

PREVALENCE AND INTENSITY OF MENINGEAL WORMS
(*PARELAPHOSTRONGYLUS TENUIS*) AND LIVER FLUKES (*FASCIOLOIDES*
MAGNA) IN ELK (*CERVUS ELAPHUS*) OF NORTHERN WISCONSIN

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PREFACE

The chapters of this thesis were written in the format of the Journal of Wildlife Diseases. Any duplication in methods or citations among chapters is intentional. Each of the two research chapters is designed for easier editing before submission to the Journal of Wildlife Diseases for publication.

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INTRODUCTION

Elk (*Cervus elaphus*) translocation has become common in the United States and herds have been successfully reintroduced into Michigan (Bender et al., 2005), Pennsylvania (Woolf et al., 1977), Oklahoma (Raskevitz et al., 1991), and Kentucky (Larkin et al., 2003). Managers often examine ecological impacts and survival and productivity in reintroduced animals, but the effect of disease on recipient or transplanted populations is not often considered (Wobeser, 2006). Diseases endemic to a system can dramatically affect reintroduced animals. Additionally, disease can be accidentally transplanted to recipient populations with translocated animals. Managers are becoming more aware of disease ecology as outbreaks that affect both native and transplanted populations become increasingly common (Corn and Nettles, 2001). This thesis focuses on disease concerns of a reintroduced elk herd in northern Wisconsin.

History of the Clam Lake Elk Herd

Elk or wapiti were once common throughout Wisconsin and were recorded in 50 of the state's 72 counties (Schorger, 1954). But similar to other elk populations in eastern North America, the Wisconsin population was extirpated by the late 1800's (Schorger, 1954; Jackson, 1961). In 1932, the Wisconsin Conservation Commission attempted to reintroduce elk into the state, but the attempt failed by the 1950's primarily because of poaching (Schorger, 1954; Anderson, 1999). In 1989, the Wisconsin state legislature directed the Wisconsin Department of Natural Resources (WDNR) to explore the feasibility of restoring elk, caribou (*Rangifer tarandus*), and/or moose (*Alces alces*) to Wisconsin in order to promote education, recreation, and economic benefits such as tourism and hunting in the state (Anderson, 1999; Pils, 2000). Moreover, the WDNR

aimed to reestablish a native ungulate into northern Wisconsin (Pils, 2000). It was determined that elk reintroduction would be most likely to succeed because moose and caribou would potentially succumb to meningeal worm (*Parelaphostrongylus tenuis*) more readily than elk (Parker, 1990). As a result, the University of Wisconsin-Stevens Point (UWSP) acquired 25 elk from Michigan in 1995 and released them into the Chequamegon National Forest near Clam Lake, Wisconsin.

The 25 Wisconsin founder animals underwent extensive health testing, treatment for internal and external parasites, and a three-month quarantine before spending two weeks in an acclimation pen (Anderson, 1999; Pils, 2000). Following their release, the elk herd was monitored by UWSP, but in 1999 management responsibilities for the herd were transferred from UWSP to the WDNR. Currently, a herd of approximately 125 animals is located in the Chequamegon National Forest near Clam Lake in Ashland, Bayfield, and Sawyer Counties (Fig. 1, L. Stowell, WDNR, personal communication). About 70 adults and calves are radio-collared and monitored routinely to assess population size, distribution, productivity, and survival (L. Stowell, WDNR, personal communication). In addition, newborn calves are captured in spring of each year and collared to monitor birth rates and calf survival. Using radio-collared animals, the WDNR monitors elk year-round. Elk tend to form large aggregations of both sexes in winter, split into cow/calf groups in the spring/summer, and remain in small groups of females accompanied by defending males during the fall rut (Wisdom and Cook, 2000). Intense year-round monitoring is necessary because the WDNR has determined that limited hunting will be allowed when the population of elk reaches 200 animals (L. Stowell, WDNR, personal communication).

Overall, the elk herd has steadily increased since 1995. The herd increased by approximately 15 to 25% annually from 1997-2003, but a growth plateau was detected during 2004 and 2005 (L. Stowell, WDNR, personal communication). In 2004, the growth rate slowed to 4% and in 2005, the herd decreased by 1 to 2%, partially because of increased mortality in the herd (L. Stowell, WDNR, personal communication). The majority of mortalities were attributed to predation and vehicular collisions, but there were a number of cases where parasites contributed directly or indirectly to mortality (WDNR, unpublished data). Mortalities of collared individuals in 2004 and 2005 showed that three animals had extremely high infestations of liver flukes (*Fascioloides magna*) and wolves killed two animals infected with meningeal worm (WDNR, unpublished data). In 2004-2005, 36% of mortalities involved liver flukes or meningeal worms, and these parasites have been documented in 7 of 69 (10%) mortalities from 1995-2005 (WDNR, unpublished data). To effectively monitor the Clam Lake elk herd, the WDNR needed information on baseline prevalence of meningeal worms and liver flukes to assess their potential effects on future growth.

Parasites

Parasites have been documented in several elk mortalities in the Wisconsin herd. To understand how these parasites affect elk, I will give background on each parasite along with a thorough explanation of life cycles. Hosts for most parasites fall into three major categories: definitive hosts, intermediate hosts, and aberrant hosts. A definitive host is the host in which the parasite achieves sexual maturity (Roberts and Janovy, 2005). The intermediate host is an obligate host where parasite development occurs but does not involve sexual reproduction (Roberts and Janovy, 2005), and an aberrant host is a host

where parasite migration and development are not successfully completed within the host (Pybus, 2001).

***Parelaphostrongylus tenuis* or Meningeal Worm**

Parelaphostrongylus tenuis is a nematode parasite of cervids. In 1964, this parasite was shown to be the etiologic agent of “moose sickness,” a neurologic disease causing ataxia, weakness, paraplegia, impaired vision, hearing problems, fearlessness, listlessness, circling, emaciation, and peculiar positioning of the head (Anderson, 1964; Loken et al., 1965). Meningeal worm is common and widely distributed in white-tailed deer (*Odocoileus virginianus*), the parasite’s normal definitive host (Bindernagel and Anderson, 1972; Anderson and Prestwood, 1981; Comer et al., 1991; Lankester, 2001). *Parelaphostrongylus tenuis* does not normally cause neurologic disorders in white-tailed deer (Anderson, 1972), but even a low intensity of parasites may cause severe neurologic disorders leading to death in caribou, elk, moose, reindeer (*Rangifer tarandus tarandus*), mule deer (*O. hemionus*), and black-tailed deer (*O. h. columbianus*) (Anderson, 1964; Anderson, 1971; Carpenter et al., 1973; Trainer, 1973; Nettles et al., 1977; Tyler et al., 1980; Lankester, 2001).

Meningeal worm is prevalent in eastern and central North America (Lankester, 2001). The life cycle begins when ungulates accidentally ingest infected snails or slugs, the intermediate hosts, when feeding on vegetation (Anderson and Prestwood, 1981). Larvae are released via digestive processes and migrate to the central nervous system where they often inhabit the meninges. Males and females mature into adults, mate, and females shed unembryonated eggs into the circulatory system. Eggs migrate to the lungs where they develop into first-stage larvae and are coughed up and swallowed by the host.

Larvae then pass unharmed in the feces. When a terrestrial snail or slug encounters infected feces, larvae penetrate and complete their development into the infective stage within the intermediate host (Samuel et al., 1992; Lankester, 2001). A large variety of terrestrial gastropods, such as *Anguispira alternata*, *Cochlicopa lubrica*, *Deroceras* spp., *Discus* spp., *Pallifera dorsalis*, *Philomycus carolinianus*, *Striatura exigua*, *Strobilops labyrinthica*, and *Zonitoides* spp., have been found to be suitable intermediate hosts (Kearney and Gilbert, 1978; Upshall et al., 1986; Rowley et al., 1987; Lankester, 2001).

Parelaphostrongylus tenuis complete the life cycle in white-tailed deer, producing an asymptomatic infection that rarely causes death (Anderson and Prestwood, 1981). However, they can cause severe clinical signs when they infect abnormal hosts where larvae do not migrate normally, causing physical damage to neural tissue (Samuel et al., 1992). Meningeal worms have been found in the spinal cord of moose, caribou, and elk where they cause tissue damage, severe neurologic disease, paralysis, and death (Carpenter et al., 1973; Trainer, 1973; Woolf et al., 1977; Olsen and Woolf, 1978; Samuel et al., 1992; Lankester, 2002). In areas where white-tailed deer overlap with moose and caribou, meningeal worms have caused declines in these cervid species (Karns, 1967; Gilbert, 1974; Bergerud and Mercer, 1989). In fact, extirpation of entire reintroduced caribou populations has resulted from *P. tenuis* infection (Anderson, 1972; Bergerud and Mercer, 1989) and they have likely limited the success of elk reintroductions (Anderson and Prestwood, 1981; Raskevitz et al., 1991). In Kentucky, 23% of elk mortalities from 1997-2001 were attributed to *P. tenuis* infection (Larkin et al., 2003). However, only 3% of mortalities from 1981-1994 in the Michigan elk herd were from *P. tenuis* (Bender et al., 2005).

Meningeal worm exists in deer populations in Wisconsin. Dew (1988) examined white-tailed deer from Vilas, Oneida, and Forest Counties in northern Wisconsin and found that 58% of deer were infected with *P. tenuis*. This parasite could affect elk in northern Wisconsin but prevalence likely varies spatially and temporally.

