6273	0.6258
Richness	(Intercept)		0.70652	0.18461
$R^2_c=0.466$	Age*	0.018	-0.06269	0.02645
	Alaria Intensity***	$6.36e^{-9}$	0.32920	0.05666
	Sex*	0.046	0.22592	0.11300
	Fe^-	0.072	-0.78879	0.43907

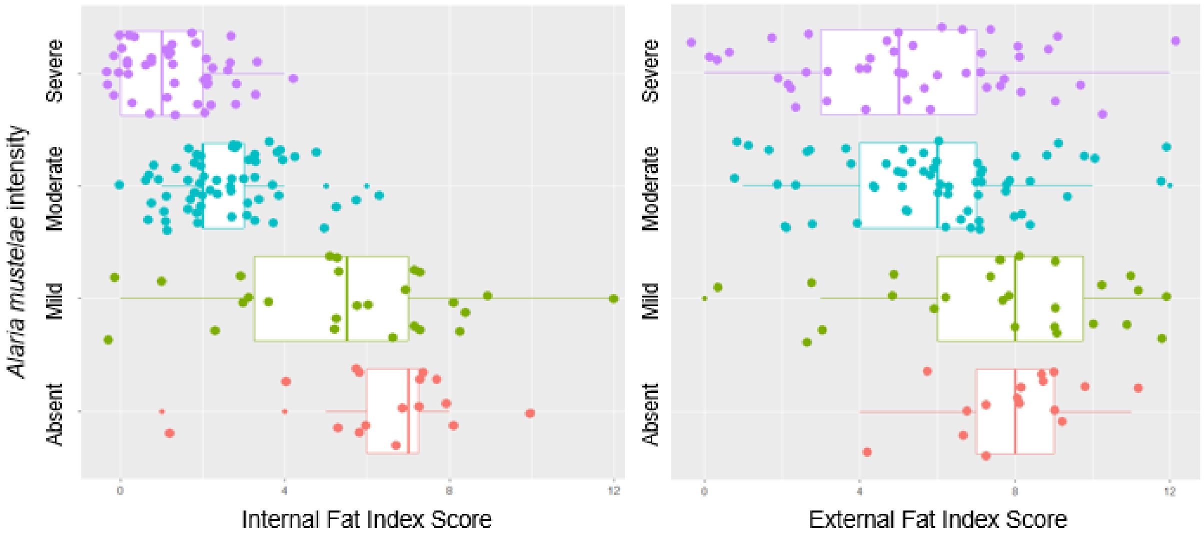

Figure 5.3 Effect of *Alaria mustelae* infection intensity on fat content. Boxplots of fat index scores, both internal and external, against infection intensity, including jitterplot of records from individual animals.

The girth-length index was negatively associated with increasing *Cu* and *Zn* concentrations (Figure 5.4B), including the interaction effect of both contaminants. When the effect of combined concentrations of Cu and Zn were considered, there was a similarly strong negative effect on GLI. Conversely, *Mo* was positively associated with GLI, larger animals more likely to have higher Mo concentrations (Table 5.1).

The model of *LSI* explained the least variance of the energetic condition models (Table 5.1), which is likely because this index is very unspecific, related in general to either hepatic or overall physiological stress (Table S2.1). This means this index is likely related to the pathological effects of contamination and parasites but could also be influenced by secondary infections or disease. Both *age* and *Mn* concentrations were negatively associated with *LSI*, younger animals, and those with low concentrations of *Mn* more likely to show a change in liver size. Only *Ga* was positively associated with the difference in the *LSI* from the expected ratio, animals with higher concentrations more likely to show changes in liver size (Figure 5.4C).

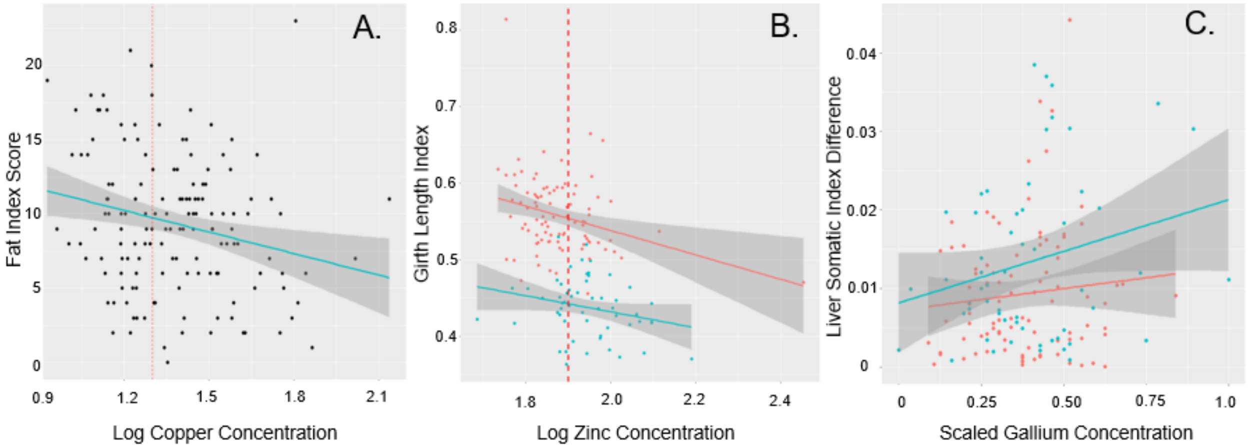

Figure 5.4 Significant moderators of energetic condition. Scatterplots of the effect of A) copper concentration on fat index score; B) zinc concentration on girth length index, by species (red=otter, blue=mink); and C) gallium concentration on the liver somatic index difference (deviation from expected ratio, 0.05 for otters, 0.035 for mink) by species (red=otter, blue=mink). Dashed red line indicates the weighted average concentration in otter and mink from the literature, log Cu and Zn used to allow for easy comparison against the weighted average.

5.3.2.2 Parasitic infection

Increasing intensities of infection by mesocercariae of *A. mustelae* were only associated significantly with elevated levels of *Cu* in our animals (Table 5.1), which when graphed by species, appeared mainly driven by concentrations in otters (Figure 5.5A). Helminth parasite species *richness* was negatively correlated with age, with older animals less likely to have multiple infections, but positively correlated with *Alaria intensity*. Males were also more likely to have a higher *richness* of helminth species related to females (Figure 5.5B).

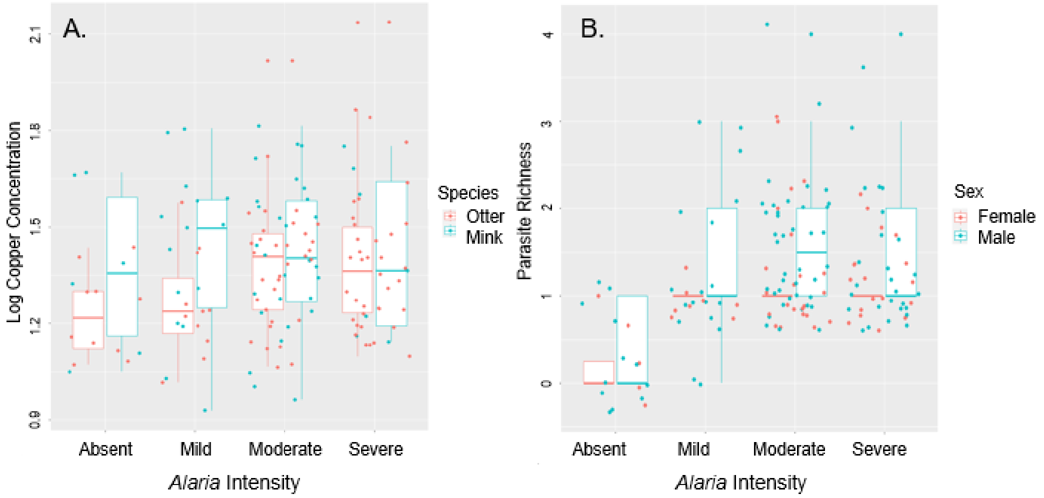

Figure 5.5 *Alaria mustelae* infection intensity in relation to Copper and helminth richness. Boxplots of increasing intensity of *Alaria* infection against A) log Cu concentration, by species (red=otter, blue=mink), and B) helminth species richness by sex (red=female, blue=male), including jitterplot of records from individual animals.

5.3.2.3 Energetic condition, parasite infection, and combined metal indices

The GLMM investigating the effect of parasitic infection and the combined number of essential and non-essential metals on FIS explained significant variance (R^2_c=0.636). The final model including a significant relationship between decreasing fat levels and increasing *Alaria* infection intensity (p=2.2e^{-16}) and higher numbers of elevated essential metals (p=0.005); sex, age, the interaction effect of *A. mustelae* intensity and richness, and the number of elevated non-essential metals did not significantly influence fat content. The models for GLI and LSI had no significant moderators.

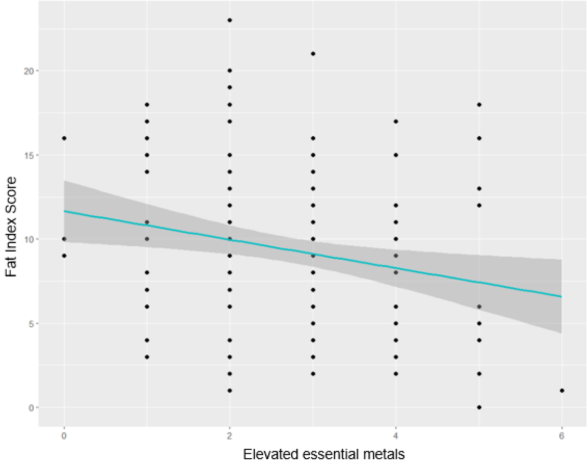

Figure 5.6 Effect of ECI on FIS. Scatterplots of the effect of elevated essential metals on fat content, with line of best fit.

5.4 Discussion

5.4.1 Effect of parasitic infection

In agreement with our hypothesis, parasitic infection was significantly correlated with energetic condition. As predicted, highly prevalent infections by mesocercarial *A. mustelae* were strongly related to decreased fat stores, though contrary to our prediction that total *richness* of helminth species and the interaction effect with *Alaria intensity* did not correlate significantly with energetic condition. This is likely due to low prevalence of parasite species other than *A. mustelae*; while there was a diversity of species identified, they were generally below prevalence reported in other otter and mink populations in North America (see Chapter 3). Interestingly, the effect of *Alaria* infection was most strongly seen in internal fat, which was decreased more than external fats suggesting that the animals were metabolizing internal fat to respond to infection. This is contrary to the general assumption that mammals use external fat first when facing energetic challenges, ruminants especially (see Chapter 2). It is possible that otter and mink are metabolizing internal fat first as it tends to be made of brown adipose tissue which is more easily changed into metabolic heat (Wu et al. 2012). Along with their thick fur, mustelids use metabolic heat as their main source of homeostasis, having some of the highest metabolic rates in the animal kingdom (Iverson 1972, Stephenson et al. 1988, Pfeiffer and Culik 1998).

To maintain their high metabolic demands, mustelids must hunt constantly and need large amounts of prey. If parasitic infection reduces energy availability, either due to the diversion of resources to immune response or a disruption in hunting efficacy due to lethargy, pain, or general malaise, animals may use up internal fat stores needed to maintain body heat in winter. The trapping season occurs during a period in which temperatures in Alberta can get as low as -40ºC. Almost all animals were trapped between November and March when they must produce constant metabolic heat, which may be a compounding stressor with infection. Month of capture was included as a random effect in the model to control potential differences between the beginning and end of winter, but analysis showed no significant difference in fat between months. Mustelids do not store fat for winter like other species and continue to hunt throughout the season (Larivière and Walton 1998, Larivière 1999)

5.4.2 Effect of metal contaminants

As predicted by their hypothesized detrimental effects, elevated metal contaminant concentrations were both significantly negatively correlated with energetic condition. Also as predicted, this effect was seen only with the essential metals and not the non-essential metals. This is likely due to the low detection rates of many of the non-essential metals like Al, Pb, and Sr, limiting sample size for comparison. The non-essential metals are also more likely to be highly toxic or fatal in high concentrations which may mean we are less likely to receive animals from trappers with high concentrations as they would be in extremely poor condition. Essential metals on the other hand are always present in the animal, meaning we have "healthy" levels for the model to compare against the elevated concentrations. Also, because mammals regulate concentrations of essential metals in their systems, differences in concentration are not necessarily good predictors of exposure. This means that even mildly elevated levels may be indicative of higher exposure and related to ongoing stress to their systems due to the energy required to constantly regulate metal levels at healthy levels (Rajotte and Couture 2002). Since this effect is less lethal, contaminated animals may also be more likely to persist, even if it is at a constant energetic challenge that is causing a significant loss of body fat or stunting growth.

Of the metal contaminants, Cu and Zn were the strongest predictors of poor condition for both fat content and chest girth in relation to length. Elevated levels of both correlated with less fat or a smaller girth to length ratio and their interaction effect was also significant. When the ratio of

Cu to Zn was graphed, there was a weak relationship showing that when there was more Zn in relation to Cu the animals tended toward better energetic condition (Figure S5.4). This is likely because elevated Zn levels may reduce uptake of Cu in mammals, which can lead to deficiencies or potentially a protective relationship in areas with Cu pollution (Kosik-Bogacka and Łanocha-Arendarczyk 2019). However, this may only be the case in animals where both were at lower concentrations and had a limited effect at high concentrations as both can be toxic, explaining the weak observed effect between Cu/Zn ratio and body fat. The combined concentrations of both Cu and Zn had a strong negative relationship with FIS, suggesting that the significance of the interaction effect in the model is due to the combined toxic effects of both metals (Figure S5.4) Copper deficiencies have also been linked with increases in Mo concentrations, which may explain why increasing Mo concentrations were significantly positively correlated with GLI in the model, higher concentrations mitigating the toxic effects of Cu (Łanocha-Arendarczyk and Kosik-Bogacka 2019).

Both Cu and Zn can have toxic effects in vertebrates. Zinc can reduce uptake of other essential trace metals like Ca, Fe, and Mg, and mimic other divalent metals, binding to metalloproteins disrupting their cellular activities. The toxic effects of Zn may be due to deficiencies in these metals or cellular disruption, leading to developmental, physiological, or growth effects (Venugopal and Luckey 1978; Kambe et al. 2004). This may explain the negative relationship with the GLI, excess Zn reducing trace elements needed for growth, especially Ca which is essential for bone growth. Copper toxicity is associated with oxidative stress and cell death which may reduce immune capacity, and chronic exposure can be related to a reduction in growth, feeding and litter size (Łanocha-Arendarczyk and Kosik-Bogacka 2019), which may explain the negative effect on growth as measured by the chest girth to body length ratio. Cu also has a role in fat metabolism and dietary excess is associated with a decrease in adipose tissue (Engle 2011), which may explain the negative relationship observed between Cu and FIS. The toxic effects of Cu may also explain why it was the only significant predictor of *Alaria* infection intensity, a reduction in immune capacity leading to more intense infections and symptoms. Both Cu and Zn were seen at elevated levels compared to historical reports across North America.

The model investigating the effect of our variables on the difference in LSI showed only Ga was related to changes in liver size, which is related to systemic stress (Penzo-Méndez and Stanger

2015). Information about Ga toxicity is limited but reported have linked chronic toxicity in mice with growth reduction and reduced female survival (Venugopal and Luckey 1978), as well as developmental effects in utero (Domingo 1994). In fish, it acts as a hepatotoxin and can damage kidney function (Yang 2014). As Ga is liver toxin, the observed correlation with changes in animal liver size suggests the levels we observed in otter and mink may be toxic. Though Ga has not been previously reported to influence liver size, significant changes in liver mass have been associated with Cd, Hg, and Pb toxicity in mink (Ljungvall et al. 2017). When the effect of Ga on LSI was visualized by age class (juveniles and adults), there was no apparent relationship for juveniles between Ga and LSI; however, for adults there was an association between Ga concentrations and changes in host liver size. This may be significant as when only age was visualized against LSI, old animals were less likely to show changes in their liver size. Gallium toxicity is poorly understood, and it is rarely tested for in mammal. These results highlight the need for further investigation about the specific pathways of toxicity and prevalence of Ga contamination in wildlife, especially in areas of strong anthropogenic change like Alberta and British Columbia.

It should be noted that due to limitations inherent in cross-sectional studies of wildlife that are opportunistically sampled, we cannot definitively conclude causal effects of metal or parasitic infection on energetic condition. As previously noted, the effects of metal toxicity and parasitic infection can be regulated by multiple variables including environmental, biological, physiological, and chemical factors. However, due to the strong, significant correlation between *A. mustelae* infection and metal contamination and poor energetic condition in this study, we are confident there is likely a causative relationship between infection, contamination, and reduced fitness.

5.4.3 Conclusion

By comparing the effect of parasitic infection and metal contaminants on energetic condition in aquatic mesocarnivores, we found that, as predicted, both parasitic infection and elevated levels of metal contaminants were related to poor energetic condition. While sub-lethal or sub-clinical effects are rarely integrated into wild mammal research and conservation, our work has shown the importance of these stressors for mammal energetic condition, which directly relates to fitness (Brown et al. 1993, Berta et al. 2015). These sub-lethal effects may be particularly important in the age of anthropogenic change, as rising temperatures and extreme weather events may have a compounding effect on mammal energetics and therefore fitness (Levesque et al. 2016). It has also been suggested that rising temperatures may increase parasitic disease and severity, benefiting parasites and reducing host immune responsivity (Paull and Johnson 2011, Marcogliese 2016). Similarly, uptake and toxicity of metal contaminants can also be temperature dependent and may be altered by climate change (Sokolova and Lannig 2008). Infections of *A. mustelae* mesocercariae infecting otter and mink and contributing to poor energetic condition has not been previously reported, and the two years of this study (2020-2022) included some of the hottest summers on record in Canada (Zeidler 2021). While no information is available about infections prior to this period, it is possible that these infections and resulting poor condition in our animals are a result of a parasite population boom due to higher temperatures. Future research is essential for us to understand how climate change will influence parasites and metal contaminant exposure and the health of our aquatic species.

