GIANT LIVER FLUKE AND MOOSE: JUST A FLUKE?

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ABSTRACT: The giant liver fluke, *Fascioloides magna*, is a possible contributing factor to moose (*Alces alces*) declines in North America, but evidence linking *F. magna* infection directly to moose mortality is scarce. This review identifies knowledge gaps about the transmission and impact of *F. magna* infection on moose and proposes new directions for research and management of this parasite. We suggest that the importance of intermediate snail hosts has been largely neglected in current management discussions and warrants greater emphasis. The intermediate hosts responsible for *F. magna* transmission likely vary by region and recent genetic evidence suggests that *F. magna* was restricted to several isolated refugia during cervid extirpation events in North America. This distributional history represents several coevolutionary and pathological implications for definitive hosts of *F. magna*. We suggest that *F. magna* infections are most ecologically significant as they relate to sublethal impacts and multiple parasitic infections. In assessing infection risk on landscapes, most models rely heavily on monitoring white-tailed deer (*Odocoileus virginianus*), but this approach only measures risk indirectly. The reliability and accuracy of models would probably improve if snail habitat in ephemeral wetlands was included as a predictor variable.

The giant liver fluke, *Fascioloides magna*, is a trematode parasite that infects the livers of white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and caribou (*Rangifer tarandus*) across North America (Fig. 1; Pybus 2001). Moose (*Alces alces*) and other cervids often become infected with *F. magna* where their range overlaps with these natural fluke hosts (Karns 1972, Lankester 1974, Pybus 2001).

Fascioloides magna is a parasite of unknown significance for host mortality. Although liver damage associated with infection appears to have little effect on white-tailed deer (Foreyt and Todd 1976, Presidente et al. 1980, Pybus 2001), its impact on other cervids is more controversial (Foreyt 1992, 1996, Pybus 2001, Lankester and Foreyt 2011). Whether damage from *F. magna* infection increases mortality in moose is of particular interest. In northwestern Minnesota, Murray et al. (2006) concluded ~20% of moose mortality was probably related to *F. magna* infection; however, cause of mortality was partly a classification by exclusion because if no other cause was evident, and the liver tissue contained an abundance of fluke damage, individuals were classified as probable fluke mortalities. Lankester (2010) notes that the prevalence of *F. magna* infection in northwestern Minnesota between 1972 (87%; Karns 1972) and the beginning

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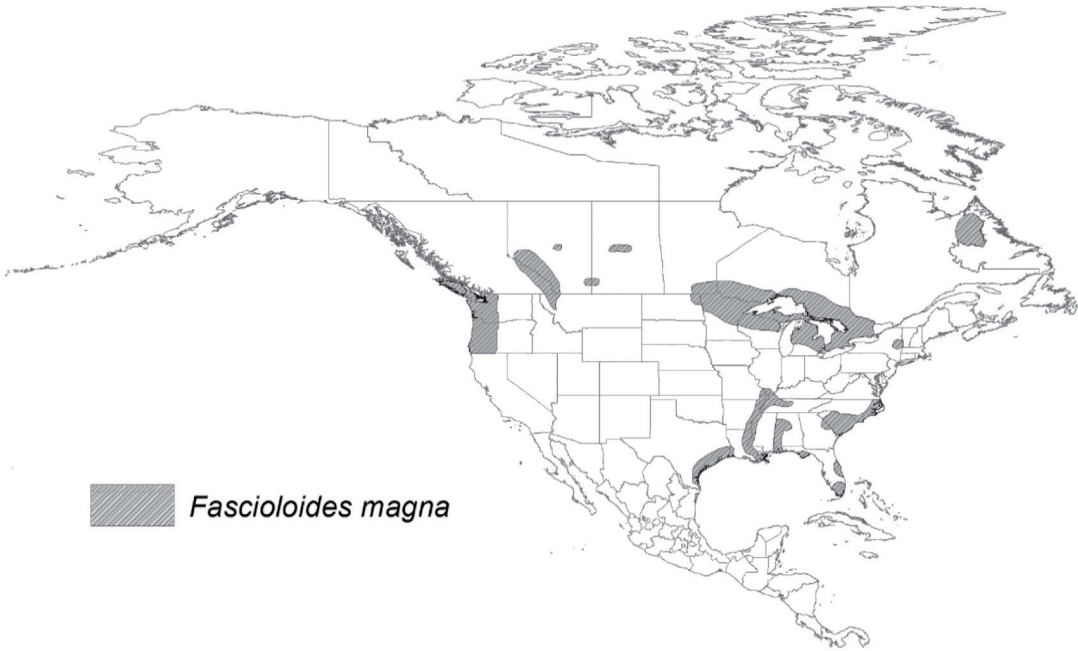
Fig. 1. North American distribution of *Fascioloides magna* redrawn from Pybus (2001). North Dakota and Tennessee distributional information added from Maskey (2011) and Pursglove et al. (1977).

of the Murray et al. (2006) study in 1995 (89%) did not change when the moose population was increasing. Additionally, captive moose infected experimentally with *F. magna* showed no outward clinical signs of infection (Lankester and Foreyt 2011), but this study only used 3 individuals with adequate food, minimal stress, and no predatory interaction. Energy expenditure in free-ranging moose is likely higher when tissue repair and immune responses occur in concert with severe malnutrition, predation pressure, and other pathogens (Pybus 2001, Lankester and Samuel 2007).

In this review, we summarize the life cycle of *F. magna* including the biology of lymnaeid snail intermediate hosts. We then draw on current concepts in parasitology including coevolution, sublethal effects, coinfections, landscape ecology, and ecological interactions to inform management strategies concerning the habitat and parasitic relationships of *F. magna* and moose.