Meningeal worm may contribute directly to mortality in the Clam Lake elk herd, but it may also have other influences on the population. *Parelaphostrongylus tenuis* infection in moose and captive elk has been shown to predispose infected animals to mortality by hunting and predation (Gilbert, 1974; Olsen and Woolf, 1978; Thomas and Dodds, 1988), and has been suggested as an indirect influence on mortality in a reintroduced elk population in Kentucky (Larkin et al., 2003). Abnormal behavior patterns could increase chances of predation or vehicular collisions, the two highest causes of mortality in the Clam Lake elk herd (L. Stowell, WDNR, personal communication). Woolf et al. (1977), found that *P. tenuis* was not a significant mortality factor in a captive elk population in Pennsylvania, but higher than average mortality was documented in younger age-classes. It has also been suggested that *P. tenuis* may affect a female elk's ability to properly care for young (Olsen and Woolf, 1978). If either improper care of calves by females or additive mortality on young occurred in the Clam Lake elk herd, population effects could be drastic, limiting the growth rate of a young and relatively small population.

***Fascioloides magna* or Giant Liver Flukes**

Giant liver flukes, *F. magna*, are common parasites of wild and domestic ruminants throughout North America (Foreyt and Todd, 1976; Foreyt, 1981; Lankester and Luttich, 1988; Pybus, 1990; Pybus, 2001). White-tailed deer, caribou, and elk are

common definitive hosts where flukes are normally benign (Foreyt and Todd, 1979; Foreyt, 1981; Foreyt, 1996; Pybus, 2001). However, these flukes have the potential to cause extensive hepatic damage in elk, moose, and cattle, although damage is usually not lethal (Foreyt and Todd, 1976; Foreyt, 1996; Pybus, 2001). Infection in domestic goats and sheep can be lethal because parasite migration severely damages hepatic tissue (Foreyt and Todd, 1976; Foreyt and Hunter, 1980; Foreyt and Leathers, 1980).

Fascioloides magna occurs in five major areas in North America: the Great Lakes region, the Gulf coast, the northern Pacific coast, the Rocky Mountain trench, and the northern portion of Quebec and Labrador (Pybus, 2001). These parasites are dependent on waterbodies with emergent vegetation and abundant snail intermediate hosts to complete their life cycle (Pybus, 1990). It is in this setting that definitive hosts, such as elk, will acquire parasites when ingesting aquatic vegetation encysted with metacercariae, an infective stage between juvenile and adult. Metacercariae are activated in the gut where they become active juveniles, which migrate across the gut wall to the liver and mature slowly into adult flukes. At this time, individuals will attempt to locate mates and encapsulate in pairs, although capsules occasionally contain more than two flukes (Foreyt and Todd, 1976; Addison et al., 1988; Pybus, 1990; Foreyt, 1996; Pybus, 2001). Adult flukes release eggs that are passed in the feces and ciliated free-swimming miracidia are released from eggs in water. These miracidia must find a suitable aquatic snail species to serve as an intermediate host. Once a host is found, miracidia penetrate the snail tissue and develop further. After numerous asexual reproductive events, cercariae emerge from the snail and encyst as metacercariae on aquatic vegetation and remain viable for prolonged periods (Pybus, 2001). The life cycle then begins again.

Although elk are definitive hosts for flukes and infections can be sustained without mortality, severe infestation could contribute to mortality. Prevalence of *F. magna* in adult elk has been reported as high as 86% in Canada (Pybus, 1990). Experimental infections of elk caused mortality when an animal was exposed to 2000 metacercariae (Foreyt, 1996). In addition, three ranched elk in Montana presumably died from high infestations of *F. magna* (Hood et al., 1997) where hemorrhaging caused by migrating flukes could have caused mortality. High liver fluke intensity and chronic infection can result in fibrosis of the liver, and this is commonly observed in elk, fallow deer (*Dama dama*), and caribou (Pybus, 2001). Furthermore, flukes can cause liver damage, abscesses, and anemia in white-tailed deer (Cheatum, 1951).

In addition to outright mortality, fluke infection may indirectly affect survival. Studies on the effects of *F. magna* in white-tailed deer have shown that the parasite influences reproductive success and output by affecting weight of females prior to breeding, timing of conception, and body condition (Mulvey et al., 1994). Liver fluke infections may also decrease winter survivorship in white-tailed deer that experience starvation during severe winters (Cheatum, 1951). Moreover, nutritional status may affect pathogenicity. The common liver fluke, *F. hepatica*, caused sheep on a lower protein ration to die more quickly and show rapid anemia, hypoalbuminemia, and weight loss when compared to sheep on a higher protein diet (Berry and Dargie, 1976). Therefore, *F. magna* infection could cause indirect mortality, especially in cold winter climates experienced in northern Wisconsin. This can be particularly important in pregnant females in the Clam Lake herd, where high liver fluke infestations and severe environmental conditions could ultimately influence offspring survival.

The objective of this study was to determine if liver flukes and/or meningeal worms could potentially play a role in decreased growth in the Clam Lake elk herd. I evaluated fecal samples for the presence of meningeal worm larvae in winter, spring, summer, and fall. In addition, I specifically sought to quantify prevalence and intensity of liver flukes during the same seasons. Chapter Two of this thesis reports results from meningeal worm investigations while Chapter Three reports results from liver fluke analyses. Chapter Three includes additional work beyond the scope of the original project examining variation of fluke egg intensity among fecal pellets from the same individual.

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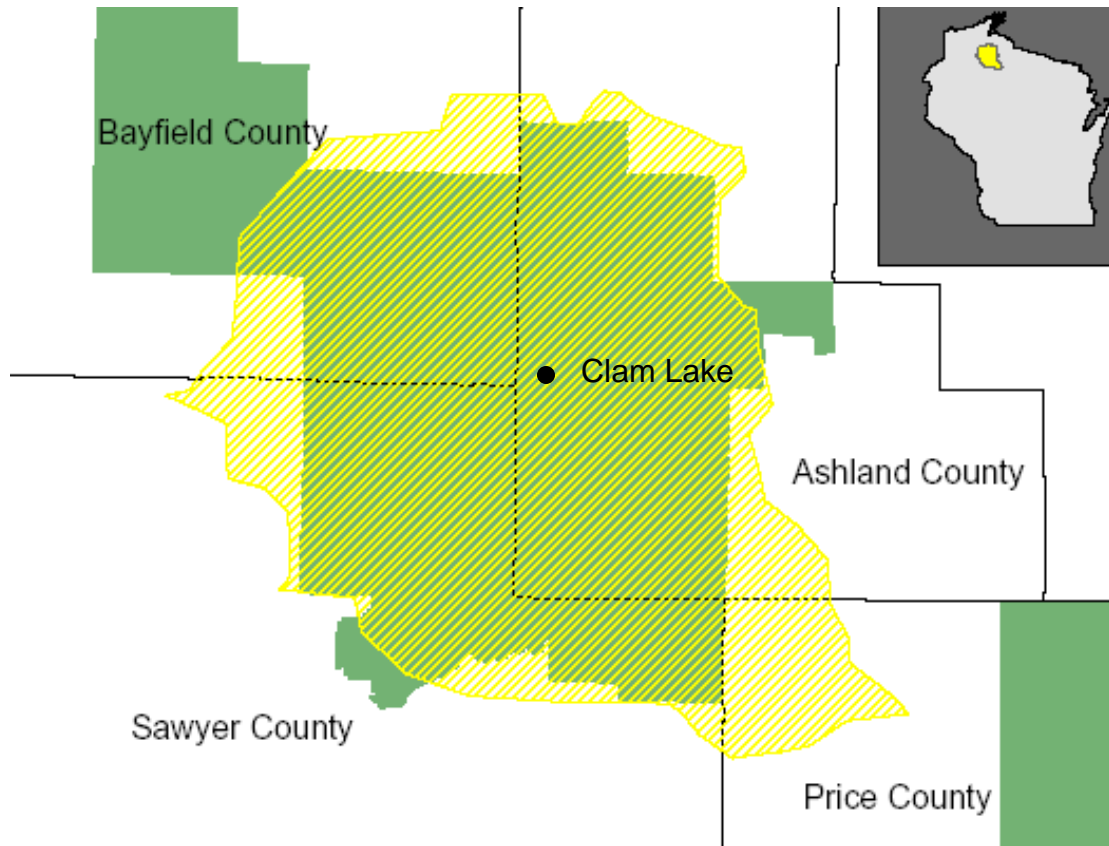
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Figure 1. Buffer range for the Wisconsin elk herd in Ashland, Bayfield, and Sawyer Counties near Clam Lake, Wisconsin, USA.



Running Head: SHORT COMMUNICATIONS

Prevalence of Meningeal Worm (*Parelaphostrongylus tenuis*) in Fecal Samples from a Reintroduced Elk (*Cervus elaphus*) Herd in Wisconsin (USA)

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ABSTRACT: A recent decline in growth of a reintroduced elk (*Cervus elaphus*) herd in Wisconsin and an increase in documented meningeal worm (*Parelaphostrongylus tenuis*) mortalities in radio-collared individuals led to concern about the parasite's potential effect on herd growth. Therefore, we used fecal samples to determine minimum baseline prevalence and intensity of meningeal worms in the elk herd. We evaluated feces for larval worms because elk are not hunted so post-mortem examination was not feasible. In 2006 and 2007, fecal samples were collected from groups of elk in winter, summer, and fall. We also collected feces from individual females during spring calving season. All samples were processed using the modified Baermann technique to determine

presence of meningeal worm larvae. We collected a total of 140 samples in 2006 and 152 samples in 2007. No *P. tenuis* larvae were detected in elk feces and no animals died of *P. tenuis* in 2006 and 2007. Therefore, it is unlikely that *P. tenuis* infection is a major mortality factor in this elk herd. Low infection rates, variable shedding of larvae in elk feces, and variation in habitat use of elk and infected white-tailed deer (*Odocoileus virginianus*) could account for the lack of larvae detected in feces.