Chapter 6: General Conclusion

The research presented in this thesis is the most comprehensive assessment of the influence of sub-lethal helminth infection in mammals, and parasitic and metal contaminant stressors in river otter and mink in Western Canada. Given the position of otter and mink as sentinel species in aquatic ecosystems, the highly prevalent infections by zoonotic parasites and elevated levels of metal contaminants reported are extremely concerning, especially considering the effect of a changing climate which may increase the severity of both parasites and contaminants (Sokolova and Lannig 2008, Marcogliese 2016). As discussed in Chapter 1, the goal of this research was to describe the cumulative effect of sub-lethal helminth infection and contaminant exposure on otter and mink energetic condition. This research showed that previously unreported mesocercarial infections caused by *A. mustelae* and metal contamination were strongly correlated with poor energetic condition in animals from across Alberta and British Columbia.

6.1 Summary of Work

Sub-lethal (or sub-clinical) infections of helminths negatively affect the energetic condition of mammals, which has implications for both individual and population fitness. Chapter 2 showed, through a meta-analysis of the literature, that mammal energetic condition is significantly negatively affected by sub-lethal helminth parasites, across all major groups (including ungulates, carnivores, primates, and rodents). Methodological limitations and disparities in wildlife research can reduce the resolution of these effects and may have driven previous assumptions of insignificance. Cross-sectional observational studies produced weaker effect sizes compared to experimental or semi-controlled studies. Methodology also explained inter-study variance in wildlife research; opportunistic sampling of carcasses led to reduced effect sizes and increased variance compared to studies using active sampling protocols of live animals. These findings are important as they will instruct future research and provide a comprehensive context for the inclusion of sub-lethal parasites into ecological and management models.

There is a broad diversity of helminth parasites in otter and mink in Western Canada, including multiple zoonotic species of concern. Chapter 3 outlined that infection by helminth parasites in otter, mink, and muskrat were characterized by four main species (*Alaria mustelae*, *Filaroides martis*, *Isthmiophora inermis*, and *Versteria rafei* n. spp.). At the top of aquatic ecosystems, otter and mink are definitive hosts to a range of parasite species. As mesocarnivores connecting aquatic and terrestrial food webs, they are also potential intermediate or paratenic hosts, sometimes for the same species such as in *Versteria rafei* n. spp. and *Alaria mustelae*. Investigation of their helminth communities revealed multiple species of concern for zoonotic transmission, including the description of a previously undescribed species of *Versteria*. This research highlighted a serious gap in knowledge around helminth communities in Western Canada and the threat related to vulnerable communities. Ongoing research is needed to assess the risk of infection by *Versteria* spp., *Toxoplasma gondii*, and *Alaria mustelae* for First Nation communities and fur trappers who handle these organisms. Furthermore, we need research into the distribution of these parasites in other intermediate and definitive hosts to understand the range of this threat to wildlife and human communities.

River otter and mink are exposed to multiple metal contaminants in Western Canada at levels above previous historical averages; exposure driven by anthropogenic sources. Chapter 4 reported elevated concentrations of trace metals in Western Canada compared to historical reports in otter and mink in North America; Ag, Al, Bi, Cd, Ga, Rb, Sr were strongly elevated in British Columbia and Ba, Fe, Ga, Rb, and Sr in Alberta. Ga and Rb, which have historically received little attention, were seen at much higher levels than previously reported and may represent emergent contaminants of concern. Anthropogenic sources of contaminants were significant drivers of these trace metal contaminants, especially industrial, mining, pulp and paper, and oil and gas activities, across both local and sub-watershed scales. Areas where populations may be more at risk included coastal populations along the Salish Sea, including Vancouver Island and the Sunshine Coast, the Columbia and Thompson River valleys and other environments impacted by coal mining, transport, and processing, and watersheds within the Alberta oil sands. Future research is needed to assess the impact of these elevated levels of trace metal contaminants and access the efficacy of policy around industrial waste management and water quality standards for the protection of aquatic species.

Both parasitic infection and pollutant exposure negatively correlate with the energetic condition of mink and otter. In Chapter 5, by comparing the effects of parasitic infection and metal contaminants on energetic condition in otter and mink, we showed that both exposure to parasites and elevated levels of contaminants were related to poor energetic condition. This work emphasizes that, though rarely integrated into conservation management, sub-lethal stressors have significant effects on mammal energetic condition, which relates directly to both individual and population fitness. These sub-lethal effects may be particularly important in the age of anthropogenic change, as rising temperatures and extreme weather events may have compounding effects on mammal energetics and therefore fitness, as well as the availability of both parasites and contaminants. Future research is essential for us to understand how climate change will influence parasites and metal contaminant exposure and the health of our aquatic species.

6.2 A paradigm shift in wild mammal research

This work outlines an important shift in thought related to the investigation of the effects of parasites and pollutants in mammals. Historically, both parasite and toxicology research has focused on the lethal and pathological effects of stressors on mammal health, while sub-lethal or sub-clinical effects have been considered unimportant (Rajotte and Couture 2002, Shanebeck et al. 2021). This work has endeavored to re-frame the paradigm around the importance of parasitic and contaminant stressors on wild mammals, by shifting the focus from mortality to fitness and pathology to energetic condition. While mortality and pathology are important threats to populations, focusing on them alone ignores the important regulatory costs of pervasive sub-lethal stressors. Exposure can lead to energetic challenges as animal fights to maintain homeostatic control, mount an immune response, repair localized damage, ensure adequate resources, and reproduce despite it all (Rajotte and Couture 2002, Berta et al. 2015). Because of this, the global meta-analysis of the literature and summaries of historical reports of parasitism and contaminant exposure in otter and mink contained in this thesis are invaluable resources for future research. This includes an exhaustive summary of methods for the analysis of energetic condition in mammals and a framework for future projects, with special emphasis on the consideration of methodological limitations in project design. We hope that these resources will instruct ongoing research and provide a comprehensive context for the inclusion of the sub-lethal effects of stressors into ecological and management models.

6.3 Implications for aquatic ecosystems

River otter, mink, and other mustelids are ideal sentinel species as key predators in aquatic and terrestrial ecosystems and are useful as indicators of contaminant exposure on a broad scale (Basu et al. 2007, Peterson and Schulte 2016, Goretti et al. 2018). As such, they are essential as advanced warning systems for threats to ecosystems and human communities (Bossart 2011). Historically, piscivorous mammals have been used as sentinels for environmental health as well as for the toxicology of contaminants, potentially predictive of the danger for humans (Basu 2012). Research on metal contaminants in otter and mink is abundant (see Chapter 4, Table 4.2), which makes them especially useful as early indicators of potential contaminant threats thanks to extensive data for comparison (Basu et al. 2007). The elevated levels of contaminants and widespread mesocercarial infections of a zoonotic parasite in otter and mink outlined in this research are causes for concern in Western Canada.

6.3.1 Parasitic infection

Widespread infections of *Alaria mustelae* have implications for fish population health and management. Not previously reported in otter and mink, infection is likely mirrored in fish species which may be concurrently experiencing increased rates of black-spot disease. Black-spot disease can be caused by a large variety of trematodes and can reduce growth, cause deformities, lower thermal tolerance, and reduce survival and reproductive success (Hockett and Mundahl 1989, Steedman 1991, Hagmayer et al. 2020, Duflot et al. 2023). Infections have been reported to damage the retina, altering visual acuity, and are associated with reduced growth in Arctic charr (*Salvelinus alpinus*) (Voutilainen et al. 2010, Padros et al. 2018). It is likely that *A. mustelae* would cause similar pathology in fish species, as black-spot disease is driven by the response of the host to migrating and encysting trematodes and is generally unrelated to the specific species of trematode. Species of *Alaria* also migrate to the vitreous fluid of the eye in human infections, suggesting they may do this in other hosts as well (Mohl et al. 2009, Portier et al. 2014). This may have implications for the health and management of salmonid species, whose decline has been driven in part by parasitic infection, as well as other vulnerable species (Schaaf et al. 2017). Further research is needed to investigate the potentially dramatic consequences of widespread *A. mustelae* infection, especially in commercially important salmonid species, which may be a higher risk for negative effects. As a zoonotic species, infection may also be a threat for humans, especially rural and indigenous communities, via undercooked fish, and monitoring of potential cases should be implemented in Alberta and British Columbia.

6.3.2 Metal Contaminants

The results of this study showed metal contaminant levels are high in British Columbia and Alberta in relation to other parts of North America when compared against historical reports going back to the 1980's. Specifically Cu, Cd, Fe, Se, and Zn, which are a threat to the health of aquatic ecosystems. Metal contaminant effects on microbes and invertebrates can prevent litter decomposition and therefore alter nutrient cycling in freshwater streams (Ferreira et al. 2016). They can also reduce coral reproductive success (Nalley et al. 2021), affect amphibian behavior, growth, development, and survival (Sievers et al. 2019), and reduce species richness across all major marine ecosystems (Johnston and Roberts 2009). Fish may be particularly at risk due to the tendency of many metals to bioaccumulate up trophic levels, with metal contamination impacting fish abundance, diversity, and richness (McKinley and Johnston 2010). These metal contaminants may be a serious and generally unknown threat for aquatic life in Western Canada. Future analysis of the samples collected in this project will include the use of stable isotopes to connect diet and contaminant exposure. Further research is needed to investigate metal contaminant concentrations in important fish and invertebrate species across British Columbia and Alberta. This is especially important as the results of our modeling suggest that high environmental concentrations, not long-term accumulation, are the main driver of some contaminants such as Co, Fe, Pb, Se, and Zn.

6.3.3 Anthropogenic Sources of Contaminants

Despite regulatory measures, our analyses reveal metal contaminants are at higher levels than previously reported averages across North America and appear to be driven by anthropogenic sources of contamination. Anthropogenic activities have been increasing rapidly in Alberta and British Columbia over the last decade. Aquatic ecosystems are subject to a combination of stressors that include urban development and waste, land clearing for forestry or agriculture, mining, and oil and gas extraction, along with the contaminants that are associated with these activities (Johnson et al. 2005, Sofield and Bollinger 2009, Huang et al. 2016, Lima and Wrona 2019, Northcote 2019, Folkerts et al. 2021). While there are federal and provincial limits set on metal contaminants in aquatic ecosystems, businesses self-report their releases to the National

Pollutant Release Inventory (Government of Canada, 2021), which can lead to underreporting and limit our ability to predict contaminant burdens in ecosystems (Walker 2022). As metal contaminant concentrations have remained consistently high in Alberta and British Columbia compared to the rest of North America, and have even increased since previous studies in some cases, mitigation efforts to protect aquatic wildlife appear not to be working. Proper remediation of abandoned oil and gas sites and mines must be the primary goal for legislation and government policy going forward, along with more accountable reporting methods. The cumulative effects of various anthropogenic stressors can contribute to negative outcomes for individuals and populations, and more research is needed to identify all the stressors in aquatic systems (Lima and Wrona 2019). Exposure to metal contaminants driven by anthropogenic activities is a serious threat to the health of our aquatic species as well as the human communities that subsist on them. Action is necessary to mitigate their effects in both freshwater and marine ecosystems.

6.3.4 Global Climate Change

6.3.4.1 Parasitism and rising temperatures

As infection by mesocercarial *A. mustelae* has not been previously reported in river otter or mink in North America, it may represent an emergent disease, potentially related to increasing temperatures due to climate change. Rising temperatures may benefit parasites, potentially due to the added stress of temperature on hosts, reducing their capacity to mount a successful immune response (Paull and Johnson 2011, Marcogliese 2016). Increases in black-spot disease have already been linked to higher temperatures in Coho salmon in Oregon (Cairns et al. 2005) and steelhead trout in California (Schaaf et al. 2017). Salmonid species may be particularly susceptible to black-spot disease (Schaaf et al. 2017). Parasitic infection and rising temperatures are suggested as major factors in wild Pacific salmon decline (Miller et al. 2014). Infections in otter and mink are likely mirrored in fish species, which may be concurrently experiencing increased rates of black-spot disease. It is possible the widespread, previously unreported infections of *A. mustelae* seen in this study are due to record-breaking temperatures in British Columbia and Alberta in recent years. Future research is needed to determine if the highly prevalent infection by mesocercarial *A. mustelae* are related to rising temperatures, and how this may affect threatened fish species in Western Canada.

6.3.4.2 Metal contaminants and climate change

Contaminant availability and severity in aquatic organisms may be affected by rising temperatures and global climate change. Uptake and toxicity are often temperature dependent (Sokolova and Lanning 2008), and global change may alter ecological function, causing range, dietary, and behavioral changes, which may expose some species to higher levels of contaminants (Dietz et al. 2019). Rising temperatures can enhance the mobility of pollutants in the ecosystems and increase toxicity in organisms due to compounding climate change stressors (Kibria et al. 2021). Global climate change has also led to an increase in the incidence of wildfires in recent years, which can further increase contaminant exposure in watersheds due to the loss of vegetation and surface runoff (Raoelison et al. 2023). Considering the high exposure of multiple metal contaminants in Alberta and British Columbia, and the recording breaking temperatures in recent years, metal contamination may be an even more serious threat. Future research is needed to investigate concentrations of contaminants in fish species of concern, as well as the energetic effects of exposure to Cu, Cd, Fe, Ga, Se, and Zn in heat-stressed organisms. This is essential to predict the potential implications of high metal contamination for at-risk fish species in Western Canada.

6.4 General conclusion

Overall, the findings of this thesis highlight serious gaps in knowledge concerning the importance of sub-lethal stressors such as parasites and metal contaminants in mammals. This work synthesized the current state of the literature regarding sub-lethal effects of helminths on mammals and historical reports of metal contamination in otter in mink to create averages for easy comparison and expanded our understanding of contaminant exposure and parasitic infection in aquatic mammals in Western Canada. The prevalence of previously unreported mesocercarial infections of *A. mustelae* and elevated levels of metal contaminants warrant ongoing investigation, especially concerning potential risks to aquatic ecosystems. While this study focused on Alberta and British Columbia, our results on the energetic effects of parasite and metal contaminant exposure are broadly applicable, especially for other mustelid or carnivore species, which have similarly high metabolic requirements. Future research is needed to investigate the prevalence and impact of *A. mustelae* in aquatic species. Furthermore, research is needed to investigate the potentially severe implications of elevated levels of metal contaminants reported, and their effects on aquatic species health and functioning in Western Canada.

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†Indicate the 143 studies that were included for data extraction in the meta-analysis.

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Appendices

Appendix A

A.1 Quotes from textbooks on the effects of helminths on wildlife health

Cestodes
[wildlife, *Tania* spp.] "no clearly established patterns of significant population impacts from this parasite" (Botzier and Brown, 2014, p. 117)
[wildlife, *Echinococcus* spp.] "…rarely has adverse effects on definitive hosts, and there is no clear evidence for poulation impacts on intermediate hosts" (Botzier and Brown, 2014, p. 119)
[wild mammals] generally "little or no problem to its host" (Samuel *et al.*, 2001, p. 150)
[marine mammals] "usually innocuous, but in extreme cases can result in debilitation and death of the host" (Geraci and St. Aubin, 1987, p. 408)
[marine mammals] "very few parasites of this group are considered disease problems" (Daily, 2005, p. 317)

Trematodes
[wildlife, liver flukes] "generally….do not cause significant population impacts" (Botzier and Brown, 2014, p. 96)
[wildlife, liver Flukes] "although death of individuals may occur, there is little evidence of population control as a result of infection" (Samuel *et al.*, 2001, p. 135)
[wildlife, liver Flukes] "..not of significant concern as a disease in wild mammals." (Samuel *et al.*, 2001, p. 136)

Acanthocephalans
[marine mammals] "usually of little pathological significance despite the occurrence in some hosts of thousands of parasites" (Geraci and St. Aubin, 1987, p. 409)
[wildlife] "most acanthocephala do not cause serious pathology in their definitive hosts, and only a limited number of groups are of concern to wildlife studies." (Botzier and Brown, 2014, p. 75)

Nematodes
[marine mammals, anisakid parasites] "despite a high intensity, may cause no apparent ill effects" (Daily, 2005, p. 316)
[marine mammals] "generally not serious to the host" (Geraci and St. Aubin, 1987, p. 410)
[ungulates, strongyles] "can represent potential and real threats to economically important wild and domestic ruminants." (Samuel *et al.*, 2001, p. 220)

Helminths in general
[Wildlife] "have little detrimental effect on their hosts for most of the time" (Delahay *et al.*, 2009, p.)
[marine mammals] "usually have little relevance on host health" (Raga *et al.*, 2009, p. 1325).
[wild mammals] some "do not have pathogenic tendencies, or are difficult to assess, but nonetheless have potential to cause problems" (Samuel *et al.*, 2001, p. vii)

A.2 Inclusion and exclusion criteria

Studies were included or excluded based on the following criteria. (*a*) The study must report data on the correlation between internal infection by parasitic helminths (acanthocephalan, cestode, nematode, trematode) and host energetics ('body condition', 'health'). (*b*) The host must be a mammal. (*c*) Studies were excluded if the directionality of effect or if sufficient test statistics or data were not reported and could not be obtained from the corresponding author. (*d*) Studies that included 10 or fewer individuals were excluded. (*e*) Studies investigating only antibody levels or only reporting observed tissue damage or mortality were excluded.