THE LIFE CYCLE

Fascioloides magna completes its life cycle using snail, plant, and cervid hosts sequentially (Swales 1935, Pybus 2001). Eggs released in deer pellets develop into miracidia (Fig. 2) that penetrate the foot of an aquatic lymnaeid snail, developing into sporocysts that give rise to rediae by asexual reproduction. Rediae then release free-swimming cercariae from the snail that encyst as metacercariae on aquatic vegetation, and if ingested by a cervid, will excyst and penetrate the intestine as juvenile flukes. Juvenile flukes migrate to the liver and continue to the liver parenchyma until they find one or more other flukes (Foreyt et al. 1977). This migration is visible as black tracks, scarring, and hemorrhaging. Paired flukes settle in the liver and become encapsulated in a pseudocyst of host origin. The stimulus for capsule formation is unknown, but may be functionally similar to the proline stimulus in the related liver fluke, *Fasciola hepatica* (Wolf-Spengler and Isseroff 1983).
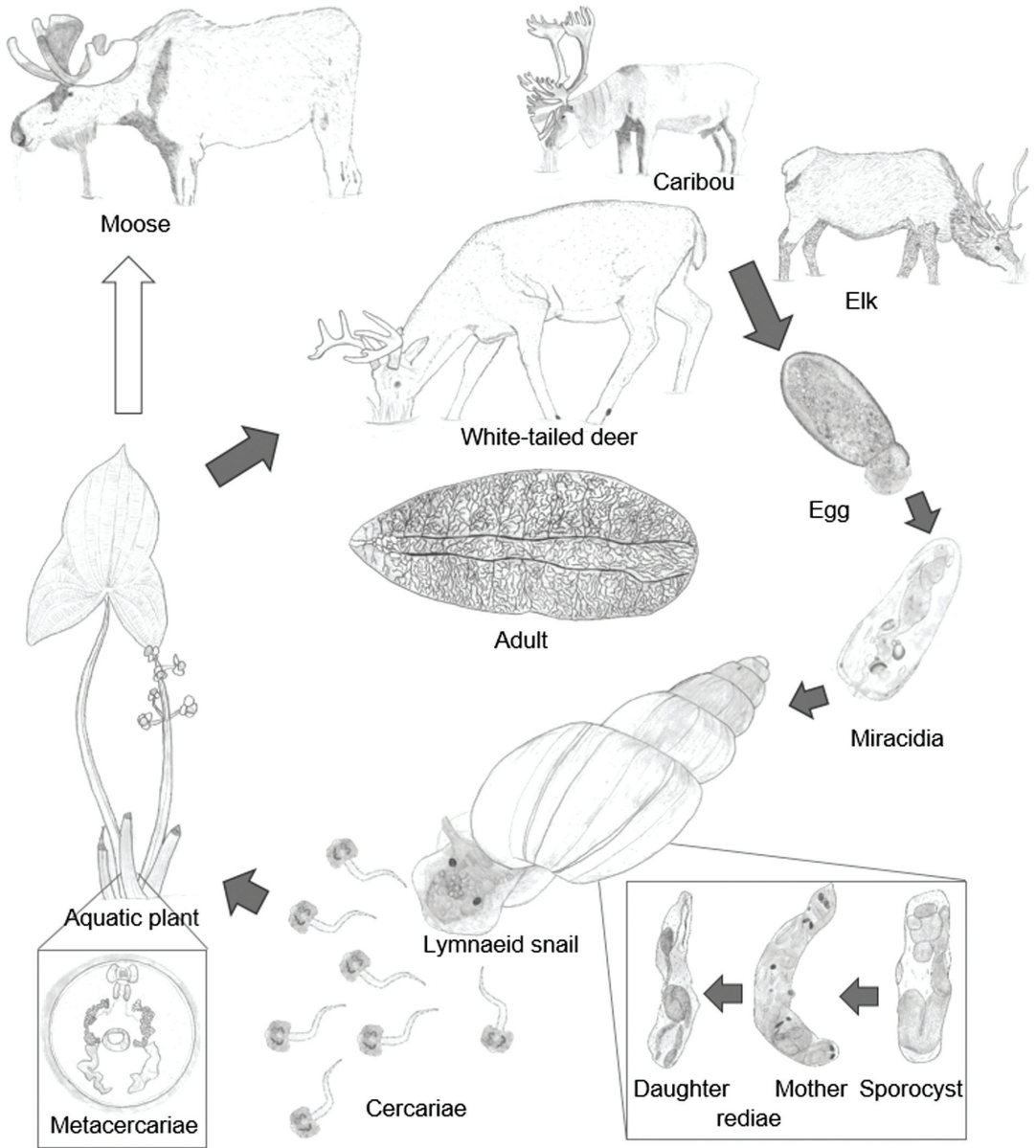
Fig. 2. The life cycle of *Fascioloides magna*. Gray arrows indicate the normal life cycle progression. The hollow arrow indicates the dead-end host, moose, in which the parasite does not successfully shed eggs (drawings by J. Trevor Vannatta).

Lymnaeid snails

The taxonomy, ecology, relative importance, and North American geographic range of specific lymnaeid snail hosts remains poorly understood and a much debated topic (Baker 1911, Hubendick 1951, Correa et al. 2010, Dillon et al. 2013). As such, we focus

our discussion on the ecology and geographic range of known *F. magna* host snails using the most current taxonomic information (Tables 1 and 2).

The taxonomy of lymnaeid snails is still debated and the varied growth patterns of lymnaeid snails in different environments

Table 1. Potential hosts of the giant liver fluke *Fascioloides magna* and their habitat associations.

F. magna host snail	General habitat description	Source
Fossaria spp.	Highly amphibious. Found on mud substrates in ephemeral wetlands and ditches.	McCraw 1959, 1961, Clarke 1981, Laursen et al. 1992, Laursen and Stromberg 1993, Dunkel et al. 1996
Lymnaea caperata	Found in floodplains, marshes, and other temporary waters. Can be tolerant of dry conditions.	Clarke 1981, Laursen et al. 1992, Laursen and Stromberg 1993, Dunkel et al. 1996
Lymnaea elodes	Found among vegetation in many water bodies, primarily lakes, streams and ponds.	Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996
Lymnaea stagnalis	Found on vegetation (e.g., *Typha*) and rocks in large, permanent lakes with diverse substrates.	Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996
Lymnaea catascopium	In lakes or large, slow moving rivers on rocks or vegetation exposed to current.	Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996
Lymnaea columella	Found in lakes and other slow moving waters on vegetation or submerged sticks.	Clarke 1981
Lymnaea cubensis	Found in ponds and marshes similar to the habitat of *Fossaria* spp.	Hubendick 1951, Pointier and Augustin 1999
Lymnaea megasoma	Occurs in many habitats from large to small lakes, beaver ponds, and slow moving rivers on muddy and silty sediments	Clarke 1981, Vannatta 2016
Acella haldemani	An exceptionally rare species found in shallow water vegetation of ponds and lakes	Clarke 1981

has led to taxonomic misclassifications in the past (Hubendick 1951, Dillon et al. 2013). More recently, genetic analyses have revealed that almost all North American lymnaeid species are in a single clade, and that they may be reclassified into the genera *Catascopium* and *Hinkleyia* to reflect shared ancestry in North America (Correa et al. 2010). Snails in the *Stagnicola* genus (*Lymnaea elodes* and *L. catascopium*, Table 2) may actually represent a single species of the new genus *Catascopium*. As a final taxonomic note, there is likely no group of lymnaeids as taxonomically confused as the *Fossaria* genus (Stewart and Dillon 2004); hence, we refer to all *Fossaria* group snails as *Fossaria* spp., except for *L. caperata* and the circum-Caribbean snail, *L. cubensis*, which are long recognized as distinctive (Baker 1911, Hubendick 1951, Dillon et al. 2013).