Key words: *Cervus elaphus*, elk, fecal samples, meningeal worm, *Parelaphostrongylus tenuis*, Wisconsin

Meningeal worms (*Parelaphostrongylus tenuis*) are common parasites of white-tailed deer (*Odocoileus virginianus*) in eastern and central North America (Lankester, 2001). White-tailed deer serve as the definitive host for the parasite where it rarely causes clinical disease (Anderson, 1972; Anderson and Prestwood, 1981). However, meningeal worms have the potential to cause severe clinical signs, neurologic disease, and death in caribou (*Rangifer tarandus*), elk (*Cervus elaphus*), moose (*Alces alces*), reindeer (*Rangifer tarandus tarandus*), mule deer (*O. hemionus*), and black-tailed deer (*O. h. columbianus*) (Anderson, 1964; Anderson, 1971; Carpenter et al., 1973; Trainer, 1973; Nettles et al., 1977; Tyler et al., 1980; Lankester, 2001). The parasite is of concern to managers in areas where susceptible cervids are sympatric with white-tailed deer because mortality from *P. tenuis* has been documented in cervids in these areas (Anderson, 1972; Gilbert, 1974; Thomas and Dodds, 1988; Bergerud and Mercer, 1989; Samuel et al., 1992).

Twenty-five elk were reintroduced to Wisconsin (USA) from Michigan (USA) in 1995 (Anderson, 1999; Pils, 2000). Currently, the Wisconsin Department of Natural Resources (WDNR) manages a herd of approximately 125 animals located in northern Wisconsin near Clam Lake in Ashland, Bayfield, and Sawyer Counties (L. Stowell, WDNR, personal communication). About 70 adults and calves are radio-collared and monitored year-round to assess population size, distribution, productivity, and survival (L. Stowell, WDNR, personal communication). The elk population increased by approximately 15 to 25% annually from 1997-2003, but a growth plateau was detected during 2004 and 2005 (L. Stowell, WDNR, personal communication). In 2004, the growth rate slowed to 4% and in 2005, the herd decreased by 1 to 2%, partially because of increased mortality in the herd (L. Stowell, WDNR, personal communication). The majority of mortalities were attributed to predation and vehicular collisions, but parasites contributed directly or indirectly to mortality in several cases (WDNR, unpublished data). Recent mortalities of collared individuals showed that one animal died directly from *P. tenuis* and wolves killed two other infected elk (WDNR, unpublished data). Increased meningeal worm infection combined with decreased herd growth motivated the WDNR to evaluate infection. In order to effectively monitor the Clam Lake elk herd, we initiated a project to determine baseline prevalence and intensity of meningeal worms in the herd. We hypothesized that few elk would be infected with low numbers of meningeal worm larvae.

Parasite larvae may be shed at various rates depending upon time of year (Foreyt and Trainer, 1980; Thomas and Dodds, 1988; Slomke et al., 1995; Nankervis et al., 2000; Lankester, 2001; Duffy et al., 2002). We used herding tendencies of elk in different

seasons to aid in sampling (Wisdom and Cook, 2000). In 2006 and 2007, we collected fecal samples from groups of elk during winter, summer, and fall. Since approximately half of the Clam Lake herd is radio-collared, we located groups of animals using radio-telemetry (Fuller et al., 2005). We also collected samples from individual females with radio-collars during calving season in May and June. Pregnant female elk leave groups just before giving birth and stay separated for up to three weeks after calves are born (Wisdom and Cook, 2000). Each year, WDNR employees locate cow elk just after birthing to radio-collar calves and monitor birth rates and calf survival (Pils, 2000). We collected fresh fecal samples from known individuals since a female will stay in seclusion with her calf during this time period (Wisdom and Cook, 2000).

When groups of animals were located, each radio-collared individual in the group was recorded. The number of samples collected was based on group size. We collected no more samples than the number of known individuals in the group because group numbers are strictly monitored by the WDNR (L. Stowell, WDNR, personal communication). We searched for fresh samples because larvae die as samples desiccate (Thomas and Dodds, 1988). In winter, all pellet groups were frozen and it was difficult to distinguish between recent and older samples. Therefore, we collected samples after recent snowfall to find the freshest samples on top of snow. In spring, summer, and fall, fresh samples were easily identified from older samples. Rain water may also disperse *P. tenuis* larvae from feces of infected animals (Duffy et al., 1999), so we did not collect feces during or immediately following rainfall.

To minimize the possibility of double sampling during winter, we collected fecal pellets on an elk path from an individual animal. Once a sample was collected from a

path, we ceased sampling on that path. In summer, elk paths were generally not as visible, but we attempted to collect from separate paths. Again, we minimized double sampling by collecting fewer fecal samples than known elk in a group.

We collected entire fecal pellet groups and weighed each sample in order to determine prevalence and intensity of parasites per gram of feces. All samples were placed in individual plastic bags, labeled, and frozen at $\leq -17^{\circ}\text{C}$ until processed in the laboratory. For individual elk, we noted the WDNR identification number on the bag.

We used 15 g of each sample to evaluate presence of *P. tenuis* larvae using the modified Baermann technique as described by Forrester and Lankester (1997). Briefly, we used uncut pellets and placed them in window screening. The pellets were submerged in 250 ml glass beakers filled with tap water for 24 hrs. After 24 hrs, pellets were discarded, fluid was allowed to settle for 1 hr, and all but 50 ml of fluid was discarded. We examined the remaining fluid using a dissecting scope at 40X. Additionally, melted snow and ice in bags from winter samples was evaluated for larvae on the chance that they dispersed from feces during melting. Since residual *P. tenuis* larvae have been found on Baermann apparati cleaned with soap and tap water (McCollough and Pollard, 1993; Whitlaw and Lankester, 1995), all equipment was rinsed with 95% ethanol between uses.

In 2006, group collections consisted of 47 winter samples, 51 summer samples, and 21 fall samples with an additional 21 individual samples collected in spring. In 2007, group collections consisted of 47 winter samples, 29 summer samples, and 58 fall samples with an additional 18 individual samples collected in spring. This resulted in 140 samples in 2006 and 152 samples in 2007 for a total of 292 samples. Meningeal

worm larvae were not detected in elk fecal samples using the modified Baermann technique. No elk mortalities were attributed to *P. tenuis* infection in 2006 or 2007.

WDNR biologists were concerned about meningeal worm infection and associated slow growth in the elk herd in 2004 and 2005. This free-ranging herd is closely monitored with a large number of radio-collared animals, so mortalities are documented more thoroughly than in many other free-ranging herds. Fecal samples were used as a way of determining baseline prevalence and intensity of meningeal worms. Since hunting is not allowed in the Clam Lake elk herd, post-mortem examination only occurs when WDNR employees collect animal carcasses from the field.

Meningeal worm larvae were not detected in any of the 292 elk fecal samples collected in 2006 and 2007. Elk are inadvertent hosts of the parasite and larvae can be shed intermittently and at low levels (Welch et al., 1991). Welch et al. (1991) experimentally infected elk calves with *P. tenuis* and found that larval density in feces varied among animals as well as among samples from the same individual and larvae were shed inconsistently. Larvae have been documented in elk feces from experimental and free-ranging *P. tenuis* infections (Pybus et al., 1989; Welch et al., 1991; Samuel et al., 1992; Duffy et al., 1999); however, elk do not always shed larvae (Samuel et al., 1992; Welch et al., 1991). We realize the limitations of using elk feces to evaluate prevalence, but as noted above, we could not examine carcasses. Accordingly, we aimed to determine minimum infection rates because not all infected individuals would be detected using feces. We hypothesized that meningeal worm would be at a low level since large numbers of elk mortalities were not occurring in this highly monitored herd.

Meningeal worm can have variable effects on mortality. In Kentucky, 23% of elk mortalities from 1997-2001 were attributed to *P. tenuis* infection (Larkin et al., 2003), but in Michigan, only 3% of mortalities in the elk herd from 1981-1994 were from the parasite (Bender et al., 2005). Environmental factors could account for these differences, including differential habitat use of elk and white-tailed deer (Raskevitz et al., 1991). Shortly after the reintroduction in 1995, winter feeding ecology of the Clam Lake elk herd was intensively studied with some focus on the relationship between elk and deer. Lizotte (1998) compared food habits of both species and found that elk selected similar food items as deer in winter. However, he also examined 120 winter elk feeding sites for the presence of deer by looking for tracks and fecal pellets and examined 159 additional feeding sites after snowmelt in spring. He found that deer and elk used similar feeding sites only 8.2% of the time, indicating that there was little seasonal dietary overlap in Wisconsin. Elk and deer may be using different habitats for feeding, which would minimize elk contact with infected gastropods resulting in minimal infection.

Recent information suggests that *P. tenuis* mortality in Wisconsin is similar to Michigan. The free-ranging Michigan elk herd currently contains approximately 1100-1200 animals with a yearly harvest (MIDNR, 2008). Michigan served as the source of the Wisconsin elk herd; therefore, it is likely that the two populations are genetically similar. If prevalence of *P. tenuis* in the Wisconsin elk herd does not change drastically, it is feasible that positive growth could continue as in Michigan and the herd could sustain limited hunting, a management goal of the WDNR once the Clam Lake population reaches 200 animals (L. Stowell, WDNR, personal communication).

Few data exist regarding prevalence and intensity of meningeal worm in free-ranging elk in areas sympatric with infected deer. Therefore, we believe that these results warrant reporting. The parasite exists in Wisconsin deer herds and several studies reporting *P. tenuis* in white-tailed deer have been conducted throughout various parts of the state (Samuel and Trainer, 1969; Trainer, 1973; Foreyt and Trainer, 1980). Dew (1988) reported that 58% of deer harvested in Vilas, Oneida, and Forest counties in northern Wisconsin were infected. These counties are < 100 km from the elk herd range, so infection rates of white-tailed deer in areas where elk reside may be similar. We did not detect larvae in fecal samples, but the parasite has caused direct and indirect mortality of radio-collared individuals in the herd (WDNR, unpublished data). However, since the onset of this study, the growth rate in the elk herd has increased to 13% in 2006-2007 (L. Stowell, WDNR, personal communication). Based on WDNR mortality data and lack of larvae in feces, *P. tenuis* does not seem to be a major mortality factor in the Clam Lake elk herd.