For example, during full-text review, five papers that were included met the criteria as follows: Ceriac *et al.* (2019) reported on the interaction between infection by the intestinal helminth *Haemonchus contortus* and nutritional status in goats (food digestibility and blood parameters). Debeffe *et al.* (2016) investigated the effect of parasitic infection by intestinal helminths on body condition in a population of feral horses. Deenonpoe *et al.* (2015) analyzed the effect of infection by the liver fluke *Opisthorchis viverrini* on immune function in hamsters, observing an increased prevalence of the bacteria *Helicobacter pylori*. Ponlet *et al.* (2011) compared intestinal helminth infection (richness and diversity) and energetic condition in rodents *via* splenic (immune defense) and testes mass (reproduction). Seguel *et al.* (2019) investigated the correlation between infection by hookworm (*Ucinaria* spp.) and stress and core systemic functioning in South American fur seals (*Arctocephalus australis*) *via* cortisol and haematological parameters.

For comparison, five papers that were excluded based on our selection criteria were (1) Barnes *et al.* (2007) reported mortality and tissue damage by cyst development from *Echinococcus granulosus* infection in wallaby lung tissue, but with no mention of host energetics. (2) Wu *et al.* (2012) included nine experimental (infected with *Trichuris suis*) and five control pigs, however only three of the experimental pigs were successfully infected in the end, so the study was excluded for low sample size. (3) Kavaliers, Colwell and Choleris (1998) investigated the correlation between infection by the gastrointestinal parasite *Heligmosomoides polygyrus* and female avoidance of the odour of infected males, with no data on parasite effects on male or female condition. (4) Irvine *et al.* (2001) looked at the gastrointestinal parasites of reindeer, and how seasonality and infection intensity affected worm fecundity, but presented no analysis of energetic effects on hosts. (5) Snaith *et al.* (2008) reported the correlation between primate group size and stress (cortisol), then group size and GI helminth prevalence, but did not analyze the correlation between parasite prevalence and stress

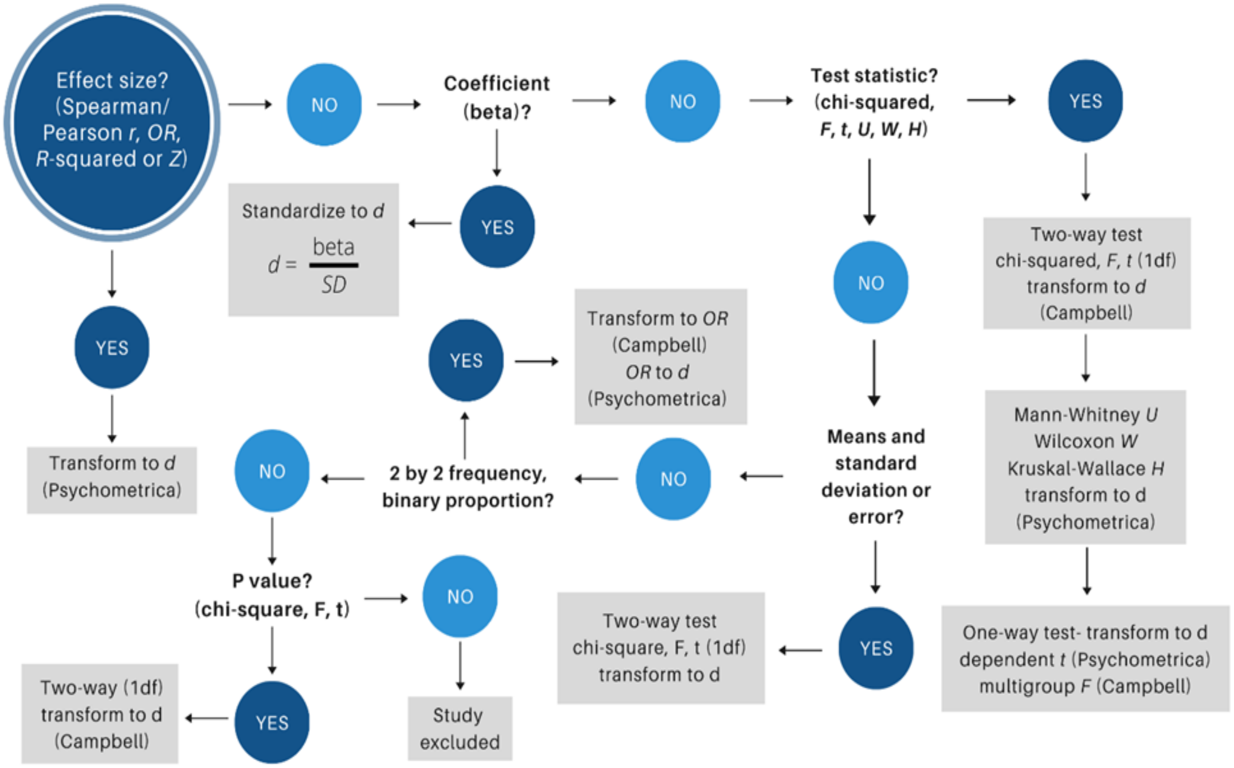

Figure S2.1 Hierarchy decision tree for calculation of Cohen's *d*. Protocol for the calculation of Cohen's *d* from the included studies to address inconsistencies in reporting of statistical results and data, including how the calculations were performed; through Psychometrica (Lenhard and Lenhard, 2016) and Campbell (Lipsey and Wilson, 2001; Wilson, 2001), online open-source calculation software, or by a predetermined formula. OR, odds ratio.

Table S2.1 Energetic proxies. All energetic proxies included in the meta-analysis and binned by categories of scale: (A) morphological; (B) physiological, and (C) observational (external). The table details the systems and processes each energetic proxy is related to according to the literature, the type of data with which it is commonly recorded, the energetic condition category it was nested within for this study, and the average period within which that proxy can be expected to change in response to energetic challenges. We also provide general information from the literature about the strengths and limitations of their use as a proxy for host energetic condition (fitness).

A. Morphological

Energetic proxy	**Related to**	**Data types**	**Energetic condition category**	**Change period**	**Strengths**	**Limitations**	**References**
Body mass	Size, sex, growth, fat, organ mass	Direct numerical: kg	Nutritional status	Days to weeks	Ease of measurement and analysis, non-lethal	Can change rapidly, and is related to sex, age, time of year, and individual variation	Noyce and Garshelis (1994); Brown et al. (1993); Peig and Green (2009, 2010); Sanchez et al. (2018)
Change in mass	Growth rate, nutrition, water retention	Direct numerical: kg	Nutritional status	Days to weeks	Relates mass to the individual, good for experimental projects, non-lethal, more important in juvenile animals	Can be affected by individual variation and multiple systems, slower growth may not necessarily be indicative of worse health	Reif et al. (2006); Peig and Green (2010); Hoste et al. (2016); Brown et al. (1993); Sanchez et al. (2018)
Body mass index (BMI)	Growth, fat, muscle mass, organ mass	Scaled numerical: kg/m^2	Nutritional status	Days to weeks	Ease of measurement and analysis, accounts for effect of body length on mass (limits effect of age and sex related to size), non-lethal	Differences in mass/length ratios may not necessarily be indicative of fitness, and may be affected by age/sex	Peig and Green (2010); Sanchez et al. (2018); Hoste et al. (2016)

Body part length	Growth	Proportional, scaled or direct numerical: m	Nutritional status	Months to years	Ease of measurement and analysis, often non-lethal, can be body part related to specific species fitness	Related to sex, age, sub-species variation	Peig and Green (2010); Sanchez et al. (2018)
Body condition score (BCS)	Fat, muscle tone, nutrition	Scaled numerical, ordinal	Nutritional status	Days to weeks	Can combine multiple factors to give broad overall scale	Subjective, difficult to model statistically, and still may be affected by age/sex	Peig and Green (2010); Sanchez et al. (2018)
Standardized mass index (SMI)	Fat, muscle mass, organ mass	Scaled numerical	Nutritional status	Days to weeks	Adjusts mass/length ratio by what average mass should be for that length, accounts for sex, age size variation	Can vary between populations (models need to account for sub-sampling), may produce similar scores for animals with vastly different mass/length measurements	Peig and Green (2009, 2010); Sanchez et al. (2018)
Kidney fat index (KFI)	Nutrition	Ordinal, proportional	Nutritional status	Days to weeks	Does not require whole carcass	Subjective if ordinal, has been shown in some mammals to be inaccurate under a certain threshold	Suttie (1983); Hoste et al. (2016); Sanchez et al. (2018); Corbin et al. (2008)
Fat (subcutaneous, bone marrow, omental subcutaneous)	Nutrition, energy availability, systemic stress	Proportional, ordinal, direct numerical: kg	Nutritional status	Weeks to months	Generally an excellent measure of body condition and health of mammals	Fat may not indicate health or reproductive success, not all species use fat from the same locations in the same order, may need to compare several sites	Suttie (1983); Hoste et al. (2016); Sanchez et al. (2018)

Liver mass (or index)	Increased energy demands [basal metabolic rate (BMR)], digestive stress, systemic stress, liver damage, systemic inflammation	Direct/scaled numerical: kg	Core systemic functioning	Days to weeks	Liver mass is tightly controlled to around 5% of mammal body mass (even when transplanted larger or smaller, the body adjusts the liver to 5%)	5% rule is not universal, so needs standardizing for species, and may change according to environmental factors that affect BMR (like cold stress)	Konarzewski and Diamond (1995); Kristan (2004); Zhu et al. (2013; Penzo-Méndez and Stanger (2015)
Spleen mass (or index)	Immune investment, systemic stress [increased storage of red blood cells (RBCs)]	Direct/scaled numerical: kg	Defense/stress	Days to weeks	Simple way without laboratory tests to quantify immune burden; mass gain versus spleen mass can provide a metric of trade-off between body condition and immune health	Tends to be larger in animals in good condition as they have excess energy to invest in immune health; storage of RBCs may be short term	Schulte-Hostedde and Elsasser (2011); Corbin et al. (2008)
Reproductive organ mass	Investment in reproductive output	Direct numerical: kg	Reproduction	Weeks to months	Easy method to quantify differences in energy allocation to reproductive output	Smaller organs not necessarily indicative of poor health, more helpful on a population rather than individual level	Schulte-Hostedde and Millar (2004)
Kidney mass	Increased energy demands (BMR), stress, digestive disruption, kidney damage	Direct numerical: kg	Core systemic functioning	Weeks to months	Can allow assessment of effects of infection on digestive health, stress hormones, and breakdown of muscle tissue (creatinine) due to nutrient limitation	Can be changed by a variety of factors, and individual variation must be accounted for	Konarzewski and Diamond (1995); Kristan and Hammond (2004)
Heart mass	Increased energy demands (BMR), systemic stress, heart damage	Direct numerical: kg	Core systemic functioning	Weeks to months	Very important for survival, and changes may be indicative of serious stress on cardio-pulmonary health	Tends to have lower variability than other organs like liver or spleen	Konarzewski and Diamond (1995)

Adrenal gland mass	Chronic stress,	Direct numerical: kg	Defense/stress	Months to years	Good proxy for stress/cortisol levels without needing to perform laboratory tests on blood/feces	Individual variation must be considered, including differences in size, sex and population	Ulrich-Lai et al. (2006); Blackburn et al. (2015)
Intestinal mass	Immune response, increased energy demands (BMR), intestinal damage	Direct numerical: kg	Core systemic functioning	Weeks to months	Quantitative measure that can be related to observations of thickening, ulceration, or histological examination	Response to energy demand tends to be less significant than in other organs	Konarzewski and Diamond (1995); Kristan and Hammond (2004)
Milk production	Nutrition, reproductive effort	Direct numerical, proportional: l	Reproduction	Days to weeks	Direct connection between mother body condition and energy allocation for reproductive success	Milk production can be related to age, season, and food availability; analysis must account for these variables	Landete-Castillejos et al. (2005); Hoste et al. (2016); Renaud et al. (2020)
Muscle depth	Nutrition, overall health	Direct numerical: m	Nutritional status	Months to years	Longer term metric of body condition and not as variable as fat, excellent to compare in growing individuals	Many muscles can be used, but may be affected differently by nutritional burdens, which also can be species specific	Beasley et al. (2010); Blackburn et al. (2015)

B. Morphological

Energetic proxy	Related to	Data types	Energetic condition category	Change period	Strengths	Limitations	References
Hematocrit (HCT)/packed cell volume (PCV)/red blood cell count (RBC)	Fluid balance, systemic stress, anaemia, nutrition, blood loss	Proportional: RBC/volume	Core systemic functioning	Days to weeks	Common blood test, does not require species-specific validation, related to both survival and reproduction	May be affected merely by fluid retention/ dehydration, also may change with sex, age, and season, haematological parameters must be analyzed carefully as they can be affected by multiple factors. Varies seasonally in some species, reduction may be due to blood loss or nutrient deficiency	Trumble et al. (2006); Reif et al. (2006); Beldomenico et al. (2008b); Behnke et al. (2011); Budischak et al. (2012); Maceda-Veiga et al. (2015)
Hemoglobin (HG)	Aerobic health, nutrition, blood loss	Proportional: HG/volume, g/dl	Core systemic functioning	Days to weeks	Common blood test, does not require species-specific validation, related to both survival and reproduction	Varies by species and sex, need standard levels for comparison, reduction is often due to blood loss	Budischak et al. (2012); Murphy (2014)
Mean corpuscular volume (MCV)	Aerobic health, RBC age/ regeneration, systemic stress	Proportional: HCT × 10/RBC	Core systemic functioning	Days to weeks	Common blood test, does not require species-specific validation, related to reproduction	May decrease or increase with parasitic infection	Noyce and Garshelis (1994); Budischak et al. (2012)
Corticoid levels	Physiological stress, behavioral change, immuno-modulation	Proportional: mol/l	Defense/ stress	Hours to days	Non-lethal (blood or feces), easy to compare to faecal egg count (FEC) and monitor over time	Since it is a systemic response to stress, can be triggered by a variety of stimuli, including capture	Defolie et al. (2020); Milner et al. (2003); Pawluski et al. (2017); Milner et al. (2003)

Milk protein/ fat levels	Nutrition, body condition	Proportional	Reproduction	Days to weeks	Mothers in poor condition produce poorer quality milk, and is a good indicator of trade-off between personal survival and reproduction	Small changes may be indicative of differences in diet, may also be affected by systemic stress; changes can also be seasonal due to changes in food availability	Landete-Castillejos et al. (2005); Renaud et al. (2020)
Gut microbiota	Digestive health, immune health	Proportional, scaled or direct numerical	Core systemic functioning	Days to weeks	Direct effect, can easily monitor specific species/ changes; linked to many important processes (like nutrition and behavior), reports link species richness to better health	Requires knowledge of ‘normal’ gut flora, may include complex interactions between environmental factors, immune response, diet (herbivores may be more affected), and specific species of bacteria	Reynolds et al. (2015); Sampson and Mazmanian (2015); Aivelo and Norberg (2018); Suzuki (2017)
Reproductive hormones	Reproductive fitness, stress	Direct numerical, proportional: mol/l	Reproduction	Days to weeks	Non-lethal (blood or feces), directly related to reproductive behavior and output	Highly seasonal depending on species	Courot and Ortavant (1981); Wade and Schneider (1992); Milner et al. (2003); Mauget et al. (2007); Mills et al. (2009)
Creatinine	Stress, excretory health	Direct numerical: g/dl	Core systemic functioning	Hours to days	Non-lethal (blood), general indicator of systemic stress	May be affected by multiple factors, and can change quickly, small changes may be related to diet differences	Milner et al. (2003); Trumble et al. (2006)
Albumin	Protein supply and nutrition	Direct numerical: g/l	Core systemic functioning	Hours to days	Good non-lethal indicator of protein nutrition, low albumin is an indicator of low protein digestion or metabolism	May be affected by a variety of factors including changes in diet and age, which need to be controlled for	Meagher (1998); Milner et al. (2003)
Liver enzymes (various)	Systemic stress, liver damage	Direct numerical-amount/l	Core systemic functioning	Hours to days	Good indicator of direct damage to liver	Affected by stress, so may be heightened during capture or by traumatic death	Meagher (1998); Trumble et al. (2006)

Metabolic rate	Nutrient availability, aerobic health	Direct numerical, proportional, scaled	Core systemic functioning	Hours to days	Effective way to measure host's ability to generate energy to travel, forage, maintain homeostasis, and reproduce; changes may be particularly important in species that live in cold environments and require high BMR	Requires laboratory setting or complex field equipment for accurate analysis; metabolic rate may also be increased due to immune burden	Munger and Karasov (1989); Meagher and O'Connor (2001); Lutermann et al. (2013)
Sperm cell count	Reproductive health and energy allocation	Direct numerical	Reproduction	Days to weeks	Directly linked to fitness as higher sperm counts are associated with reproductive success	Time consuming, invasive, most often requires live specimen as sperm decomposes quickly	Schulte-Hostedde and Millar (2004); Rouatbi et al. (2016)
Placental scars/ shed ova	Reproduction	Direct numerical	Reproduction	Years	Good indicator of number of pregnancies in the animal's life or output of ova during the season for some species	Age must be controlled for, clumping by age class may lead to false correlation as in many species' infection increases with age, as does number of pregnancies; retention of corpa lutea from shed ova persists in some species over a whole breeding season, but this is species specific	Iason and Boag (1988); Nelson et al. (2003); Newey and Thirgood (2004)
Serum glucose	Fat breakdown, carbohydrate metabolism	Direct numerical: g/l	Core systemic functioning	Hours to days	Normally strictly controlled, decrease is often associated with fasting or nutrient loss	Not directly related to fitness, best used as supplementary metric	Noyce and Garshelis (1994); Milner et al. (2003)
Serum cholesterol	Lipid metabolism, metabolic stress	Direct numerical: g/l	Core systemic functioning	Days to weeks	Changes in times of high metabolic demand like pregnancy and lactation, may be best to compare infected/uninfected groups during such periods	Not directly related to fitness, best used as supplementary metric	Seguel et al. (2019); Noyce and Garshelis (1994)