Three lymnaeid snail intermediate hosts have been found naturally infected with *F. magna*: *Fossaria* spp., *Lymnaea caperata*,

and *L. elodes* (Table 2; Swales 1935, Laursen and Stromberg 1993). However, when the geographic range of *F. magna* is overlaid with the ranges of intermediate snail hosts, no combination of known, natural intermediate hosts fully explains its distribution (Fig. 3). Of primary interest are the *F. magna* populations in Labrador, Canada and Florida, USA. It is most likely that natural infections in *L. elodes* explain the presence in Labrador, despite this parasite population residing on the very northern range edge of *L. elodes*. In Florida, it is most likely that *L. cubensis* is an undocumented natural host as it is also a host of the closely related liver fluke, *Fasciola hepatica,* in Latin America (Cruz-Reyes and Malek 1987). Not only is *L. cubensis* closely related to other *Fossaria* spp. snails (Correa et al. 2010), it also shares similar habitat characteristics (Table 1). The natural hosts *Fossaria* spp. and *L. caperata* are amphibious snails common to ephemeral water habitats. *Lymnaea caperata, L. elodes,*

Table 2. Known intermediate snail hosts for *Fascioloides magna* in North America, common synonyms[a], and whether snails are known natural or experimental (e.g., infected with miracidia in the laboratory) hosts.

F. magna host snail	Common synonyms	Natural or Experimental host (Source)
Fossaria spp.	*Lymnaea humilis; L. bulimoides; L. modicella; L. parva; L. ferruginea; L. obrussa; L. umbilicata; L. desidiosa; L. dalli*	*Natural* (Swales 1935, Laursen and Stromberg 1993) *Experimental* (Sinitsin 1930, Krull 1933, Dutson et al. 1967, Griffiths 1973, Foreyt and Todd 1978)
Lymnaea caperata	*Stagnicola caperata; S. caperatus*	*Natural* (Griffiths 1962, Laursen and Stromberg 1993) *Experimental* (Griffiths 1962, Foreyt and Todd 1978, Laursen 1993)
Lymnaea elodes	*Stagnicola elodes; S. palustris; S. exilis; Lymnaea palustris; L. reflexa; L. umbrosa; S. reflexa*	*Natural* (Swales 1935) *Experimental* (Campbell and Todd 1955, 1956, Griffiths 1962, Dutson et al. 1967, Foreyt and Todd 1978, Laursen 1993)
Lymnaea stagnalis	No relevant synonyms	*Experimental* (Wu and Kingscote 1954, Griffiths 1973, Foreyt and Todd 1978)
Lymnaea catascopium	*Stagnicola catescopium; Lymnaea emarginata; L. woodruffi; L. walkeriana*	*Experimental* (Laursen 1993)
Lymnaea columella	*Pseudosuccinea columella*	*Experimental* (Krull 1933, Dutson et al. 1967[b], Laursen 1993, Flowers 1996)
Lymnaea cubensis	No relevant synonyms	Host of closely related fluke, *Fasciola hepatica*, in Latin America (Cruz-reyes and Malek 1987)
Lymnaea megasoma	*Bulimnea megasoma*	12 specimens were found shedding gymnocephalus cercariae in Minnesota, presumably *F. magna* or *F. hepatica*. (Gilbertson et al. 1978)
Acella haldemani	*Lymnaea gracilis*	Not evaluated

[a] Synonyms derived from Baker 1911, Hubendick 1951, Clarke 1973, Dillon et al. 2013.
[b] This account is far outside the known range of *L. columella* and in doubt.

and *L. catascopium* are very closely related, yet natural infections in *L. catascopium* have not been documented (Table 2).

The importance of snail intermediate hosts has been greatly overlooked to best understand the ecology of *F. magna* in North America. The lymnaeid intermediate hosts of *F. magna* are potentially a critical bottleneck at which *F. magna* might be effectively managed. Whereas white-tailed deer and elk disperse widely and occupy a variety of habitats, the snail hosts of *F. magna* are restricted to ephemeral wetlands or similar habitats (Table 1).

The habitat preferences of lymnaeid snails are most pertinent for definitive cervid hosts relative to their exposure to metacercariae while foraging on aquatic plants. In *Fasciola hepatica,* snail-plant associations and other factors have been used to predict where snails, and thus infection risk, are more common (Rondelaud et al. 2011). Similar methodology may be useful to predict *F. magna* infection risk. The presence of *Fossaria* spp., *L. caperata*, and *L. elodes* snails in ephemeral wetlands points to emergent aquatic vegetation as the primary source of giant liver fluke infection.

However, regional adaptation of parasites to their intermediate hosts is common (Lively 1989, Vera 1991 as cited by Combes
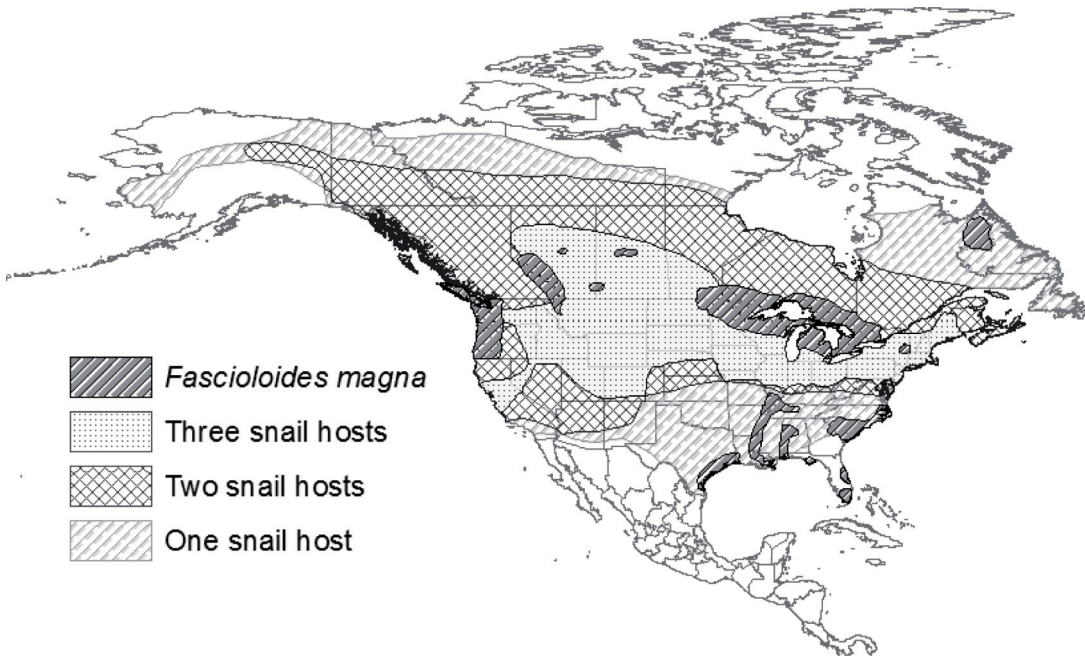
Fig. 3. Geographic range of lymnaeid snail intermediate hosts in North America. Different patterns represent the number of known, natural intermediate host snails in an area. Maps derived from Baker 1911, Hubendick 1951, Clarke 1973, 1981, Jokinen 1992, Laursen et al. 1992, Pybus 2001, Maskey 2011, and the 2016 IUCN Red List of Threatened Species.