Despite the apparent low level of meningeal worm infection in Wisconsin elk, we recommend periodic monitoring since the parasite has the potential to cause serious neurologic disease in elk (Carpenter et al., 1973; Olsen and Woolf, 1978; Samuel et al., 1992). As elk serum testing for *P. tenuis* advances, it would be possible to collect serum samples during yearly WDNR elk trapping, which would give a much better estimate of prevalence. Even though the parasite is not currently causing significant problems in the elk herd, prevalence could change as the elk herd grows or if deer densities in the elk range increase. In 2005-2006, deer densities in the core elk habitat were estimated at 9 deer/km² and were lower than average statewide density of 14 deer/km² (Rolley, 2006).

This is worth noting since deer density can predict infection in sympatric ungulates (Karns, 1967; Gilbert, 1974; Bergerud and Mercer, 1989). Even though meningeal worm may not cause numerous direct mortalities in the herd, it may limit herd growth and expansion. However, at this time, only occasional monitoring is necessary and minimal management for *P. tenuis* is required.

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Running Head: LIVER FLUKES IN FECES FROM WISCONSIN ELK

Prevalence and Intensity of Liver Fluke (*Fascioloides magna*) Eggs in Fecal Samples from a Reintroduced Elk (*Cervus elaphus*) Herd in Wisconsin (USA)

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ABSTRACT: In 1995, 25 elk (*Cervus elaphus*) were reintroduced into Wisconsin (USA) from Michigan (USA). The population has been closely monitored since that time and was estimated at more than 120 animals in 2004. However, in 2004-2005, population growth slowed and numerous liver flukes (*Fascioloides magna*) were identified in elk carcasses. Our objective was to obtain baseline prevalence and intensity information on liver fluke eggs in elk fecal samples over a 2-year period. Additionally, we determined mean intensity and variation in eggs shed in fecal pellet piles from individual elk in order to determine the minimal number of pellets that need to be processed to obtain accurate intensity data. We found that a minimum of four samples should be examined when using the formalin-ethyl acetate sedimentation technique to determine egg intensity. In

2006 and 2007, we collected feces from groups of elk in winter, summer, and fall and from individual females during elk calving season. Average prevalence of liver fluke eggs was 33.1% and 45.9% in 2006 and 2007, respectively and prevalence did not vary significantly with season. Fluke egg intensity in 2006 ranged from 0.2-28.4 eggs/g with a mean of 4.0 eggs/g. In 2007, intensity estimates ranged from 0.2-18.4 eggs/g with a mean of 3.3 eggs/g. We used two-way ANOVA to evaluate the effects of group and season on intensity and found no effect of group ($P=0.453$) or season ($P=0.785$) and the interaction between group and season was not significant ($P=0.145$). However, a second two-way ANOVA using year and season showed that intensity varied with season ($P=0.008$) as well as with year ($P=0.023$) and there was a slight interaction ($P=0.005$). Mean yearly intensity in 2006 was significantly greater than intensity in 2007 with the exception of intensity during spring. Liver fluke infection seems to be common in the Clam Lake elk herd and is not likely causing a population decline. Moreover, the growth rate of the elk herd has rebounded since 2005 and there have been no documented mortalities where *F. magna* contributed to mortality. Because *F. magna* infection is relatively common in these elk, we recommend minimal management in the form of periodic monitoring to evaluate prevalence trends over time.

Key words: *Cervus elaphus*, elk, formalin ethyl-acetate sedimentation technique, *Fascioloides magna*, fecal samples, liver flukes, Wisconsin

INTRODUCTION

Fascioloides magna or the giant liver fluke is a common parasite of elk (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*) in many regions of North America (Foreyt, 1981; Pybus, 2001). In these definitive hosts, infection is usually sub-clinical (Foreyt and Todd, 1979; Foreyt, 1981; Foreyt, 1996) but may indirectly affect survival. In white-tailed deer, *F. magna* has been shown to influence reproductive success and output (Mulvey et al., 1994). Infections may also decrease winter survivorship in white-tailed deer during severe winters when starvation is common (Cheatum, 1951). High infestations of liver flukes have caused mortality in experimentally infected and game-ranched elk (Foreyt, 1996; Hood et al., 1997). In addition, an elk calf in Oregon was diagnosed with clinical disease from liver fluke infection where immature fluke migration may have caused severe hepatic and muscular damage (Bildfell et al., 2007).

Twenty-five elk were reintroduced to Wisconsin (USA) from Michigan (USA) in 1995 (Anderson, 1999; Pils, 2000). Currently, the Wisconsin Department of Natural Resources (WDNR) monitors a herd of approximately 125 animals located in northern Wisconsin near Clam Lake in Ashland, Bayfield, and Sawyer Counties (L. Stowell, WDNR, personal communication). The Clam Lake elk herd is comprised of three primary groups throughout the year, although two of these groups join together in winter (L. Stowell, WDNR, personal communication). About 70 adults and calves are radio-collared and monitored year-round to assess population size, distribution, productivity, and survival (L. Stowell, WDNR, personal communication).

Overall, the population has grown steadily since 1995 with annual growth rates of 15 to 25% from 1997-2003. However, in 2004 the growth rate slowed to 4% and in 2005, the herd decreased by 1 to 2%, partially because of increased mortality in the herd (L. Stowell, WDNR, personal communication). Most mortalities were attributed to predation and vehicular collisions, but parasites contributed directly or indirectly to mortality in several cases (WDNR, unpublished data). In 2005, high intensity infections of adult liver flukes were identified in mortalities of collared individuals and were thought to contribute to mortality (WDNR, unpublished data). Our objectives were to 1) determine baseline prevalence and intensity of liver fluke eggs from fecal samples so the WDNR could monitor parasites in the Clam Lake herd in an effective manner, and 2) determine variability in detection of fluke eggs in fecal samples from individual elk. Our hope was to determine the minimum amount of feces that should be processed to acquire accurate mean intensity data.

MATERIALS AND METHODS

Sampling

In order to obtain seasonal prevalence and intensity data in 2006 and 2007, we collected fecal samples from groups of elk during winter (February-March), summer (July), and fall (September-October) in addition to collecting samples from individual females during calving season in May and June. The Clam Lake herd stays in three main groups, Wayside, 1029, and 208, named after landmarks near their respective home ranges. We used seasonal herding tendencies of elk to aid in sampling (Wisdom and Cook, 2000). Since approximately half of the Clam Lake herd is radio-collared, we located groups of animals using radio-telemetry and collected fecal samples from areas

recently used by elk (Fuller et al., 2005). Individual female radio-collared elk were sampled during calving season. Pregnant female elk leave their groups just before giving birth and stay separated for up to three weeks after calves are born (Wisdom and Cook, 2000). Each year, WDNR employees locate females just after birthing to radio-collar calves and monitor birth rates and calf survival (Pils, 2000). Because females stay in seclusion with calves during this time period (Wisdom and Cook, 2000), we were able to collect fresh fecal samples from individuals.

We used radio-telemetry to locate groups of animals and recorded all radio-collared animals in the group. The number of fecal samples collected was based on group size. We collected no more samples than the number of known individuals in the group because group numbers are closely monitored by the WDNR (L. Stowell, WDNR, personal communication). We obtained fresh samples because moisture is crucial for liver fluke egg development (Foreyt, 1981; Pybus, 2001), and eggs may die as samples desiccate. During winter collections, all pellet groups were frozen making it difficult to distinguish between recent and older samples. Therefore, we collected samples after recent snowfall to ensure the collection of fresh samples on top of snow. In spring, summer, and fall, fresh samples could easily be identified from older samples.

To minimize the possibility of double sampling during winter, we collected fecal pellets from an individual elk along a travel pathway. Once a sample was collected from a pathway, we ceased sampling on that pathway. In summer, elk paths were difficult to determine, but we attempted to collect from separate paths. Again, we minimized double sampling by collecting fewer fecal samples than known elk in the group.

We collected entire fecal pellet groups and weighed each sample in order to determine prevalence and intensity of parasite eggs per gram of wet feces. All samples were placed in individual plastic bags, labeled, and frozen at $\leq -17^{\circ}$ C until processed in the laboratory.

Laboratory Analyses

While processing preliminary fecal samples in the laboratory, we noticed variation in egg counts within individual pellets of a sample. To determine minimum number of pellets to be processed in order to accurately estimate egg counts, we randomly selected fecal samples from 5 positive individuals. We ran each of these samples through two separate trials. In trial 1, we weighed and processed 10 pellets individually using the modified formalin-ethyl acetate sedimentation technique (Ritchie, 1948; Faler and Faler, 1984). We finely chopped a pellet, immersed it in water, and strained the solution through two layers of cheesecloth. The cheesecloth was discarded and we centrifuged the solution at 2000 rpm for one minute and decanted the supernatant. The sediment was resuspended in water and centrifuged again at 2000 rpm. Once the supernatant was decanted, we mixed 10 ml of 10% formalin with the sediment followed by 3 ml of ethyl acetate. After the sample was mixed thoroughly, we then centrifuged it at 1500 rpm for one minute. The top three resulting layers were decanted and the remaining sediment was examined under a compound microscope at 100X. Liver fluke eggs were identified by size and microscopic characteristics (Campbell, 1961). We recorded all results as eggs per gram of wet feces to determine mean intensity of infection.

In trial 2, we used the same method as above, but instead of analyzing fluke eggs in each pellet individually, we chopped and mixed 10 pellets together, and examined 10 approximately equal aliquots of mixed sample.

To calculate seasonal and yearly prevalence and intensity of liver fluke eggs in feces collected from free-ranging elk in 2006 and 2007, we weighed all fecal samples and 5 g were chosen randomly and processed for *F. magna* eggs.