Serum urea/ nitrogen	Excretory health, body condition, dehydration	Direct numerical: mmol/l	Core systemic functioning	Hours to days	Useful when comparing with creatinine, as both may be related to loss of kidney function, but urea may also be related to dehydration while increased creatinine may be linked to muscle breakdown due to starvation	Small changes may be related merely to protein content in diet	Noyce and Garshelis (1994); Milner et al. (2003); Trumble et al. (2006)
Plasma electrolytes (Na, Ca, P)	Body water, excretory health, nutrition	Direct numerical: mmol/l	Core systemic functioning	Hours to days	Related to diet and digestive health, good indicator of dehydration	Also affected by dehydration and season, may only be appropriate as secondary metric	Trumble et al. (2006)
Serum pepsinogen	Digestive health, loss of parietal cells	Direct numerical: i.u./l	Core systemic functioning	Days to weeks	Good indicator of disruption to the digestive process, nutrient intake and weight gain reported to be negatively correlated with pepsinogen levels	Mainly reported in domestic ungulates, unclear if the same relationship exists in other mammals	Arneberg (1999); Moradpour et al. (2013)
Pollutant concentration	Liver health, excretory health, systemic stress	Direct numerical: µg/g	Core systemic functioning	Weeks to years	Helpful for ecosystems or species where there is specific concern about a toxin(s), or if there are known environmental levels (i.e. exposure is consistent), for investigating host's ability to excrete toxins, or as compounding stressor	Still poorly understood, some parasites seem to reduce exposure, some seem linked to increased exposure, needs more parsing out and control of environmental and physiological factors	Sures (2008); Marcogliese and Pietrock (2011)
Oxidative stress	Systemic stress, liver damage	Direct numerical: amount/l	Core systemic functioning	Hours to days	Potential option as bioindicator for sublethal effects of parasite infection	Time consuming and potentially costly tests	Bahrami et al. (2014); Rodríguez-Estival et al. (2016)

Leptin	Immune depression, nutrient deficiency	Direct numerical: g/l	Defense/ stress	Days to weeks	Poor nutrition shown to decrease leptin rapidly in mammals, good metric of nutrition status in live animals	May require species-specific validation of assay	Lord et al. (1998); Zaralis et al. (2009)
Comorbidity (secondary infection)	Immune health/ suppression, stress	Ordinal, proportional, direct numerical	Defense/ stress	Weeks to months	Can use more obvious clinically significant infections to reveal subtle effects of parasite infection	Requires naturally occurring co-infection; without data on immune suppression, or physical condition, may lead to false correlation	Knowles (2011); Beldomenico et al. (2008a); Serrano and Millán (2014)
Digestive uptake	Digestive health, nutritional demands	Proportional, direct numerical	Core systemic functioning	Days to weeks	Can investigate direct effects of intestinal parasite on nutrient assimilation (nutrient in minus nutrient out)	Requires a laboratory setting and often novel feed options for precision	Munger and Karasov (1989); Reif et al. (2006); Ceriac et al. (2019)

C. Observational

Energetic proxy	**Related to**	**Data types**	**Energetic condition category**	**Change period**	**Strengths**	**Limitations**	**References**
Activity (movement, activity/rest,	Behavior in response to sympathetic/ somatic cues (trade-off theory)	Direct numerical, proportional	Defense/ stress	Hours to days	Effective way to compare infected/ uninfected groups and external effects of subtle internal changes, non-lethal	Animal behavior can be affected by a variety of factors, needs strong controls	Levesque, Nowack, and Stawski (2016); Hutchings et al. (2006); Collins et al. (2016)
Reproductive behavior	Trade-off between reproduction and energy conservation	Direct numerical, proportional	Reproduction	Days to months	Can related internal effects on physical fitness to reproductive fitness, non-lethal	Animal behavior can be affected by a variety of factors, needs strong modelling/controls	Hutchings et al. (2006); Heldstab et al. (2017); Berta et al. (2015)

Reproductive rates (pregnancies, litter size, interval and gestation period)	Reproductive fitness, nutrient availability	Direct numerical, proportional, scaled	Reproduction	Months to years	Pregnancy and lactation are the most energetically taxing period of a female's life, direct connection between body condition and fitness	Many factors may influence this, and it is difficult to observe in the wild	Degen (2008); Berta et al. (2015); McNab (2012); Heldstab et al. (2017)
Pup growth/ survival, litter size	Body condition, nutrient availability	Direct numerical, ordinal	Reproduction	Days to months	Like pregnancy, lactation is extremely taxing on mothers, some mammals participate in 'bet-hedging' strategies and abandon a proportion of litter based on resource availability	Many factors may influence this, and it is difficult to observe in the wild	Degen (2008); Berta et al. (2015); McNab (2012)
Physical endurance	Aerobic health, metabolic rate	Direct numerical, scaled	Nutritional status	Weeks to months	Easy, but invasive way to measure control and infected individual's physical fitness	Requires controlled laboratory settings to measure effectively	Meagher and O'Connor (2001); Berta et al. (2015); McNab (2012)
Population dynamics	Community reproductive fitness and resource availability	Direct numerical, scaled	Reproduction	Months to years	Only direct way to relate parasite infection to a specific population's fitness	Difficult to assess without known infection rates/controls, requires complex statistical modelling	Arneberg (2002); Hudson et al. (1998); Grenfell (1988)
Food intake (elective feeding, foraging activity)	Nutrient availability, digestive health	Direct numerical, proportional	Nutritional status	Days to weeks	Easy, non-invasive way to test effects of infection on nutrient requirements, via the host's response	Small variations are hard to attribute to any single factor without strong controls, must consider age, food availability and reproductive status	Ceï et al. (2016); Zaralis et al. (2009); Munger and Karasov (1989)
Food uptake	Digestive health, digestive ability	Direct numerical	Core systemic Functioning	Days to weeks	Easy, non-invasive way to test effects of infection on digestive functioning via nutrients/food in and nutrients/food out (via feces)	Requires controlled experimental settings	Ceï et al. (2017)

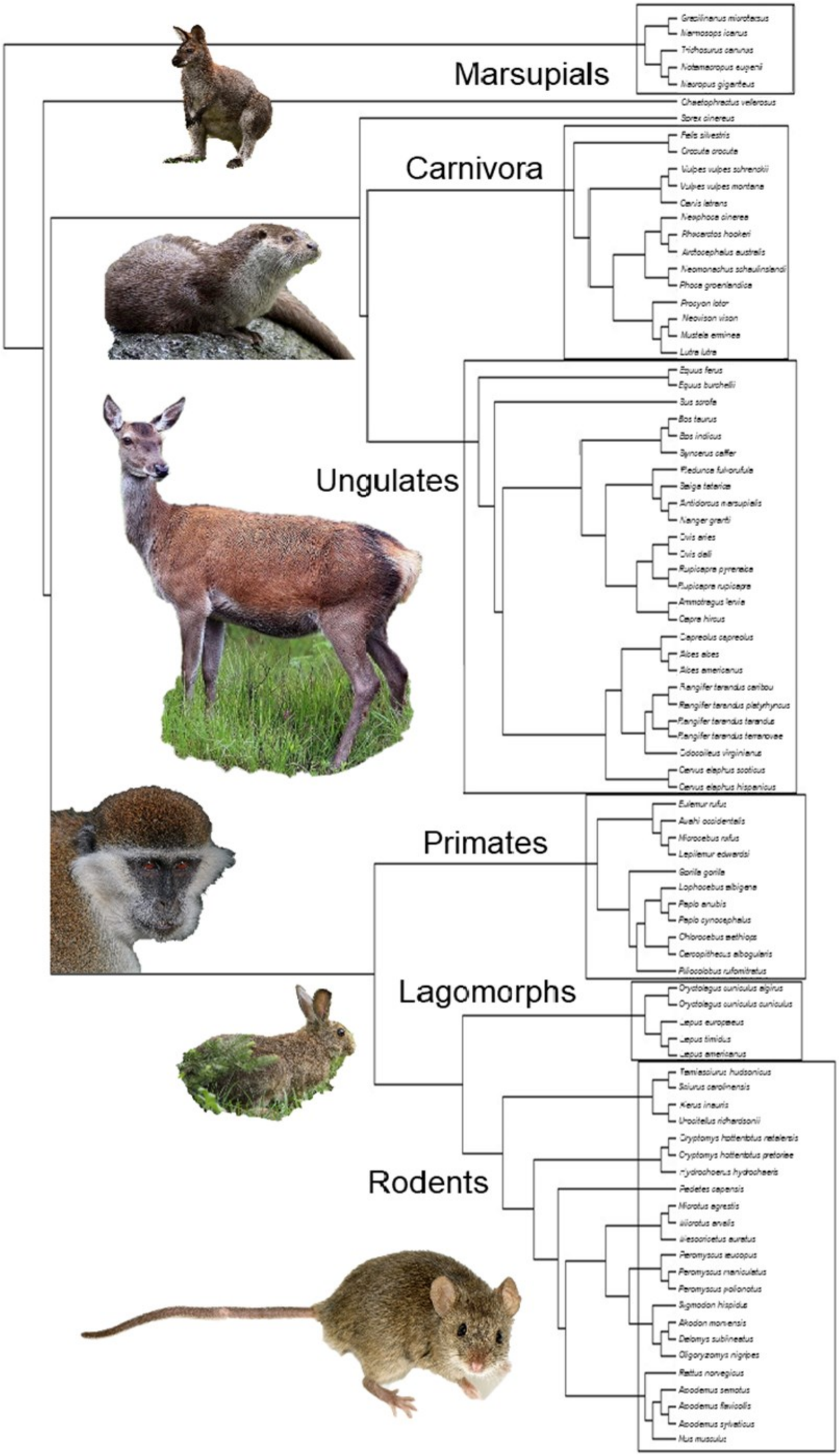
Gracilinanus microtarsus
Marmosops incanus
Trichosurus caninus
Notamacropus eugenii
Macropus giganteus
Marsupials
Chaetophractus vellerosus
Sorex cinereus
Carnivora
Felis silvestris
Crocuta crocuta
Vulpes vulpes schrenckii
Vulpes vulpes montana
Canis latrans
Neophoca cinerea
Phocarctos hookeri
Arctocephalus australis
Neomonachus schauinslandi
Phoca groenlandica
Procyon lotor
Neovison vison
Mustela erminea
Lutra lutra
Ungulates
Equus ferus
Equus burchellii
Sus scrofa
Bos taurus
Bos indicus
Syncerus caffer
Redunca fulvorufula
Saiga tatarica
Antidorcas marsupialis
Nanger granti
Ovis aries
Ovis dalli
Rupicapra pyrenaica
Rupicapra rupicapra
Ammotragus lervia
Capra hircus
Capreolus capreolus
Alces alces
Alces americanus
Rangifer tarandus caribou
Rangifer tarandus platyrhynchus
Rangifer tarandus tarandus
Rangifer tarandus terraenovae
Odocoileus virginianus
Cervus elaphus scoticus
Cervus elaphus hispanicus
Primates
Eulemur rufus
Avahi occidentalis
Microcebus rufus
Lepilemur edwardsi
Gorilla gorilla
Lophocebus albigena
Papio anubis
Papio cynocephalus
Chlorocebus aethiops
Cercopithecus albogularis
Piliocolobus rufomitratus
Lagomorphs
Oryctolagus cuniculus algirus
Oryctolagus cuniculus cuniculus
Lepus europaeus
Lepus timidus
Lepus americanus
Rodents
Tamiasciurus hudsonicus
Sciurus carolinensis
Xerus inauris
Urocitellus richardsonii
Cryptomys hottentotus natalensis
Cryptomys hottentotus pretoriae
Hydrochoerus hydrochaeris
Pedetes capensis
Microtus agrestis
Microtus arvalis
Mesocricetus auratus
Peromyscus leucopus
Peromyscus maniculatus
Peromyscus polionotus
Sigmodon hispidus
Akodon montensis
Delomys sublineatus
Oligoryzomys nigripes
Rattus norvegicus
Apodemus semotus
Apodemus flavicollis
Apodemus sylvaticus
Mus musculus

Figure S2.2 Phylogenetic Tree of included species by clade. Image sources: Wallaby image modified from an image by benjamint444, available from Wikipedia under a CC A-SA 3.0 Unported license. River otter image modified from an image by Bernard Landgraf, available from Wikipedia under a CC A-SA 3.0 Unported license. Red Deer image modified from an image by Charles J Sharp, available from Wikipedia under a CC A-SA 4.0 international license. Grivet Monkey image modified from an image by Charles J. Sharp, available from Wikipedia under a CC A-SA 4.0 international license. *Lepus americanus* image modified from an image by Walter Siegmund, available from Wikipedia under a CC A-SA 3.0 Unported license. Mouse image modified from an image by George Shuklin, available from Wikipedia under a CC A-SA 1.0 Generic license.

A.3 Additional information on the data set

Of the 1115 articles gathered from the initial literature search, the four most prevalent terms in the abstracts were “disease,” “sheep,” “nematode” and “prevalence” (Fig. S2.2), highlighting the focus of parasitological research on mammals until recently. Research came mostly from the agricultural sector and targeted identified pathogenic diseases, which then biases research toward nematodes. These parasites often cause obvious disease processes in mammals. The 142 studies eventually included in the meta-analysis were published between 1977 and 2020, with the highest count of reported effect sizes peaking around 2011 (Fig. S2.4). Experimental design and methodology varied widely among studies. However, some trends could be detected with many research projects that were cross-sectional (*n* = 83), investigated the effect of parasites on wild species captured or monitored alive in the field (*n* = 73), most often ungulates (*n* = 56), and from species considered “stable” (*n* = 68) by the IUCN (2021). The predominant energetic condition metric used was nutrition (*n* = 105), with nematodes the most studied helminth species (*n* = 113) across studies included in the meta-analysis (see Table S2.2 and Data file S1). Three studies included all the above, reporting cross-sectional sampling of live wild-caught ungulates of stable (non-endangered) species, measuring change in nutrition as a response to infection by nematodes (*k* = 13). Note that none of the studies included investigated the effects of acanthocephalans.

Reported changes in host energetic condition were mostly linked to infection by nematodes (77.1%). Cestodes (8.2%) and trematodes (4.2%) were much less commonly considered for their effects on host energetics. Some studies also reported infection by multiple parasite groups (10.5%). Records included helminth infections in various host tissues but predominantly from the gastrointestinal tract (81.0%). However, host condition was also measured in response to respiratory (6.2%), liver (5.8%), heart (2.5%), excretory (2.2%), and muscle (0.3%) infections. Few studies considered response to infection of multiple tissues (2.0%). Parasitic infection was identified by either confirmation through host necropsy (64.6%) or faecal examination and identification of parasite eggs (33.7%). In rare cases, hosts were assumed to be infected without confirmation (1.7%). When quantifying parasite infection, included records were based predominantly on presence/absence (43.2%) or intensity (42.6%). Within studies reporting presence/absence, some included parasite infection only as treated *versus* untreated groups (7.0%) or infection over time (0.7%). A subset of mixed infections quantified parasites by species richness (6.5%).

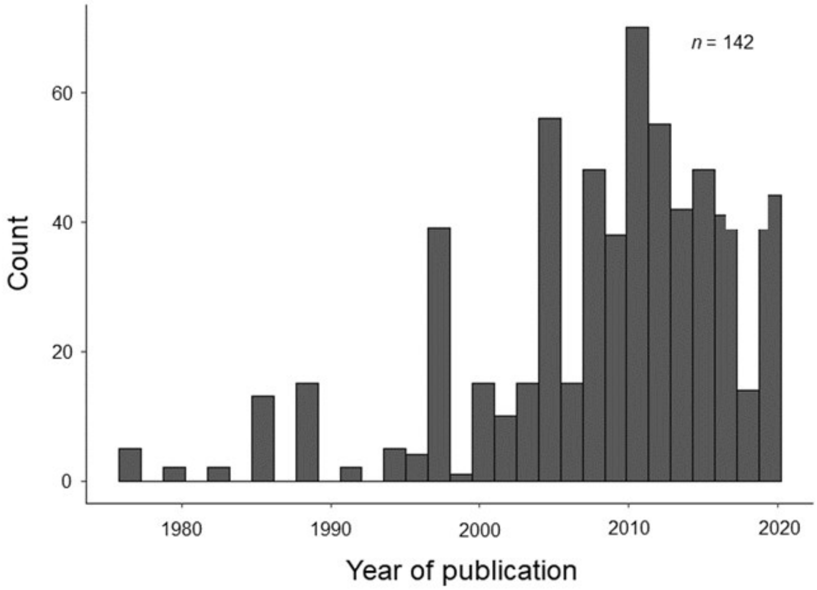

Figure S2.3 Included studies by year. Point counts of included studies by the year in which they were published.