2001). As such, management on a local scale must first identify which intermediate hosts are regionally important to identify habitats to manage accordingly. For *F. magna*, it is most likely that *Fossaria* spp. are the most important hosts at lower latitudes and *L. elodes* becomes progressively important northward.

Aquatic vegetation

Aquatic feeding is common and seasonally important for moose (de Vos 1958, Hennings 1977, Belovsky and Jordan 1981, Fraser et al. 1982, 1984), but much less so for white-tailed deer and other cervids (Skinner and Telfer 1974, Hennings 1977, Ceacero et al. 2014). Sodium requirements are hypothesized as the principal driver of aquatic feeding habits of moose in non-coastal areas (Belovsky and Jordan 1981, Fraser et al. 1982, Ceacero et al. 2014); however, the high protein

content of aquatic vegetation and seasonal, nutritional requirements arguably influence this months-long foraging behavior by moose and other ungulates (Ceacero et al. 2014). A comprehensive review of aquatic foraging by moose suggests that this behavior varies regionally and is likely influenced by foraging efficiency and sodium acquisition (Morris 2014). For example, if moose in the Great Lakes region feed on aquatic plants more than moose in other regions, they might be expected to have higher infection rates of *F. magna*.

Seasonality is also an important factor in both aquatic foraging and parasite transmission (Lepitzki 1998, Morris 2014). Early and late season feeding by moose near muddy lake and stream edges is more likely to lead to infection because as temperature increases in early spring, infected snails appear to generate a pulse of metacercariae

(Lepitzki 1998) and moose may encounter more viable metacercariae and higher risk infection; this general trend might also hold true in the fall. Snails which became infected in spring would begin shedding cercariae ~50 days post-infection when wetlands provide green vegetation during early fall senescence. In fact, known peaks in metacercarial encystment match this seasonal variation in forage availability and feeding behavior (Lepitzki 1998).

Although classic life cycle studies implicate aquatic vegetation as the only source of *F. magna* infection (Swales 1936, Pybus 2001), some cercariae may encyst as free-floating metacercariae in surface water (Morley 2015). Little research has been done with free-floating metacercariae, but in human fascioliasis, certain patients have no other reported routes of infection other than ingestion of free-floating metacercariae (Mas-Coma 2004, Morley 2015).

It is also possible that moose become infected with *F. magna* by drinking water or consuming wet soils at mineral licks (geophagy), a common behavior in cervids (Weeks and Kirkpatrick 1976, Fraser et al. 1984, Abrahams 2013, Lavelle et al. 2014). The dissolved minerals in these soils could create a rich habitat for snails and represent a shared resource between *F. magna* definitive and dead-end hosts, such as moose (Lavelle et al. 2014). We found no information about which, if any, snail species inhabit mineral lick habitats. It is possible that the shallow ephemeral habitats of *Fossaria* spp., *L. caperata*, and *L. elodes* are similar to that at mineral licks and high amounts of available calcium could facilitate high snail density (Dillon 2000).

DISTRIBUTION, COEVOLUTION, AND PATHOLOGY

The distribution of *F. magna* across North America has long been of interest (Pybus 2001). Its patchy distribution (Fig. 1)

represents the historical coevolution of *F. magna* within its definitive hosts and explains the patterns of variable pathology within these hosts. Pybus (2001) proposed 2 explanations for the patchy distribution: 1) *F. magna* was never widely dispersed and only established in isolated pockets of North America, or 2) a complex series of events involving glaciation, deer speciation, extirpation, and restocking established *F. magna* widely across the continent and it was later reduced to a patchy distribution. The latter scenario involves human colonization which extirpated the cervid hosts from many locations, followed by either re-establishment from various refugia or cervid restocking programs in current habitat pockets. Recent genetic analyses indicate that the second, more complex hypothesis is more likely (Bazsalovicsová et al. 2015).

Fascioloides magna coevolved with ancestral *Odocoileus* spp. (Pybus 2001), and in the Pliocene, the same glaciation events that generated speciation among *Odocoileus* spp. (Heffelfinger 2011) likely led to the generation of the 2 distinct western (Pacific Northwest and Alberta) and eastern clades (southern USA, Great Lakes, Labrador; Bazsalovicsová et al. 2015). Within the various habitat patches analyzed by Bazsalovicsová et al. (2015), mitochondrial haplotypes appear conserved, suggesting that *F. magna* remained within isolated refugia where white-tailed deer were not extirpated during human colonization of North America. If restocking programs had distributed *F. magna* from other infection foci, regionally conserved haplotypes would likely be less common (Bazsalovicsová et al. 2015).

We found little evidence supporting the hypothesis that the viability of miracidia and metacercariae of *F. magna* has limited range expansions from refugia (Pybus 2001). It is a robust parasite which has been introduced to Europe at least twice and expanded its European range considerably in the past

100 years (Králová-Hromadová et al. 2011, Bazsalovicsová et al. 2015). It seems more probable that natural processes and deer restocking programs near North American refugia have led to range expansion that monitoring programs have not detected and historical records cannot identify. Although *F. magna* readily expands its range as host ranges expand (Pybus et al. 2015), it is likely that abiotic factors such as precipitation, soil characteristics, and temperature have limited expansion in parts of North America (Pybus 2001, Maskey 2011). Further study is warranted, as with the related fluke *F. hepatica* (Zukowski et al. 1991).

