

Elk Abundance, Survival, and Health in the Cumberland Mountains of Tennessee

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ABSTRACT

Managing sustainable wildlife populations requires insight into population abundance and health. Since reintroduction, elk (*Cervus canadensis*) at the North Cumberland Wildlife Management Area (NCWMA) in Tennessee have shown marginal population growth using low-precision abundance estimates. Limited research investigating possible population limiting factors has occurred since evaluations conducted directly after translocation. To provide information necessary for effective population management, we estimated abundance, identified survival rates, and conducted mortality and health surveillance. Precise abundance estimates of eastern elk populations are challenging to obtain using traditional capture-recapture due to invasive handling of individuals and low detection in forested landscapes. Therefore, we used elk DNA from scat noninvasively collected in 2019 to genetically identify individuals using 16 microsatellites with sex determination and estimated abundance using a Huggins closed capture model. From 157 successfully genotyped fecal samples, we identified 85 individuals (64 females, 21 males). The abundance model estimated 159 elk (123 females, 36 males) with acceptable precision (coefficient of variation: 15.6%) and identified a female skewed sex ratio (1:5). To further investigate population status, we placed GPS collars on 29 elk (21 females, 8 males) during 2019 and 2020. We estimated annual survival rates using known-fate models and identified primary causes of death of collared elk from 2019 to 2022. We estimated an average yearly survival rate of 80.2% with primary causes of mortality including meningeal worm (*Parelaphostrongylus tenuis*) associated disease ($n=3$), poaching ($n=1$), vehicular

collision ($n=1$), legal hunter harvest ($n=1$), and unknown due to carcass degradation ($n=3$). We used blood, feces, tissue, and ectoparasites collected during elk capture to further assess population health. We conducted surveillance for pathogens based on presence in the southeastern United States, potential causes of elk morbidity and mortality, agricultural animal risk, and/or zoonotic risk. Our surveillance identified the presence of pathogens with potential negative population implications including *P. tenuis* and *Mycobacterium avium* subspecies *paratuberculosis* (Johne's disease). Our research provided precise abundance estimates, sex ratios, and increased understanding of influential parameters of elk population growth: survival and health. Identifying potential population limiting factors can aid in supporting data-based management strategies for the NCWMA elk population.

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CHAPTER ONE

INTRODUCTION

Reintroduced elk (*Cervus canadensis*) in Tennessee are an important state natural resource garnering significant economic benefits and public support for a sustainable population (Chapagain and Poudyal 2020, Chapagain et al. 2020, Watkins et al. 2021). To manage sustainable wildlife populations, insight into population status and health is imperative. Surveilling causes of morbidity and mortality provides comprehensive information on survival, which directly impacts abundance and population viability. My dissertation focuses on estimating abundance, identifying survival rates, analyzing causes of mortality, and conducting pathogen surveillance to provide information necessary for effective population management of elk in Tennessee.

Literature Review of Elk in Tennessee

Elk were prolific and well distributed throughout Tennessee until 1825 (Ganier 1928). Overharvest and habitat loss caused significant population declines, leading to the extirpation of elk in Tennessee when the last individual was reportedly killed in the mid to late 1800s (Rhoads 1896, Ganier 1928, O’Gara and Dundas 2002). Since the early 1900s, there have been numerous attempts to reestablish elk to portions of their former range in the eastern United States. Some states have successfully reestablished elk populations; however, many elk reintroduction attempts have failed or had reduced or declining population growth primarily due to poaching, disease, and human-elk conflict over crop destruction (O’Gara and Dundas 2002).

In 1996, the Tennessee Wildlife Resources Agency (TWRA) evaluated the potential of reintroducing elk to Tennessee. Considerations for reintroduction areas included the availability of suitable elk habitat, recreation potential, risk of human-elk conflict, and likelihood of disease. As public support for elk restoration grew, the Upper Cumberland Plateau was the chosen reintroduction site (TWRA 2018). The restoration zone was 271,139 ha (670,000 acres) and composed of parts of Anderson, Campbell, Claiborne, Morgan, and Scott counties (TWRA 2000).

Manitoban elk (*C. c. manitobensis*) at Elk Island National Park, Canada (EINP) are thought to be the most genetically similar elk subspecies to the extirpated eastern subspecies (*C. c. canadensis*; TWRA 2000). For this reason, and the population's extensive disease testing and lack of chronic wasting disease detection, EINP elk were selected to be released in the restoration zone (TWRA 2018). Elk were tested for pregnancy and diseases of concern prior to being selected for reintroduction (Kindall et al. 2011). Fifty EINP elk in 2000, 36 in 2001, and 50 in 2002 were released into the North Cumberland Wildlife Management Area (NCWMA). Land Between the Lakes (LBL), Kentucky provided elk originally stocked from EINP for reintroduction in 2003 ($n=31$; Kindall et al. 2011). In 2008, 34 more elk from LBL were released for a total of 201 elk (Kindall et al. 2011). All elk were released in the NCWMA without a period of acclimation (Kindall et al. 2011). A 12% population growth rate was projected prior to translocation, with the goal of achieving a huntable elk population within as few as 7 years of reintroduction (Wathen et al. 1997, TWRA 2000).