Data Analyses

For validation trials, we used two-way analysis of variance (ANOVA) on SAS software (SAS Institute Inc., Cary, North Carolina, USA) to evaluate if fluke egg intensity in each sample varied with trial or among individuals. Trial method was set as a fixed factor while individual was set as a random factor. Because variance increased with intensity, we log transformed intensity data. In order to compare how variance in intensity estimates differed between trails, we used a two-tailed variance ratio test (Zar, 1999). We also calculated a running mean of intensity over the 10 subsamples from each individual to determine the number of samples needed for intensity to stabilize.

Prevalence of liver fluke eggs in elk feces are presented as proportion of positive samples with 95% confidence intervals (Zar, 1999). We used two-way ANOVA to evaluate egg intensity in season by herd subgroup. We also used two-way ANOVA to evaluate egg intensity in season by year. Data analyses were performed with SPSS software (SPSS Inc., Chicago, Illinois, USA) with statistical significance assessed at $P < 0.05$.

RESULTS

Sampling

In 2006, we collected 47 winter fecal samples, 51 summer fecal samples, and 21 fall fecal samples. We also collected 21 samples from individual elk in spring. In 2007, we collected 47 winter samples, 29 summer samples, and 58 fall samples and pellets from 18 known individuals in spring. We collected 140 samples in 2006 and 152 samples in 2007 for a total of 292 samples over a two-year period. Although samples were collected from three groups in some seasons, elk groups Wayside and 1029 combine in winter; therefore, these groups were pooled for consistency.

Validation Trials

Variance among subsamples did not differ between trials. F statistics for each individual are as follows: sample 1 = 1.67, sample 2 = 1.35, sample 3 = 1.45, sample 4 = 1.06, and sample 5 = 1.69 (critical $F_{0.05(2), 9, 9} = 4.03$). Mean egg intensity did not differ between trials ($F_{1,90}=2.523$, $P=0.187$) but did differ across individuals ($F_{4,90}=113.786$, $P=0.000$). The interaction term was not significant ($F_{4,90}=1.554$, $P=0.194$, Fig. 2, Appendix 1). In general, higher intensities were calculated for individuals when single pellets were analyzed individually during trial 1 than when pooled in trial 2 (Fig. 2). Plots of the running means suggest that approximately 4 subsamples are necessary for a stable estimate of intensity (Figs. 3 and 4). Additionally, pellet groups with low intensity showed less variation than those with higher intensity (Figs. 3 and 4).

Prevalence Analyses

Overall, there was little variation in prevalence by season because 95% confidence intervals overlapped for all seasons except winter 2006 and winter 2007 (Fig.

5, Appendix 2). Average prevalence across seasons for 2006 was 33.1% and for 2007 was 45.9%. We observed differences in prevalence by group because elk group 208 had 0% prevalence during some seasons whereas some individuals in Wayside/1029 were infected each season (Appendix 2). Wayside/1029 was much less variable while 208 varied with season (Appendix 2).

Intensity Analyses

Intensity in 2006 ranged from 0.2-28.4 eggs/g with a mean of 4.0 eggs/g and in 2007 intensity ranged from 0.2-18.4 eggs/g with a mean of 3.3 egg/g. Fluke egg intensity did not vary by group ($F_{1,113}=0.566$, $P=0.453$) or by season ($F_{3,113}=0.356$, $P=0.785$), and there was no season by group interaction ($F_{3,113}=1.835$, $P=0.145$, Fig. 6, Appendix 3). Elk in the Wayside/1029 group had higher intensities of eggs in feces in spring and fall (Fig. 6) but differences were not significant overall. Because of the lack of difference between groups, we pooled the data across groups and tested for year and season effects. There were significant differences between years ($F_{1,113}=5.296$, $P=0.023$) and seasons ($F_{3,113}=4.185$, $P=0.008$) as well as a significant interaction ($F_{3,113}=4.470$, $P=0.005$, Fig. 7, Appendix 4). Mean intensity was higher in spring, summer, and fall 2006 than the same seasons in 2007, but intensity of fluke eggs in winter samples showed the opposite trend because intensity was higher in 2007 than 2006 (Fig. 7).

DISCUSSION

Laboratory Protocol

We found that individual pellets within a pellet pile varied in number of fluke eggs. Variation in liver fluke egg intensity in pellets from a sample could lead to inaccurate intensity estimates if adequate amounts of feces are not evaluated. We found

that variance in intensity by individual did not differ between the two laboratory trials. In addition, when 10 samples from the same fecal pellet pile were analyzed, we found no difference in mean intensity when single pellets or pooled pellets were used for analysis, so it did not matter if samples were run as single or mixed pellets when calculating eggs/g of feces. This is important because it is much more time consuming to run pellets individually. Mixing pellets would also be more cost effective because smaller amounts of ethyl acetate and formalin would be required for processing. Intensity differed across individuals, allowing us to compare variation in low and high intensity samples. We found that samples with high intensity of liver fluke eggs also showed highest variation in mean intensity with repeated analyses.

Running means for both trials showed that mean intensity stabilized when approximately four samples were evaluated (Figs. 3 and 4). If only one or two samples are used, intensity data could prove inaccurate and imprecise, especially for fecal samples with high intensity. Although a four pellet or four subsample minimum cannot equate to an exact amount of feces, some researchers use a 5 g standard in this process (W. J. Foreyt, Washington State University, personal communication). This standard would be sufficient since most adult elk fecal pellets range between 1-2 g (T. M. Weiland, University of Wisconsin-Stevens Point, unpublished data) and at least four pellets would be needed to obtain 5 g. Calf pellets are smaller in size and weight so increased numbers of pellets would be needed. In order to achieve accurate intensity results for adult elk, we recommend sampling at least 4 pellets or 4 subsamples from a pooled mixture of 4 pellets.

Prevalence

We realize the limitations of using elk feces to evaluate intensity and prevalence of liver fluke infection. However, since hunting is not allowed in this population, we were not able to evaluate livers from harvested animals. As a result, we used fluke eggs in feces to acquire baseline data. We also used this technique as a way of comparing among groups and examining an index of trends over time. Prevalence of *F. magna* eggs in elk fecal samples ranged from a low of 27.6% (8/29) in summer 2007 to a high of 61.7% (29/47) in winter 2007. Prevalence may not have varied statistically by season because of low sample size. Since spring is calving season for elk, individual female elk were sampled and it was not feasible to collect large numbers of samples. Fall 2006 and summer 2007 collections were smaller than expected because weather conditions were not conducive to sampling.

Prevalence of liver flukes in elk varies greatly by area, making prevalence comparisons difficult (Pybus, 2001). Additionally, most prevalence data are reported by identifying adult flukes in livers of harvested animals (Kingscote et al., 1987; Pybus, 1990; Pybus et al., 1991). From fecal sample analyses, we found that prevalence is variable and not extremely high in the Wisconsin elk herd and no recent mortalities have been attributed to liver flukes (WDNR, unpublished data).

We observed a seasonal difference in prevalence in winters 2006 and 2007 (Fig. 5). A peak in transmission may explain the high prevalence in winter 2007. Foreyt (1975) found that *F. magna* eggs were transmitted in high numbers in May and October-November in Texas and it is possible that Wisconsin has similar peaks in transmission in spring and fall. Since the prepatent period for elk is six months (Foreyt, 1996), an elk

infected in May would begin shedding eggs in early winter. Northern Wisconsin experienced a mild drought for most of 2006 (NCDC, 2008). Precipitation increased to approximately 12.7 cm during July and August but only 4.1 cm precipitation fell in June (NCDC, 2008). The increase in precipitation in July and August after a drought may have caused an increase in snail populations, which could potentially increase parasite transmission. Considering the prepatent period of flukes, an increase in transmission during these months could account for an increase in prevalence in winter 2007.

Prevalence of fluke eggs in feces differed with group because elk group 208 showed 0% prevalence during some seasons (Appendix 2). This group had an overall lower prevalence than the rest of the herd, with the exception of fall 2007 (Appendix 2). Elk group 208 in the Clam Lake herd stays secluded from the other groups throughout the year (L. Stowell, WDNR, personal communication). Difference in seasonal and overall prevalence may be related to habitat used by this group. Habitat can affect development of the parasite and abundance of intermediate hosts (Pybus, 2001). Although our study does not allow us to explore habitat differences, future research on liver flukes in this herd could focus on the habitat use of group 208 compared to other groups of elk.

Intensity

Intensity did not vary by elk group, but differences could have been masked because of the herding tendencies of Wisconsin elk (L. Stowell, WDNR, personal communication). Elk tend to form large aggregations in winter (Wisdom and Cook, 2000) and two of the main groups in Clam Lake (Wayside and 1029) join up in winter while the third (208) remains separate (L. Stowell, WDNR, personal communication). As a result, we pooled Wayside/1029 in analyses. Fewer fecal samples from elk group 208 were

positive for fluke eggs, so any group effect may not be evident because of small sample size (Appendix 2). Seasonal group effects were also not significant, but Wayside/1029 had higher liver fluke egg intensity in two of the four seasons evaluated (Fig. 6).

We found significantly greater fluke egg intensity in 2006 than 2007, but intensity also varied with season. Higher intensity in 2006 may be a result of environmental factors. Liver flukes depend upon waterbodies with vegetation and abundant snail intermediate hosts to complete their life cycle (Pybus, 1990). It is possible that in 2006, the primary elk groups spent more time in wetter habitats with ample emergent vegetation. These sites also support higher snail populations (Pybus, 2001). This could result in infected animals acquiring more intense infections. Additionally, changes in precipitation could also account for yearly differences in intensity. Generally, less precipitation fell in 2007 than 2006 (NCDC, 2008). Also, drought-like conditions were common in 2007 with 3 months in moderate drought, 5 months in mild drought, and 4 months in incipient drought meaning drought conditions were beginning to develop (NCDC, 2008). Comparatively, in 2006, 7 months were classified as mild drought, 1 month was classified as incipient drought, and 3 months had mild wet conditions (NCDC, 2008). Overall drier conditions experienced in 2007 may explain lower mean intensities recorded that year.