Coevolution between *F. magna* and regional hosts would be expected assuming that *F. magna* resided in isolated refugia. As a consequence, we cannot assume that one regional population will behave similarly as other parasite populations that coevolved with different hosts. In much of North America, only white-tailed deer have a long coevolutionary history with *F. magna* (Pybus 2001); however, western populations of *F. magna* likely evolved with both deer and elk (Bazsalovicsová et al. 2015). These evolutionary histories may manifest as the proportion of susceptible individuals within a population (Pybus 2001). The prevalence of infection appears to plateau at ~70% of individuals in some white-tailed deer populations, suggesting that ~30% develop resistance to infection (Foreyt et al. 1977). But, infection rate can be higher as elk in Kootenay National Park, Ontario plateau near 80% infection prevalence, and moose at ~90% prevalence in northwestern Minnesota (Pybus 2001, Murray et al. 2006, Pybus et al. 2015, Wünschmann et al. 2015).

When examining the coevolutionary history of *F. magna* and its definitive hosts, the concept of host spectra 'filters' is useful to consider (Euzet and Combes 1980 in Combes 2001), specifically, that 2 filters facilitate

the acquisition of a host spectrum: an encounter filter and a compatibility filter. The encounter filter dictates that potential hosts must overlap spatially and temporally with the parasite, as well as be exposed to the parasite by some aspect of behavior. The compatibility filter relates to whether the host is able to defend itself from the parasite, immunologically or otherwise, and stipulates that the host must provide suitable resources to the parasite. For example, foraging and/or drinking by white-tailed deer in aquatic habitats leads to encounters with *F. magna*, and the successful establishment and reproduction of *F. magna* within the liver with minimal hepatic damage verifies that the compatibility filter is open (Pybus 2001). Further, the damage during the initial stages of infection does not appear to substantially reduce fitness in deer (Pursglove et al. 1977, Presidente et al. 1980, Mulvey and Aho 1993, Mulvey et al. 1994), indicating a finely tuned host-parasite coevolutionary history (Combes 2001).

Elk present a slightly different scenario because although both filters are open, the heightened severity of hepatic damage suggests that the compatibility filter has only recently opened (Pybus 2001). Elk infected with *F. magna* often survive, but infection intensity can be high (Pybus 2001, Pybus et al. 2015) and mortality occurs in certain animals (Foreyt 1996, Pybus et al. 2015). It is believed that elk shed larger quantities of fluke eggs than white-tailed deer (Swales 1936, Pybus 2001).

F. magna infections often lead to death in mule deer (*Odocoileus hemionus*), yet mule deer successfully shed *F. magna* eggs (i.e., the compatibility filter is open; Foreyt 1992). The difference in mortality between white-tailed and mule deer is probably a function of habitat use where barriers have prevented common exposure of *F. magna* to mule deer (i.e., the encounter filter is closed;

Pybus 2001), suggesting that exposure history, not relatedness, is the principle explanation for the host spectrum.

In the case of moose, the compatibility filter is closed. Moose are dead-end hosts that display a strong immune response, which some authors have suggested may contribute substantially to mortality (Murray et al. 2006). It seems possible that if infections lead to death in some elk, they may also lead to death in moose. The heightened immune response in moose may contribute to this mortality through thickening of fluke capsule walls, the inability of flukes to release eggs from capsules, and the eventual death of the parasite (Lankester 1974). Remnant capsules are often filled with dead flukes and other waste which may impair liver function (Pybus 2001). In the migratory phase of their life cycle, immature *F. magna* appear to migrate extensively in moose, sometimes damaging >50% of the liver tissue and possibly causing secondary infection (Karns 1972, Lankester 1974, Pybus 2001). The parasite population may be responding to an incompatible host population by increasing virulence, defined as damage to the host (Gandon and Michalakis 2000, Combes 2001). An increase in virulence associated with parasite migration and hepatic tissue damage may allow fluke eggs to make their way into pellets. Cattle also do not shed fluke eggs, but in severe infections with substantial hepatic damage, some eggs escape in feces (Foreyt and Todd 1974). If the identification of *F. magna* eggs in moose pellets is accurate (Kingscote 1950, Wünschmann et al. 2015), successful reproduction of the parasite in this host could open the compatibility filter, possibly leading to selection for more virulent parasite strains (Gandon and Michalakis 2000).

Virulent parasite strains may also become more common if natural hosts gain an indirect benefit through parasite-mediated competition (Price et al. 1988, Schmitz and Nudds 1994, Hudson and Greenman 1998). White-tailed deer habitat overlap with moose and elk is increasing with climatic and anthropogenic habitat alterations (Waller and Alverson 1997, Galatowitsch et al. 2009, Heffelfinger 2011, Minnesota Department of Natural Resources 2015, Dawe and Boutin 2016). Heightened virulence of white-tailed deer parasites could facilitate this range expansion through reduced competition with sympatric moose and elk, and benefit both deer and their parasites (Schmitz and Nudds 1994). Selection for virulence may also happen rapidly, as rapid evolution has been identified in other parasites expanding along range edges (Kelehear et al. 2012).

INDIRECT MORTALITY AND SUBLETHAL EFFECTS

Viewing parasitic diseases only as direct mortality factors presents a narrow perspective about wildlife disease. Many parasitic impacts, especially in mammalian hosts, are subtle and may indirectly influence mortality (e.g., a weakened host exposed to greater predation risk) or sublethal fitness impacts (e.g., reduced reproductive success; Yuill 1987). Identifying such relationships in mammalian systems is often difficult because they require substantial time, large sample sizes, and reliable metrics of host fitness. Given the complex interacting factors evident in moose declines along their southern range (Rempel 2011, Murray et al. 2012), examining only direct mortality factors probably overlooks other indirect and sublethal factors impacting the fitness of individual moose.

The most important question concerning *F. magna* in moose is if and how much direct mortality is caused by infection. Some studies suggest that white-tailed deer, a natural host which is thought to sustain little to no harm from infection (Foreyt and Todd 1976, Presidente et al. 1980, Pybus 2001), may suffer increased mortality when infected

with *F. magna* (Cheatum 1951, 1952, Addison et al. 1988). Cheatum (1951) found a two-fold higher fluke infection prevalence in winter-killed deer as compared to deer collected by researchers. The mechanism by which liver fluke infections in deer can cause increased mortality are not fully understood, but immune response, anemia, eosinophilia, and hepatic tissue damage associated with fluke migration likely increase energetic costs in winter when deer are in an energy deficit (Cheatum 1951, Presidente et al. 1980, Marcogliese and Pietrock 2011).

Moderate to high liver damage from fluke infection was not correlated with body condition in Minnesota moose found dead or euthanized by wildlife officials (Wünschmann et al. 2015). However, 42% of malnourished moose had high liver damage (>50% of the tissue altered by flukes) compared to 13% of animals in moderate condition; conversely, 33% of individuals in good condition had high liver damage. Sample size of individuals in good condition was small (n = 8) compared to moderate (24) and malnourished individuals (28).