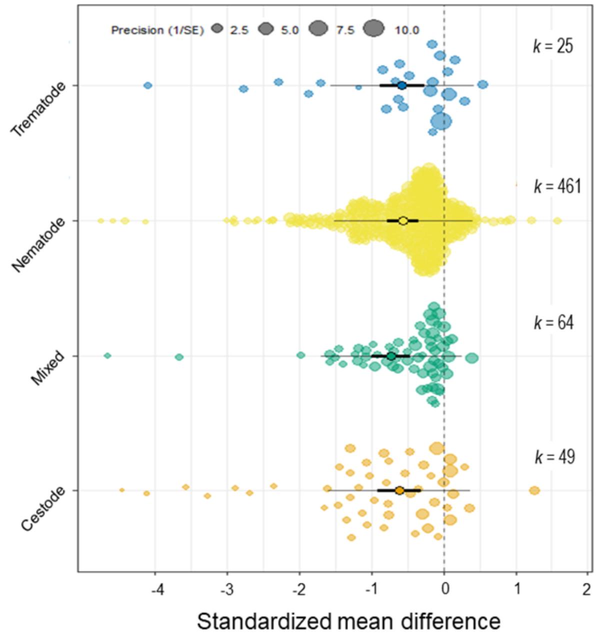

Figure S2.4 Effect of infection by parasite taxa. Orchard plot showing effect of infection on host energetics according to parasite taxonomic group: trematodes, nematodes, cestodes and mixed infections (i.e. hosts infected by more than one taxonomic group). Average effect sizes (point, 'trunk') including 95% confidence interval (bold line, 'branch') and precision interval (small line, 'twig'), individual effect sizes scaled by precision ('fruit') spread on the *y*-axis based on quasi-random noise (Nakagawa *et al.*, 2021).

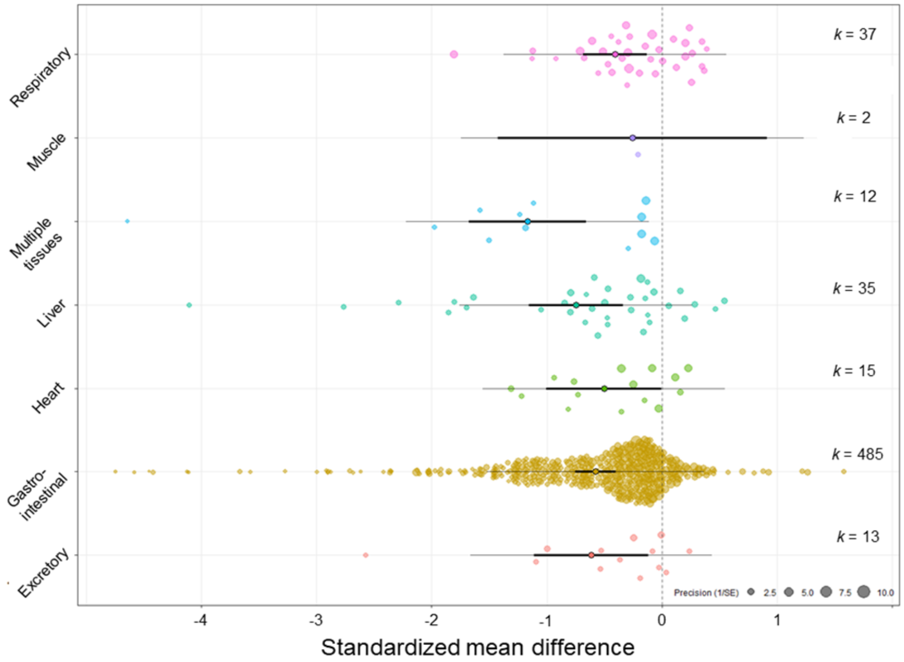

Figure S2.5 Effect of infection by tissue of infection. Orchard plot showing effect of infection on host energetics according to tissue location. Studies included analysis of parasites that infected the respiratory tract (lung, bronchioles, trachea, sinuses), muscle tissue, multiple tissues, liver, heart, gastro-intestinal tract (oesophagus, stomach, intestine, colon), and excretory system (kidney, bladder). Other details of orchard plot are as in Fig. S2.3.

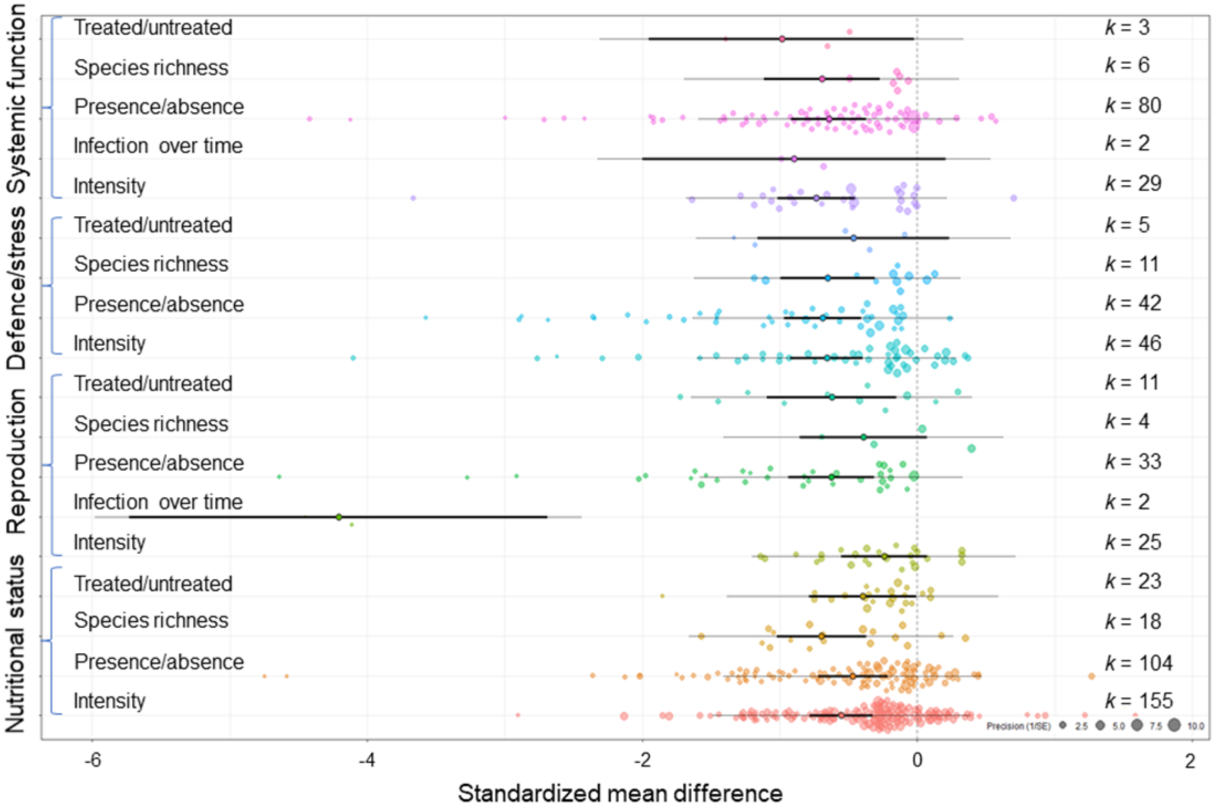

Figure S2.6 Effect of infection by energetic proxy and parasite quantification. Orchard plot showing effect of infection on host energetics according to energetic condition proxy and parasite quantification. Infection could be assessed/quantified as treated with anti-helminthics *versus* untreated (treated/untreated), infected or uninfected (presence/absence), artificially infected individuals that were then monitored over time (infection over time), total number of parasites per individual or average per individual in a population (intensity), or a metric of the diversity of parasites for infections with multiple species or taxa (species richness). Other details of orchard plot are as in Fig. S2.3.

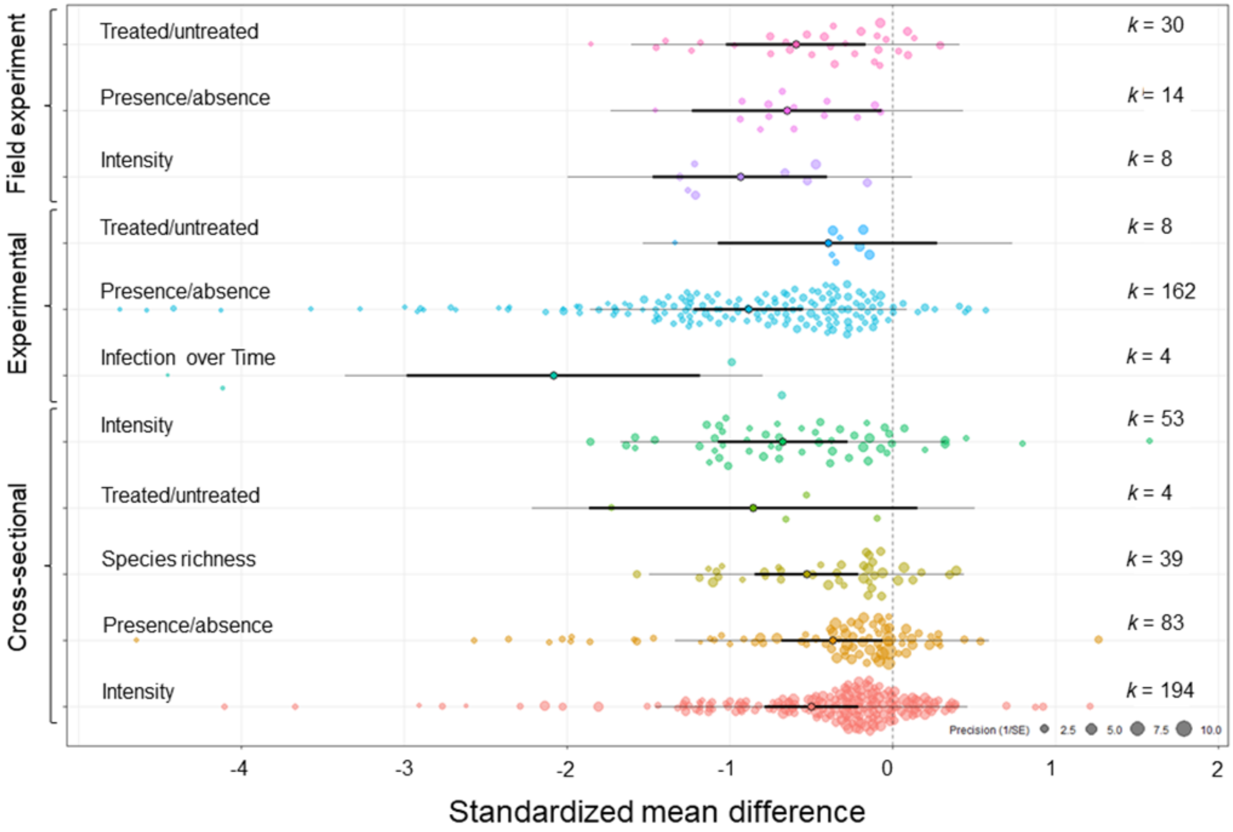

Figure S2.7 Effect of infection by study design and parasite quantification. Orchard plot showing effect of infection on host energetics according to study design and parasite quantification. Average effect size in semi-controlled field experiment, controlled experiment and cross-sectional studies and across parasitic infection measures. Infection could be assessed/quantified as treated with anti-helminthics *versus* untreated (treated/untreated), infected or uninfected (presence/absence), artificially infected individuals that were then monitored over time (infection over time), total number of parasites per individual or average per individual in a population (intensity), or a metric of the diversity of parasites for infections with multiple species or taxa (species richness). Other details of orchard plot are as in Fig. S2.3.

Table S2.2 Model results of the gastrointestinal infection subset. Effect sizes and meta-regression statistics for infections by gastrointestinal helminths using only the common body condition metrics subset. Body condition index (BMI): an ordinal rating of overall condition, body condition score (BCS): a scaled proportion of mass over length scaled by the population average and regression model exponent, body mass index (BMI): proportion of mass over length, superficial factors: various unique morphological features like antler length or coat quality that were grouped together.

Model	I^2	AIC	R^2_C	R^2_M	Moderator	Cohen's d	se	CI Lower	CI Upper	p-value	k
Intercept	52.06%	414	88.90	–	Intercept	–0.43	0.10	–0.62	–0.24	<0.0001	239
Condition proxy	47.93%	408	85.71	16.27	Body condition index (BCI)	–0.38	0.17	–0.73	–0.04	0.0298	17
					Body condition score (BCS)	–0.30	0.14	–0.56	–0.03	0.0297	45
					Body mass index (BMI)	–0.42	0.14	–0.70	–0.13	0.0039	34
					Body mass	–0.40	0.12	–0.64	–0.16	0.0011	58
					Body size/length	–0.02	0.21	–0.43	0.38	0.9070	12
					Fat	–0.43	0.14	–0.71	–0.16	0.0019	36
					Growth rate	–0. 83	0.18	–1.18	–0.47	<0.0001	21
					Muscle	–0.60	0.26	–1.05	–0.10	0.0185	9
					Superficial factors	–0.57	0.20	–0.96	–0.19	0.0036	7

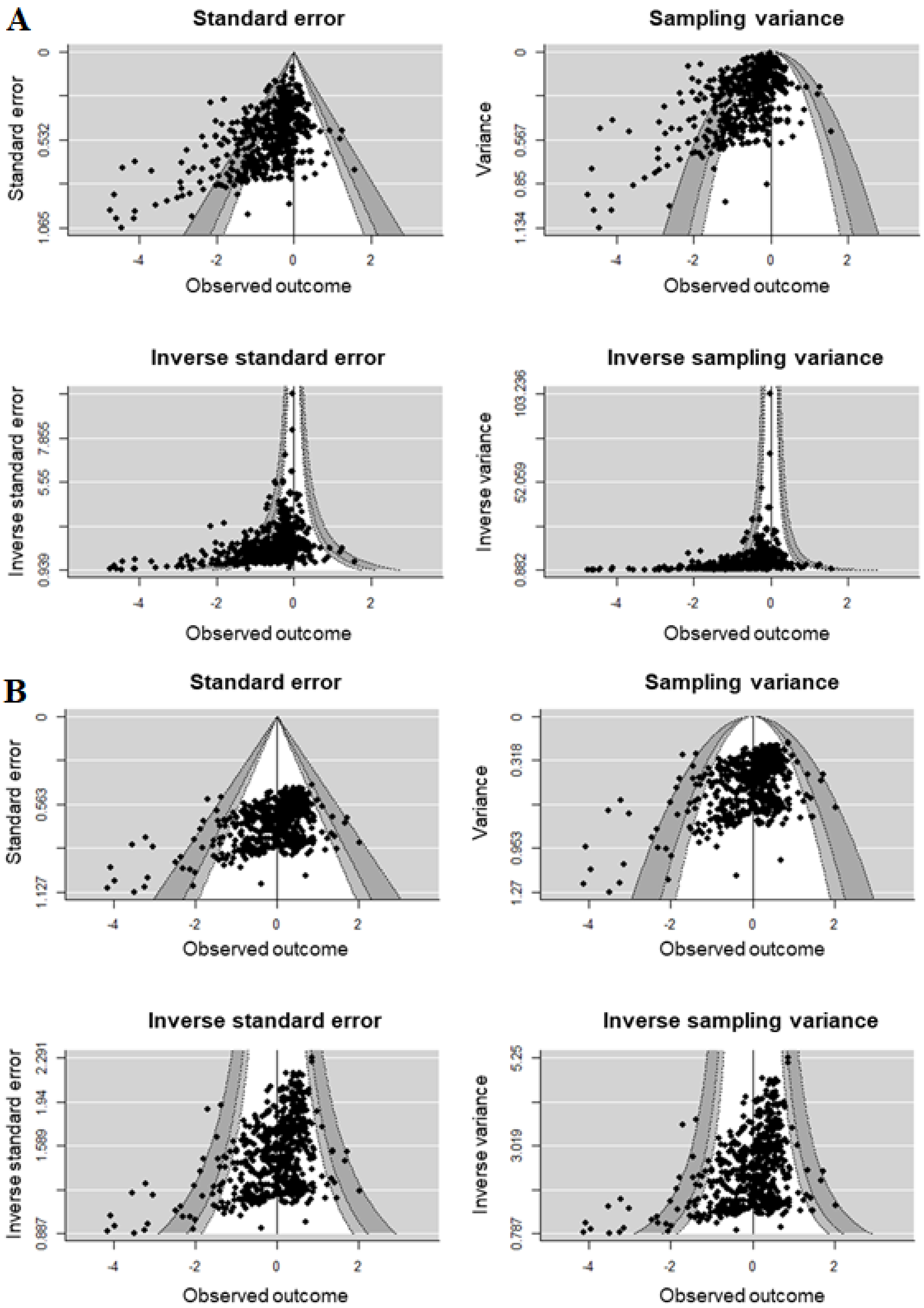

Figure S2.8 Analysis of publication bias. Contour funnel plots: (A) intercept-only model, (B) best-fit meta-regression model (host sampling origin: energetic proxy).

Figure S2.9 Word cloud of literature search abstracts. Produced by open-source software of the abstracts of all publications resulting from the original literature search (n = 1115). Excluded terms: *study, analysis, DOI, animals, parasite, index, abstract, keywords, non-host, Sp, may, article, study, also, infected, infection(s), species, female, male, worm, found, host(s).* The program also automatically excludes common filler words such as “the,” “and,” “but,” etc.

Appendix B

Table S3.1 Parasite Identification Justification. Reports of identified parasites A) Trematodes, B) Cestodes, C) Nematodes, D) Acanthocephalans by host ID, which include location and year (BC= British Columbia, AB= Alberta, 21=2020-21 season, 22=2021-22 season), species (NEVI= *Neogale vison*, LOCA= *Lontra canadensis*, ONZI= *Ondatra zibethicus*) and location the parasite was removed from. Parasite species identified is provided as well as a reference for the description morphological identification was based upon. Information for molecular identification also provided, including the region of DNA sequenced, the closest match to sequences available in the NCBI database using the BLASTn tool, the percent identity of that match, and the ascension number for the matched sequence.