The significant interaction between year and season likely resulted from increased intensity in winter 2007 (Fig. 7). As with prevalence, a peak in parasite transmission in July and August of 2006 may explain this increase. Even though intensity varied statistically in all seasons except spring, this could result from smaller sample sizes

during this season. However, with the exception of winter 2007, seasonal trends suggest that liver fluke egg intensity peaks in spring and fall in northern Wisconsin (Fig. 7).

Liver fluke intensity in other areas is not often reported as eggs/g but as adult flukes in the liver. Feces are often used to detect positive experimental infections and intensity is not reported (Foreyt and Todd, 1976; Foreyt, 1992; Foreyt, 1996). In published reports of infected elk and white-tailed deer, intensities in feces are higher than what was observed in the Clam Lake elk herd (Hood et al., 1997; Qureshi et al., 1989). However, caution must be used when interpreting these results since there is no apparent correlation between intensity of eggs in feces and number of adult flukes in the liver. Qureshi et al. (1989) reported *F. magna* intensity from fecal analyses as well as adult parasites recovered from necropsied white-tailed deer. These data showed considerable variation between adults and eggs recovered in feces.

Although it appears that liver flukes are common in the Wisconsin herd, some animals may harbor many adult flukes in the liver because liver flukes have an aggregated distribution in their hosts with few animals carrying the majority of parasites (Pybus, 2001). This may be the case in the Clam Lake herd where necropsies have shown high infestations of liver flukes with massive liver damage (WDNR, unpublished data). Fecal analyses seemed to indicate that most groups of elk are infected during some portion of the year, but intensity of eggs in feces varies with year and season.

Management Implications

Fascioloides magna have been historically found in white-tailed deer in northern Wisconsin (Campbell, 1954), including areas that encompass the core elk range. Despite rigorous health checks and parasite screening in the 25 Wisconsin founder animals

(Anderson, 1999; Pils, 2000), they were likely to acquire fluke infection from the surrounding area after release.

Both prevalence and intensity of liver fluke eggs in feces from the Clam Lake elk herd were found to be at manageable levels and no elk mortalities have been attributed to liver fluke infection in several years. Feces are not the optimal method of detection; however, since hunting is not allowed in this population, this was the best method to acquire baseline data on the population. Since elk are definitive hosts for liver flukes and shed eggs via feces, we know that these elk are commonly infected with liver flukes.

Since liver flukes have been found in the population, the WDNR could continue monitoring the elk using our protocol in order to detect changes in prevalence or intensity over time. If parasite-related mortality does not increase, we recommend continued monitoring as a minimal management strategy. Since the onset of our study, the growth rate in the elk herd has increased to 13% in 2006-2007 and there have been no recorded liver fluke related mortalities (L. Stowell, WDNR, personal communication). Prevalence could increase if density of elk and/or white-tailed deer increases or if wetter weather conditions favor parasite transmission. If prevalence or intensity increases or if death occurs from fluke infections, the WDNR could chemically treat animals during yearly trapping in January. Triclabendazole has shown to be effective against infections of both mature and immature flukes in elk and white-tailed deer and treatment can be administered directly or through medicated corn bait (Qureshi et al., 1989; Pybus et al., 1991; Qureshi et al., 1994).

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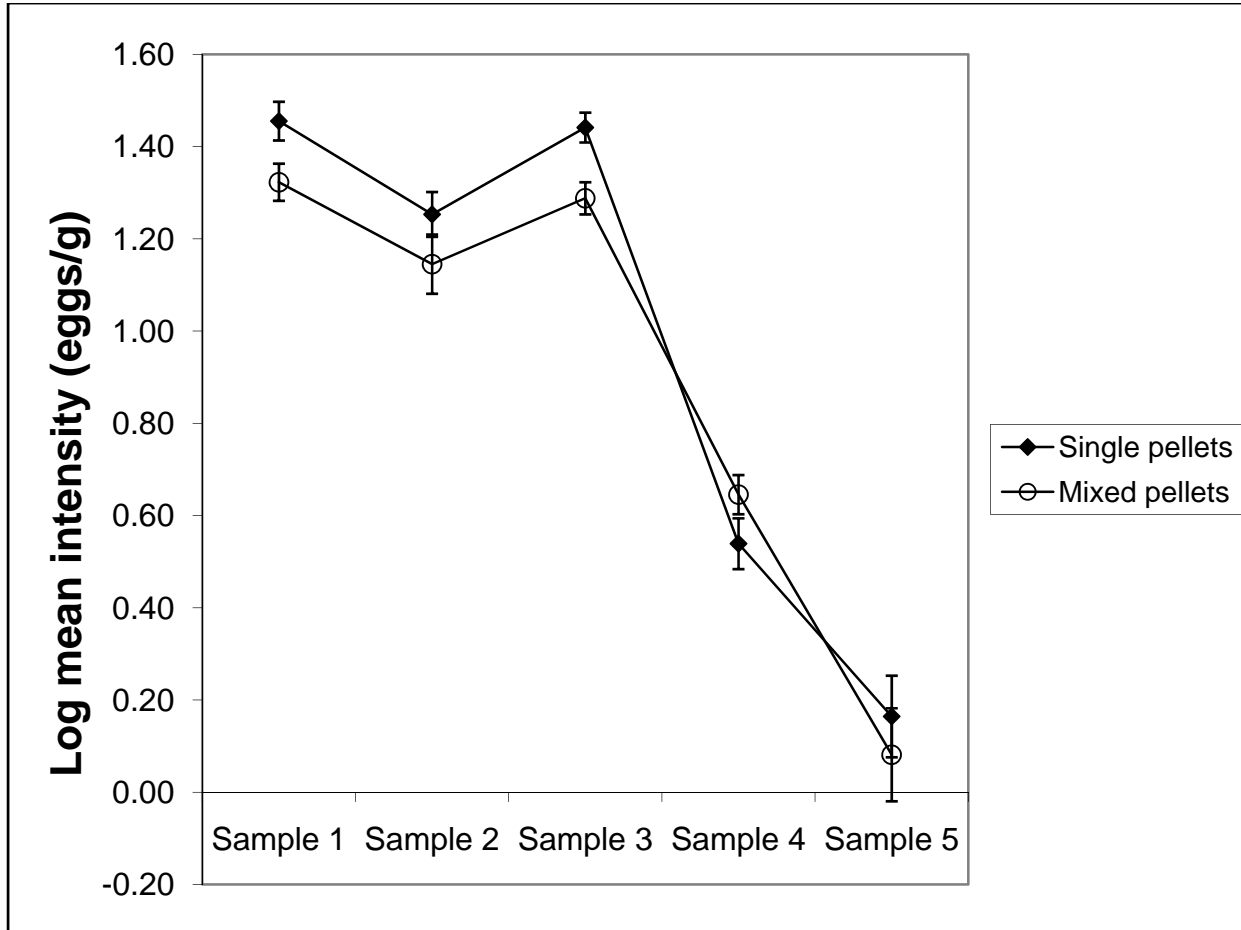


Figure 2. Interaction plot for trials showing variation in log transformed mean liver fluke egg intensity (with SE) in five elk fecal samples collected from Clam Lake, Wisconsin, USA. We used 10 samples in each trial.