When infected cervids are attacked by predators, flukes may indirectly contribute to mortality through hepatic hemorrhage as fluke capsules are often near major blood vessels in the liver (Vannatta 2016). Although anecdotal, 9 black-tailed deer (*Odocoileus hemionus columbianus*) died after being chased by dogs (Cowan 1946) and subsequent necropsies suggested a combination of heavy fluke infestation and exertion caused the hepatic portal system to hemorrhage. Hepatic hemorrhage during chase events might partially explain some differences between the northwestern and northeastern Minnesota moose populations. Moose in northwestern Minnesota experienced little direct predation mortality (~3%; Murray et al. 2006), whereas predation accounted for ~34% of mortality in northeastern Minnesota (Minnesota Department of Natural Resources 2016a). One

might further speculate that the lack of predation stress in the northwestern population allowed it to increase despite fluke prevalence near 90%.

A direct study measuring the energetic cost of *F. magna* infection in moose should be a research priority. Immune responses and tissue repair costs are difficult to measure directly, but comparison of the resting metabolic rate of infected and uninfected moose might provide an estimate of the associated energetic cost of infection (Yuill 1987, Robar et al. 2011). Further, neonatal survival and fitness (productivity) might be impacted by reduced milk production as Ross (1970) found a significant negative impact by the closely related fluke, *Fasciola hepatica*, on the milk yield of cattle.

Parasitic disease can also affect the investment that host organisms partition to their offspring (Schwanz 2008). In a study with deer mice (*Peromyscus maniculatus*), chronic trematode infection was related to an investment in fewer, larger offspring (Schwanz 2008). Lower twinning rates have been observed in white-tailed deer infected with *F. magna*, but unfortunately, weight and health of fawns were not assessed (Mulvey 1994). Termed fecundity compensation (Minchella 1985), this strategy represents the host effort to maintain fitness while under disease stress. Although examples of this phenomenon are rare in mammalian systems (Schwanz 2008), testing this hypothesis in moose is theoretically possible. If parasitic disease leads to fecundity compensation in moose, heavily diseased moose populations should have a lower calf:cow ratio at birth, albeit multiple factors influence productivity and single births, not twinning as in deer, are the norm for moose.

Other fitness effects have been documented in *F. magna*-infected white-tailed deer in South Carolina (Mulvey and Aho 1993, Mulvey et al. 1994) where, after controlling for habitat and year-to-year

variation, males with heavy fluke infections had fewer antler points and lower body weight; however, these effects were less dramatic or absent in older males. Older males with heavy infections did lose more weight during the rut which could reduce their winter survival (Mulvey and Aho 1993). Fluke-infected female deer had higher body weights before the onset of breeding season and, thus, conceived earlier and outside of the optimal breeding window. Mulvey et al. (1994) suggested that the lower probability of twinning in infected does allowed for rapid weight gain, earlier breeding receptivity, and earlier conception; however, these data and that of older males can also be interpreted as conflicting. In totality, these data are somewhat supportive of an impact on fitness and may point to more effect on younger-aged animals.

COINFECTION AND MULTIPLE PARASITIC INFECTIONS

Many wildlife disease studies are viewed from a single-host, single-pathogen perspective. However, complicated within-host environments can have significant impacts on disease dynamics and outcomes (Jolles et al. 2008, Ezenwa et al. 2010, Hoverman et al. 2013, Viney and Graham 2013, Garza-Cuartero et al. 2014). The within-host community is comparable to symbioses in larger ecosystems with some infracommunity (within-host community) members remaining neutral towards one another (Telfer et al. 2010), some appearing as antagonists (Canning et al. 1983, Jolles et al. 2008, Telfer et al. 2010, Johnson and Buller 2011), and others benefitting one another (Behnke et al. 2009, Karvonen et al. 2009, Ezenwa et al. 2010, Telfer et al. 2010).

The concept of infracommunities is particularly pertinent in declining moose populations where *F. magna* may interact with other pathogens such as brainworm (*Parelaphostronglyus tenuis*), *Echinococccus* spp.,

and bacterial and viral microparasites. Various helminths have been implicated in immune suppression (Maizels et al. 1993, Behnke et al. 2009, Ezenwa et al. 2010), and it is likely that the complex proteome of *F. magna* contains some immune suppressing functions (Cantacessi et al. 2012).

It is often difficult to identify the underlying causes of parasitic coinfections (Viney and Graham 2013). In white-tailed deer in Minnesota, a positive association was found between *F. magna* and a tapeworm larvae (*Taenia hydatigena*) that inhabits the liver (Vannatta 2016). This association was most likely related to abiotic conditions necessary for parasite survival, immunological differences in hosts, and/or behavioral differences between host individuals. In order to tease apart these variables, quality GIS or habitat data, immunological profiles of individuals, and metrics of space use are required. These logistical difficulties likely underlie our lack of knowledge about coinfection processes.

A question of paramount importance to moose management is: how do the potentially pathogenic helminth parasites *P. tenuis* and *F. magna* interact in moose? Using data from opportunistically collected free-ranging moose (Wünschmann et al. 2015), we found a signifcant negative association between *P. tenuis* and *F. magna* (Fisher's exact test $P = 0.022$), with 12 mortalities positive for *P. tenuis* and *F. magna*, 16 positive for *P. tenuis* only, 24 positive for *F. magna* only, and 9 negative for *P. tenuis* and *F. magna*. Since the moose in this study were found dead, accidentally killed, or euthanized, actual proportions of infected or coinfected individuals may be lower in the population than those in the sample population.

Three primary variables may affect the rates of coinfection of *F. magna* and *P. tenuis* in moose: 1) differences in parasite habitat outside the host, 2) space use differences between individual moose hosts, or 3) the presence of one parasite stimulating an immune

defense in the host. It is unlikely that the habitat of these parasites overlaps as *F. magna* is most commonly associated with lowland and wetland habitat (Mulvey et al. 1994, Pybus 2001, Pybus et al. 2015, VanderWaal et al. 2015, Vannatta 2016), whereas *P. tenuis* is mostly found in upland forest habitats frequented by terrestrial gastropod hosts (Cyr et al. 2014). Beyond non-overlapping habitat, a lack of coinfections could represent the habitat of specific individual moose and/or the relative density of white-tailed deer. Moose which are infected with *P. tenuis* may have home ranges with higher proportion of upland forest, moose with fluke infections could have higher proportion of wetland habitat and moose with coinfections may have high proportions of wetland and upland habitat (Cyr et al. 2014). Lastly, it is possible that high intensity fluke infections trigger an immune response preventing other infections from establishing (Lankester 2010).