A)

Host ID	Host Species	Location in Host	Identified as	Morphology Reference	Region	Closest match	Percent Identity	Ascension Number
BC21-0371	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.5	MH581270.1
BC21-0373	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.31	MH581270.1
BC21-0431	LOCA	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.18	MH581270.1
BC21-0373	NEVI	Intestine	*Alaria mustelae* (adult)	(Johnson 1979)	CO1	*Alaria mustelae*	100	MH581270.1
BC21-0395	LOCA	Intestine	*Alaria mustelae* (adult)	(Johnson 1979)	CO1	*Alaria mustelae*	100	MH581270.1
BC22-0489	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.38	MH581270.1
AB21-024	LOCA	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.49	MH581270.1
AB21-058	LOCA	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.32	MH581270.1
AB22-005	LOCA	Kidney tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.49	MH581270.1
AB22-008	NEVI	Liver tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.31	MH581270.1
AB22-016	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.18	MH581270.1
AB22-029	LOCA	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.48	MH581270.1
BC21-0387	LOCA	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.31	MH581270.1
BC22-0418	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	99.67	MH581270.1
BC22-0485	NEVI	Intestinal tissue	*A. mustelae* (mesocercaria)	(Johnson 1970)	CO1	*Alaria mustelae*	96.86	MH581270.1
BC22-0554	LOCA	Stomach	*Clinostomum poteae*	(Rosser et al. 2018)	CO1	*Clinostomum poteae*	99.8	MH282553.1
BC22-0491	LOCA	Intestine	*Isthmiophora inermis*	(Kostadinova and Gibson 2002)	28S	*Isthmiophora* spp.	99.83	KT956920.1
BC22-0491	LOCA	Intestine	*Isthmiophora inermis*	(Kostadinova and Gibson 2002)	CO1	*Isthmiophora hortensis*	91.56	MT577590.1
AB21-063	LOCA	Stomach	*Isthmiophora inermis*	(Kostadinova and Gibson 2002)	CO1	*Isthmiophora hortensis*	91.50	MT577590.1
AB21-057	LOCA	Stomach	*Isthmiophora inermis*	(Kostadinova and Gibson 2002)	CO1	*Isthmiophora hortensis*	90.66	KR062182.2
AB21-110	ONZI	Intestine	*Plagiorchis proximus*	(Barker 1915)	CO1	*Plagiorchis* spp.	100	MH369438.1
AB21-127	ONZI	Intestine	*Plagiorchis proximus*	(Barker 1915)	CO1	*Plagiorchis* spp.	100	MH369438.1

B)

Host ID	Host Species	Location in Host	Identified as	Morphology Reference	Region	Closest match	Percent Identity	Ascension Number
BC21-0396	LOCA	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.13	MK681866.1
BC21-0370	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.33	MK681866.1
BC21-0416	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.56	MK681866.1
AB21-033	LOCA	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	97.93	MK681866.1
AB21-087	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	98.36	MK681866.1
AB21-083	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	98.28	MK681866.1
AB21-033	LOCA	Intestine	*Versteria* spp.	(Lee et al. 2016)	CO1	*Versteria mustelae*	98.88	MK681866.1
AB21-087	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.35	MK681866.1
AB21-083	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	98.91	MK681866.1
AB21-033	LOCA	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.07	MK681866.1
BC21-0370	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.12	MK681866.1
BC21-0384	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.35	MK681866.1
AB21-039	NEVI	liver cyst	*Versteria* spp.	(Niedringhaus et al. 2022)	NAD1	*Versteria mustelae*	98.81	MK681866.1
AB21-085	NEVI	liver cyst	*Versteria* spp.	(Niedringhaus et al. 2022)	NAD1	*Versteria mustelae*	99.14	MK681866.1
AB21-084	NEVI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.14	MK681866.1
AB21-080	LOCA	Intestine	*Mesocestoides* spp.	(Padgett et al. 2005)	NAD1	*Mesocestoides vogae*	88.51	AP017667.1
AB21-126	ONZI	Intestine	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.14	MK681866.1
AB21-109	ONZI	liver cyst	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	98.51	MK681866.1
AB21-113	ONZI	liver cyst	*Versteria* spp.	(Lee et al. 2016)	NAD1	*Versteria mustelae*	99.21	MK681866.1
AB21-115	ONZI	liver cyst	*Hydatigera taeniaeformis*	(Aydin et al. 2014)	NAD1	*Hydatigera taeniaeformis*	98.71	NC_037071.1

C)

Host ID	Host Species	Location in Host	Identified as	Morphology Reference	Region	Closest match	Percent Identity	Ascension Number
BC21-0217	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.82	AY292795.1
BC21-0374	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.81	AY292795.1
AB21-049	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC21-0384	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC21-0384	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	95.75	AY292795.1
BC21-0371	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.68	AY292795.1
BC21-0371	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC22-0532	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC22-0532	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	100	AY292795.1
BC22-0544	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC22-0543	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC22-0534	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1

AB22-016	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.8	AY292795.1
BC22-1148	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC22-1147	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
AB22-013	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.82	AY292795.1
BC22-1147	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
AB22-031	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC21-0371	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.84	AY292795.1
BC21-0373	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.64	AY292795.1
BC21-0382	NEVI	Lung cyst	*Filaroides martis*	(Ko and Anderson 1972)	28s	*Filaroides martis*	99.82	AY292795.1
AB21-089	NEVI	Intestine	*Baylisascaris devosi*	(Anderson et al. 1974)	28s	*Baylisascaris devosi*	100	MG937776.1
BC22-0555	LOCA	Intestine	*Anisakis simplex*	(Anderson et al. 1974)	28s	*Anisakis simplex*	100	MF094292.1
BC22-0486	NEVI	Intestine	*Anisakis simplex*	(Anderson et al. 1974)	28s	*Anisakis simplex*	100	MF094292.1
AB21-066	LOCA	Stomach	*Contracaecum* spp.	(Anderson et al. 1974)	28s	*Contracaecum multipaillatum*	95.21	MH398587.1
AB21-059	LOCA	Stomach	*Contracaecum* spp.	(Anderson et al. 1974)	28s	*Contracaecum multipaillatum*	94.69	MH398587.1
AB22-013	NEVI	Stomach	*Porrocaecum depressum*	(Morgan and Schiller 1950)	28s	*Porrocaecum depressum*	98.58	U94765.1
BC22-1145	NEVI	Intestine	*Molineus mustelae*	(Schmidt 1965)	28s	*Molineus* spp.	90.08	MW853689.1

D)

Host ID	Host Species	Location in Host	Identified as	Morphology Reference	Region	Closest match	Percent Identity	Ascension Number
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	98.42	LC465332.1
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	98.83	LC465332.1
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	98.57	LC465326.1
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	98.83	LC465379.1
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	99.21	LC465346.1
BC21-0421	NEVI	Intestine	*Corynosoma strumosum*	(Nickol et al. 2002)	CO1	*Corynosoma strumosum*	94.91	LC465402.1
BC21-0397	LOCA	Intestine	*Pseudocorynosoma anatarium*	(Garcia-Varela et al. 2017)	18s	*Pseudocorynosoma anatarium*	99.58	EU267801.1
BC21-0200	NEVI	Intestine	*Centrorhynchus conspectus*	(Richardson and Nickol 1995)	18s	*Centrorhynchus conspectus*	99.76	U41399.1
BC22-1145	NEVI	Intestine	*Centrorhynchus conspectus*	(Richardson and Nickol 1995)	CO1	*Centrorhynchus aluconis*	78.82	KT592357.1
BC22-1146	NEVI	Intestine	*Centrorhynchus conspectus*	(Richardson and Nickol 1995)	CO1	*Centrorhynchus aluconis*	78.96	KT592357.1
BC22-1144	NEVI	Intestine	*Centrorhynchus conspectus*	(Richardson and Nickol 1995)	CO1	*Centrorhynchus aluconis*	79.15	KT592357.1
AB21-105	ONZI	Intestine	*Polymorphus paradoxus*	(Connell and Corner 1957)	CO1	*Polymorphus cf. paradoxus*	100	MN744785.1
AB21-131	ONZI	Intestine	*Polymorphus paradoxus*	(Connell and Corner 1957)	CO1	*Polymorphus cf. paradoxus*	99.84	MN744785.1

Table S3.2 Comparison of measurements for *Versteria*. Morphometric ranges for diagnostic characteristics for species of *Versteria* compared against *V. rafe*. Measurements given in micrometers. Abbreviations: LH, large hooks, SH, small hooks, L, length, W, width.

Species	***V. rafei***	***V. cuja***	***V. mustelae* (syn. *T. tenuicollis*)**							***V. brachyacantha***
Location	Canada	Argentina	North America			Eurasia				Central Africa
			Minnesota	Domestic	Ontario	Germany	Switzerland	Russia	Japan	
Source	This study	(Bagnato et al. 2022)	(Freeman 1956)	(Freeman 1956)	(Skinker 1935)	(Thienemann 1906)	(Wahl 1967)	(Abuladse 1964)	(Iwaki et al. 1995)	(Baer and Fain 1951)
Width	384-1003	1117-1941	1710	1630	2400	2000	1900		2000	
Scolex	170-220	276-345	230-310	200-350	237-303	260-550	300	449-477	351-593	480
Rostellum	39-67	39-75	70-90	70-97	61-77	133	91	108	74-120	126
Sucker	81-101	87-151	97- 125	97	77-110	100-133	130-150	167-186	118-186	176
Sucker/Scolex	0.48-0.46	0.32-0.49	0.42-0.40	0.41-0.28	0.32-0.36	0.38-0.24	0.43-0.5	0.37-0.39	0.34-0.31	0.37
Hooks	c.42-48	c.48	c.47-59	c.48-66	c.42	c.36-72	c.37-43	50	c.44-58	c.54
Hook length (LH/SH)	10-17	12-17	14-17	15-20	15-16	16-24 / 13-21	19-20	18-21/ 12-15	17-22	26-28
Testes	c.84-117	c.54-85			c.90-125	c.60-114	c.100-110	c.114	c.80-141	c.100-145
Testes size (L/W)	16-36	-	51-76/ 28-49	49-58/ 42-51	39-55	42-67/ 37-52	26-39/ 17-27	-	31-75/ 26-75	
Genital Atrium (L/W)	105-157/ 55-115	170-420 (L only)	-	-	193-225/ 127-155	-	68-91	-	-	-
Cirrus	armed, hairlike bristles	unarmed, smooth	-	-	-	-	-	-	unarmed	armed, hairlike bristles
Cirrus-sac L	105-176	210-311	-	-	193-220	-	229-274	352-369	187-319	240-280
Cirrus-sac W	46-95	130-185	-	-	130-154	-	123-146	158-176	64-201	120
Uterine branches	c.18-28	c.12-28	c.10-15	c.16-23	c.10-19	c.12-18	c.28 (avg)	c.14-16	c.17-25	14-17
Eggs (L/W)	18-22/ 15-18	-	-	-	17-20	20-23/ 24-28	22.4-24.6/ 17.9-21.3	-	25-27/ 20-25	-
Host	*N. vison*, *L. canadensis*	*Galictis cuja*	*M. erminea*	*N. vison*	*N. vison*	*M. nivalis*	*M. erminea*	-	*M. nivalis*	*Poecilogale albinucha*

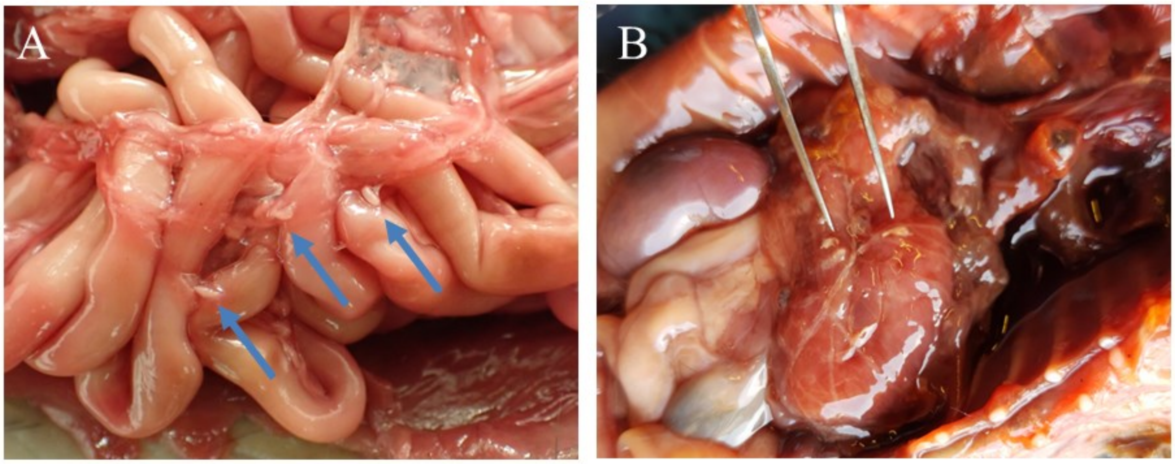

Plate S3.1 Acanthocephalan induced peritonitis in juvenile mink. Infections of two young female juvenile mink from Vancvouer island British Columbia including infection by A) immature *Centroryhynchus conspectus* seen here caught by the omentum (parasites indicated by blue arrow), and B) *Corynosoma strumosum* seen here also in the omentum (parasites indicated by forcep tips).

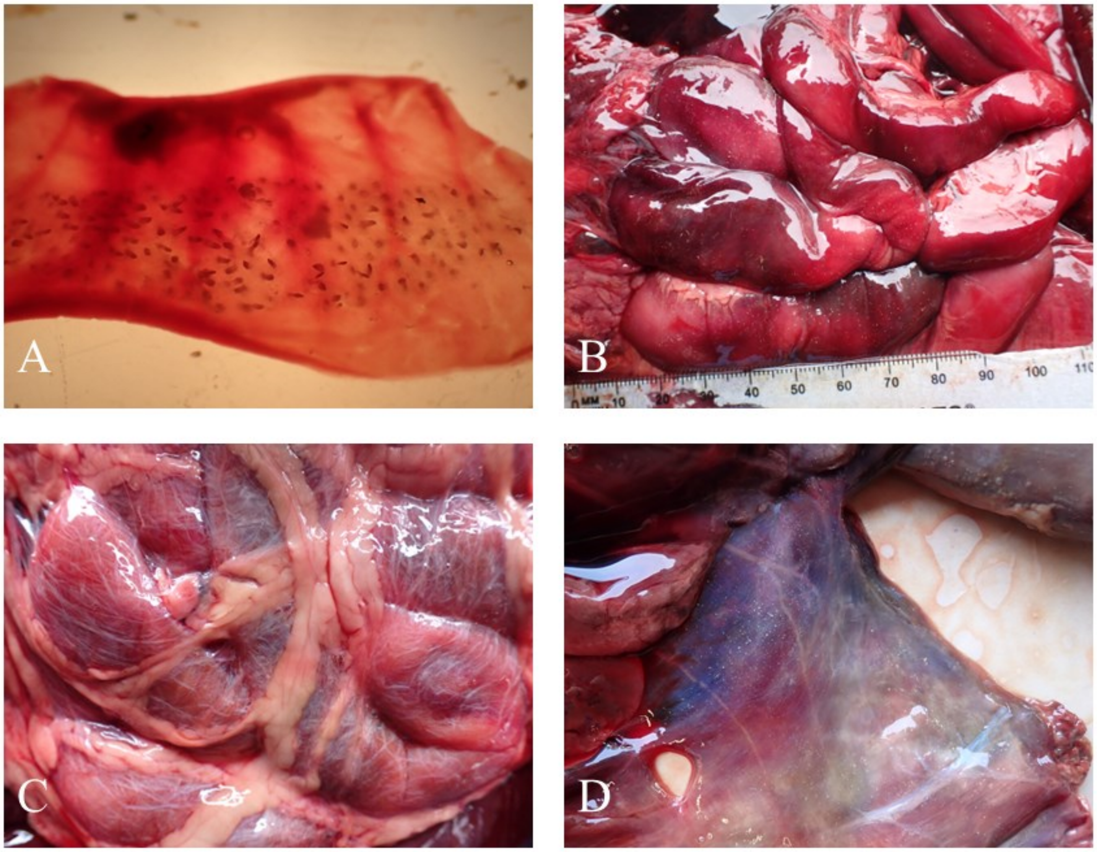

Plate S3.2 Gross pathology of *Alaria mustelae* infection. Photos of A) adult *Alaria mustelae* embedded into the intestinal wall, B) white nodules throughout the small intestine associated with serosal hemorrhaging, C) hyperemia (increased vascularity) of the omentum, D) white granules in the musculature of the abdominal wall.

B.1 *Versteria* spp. named

B.1.1 Description of new species

Family. Taeniidae Ludwig, 1886
Genus. *Versteria* Nakao, Lavikainen, Iwaki, Haukisalmi, Konyaev, Oku, Okamoto, and Ito (2013)
Species. *Versteria rafei* n. spp.
Authors. Shanebeck, KM, J Bennett, SJ Green, C Lagrue, B Presswell

Material studied. 10 complete adults, and strobilar fragments with and without scolices from 10-20 adults, which included immature, mature and gravid proglottids.