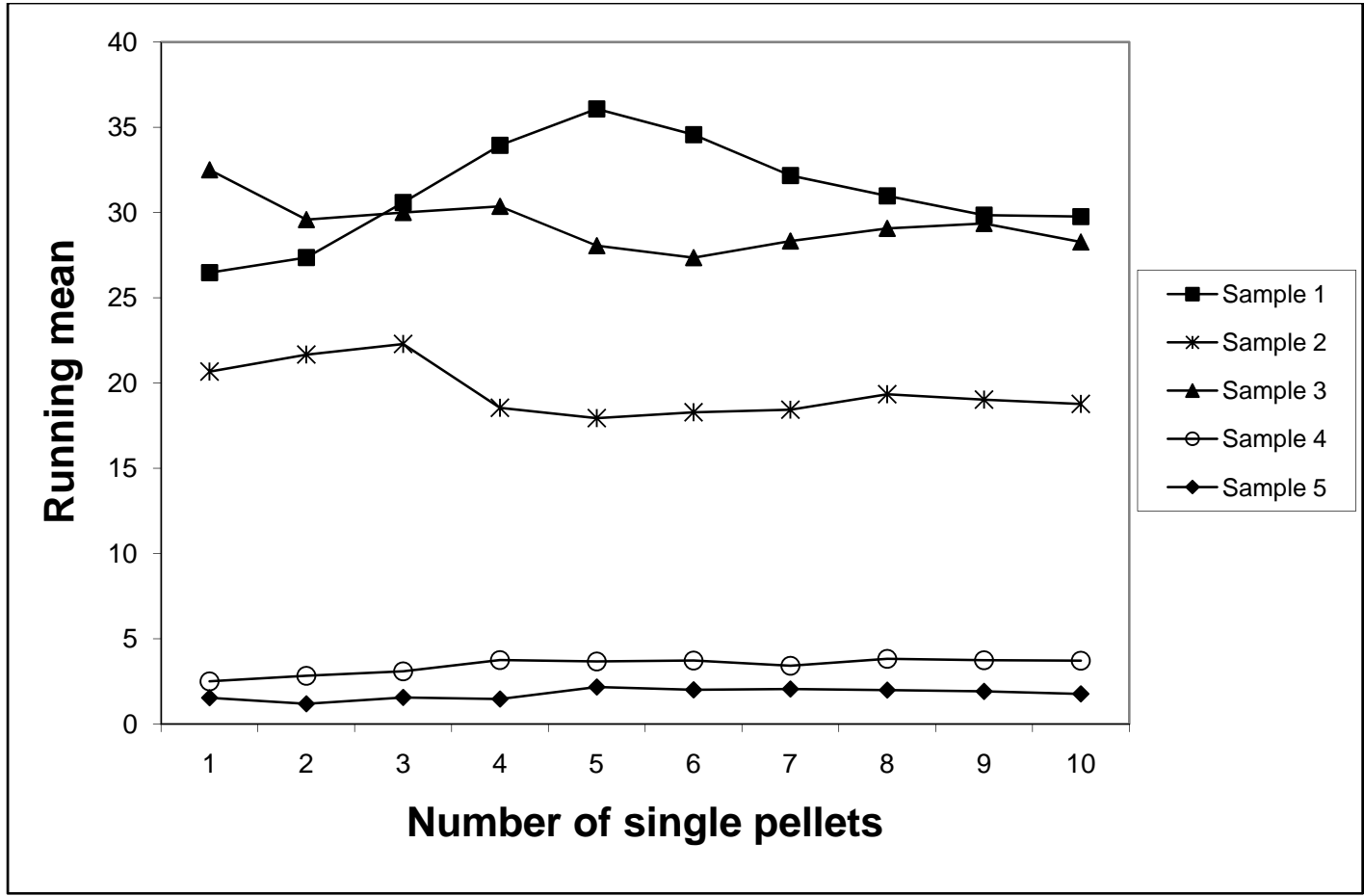


Figure 3. Running means for five randomly chosen elk fecal samples containing liver fluke eggs. Intensity was measured by analyzing eggs in 10 individual pellets.

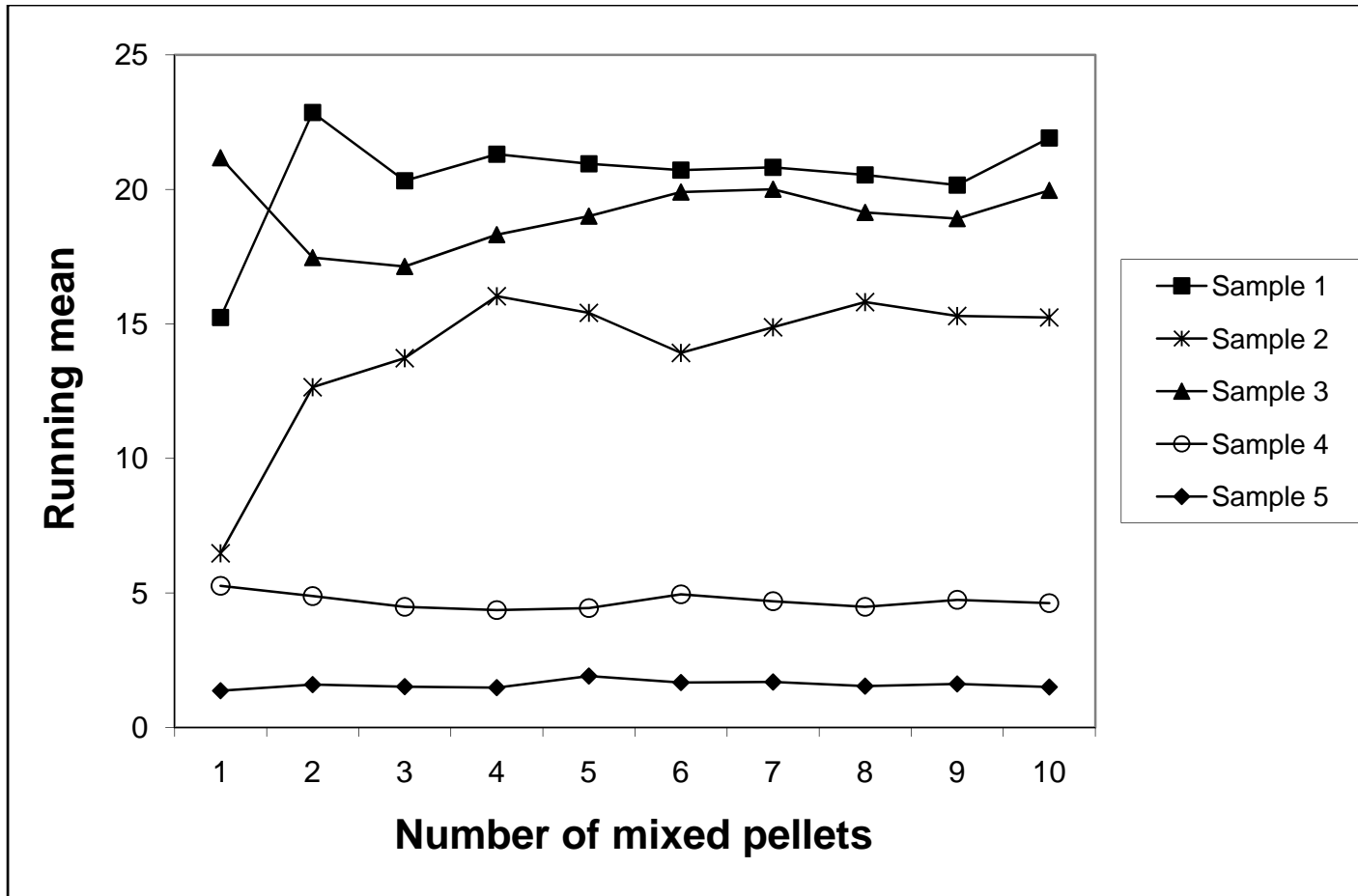


Figure 4. Running means for five randomly chosen elk fecal samples containing liver fluke eggs. Intensity was measured by analyzing eggs in 10 pellets by pooling pellets and analyzing 10 approximately equal aliquots of sample.

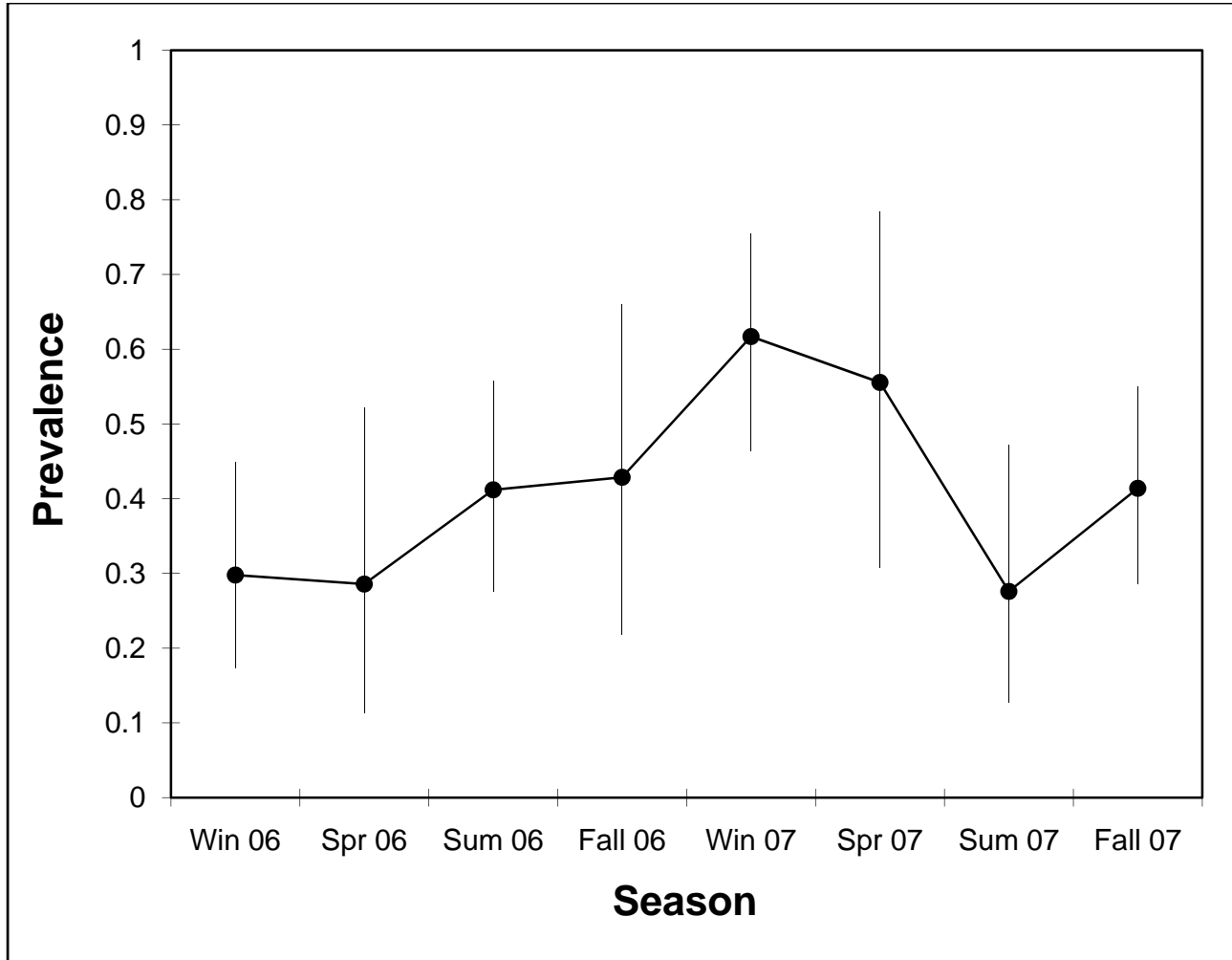


Figure 5. Seasonal prevalence and 95% confidence intervals of liver fluke eggs in elk fecal samples from Clam Lake, Wisconsin, USA in 2006 and 2007.