In considering coinfections, it is important to remember that managing one parasitic infection will likely impact another (Telfer et al. 2010). Even exposure to other infections can increase the virulence of pre-existing diseases (Sandland et al. 2007). In the case of moose, further research is needed to determine how *F. magna* and *P. tenuis* infections interact.

INFECTION ON LANDSCAPES

Possible relationships between *F. magna* infection risk and landscape characteristics have been investigated in multiple studies (e.g., Mulvey et al. 1994, Peterson et al. 2013, VanderWaal et al. 2015). Two studies analyzed coarse metrics of upland/lowland or soil moisture classes (Mulvey et al. 1994, Pybus et al. 2015), and two others found a direct relationship between certain cover types and increased infection risk or *F. magna* biomass (VanderWaal et al. 2015, Vannatta 2016). VanderWaal et al. (2015)

linked infection risk to rooted and floating aquatic marshes which contain many aquatic plant species palatable to deer and moose (Hop et al. 2001). Using similar techniques (VanderWaal et al. 2015) as employed in Voyageurs National Park, VanderWaal et al. (unpublished data from winter 2014-2015) found that *F. magna* prevalence was highest around Duluth, Minnesota and declined to the northeast (Fig. 4A, B). These data match well with moose necropsy data from the region, purportedly due to higher deer densities and, to a lesser extent, more wetland habitat (Peterson et al. 2013). Despite a limited sample size, Vannatta (2016) found a strong correlation between *F. magna* biomass and emergent herbaceous wetlands using the National Land Cover Dataset. The infection risk on these landscapes was attributed to the combination of palatable aquatic forage for deer and suitable habitat for intermediate host snails (VanderWaal et al. 2015, Vannatta 2016).

The primary issue with all current landscape-level risk assessments is that infection risk for moose is measured by the occurrence of *F. magna* within white-tailed deer or elk (Peterson et al. 2013, Pybus et al. 2015, VanderWaal et al. 2015, Vannatta 2016). These models have some success because infected deer or elk are necessary for *F. magna* persistence; however, this is only an indirect measure of the risk landscape. The risk for moose is a very narrow habitat which must include: 1) suitable intermediate hosts, 2) palatable emergent vegetation, and 3) overlapping use with infected definitive hosts. Past emphasis has been placed on the distribution of white-tailed deer, but other factors necessary for transmission are important. Although the landscape must contain infected deer, additional information about suitable snail habitat and emergent vegetation will improve the accuracy of infection risk assessments for moose.
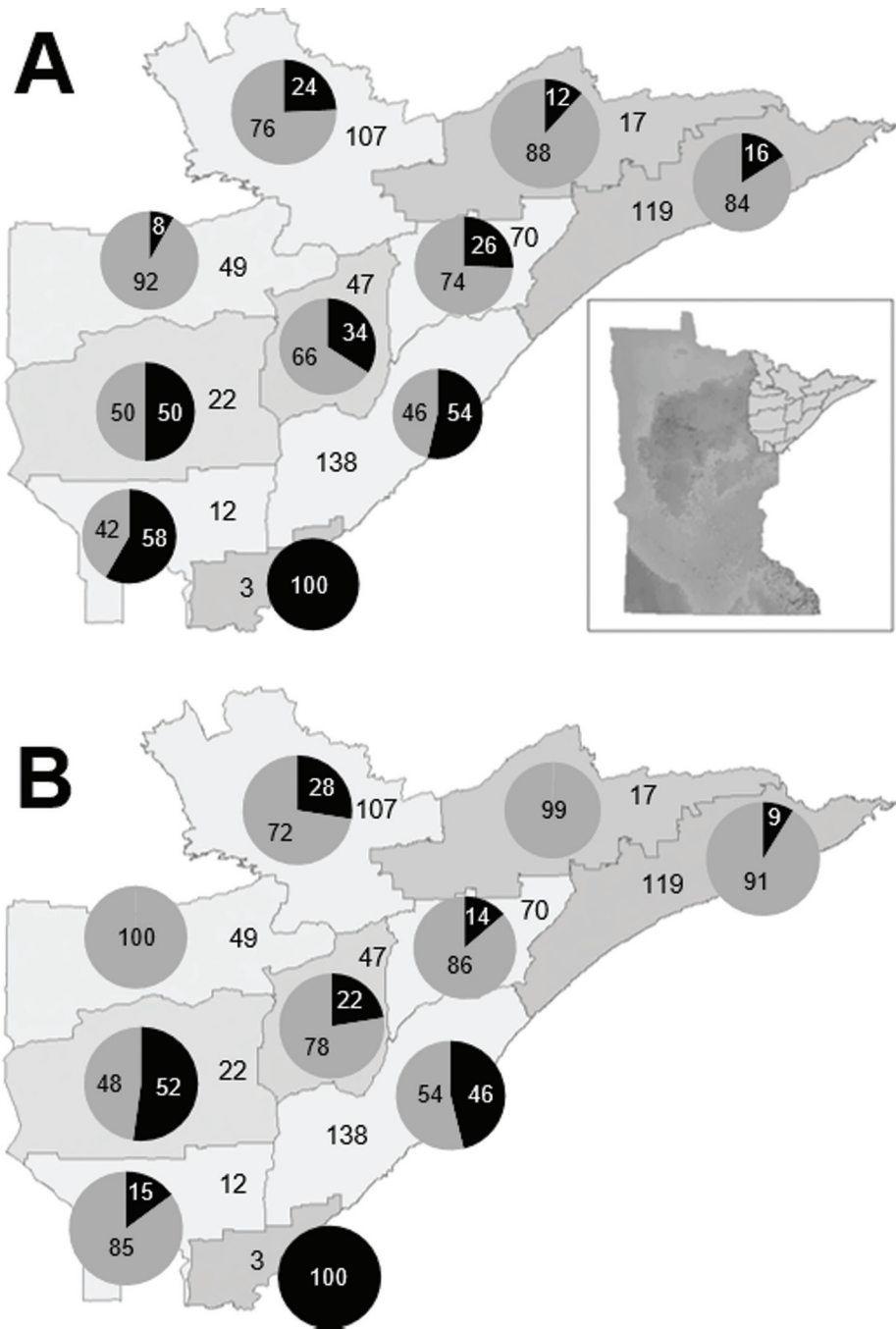
Fig. 4. Deer permit areas (DPA) sampled for *Fascioloides magna* within pellet groups in 2015 in Minnesota's arrowhead region (inset: Minnesota digital elevation model with arrowhead region DPAs highlighted). DPAs are labelled with their respective sample sizes and pie charts are labelled with the percentages represented by each slice. (A) Infection prevalence decreases from the southwest to the northeast (Black = proportion infected). (B) Mean infection intensity is less consistent across the region (Black = mean infection intensity in each DPA as a proportion of the greatest mean, 787 eggs per gram dry weight feces; VanderWaal et al. unpublished data).