Description. Strobila short, complete gravid specimens ranging from 200 to over 1000 mm. Scolex small, 170-220 (188±16 SD) µm wide, with simple rostellum 39-67 (54±9 SD) µm in diameter at widest point (n=14). Suckers large in relation to scolex, 81-101 (90±6 SD) µm wide (Plate 3.5). Neck tapering immediately posterior to suckers, 88-120 (100±9 SD) µm wide (n=14). Rostellum with double crown of very small hooks, 10-17 µm in length, ranging in number from 21-24 per row (42-48 total) (n=5); scolices almost always found without hooks, or with only a few hooks remaining. Hooks with sharp curved blade, long and stout guard with bulbous epiphyseal thickening, and a short or long, straight handle with narrow epiphyseal thickening (Plate 3.5). Proglottids craspedote: immature proglottids broader than long, mature and gravid proglottids longer than wide. Mature proglottids with length/width ratio of 1.07-1.91 (1.51±0.22 SD), gravid proglottids with length/width ratio of 1.03-4.37 (1.97±0.70 SD). Mature proglottids 629-1323 (904±152 SD) µm long and 384-1003 (610±120 SD) µm wide (n=71). Gravid proglottids 1203-3489 (1860±582 SD) µm long, 726-1241 (979±187 SD) µm wide; 18-28 uterine branches (n=30). Genital pores alternate irregularly, usually slightly anterior of the middle of proglottid, protruding in older proglottids. Genital atrium well developed with muscular sphincter, rounder than oval when relaxed, in mature proglottids 57-162 (122±24 SD) µm deep and 52-141 (95±586 SD) µm wide when relaxed (n=71), in gravid proglottids 97-201 (146±29 SD) µm deep and 65-180 (130±25 SD) µm in width when relaxed (n=30).

Male reproductive system. Testes 85-117 in number (n=71), generally sub-spherical in shape, 16-36 (23±4 SD) µm in diameter (n=131). Testicular fields confluent anteriorly, situated between longitudinal osmoregulatory canals, from anterior margin of proglottid to anterior margin of ovary. Antero-poral field with fewest testes; antero-poral and postero-poral fields interrupted by vagina and cirrus sac. (Plate 3.5). Cirrus sac ovoid in shape and relatively small, 105-195 (150±21 SD) µm long and 46-113 (82±29 SD) µm wide in mature proglottids (n=71), and 145-242 (183±22 SD) µm long and 87-139 (109±15 SD) µm wide in gravid proglottids (n=30). Vas deferens forming loops inside and outside cirrus sac, surrounded by prostatic cells. Cirrus armed with hair-like bristles in a spiral pattern.

Female reproductive system. Ovary bilobed, lobes roughly equal in size. Shape and size vary depending on stage of development; in mature proglottids at posterior, 248-390 (293±62 SD) µm wide (n=10); in mature to gravid proglottids, round and strongly staining, often just posterior of center (Plate S3.3). Vitellarium just posterior of ovary and seminal receptacle, at posterior edge of proglottid, averaging 337 (±34 SD) µm wide; ovary width to proglottid width ratio 0.29-0.49 (0.41±0.08 SD) (n=10). Vagina wide, enters genital pore behind opening of cirrus sac and migrates centrally and posteriorly before connecting with seminal receptacle. Uterus fills medium portion of proglottid, with outpocketings at irregular intervals along its length, largest at anterior end of uterus (Plate 3.5). Gravid proglottids with 18-28 lateral branches (n=30), often with secondary and sometimes tertiary bifurcations. Eggs (embryophores) sub-ovoid 18-22 µm in length and 14-18 µm in diameter (n=9).

B.1.2 Taxonomic summary

Type host: *Neogale vison* (Carnivora: Mustelidae).

Other hosts: definitive***,*** *Lontra canadensis* (Carnivora: Mustelidae); intermediate *N. vison*, *Ondatra zibethicus* (Rodentia: Cricetidae).

Type locality: Southeast Vancouver Island, British Columbia, Canada.

Known distribution*:* Western Canada: Alberta and British Columbia.

Site of infection: Small intestine (definitive host), liver (intermediate host).

Prevalence and intensity of infection: *N. vison*: 7.5% (n=106), ranging in intensity from 5-54 per host; *L. canadensis*: 3.2% (n=155), ranging in intensity from 3-23 per host.

Type specimens: voucher specimen will be deposited at the Canadian Museum of Nature's parasite collection, syntypes at the Department of Biological Sciences, University of Alberta.

Etymology: The specific epithet (a noun in the genetive case) honours Dr. Rafael "Rafe" R. Payne, a parasitologist who dedicated almost 50 years of his life to the education of undergraduate students at Biola University and the Au Sable Institute of Environmental Studies.

GenBank accession numbers: OR448764

ZooBank access number: DBD28DD4-DBB4-4931-92A5-F5D0BD897510

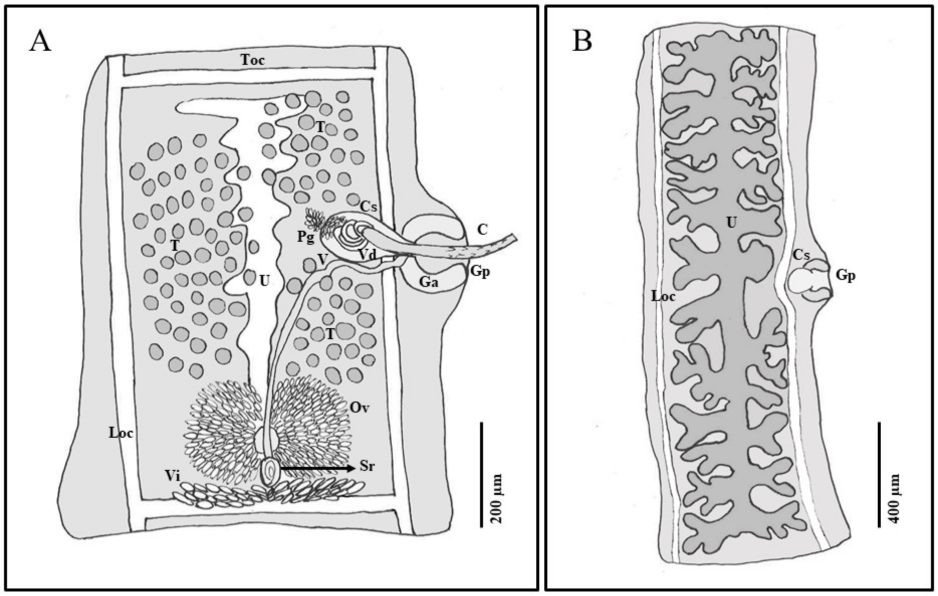

Figure S3.1 Line drawings of *Versteria rafei* n. spp. (Cestoda:Taeniidae). Examples from the North American Mink (*Neogale vison*) from British Columbia, Canada. A) mature proglottid; B) gravid proglottid. **Abbreviations**: C, cirrus; Ci, cirrus sac; Ga, genital atrium; Gp, genital pore; Loc, longitudinal osmoregulatory canal; Ov, ovary; Pg, prostatic gland; Sr, seminal receptable; T, testes; Toc, transverse osmoregulatory canal; U, uterus; V, vagina; Vd, vas deferens, Vi, vitellarium.

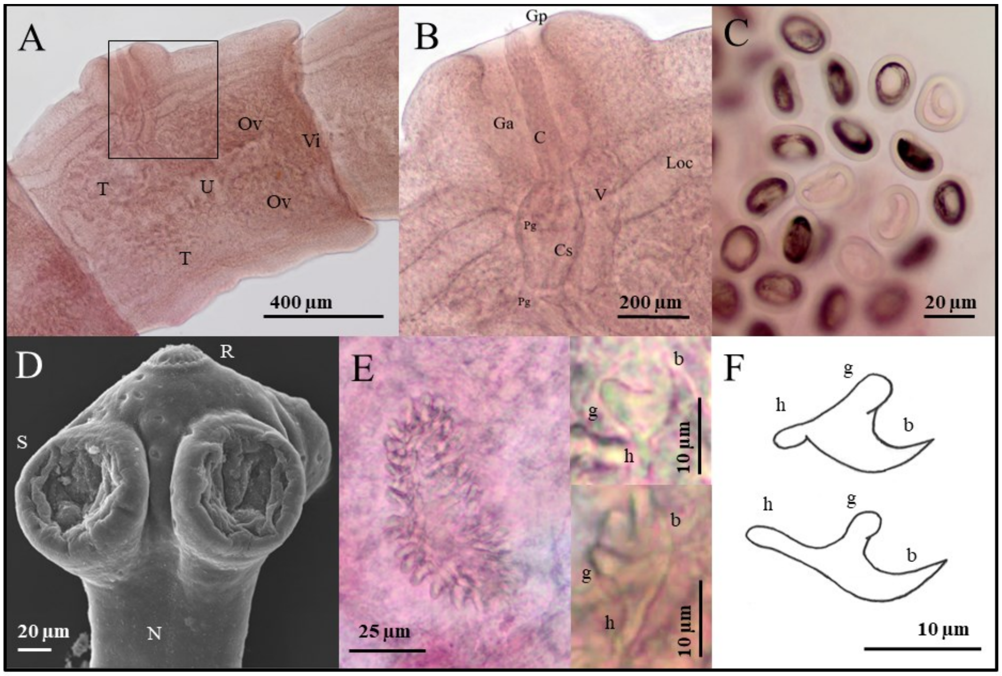

Plate S3.3 Specimens of *Versteria rafei* n spp. A) mature proglottid of an adult worm, stained with acetic acid carmine, cleared in clove oil and mounted permanently in Canada balsam; B) close up of cirrus sac and genital pore; box from panel A; C) eggs from a gravid adult; D) scanning electron microscope image of scolex (missing hooks), showing the rostellum and suckers, magnification at 995x; E) double crown of rostellar hooks, prepared by staining in acetic acid carmine then squashing the scolex on a glass slide with a cover slip and rotating, with close ups of hooks with short and long guards; F) line drawing of hooks. **Abbreviations**: b, blade; C, cirrus; Ci, cirrus sac; g, guard; Ga, genital atrium; Gp, genital pore; h, handle; Loc, longitudinal osmoregulatory canal; N, neck; Ov, ovary; Pg, prostatic gland; R, rostellum; S, sucker; T, testes; U, uterus; V, vagina; Vi, vitellarium.

B.1.3 Remarks

According to molecular evidence and the diagnosis criteria given by Nakao et al. (2013), *Versteria rafei* n. spp. belongs to the genus *Versteria* due to its short strobila, elongate gravid proglottids, small scolex, rostellum, suckers, and double crown of very small hooks; genital pores that alternate irregularly roughly at the middle of the proglottid; terminal genital ducts that pass longitudinally over the osmoregulatory canals; median, posterior female glands with bilobed ovaries, and transversely elongated vitellarium posterior to the ovary; median uterus a longitudinal stem, laterally branched when gravid; and relatively small amount of testes almost entirely anterior and lateral to the female organs. It differs from the diagnosis only in that its mature proglottids are not wider than they are long. Mature proglottids generally close to equal in length and width, though more often longer than they are wide. Descriptions of *V. mustelae*, *V. bradyacantha*, and *V. cuja* all report mature proglottids wider than long, however, the recent description of *V. cuja* also reports ranges for length and width that overlap in mature proglottids (476-2,027 long by 1,117-1,941 wide) and must have included proglottids that were longer than wide, in fact the author's line drawing shows a mature proglottid longer than wide (Bagnato et al. 2022). Skinker (1935) notes that that mature proglottids were *generally* wider than long, but that the ratio also varied. Descriptions of *V. mustelae* by Wahl (1967) and Joyeux and Baer (1936) do not provide the length-width ratio for mature proglottids. In the description by Freeman (1956) of natural and experimental infections in weasel and mink from North America, the author reports a length-width ratio of 0.54-1.71 in weasel and 1.63-1.22 in mink, showing a range of ratios in weasels while all examples reported from mink were longer than wide. It is likely that this ratio is dependent on the age of the mature proglottid, wider when young as it transitions from an immature stage but then gradually longer than wide as it matures. This ratio may also be host dependent as Freeman (1956) reported ratios in mink that were similar to those we report from mink here. We suggest the diagnostic criteria for *Versteria* be amended to state that immature proglottids are wider than long, gravid proglottids longer than wide, and that mature proglottids vary depending on their level of maturity and possibly host.

Since historically *V. mustelae* was a catchall species for the 'small-hooked taeniids of mustelids', and morphological descriptions vary widely over the years and between North America and Eurasia (Table S3.2), is difficult to accurately compare the species described herein to the rest of the genus. Modern molecular analysis has identified one or more distinct lineages of *Versteria* in North America and fossil records suggest others likely exist in Eurasia (Lee et al. 2016, Niedringhaus et al. 2022), which may explain the large variation in morphological characteristics previously reported for *V. mustelae*. However, there are several distinct features that characterize *V. rafei* n. spp. from the three other nominal species of *Versteria*.

For the main diagnostic characteristics (scolex, rostellum, suckers, hook size and number, cirrus sac, genital atrium, and uterine branches) *V. rafei* n. spp. is distinct in the size of its scolex and sucker to scolex ratio, the size of its cirrus sac, and the depth of its genital atrium. It has the smallest scolex of *Versteria* species, with a high sucker to scolex ratio (170-220 µm, and 0.46-0.48) when compared against *V. cuja* in South America (276-345 µm, 0.32-0.49) (Bagnato et al. 2022), *V. bradyacantha* in Central Africa (480 µm, 0.37) (Baer and Fain 1951), and species reported as *V. mustelae* from Europe (260-550 µm, 0.24-0.50) (Thienemann 1906, Abuladse 1964, Wahl 1967). It also has the smallest cirrus sac (105-176 µm long, 46-95 µm wide) ovoid in shape, compared to the more spherical *V. cuja* (210-311 µm long, 130-185 µm wide) (Bagnato et al. 2022), *V. bradyacantha* (240-280 µm long, 120 µm wide) (Baer and Fain 1951), and *V. mustelae* in Europe (229-369 µm long, 123-176 µm wide) (Abuladse 1964; Wahl 1967). The depth of its genital atrium (105-157 µm) is larger than that reported in *V. mustelae* in Europe (68-91 µm) (Wahl 1967), but smaller than *V. cuja* (170-420 µm) (Bagnato et al. 2022); there is no report of the depth for *V. bradyacantha* however, in the authors line drawing, they present the genital atrium as equal to or slightly larger than the length of the cirrus sac (240-280 µm) (Baer and Fain 1951).

Besides these diagnostic characteristics, what distinguishes *V. rafei* n. spp. from *V. mustelae* and *V. cuja* is its armed cirrus (Iwaki et al. 1995; Bagnato et al. 2022), which has hair-like bristles in a spiral pattern, similar to *V. bradyacantha* (Baer and Fain 1951). The hooks of *V. rafei* n. spp. (10-17 µm) are similar in size to *V. cuja* (12-17 µm), but they are different in shape, *V. cuja* having only hooks with long handles and a short blade (Bagnato et al. 2022), similar to *V. mustelae* which also has a short blade and no difference in handle size (Verster 1969). They are much smaller than the hooks of *V. bradyacantha* (26-28 µm, c.54) with fewer hooks, though there is some similarity in shape with an enlarged flattened guard, long thorn like blade, and smallish handle even in the long-handled specimens (Baer and Fain 1951). While there is no molecular information available for *V, bradyacantha* to determine its phylogenetic relationship to the species described herin, due to similarities between them it is possible that *V. rafei* n. spp. and *V. bradyacantha* are from a related lineage. They both have armed cirrus, unlike the other described species, and they have similar hook shapes, different from the shared hook shapes of *V. mustelae* and *V. cuja*.

Appendix C

C.1 Reference

Table S4.1 Detection parameters and analytes. Measured metal and its minimum detection limit (MDL), percent uncertainty, and analyte (CAS number).