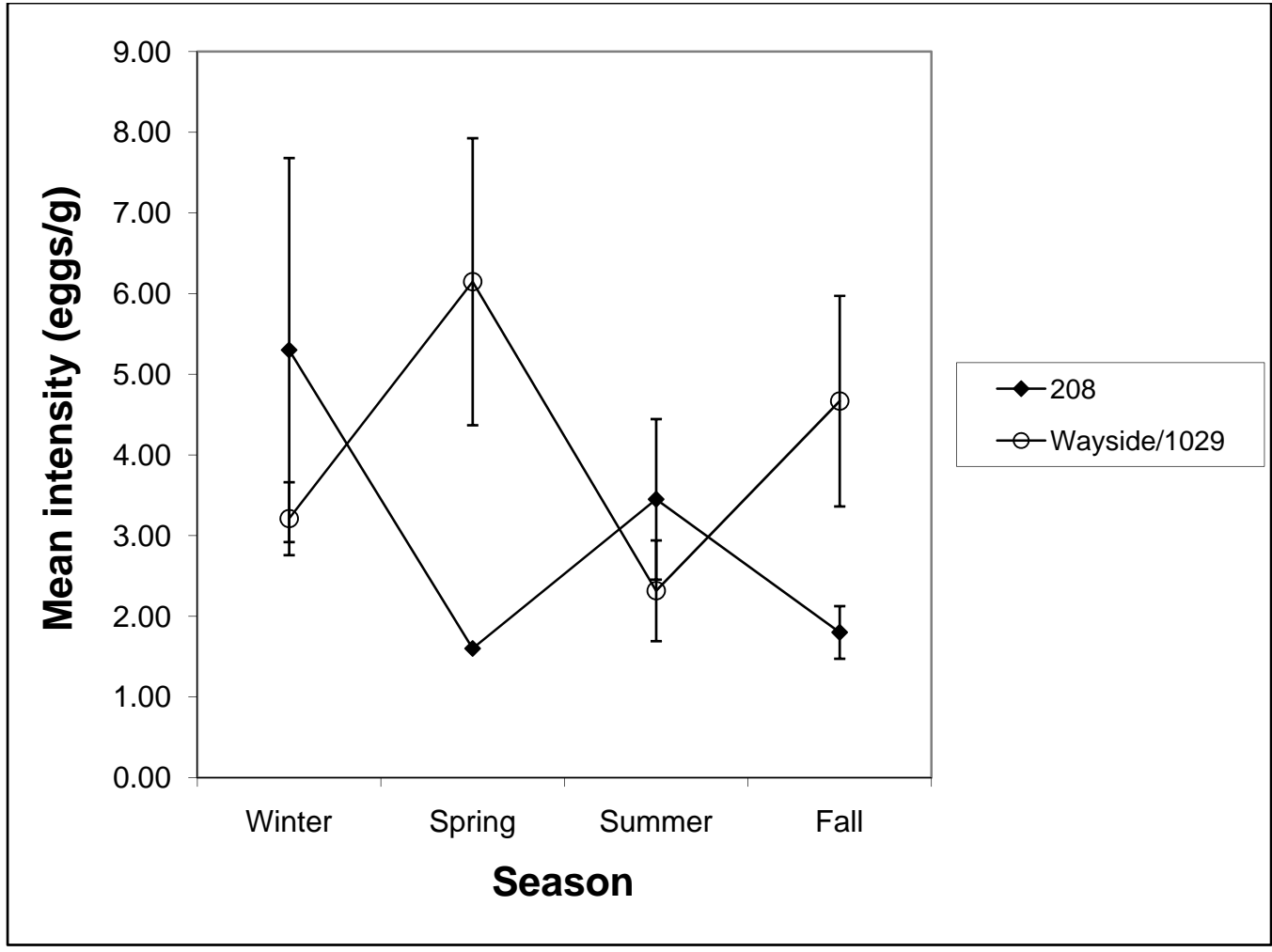


Figure 6. Seasonal intensity (and SE) of fluke eggs identified in fecal samples from groups of elk in the Clam Lake herd, Wisconsin, USA.

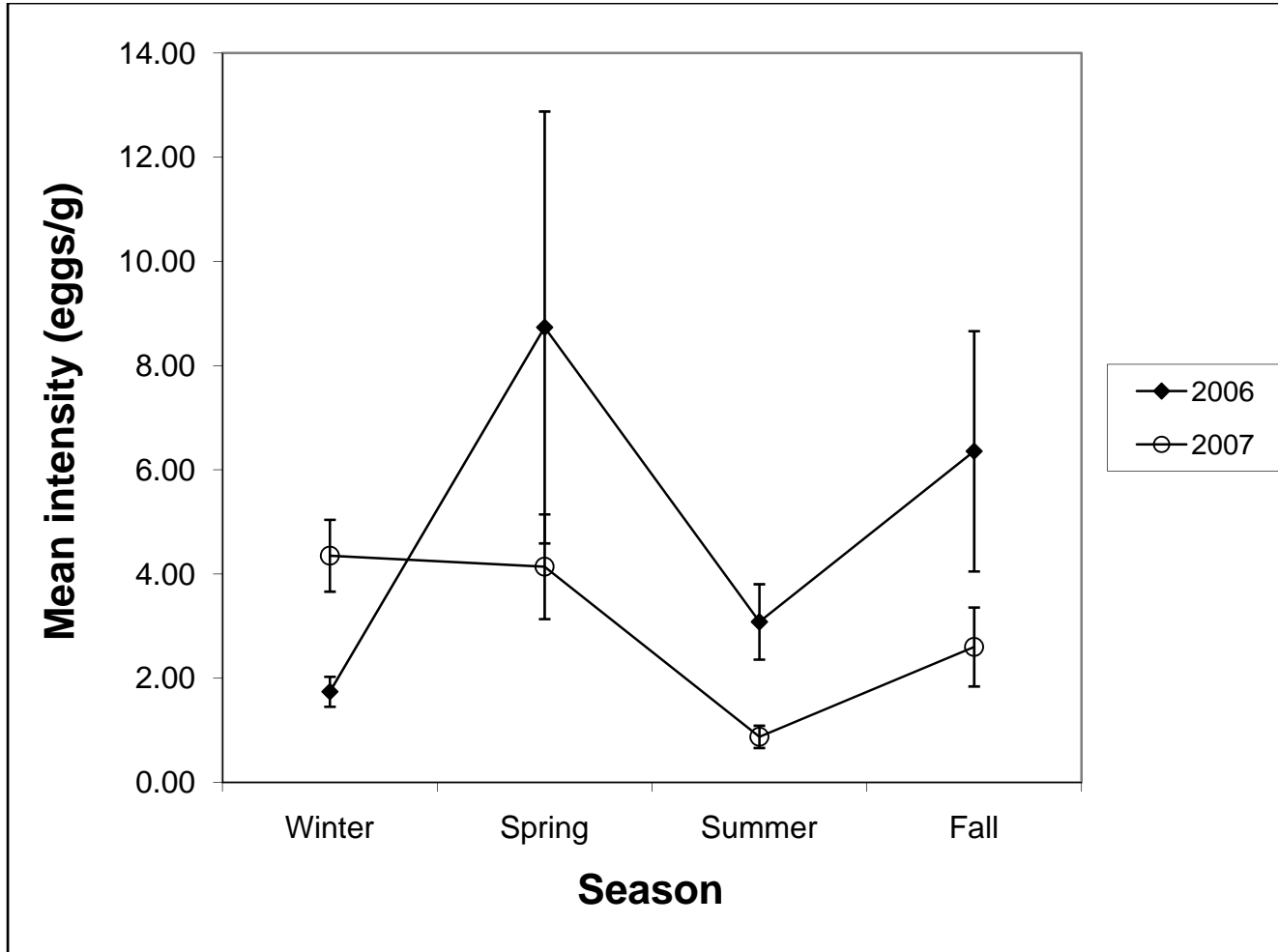


Figure 7. Seasonal intensity (and SE) of fluke eggs identified in fecal samples from elk by year in the Clam Lake herd, Wisconsin, USA.

Appendix 1. Two-way ANOVA evaluating if differences in intensity existed among five elk fecal samples and using two trial sampling schemes.

Factor	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i> -value
Method	1	0.137	0.137	2.523	0.187
Subject	4	24.748	6.187	113.786	0.000
Interaction	4	0.217	0.054	1.554	0.194
Error	90	3.149	0.035		
Total	99	28.252			

Appendix 2. Prevalence of liver fluke eggs in fecal samples from elk near Clam Lake Wisconsin, USA by group and season. Samples collected in spring were from individual animals.

Year	Season	Group	Prevalence
2006	Winter	208	0.0% (0/14)
		Wayside/1029	42.4% (14/33)
	Spring	208	0.0% (0/6)
		Wayside/1029	40.0% (6/15)
	Summer	208	28.6% (4/14)
		Wayside/1029	45.9% (17/37)
	Fall	Wayside/1029	42.9% (9/21)
	2007	Winter	208
Wayside/1029			69.7% (23/33)
Spring		208	33.3% (1/3)
		Wayside/1029	60.0% (9/15)
Summer		208	0.0% (0/8)
		Wayside/1029	38.1% (8/21)
Fall		208	54.5% (12/22)
		Wayside/1029	33.3% (12/36)

Appendix 3. Two-way ANOVA evaluating intensity of liver fluke eggs by season and group in the Clam Lake elk herd in northern Wisconsin, USA.

Factor	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i> -value
Season	3	19.715	6.572	0.356	0.785
Group	1	10.438	10.438	0.566	0.453
Interaction	3	101.488	33.829	1.835	0.145
Error	113	2083.765	18.440		
Total	120	2312.097			

Appendix 4. Two-way ANOVA evaluating intensity of liver fluke eggs by season and year in the Clam Lake elk herd in northern Wisconsin, USA.

Factor	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i> -value
Season	3	212.939	70.980	4.158	0.008
Year	1	90.409	90.409	5.296	0.023
Interaction	3	228.909	76.303	4.470	0.005
Error	113	1928.884	17.070		
Total	120	2312.097			