The riskiest habitats for moose are shallow, ephemeral water bodies which support natural infections in *Fossaria* spp. and other ecologically similar snails (Tables 1 and 2). Models in which all wetland habitat is considered indiscriminately (Peterson et al. 2013, VanderWaal et al. 2015, Vannatta 2016) can be improved with measures of wetland seasonality, water flow, and depth that influence the location of intermediate hosts and metacercarial encystment by the parasite (Laursen and Stromberg 1993, Pybus 2001). A discriminant model that buffers the inward edges of wetlands to reflect appropriate water depths would provide a better measure of infection risk (Peterson et al. 2013, VanderWaal et al. 2015, Vannatta 2016). The inclusion of all wetland habitats as an infection risk may have led Peterson et al. (2013) to conclude that deer density, not wetland habitat, was the primary driver of *F. magna* infection in northeastern Minnesota.

Unfortunately, the wetlands inhabited by the intermediate host snails present a significant barrier to developing improved risk assessment models (Malone and Zukowski 1992, DeRoeck et al. 2014). *Fossaria* spp. and *L. caperata* inhabit ephemeral wetlands that are more common in woodlands and other water features not typically visible on aerial imagery (Laursen et al. 1989, 1992, Van Meter et al. 2008). Identifying these habitats to accurately predict landscape risk requires extensive GIS modeling and field confirmation of ephemeral wetland presence (Van Meter et al. 2008), a collaborative effort for researchers, managers, and wetland ecologists.

ECOLOGICAL INTERACTIONS AND *FASCIOLOIDES MAGNA* CONTROL

The complex life cycle of *F. magna* creates logistical issues when trying to manage disease risk. Control measures such as vaccination, medicated baits, molluscicides, wetland draining, removing aquatic vegetation, introducing competitor snails, or selective breeding are not ecologically responsible, nor cost effective. However, ad-vances in our understanding of ecological interactions and their impacts on parasites may present novel management strategies in line with current topics, such as One Health (American Veterinary Medical Association 2008). Ecological interactions associated with biodiversity have stark implications for parasitic diseases (Thieltges et al. 2008, Civitello et al. 2015) and several reviews have recently emphasized the implications of biodiversity for parasites (Thieltges et al. 2008, Johnson and Thieltges 2010, Johnson et al. 2010).

Free-living larval stages, such as the miracidia, cercariae, and metacercariae of *F. magna*, are prey for many organisms. Oligochaetes may protect some snails from trematode infections by ingesting miracidia or by limiting the parasites' access to the mantle cavity (Ibrahim 2007). In a study of *Ribeiroia ondatrae*, cercariae were consumed by *Hydra* spp., copepods, and damselfly and dragonfly larvae, often at high rates (Schotthoefer et al. 2007). In marine systems, some fish (Kaplan et al. 2009) and filter-feeding barnacles (Prinz et al. 2009) predate parasite cercariae, and gastropods may ingest metacercariae (Campbell and Todd 1956, Prinz et al. 2009).

Competition for hosts and within hosts is another example of how ecological interactions may impact parasite success. The recent discovery of soldier castes in some trematode species (Hechinger et al. 2011, Garcia-Vedrenne et al. 2015) indicates that competition for and within snail hosts is pervasive and likely explains why some trematodes are stronger within host competitors (Kuris and Lafferty 1994). However, some snails may act as decoys for parasite miracidia, decreasing their transmission rates; for example, the mucus of the snail *Helisoma trivolvis* is toxic to *F. magna* miracidia (Coyne et al. 2015). Parasites can also be

sensitive to pathogens within the snail host. The fungal group Microspora in the family Unikaryonidae can act as a hyperparasite on trematode larvae when ingested by a snail host, and microsporidia can reduce *Fasciola hepatica* infections in snails (Canning et al. 1983).

Whereas most larval stages of *F. magna* are best controlled by increasing non-vertebrate biodiversity within aquatic habitats, limiting the diversity of the definitive vertebrate hosts may be a potential control measure. Elk are suspected of shedding large quantities of *F. magna* eggs when infected (Swales 1936, Pybus 2001), and the role of elk in *F. magna* infection of sympatric moose needs to be evaluated as Minnesota plans to expand its elk population (Minnesota Department of Natural Resources 2016b). In the northeastern part of Minnesota where moose are declining, the Department of Natural Resources is proposing changes in harvest strategies of white-tailed deer to limit exposure of moose to *F. magna* and other pathogens carried by deer (Minnesota Department of Natural Resources 2015).

CONCLUSIONS

Fascioloides magna is a parasite with unknown significance in its definitive hosts (white-tailed deer, elk, and caribou) and the dead-end host, moose. Recent evidence suggests *F. magna* populations were divided into eastern and western populations and subsequently limited to isolated refugia. This distributional pattern impacted the coevolutionary history of the parasite with all of its definitive hosts. White-tailed deer have a lengthy co-evolutionary history with *F. magna*, which may help explain the lessened severity of pathology in this species; however, different coevolutionary histories with other hosts appear to influence pathological severity. Moose and elk often contain high intensity fluke infections, which may represent a form of parasite-mediated competition, and this aspect

of the host-parasite relationship warrants careful consideration in areas where overlap of elk, moose, and white-tailed deer will increase in the future. Impacts of *F. magna* infection beyond direct mortality are also of increasing relevance because infection may alter host fitness, predation risk, and other stressors (e.g., climate change, competition, other pathogens) in deer, elk, and moose.

Viewing *F. magna* and moose as a single-host, single-parasite system limits our understanding of the impact of this parasite. Although these relationships are often cryptic, the interactions of multiple parasites within a host likely lead to a number of impacts on moose. For example, *F. magna* may act to facilitate other parasitic infections, or conversely, prevent infection by stimulating the host immune system. For moose, the relationship between *F. magna* and *P. tenuis* specifically warrants further examination.

Past modeling efforts to predict *F. magna* infection risk tended to ignore the importance of intermediate snail hosts. Because these hosts inhabit a very narrow range of habitats, they are an important aspect to include in *F. magna* management. Employing landscape-level GIS tools in concert with One Health and biodiversity conservation/restoration concepts should improve both understanding and prediction of infection risk. Arguably, this strategy will help minimize infection risk to moose while avoiding negative environmental impacts.

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