Analyte	Units	MDL	Uncertainty	CAS Number
Lithium	µg/g	0.015	0.010	7439-93-2
Beryllium	µg/g	0.010	0.007	7440-41-7
Aluminum	µg/g	4.340	2.89	7429-90-5
Vanadium	µg/g	0.004	0.003	7440-62-2
Manganese	µg/g	0.038	0.025	7439-96-5
Iron	µg/g	2.480	1.65	7439-89-6
Cobalt	µg/g	0.067	0.045	7440-48-4
Nickel	µg/g	0.113	0.075	7440-02-0
Copper	µg/g	0.192	0.128	7440-50-8
Zinc	µg/g	0.760	0.507	7440-66-6
Gallium	µg/g	0.014	0.009	7440-55-3
Arsenic	µg/g	0.043	0.029	7440-38-2
Selenium	µg/g	0.438	0.292	7782-49-2
Rubidium	µg/g	0.020	0.013	7440-17-7
Strontium	µg/g	0.350	0.233	7440-24-6
Molybdenum	µg/g	0.046	0.031	7439-98-7
Silver	µg/g	0.014	0.009	7440-22-4
Cadmium	µg/g	0.006	0.004	7440-43-9
Tin	µg/g	0.118	0.079	7440-31-5
Antimony	µg/g	0.017	0.011	7440-36-0
Barium	µg/g	0.041	0.027	7440-39-3
Thallium	µg/g	0.004	0.003	7440-28-0
Lead	µg/g	0.011	0.007	7439-92-1
Bismuth	µg/g	0.003	0.002	7440-69-9
Uranium	µg/g	0.002	0.001	7440-61-1

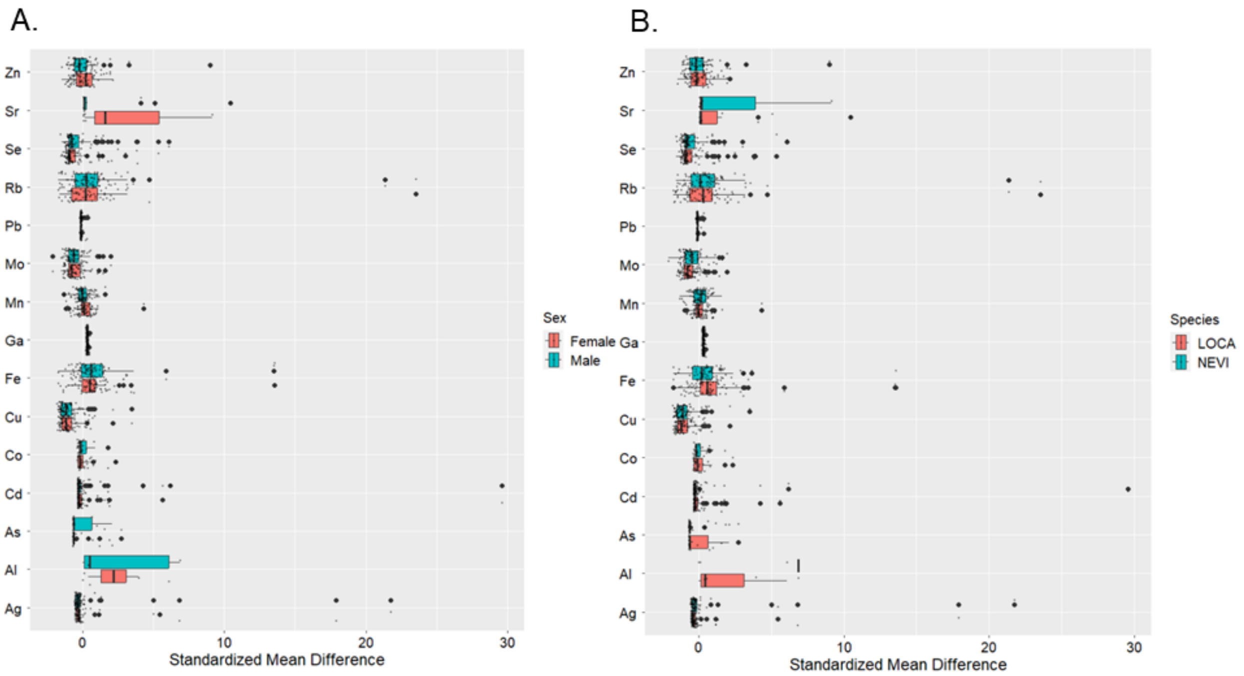

Figure S4.1 Effect of species and sex on metal levels. The standardized mean difference between the measured concentrations of metals (y-axis) and the weighted mean of that metal from previous reports in the literature. Including by, A) sex, between female and male animals, and B) between river otters (LOCA) and mink (NEVI).

Table S4.2Variance explained by the best fit models. Measure of variance for the final models of the observed metal concentrations for both the subwatershed and home range buffer scales. Variance explained presented as R^2 conditional, which accounts for both the fixed and random effects, calculated using the 'r.squaredGLMM' function from the *MuMIn* package.

Metal	Subwatershed	Home Range Buffer
Ag	0.5073783	0.5236355
Cd	0.9911226	0.992287
Co	0.4163977	0.3938421
Cu	0.4393568	0.4798455
Fe	0.1741391	0.2269146
Hg	0.286866	0.2641842
Mn	0.3255569	0.3230505
Pb	0.1916099	0.1916099
Rb	0.1342981	0.1247756
Se	0.615507	0.6130826
Zn	0.3527082	0.3114393

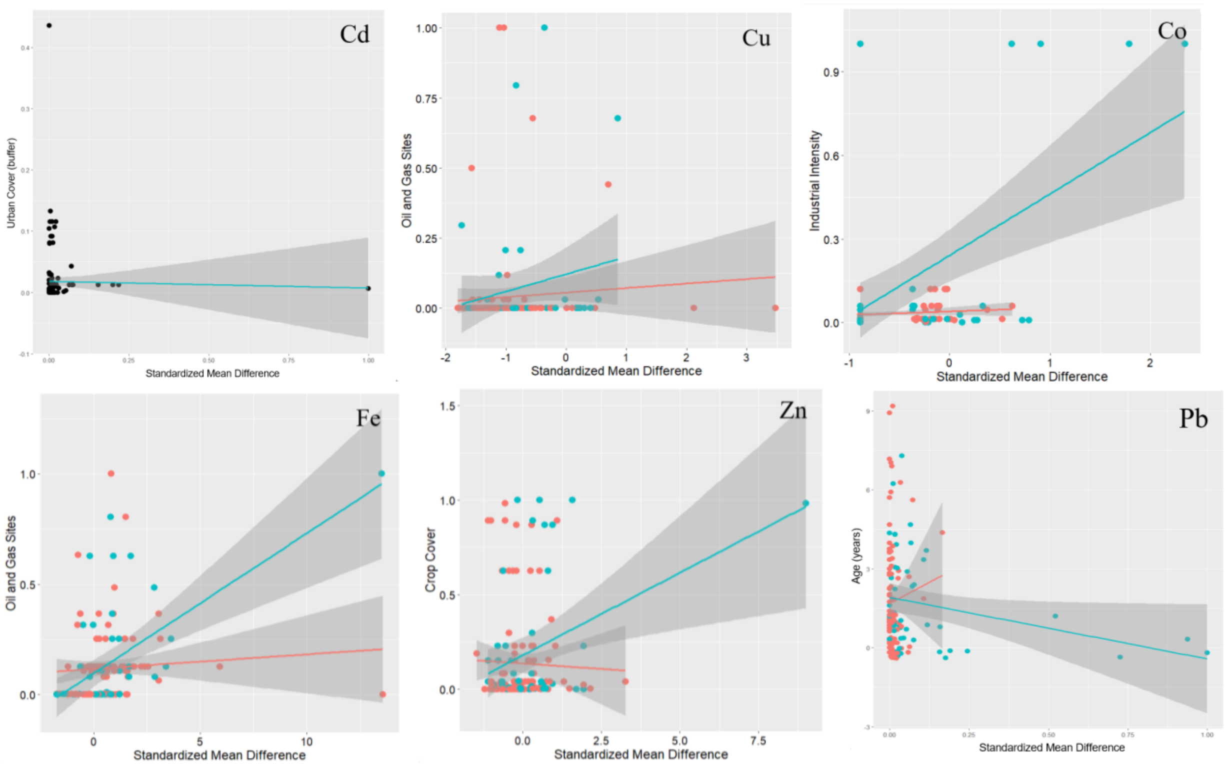

Figure S4.2 Effect of significant moderators on metal concentrations. Scatterplot of the standardized mean difference of liver metals (x axis) against scale transformed significant anthropogenic drivers (y axis), color indicates age class, juveniles in blue (< 1 year) and adults in red (> 1 year), except for Pb where the y-axis is the age in years of the animals, and colored by animals from subwatersheds where pulp and paper mills were absent are in red and present in blue. Metal indicated in the right-hand corner of the graph.

Appendix D

D.1 Systemic review search parameters

(mink* OR ‘river otter*’ OR otter* OR “lontra canadensis” OR ‘neogale vison’ OR ‘neovison vison’ OR mustelid*) AND (pollut* OR metal* OR ‘heavy metal*’ OR ‘trace metal*’ OR contaminant* OR ‘trace element*’) AND (silver OR aluminum OR cadmium OR copper OR cobalt OR mercury OR iron OR selenium OR manganese OR molybdenum OR nickel OR lead OR rubidium OR strontium OR tin OR arsenic OR thallium OR vanadium OR PCB OR PCH)

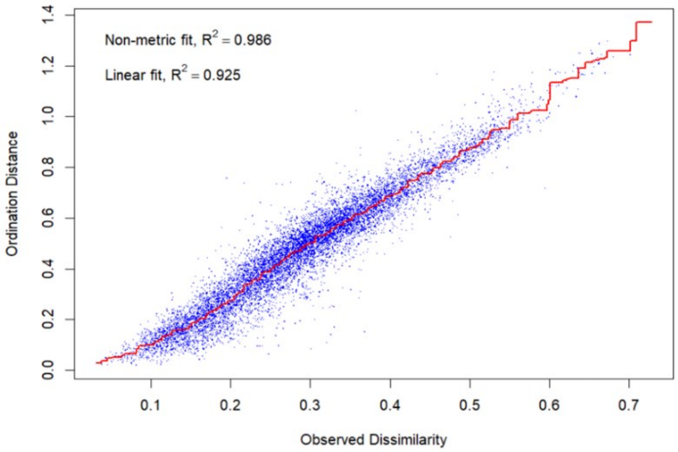

Figure S5.1 Stressplot for NMDS modeling. Plotting the goodness of fit for the NMDS modeling of parasite infection, essential elements, and energetic condition, including variance explained by the model.

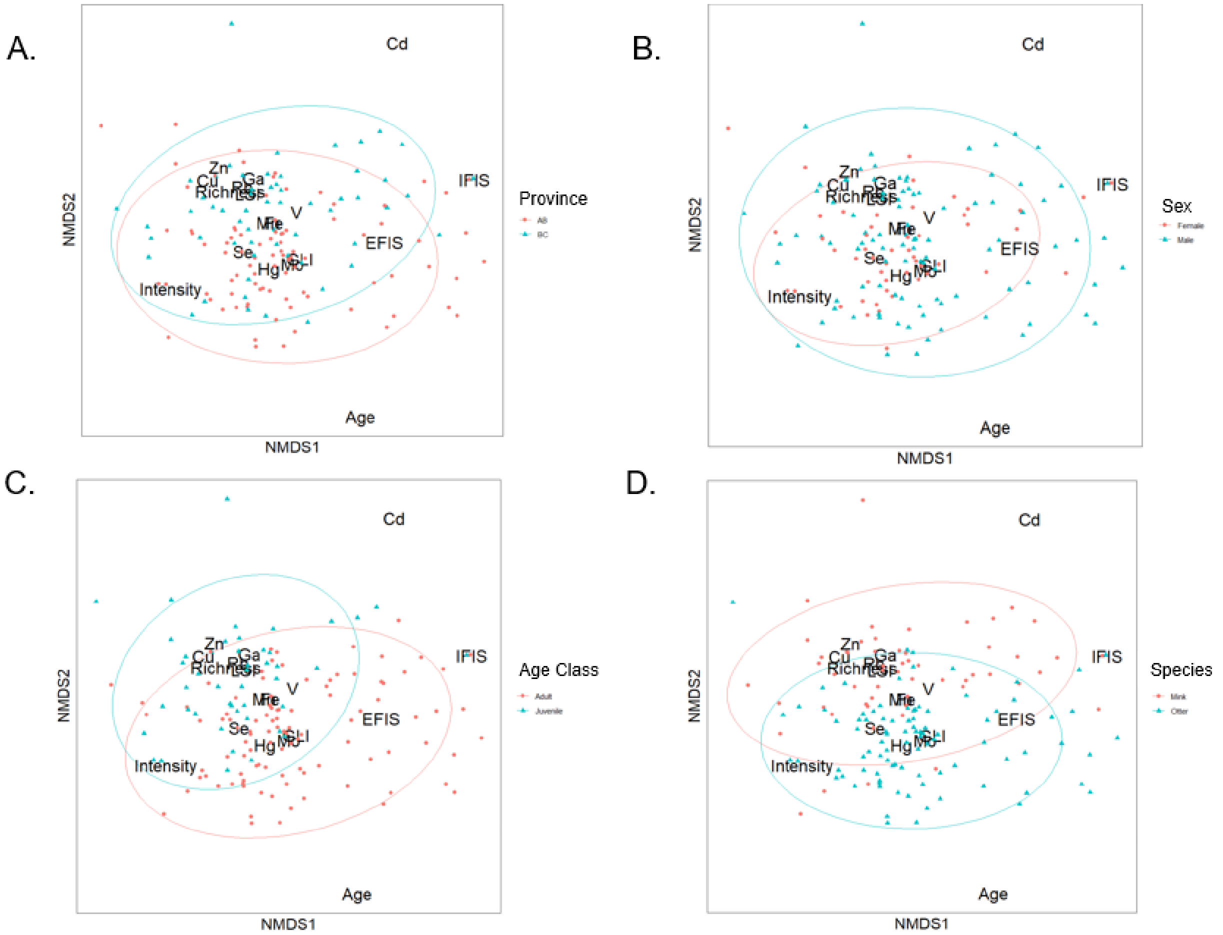

Figure S5.2 NMDS ordination results related to biological factors. Differences between animals related to their parasitic infection, liver concentrations of essential elements, and energetic condition (NMDS of non-scaled data, Bray-Curtis similarity). Relationship between biological factors shown by color and clustering identified by ellipse ("stat_ellipse" function, *ggplot2*) for A) Province, B) sex of the animal, C) age class of the animal (juvenile<1 year of age), and D) species (LOCA= *L. canadensis*, NEVI= *N. vison*).

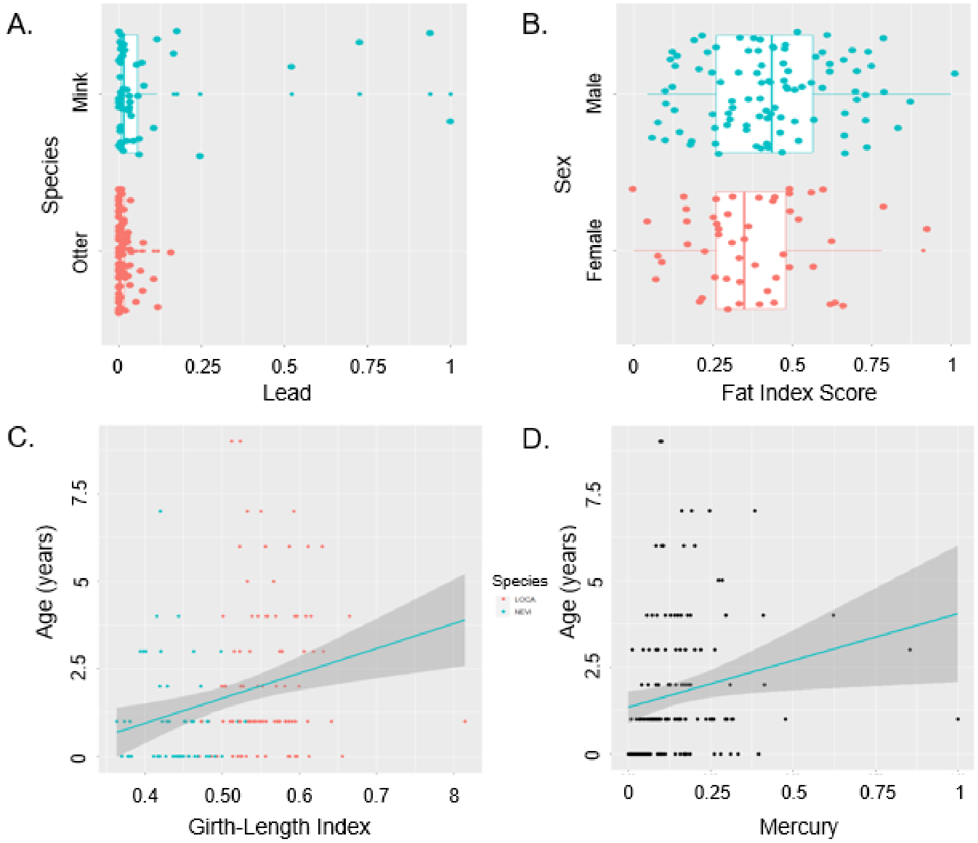

Figure S5.3 Correlation of Significant predictors in CCA analysis. A) boxplot of the scaled index of lead concentrations in the liver of otter and mink, B) boxplot of the scaled index of fat index scores from males and females, C) scatterplot and best fit line of the interaction between girth-length index and age in years, by species (red=otter, blue=mink) and D) scatterplot and best fit line of scaled index of mercury concentrations against age in years of the animal.

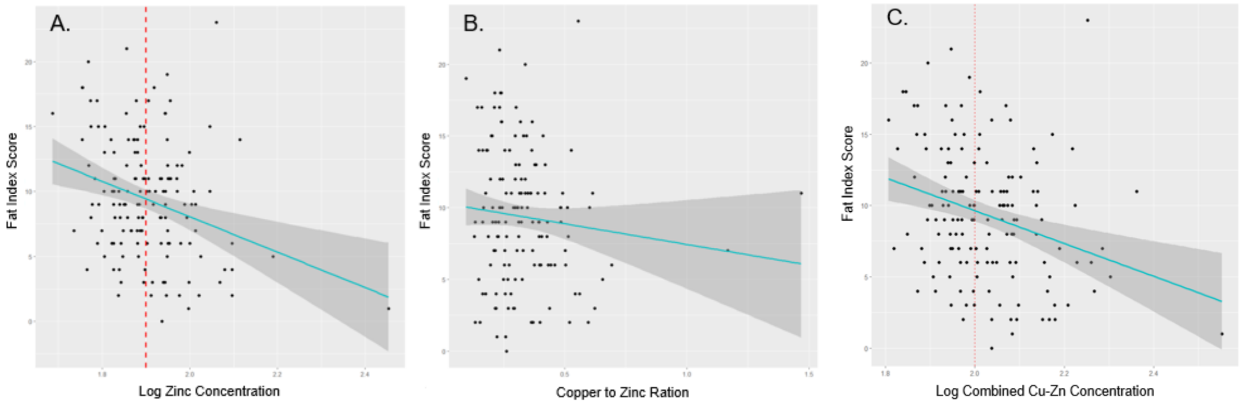

Figure S5.4 Interaction between Copper and Zinc and correlation with fat content. Scatterplots of the effect of A) log Zn concentration on fat index score, red dashed line indicates the historical weighted average; B) Cu-Zn ratio (concentration of Cu divided by concentration of Zn) on FIS, and C) the effect of combined concentrations of Zn and Cu, log transformed, on FIS, red dashed line indicates the combined weighted averages of Cu and Zn.