Population Monitoring

Estimating the population size of a species is necessary for population management and conservation, and elk abundance has been estimated using many techniques with differing success and precision (Bear et al. 1989, Skalski et al. 2005, Royle et al. 2013, TWRA 2018, Bristow et al. 2019). Precise population estimates (<20% coefficient of variation, CV; Pollock et al. 1990, Williams et al. 2002, Morin et al. 2022) following elk release have been difficult to obtain using traditional methods due to the mountainous terrain, substantial forest cover, and uneven elk distribution (TWRA 2018). Mark-resight techniques have been utilized annually since 2006 to estimate NCWMA elk abundance with elk capture and GPS collars used to mark individuals (Anderson 2009a, TWRA 2018). Collared elk were visually identified and counted along with non-marked elk. Program NOREMARK and the Bowden estimator were used to estimate elk abundance from the data (White 1996, Bowden and Kufeld 1995, Anderson 2009a).

Mark-resight population surveys in 2006 and 2007 produced population estimates of 203 and 231 elk, respectively. Following the surveys, the elk herd was considered sustainable and possibly growing. Since that time, annual mark-resight surveys have indicated limited population growth; however, the results have wide confidence intervals reflecting uncertainty in the estimates (TWRA 2018). Low-precision estimates can lack the power needed to detect population changes over time and lead to an inability to meet management objectives (Hagen et al. 2014, Morin et al. 2022).

Harvest scenarios were considered using decision making models. Under multiple hunting limit strategies, the models indicated slow elk population growth at best

or population decline (Anderson 2009b). The first elk hunt was planned for 2009 with an allowable total harvest of 5 bulls (Anderson 2009a). A harvest limit of 5 adult bulls was anticipated to have a negative, but minimal, impact on the population's growth rate (Anderson 2009a, Anderson 2009b). It was recommended that harvesting cows should not be considered until the herd grew naturally or through more reintroduction efforts (Anderson 2009b).

Accurate and precise abundance estimates are critical to establishing population management strategies including hunting quotas. Invasive mark-resight techniques using captured and marked individuals can be improved using noninvasive techniques (Goode et al. 2014). Analyzing DNA from scat noninvasively collected on the landscape has been a technique used to estimate population sizes (Kohn et al. 1999, Lukacs and Burnham 2005, Royle et al. 2013, Goode et al. 2014). The technique has been applied to cervids and has successfully identified individuals with sex determination while reducing capture bias, increasing capture probability, ensuring identification accuracy, and improving estimate precision (Brinkman et al. 2011, Goode et al. 2014). We used noninvasive scat sampling to improve previously used invasive mark-resight techniques with the goal of estimating abundance with increased precision.

One of the most significant determinants of elk population size and growth is survival (Nelson and Peek 1982, Murrow 2009, Kindall et al. 2011). Kindall et al. (2011) monitored collared NCWMA elk for 4 years following reintroduction to determine causes of mortality and estimate annual survival to better understand the population viability of the newly translocated elk. Poaching, vehicular collisions, environmental hazards, and

meningeal worm (*Parelaphostrongylus tenuis*) infection were the primary causes of elk mortality during the study; however, cause of death information for 41.9% of mortalities were undetermined due to carcass degradation. Kindall et al. (2011) used the nest survival model in Program MARK to estimate a mean annual adult survival rate of 0.79 (Total SE = 0.02) from 2000 to 2004 (Kindall et al. 2011). With the lower survival rate, the NCWMA elk population was predicted to stay constant or possibly decline (Kindall et al. 2011, Anderson 2009a). In 2015, TWRA increased penalties for poaching to address one of the significant causes of elk mortality (TWRA 2018). However, no thorough population health analyses nor survival estimates have been conducted since 2004, warranting updated research.

Literature Review of Pathogens of Concern

Translocated wildlife may have greater risk of morbidity and mortality as individuals are immunologically naïve to novel pathogens at reintroduction sites (Viggers et al. 1993, Corn and Nettles 2001, Matthews et al. 2006). High levels of elk morbidity and mortality negatively influence survival rates, potentially resulting in population limiting effects and decreased abundance. We conducted surveillance for pathogens and parasites within the NCWMA elk population based upon previous identification of the causative agent in the southeastern United States, potential for pathogens to cause elk morbidity and mortality, agricultural animal risk, and/or zoonotic risk.

Meningeal Worm

Parelaphostrongylus tenuis, a neurotropic parasitic nematode, was one of the primary causes of NCWMA elk death identified by Kindall et al. (2011). Endemic to central and eastern North America, *P. tenuis* is commonly found where the definitive host, white-tailed deer (*Odocoileus virginianus*), resides (Anderson and Prestwood 1981, Comer et al. 1991). Intermediate hosts for the nematode are many species of terrestrial gastropods (Davidson 2006). Elk are also viable hosts for the completion of the *P. tenuis* life cycle; however, elk are less suitable hosts and generally shed fewer larvae compared to white-tailed deer (Samuel et al. 1992). While *P. tenuis* infection is asymptomatic in white-tailed deer, elk and multiple other species are susceptible to neurologic disease and death (Anderson and Prestwood 1981, Samuel et al. 1992, Davidson 2006).

When excreted from the host, *P. tenuis* larvae are primarily located in the mucosal layer surrounding the fecal pellet. Dispersion of larvae on scat into the environment occurs more readily when there is heavy rainfall or pooling water. Gastropods, such as snails and slugs, become infected when inadvertently encountering larvae (Lankester and Anderson 1968). Low numbers of larvae are found in infected gastropods, indicating that gastropods primarily become infected through dispersed larvae in the soil rather than direct contact with scat. Other hosts, including elk, become infected by inadvertently eating infected gastropods when grazing (Lankester and Anderson 1968, Samuel et al. 1992). Larvae are released into the host as the snail or slug is digested. Larvae penetrate the abomasum wall of the host and migrate to the spinal cord and brain (Samuel et al. 1992).

In infected elk, *P. tenuis* can reside throughout the central nervous system including the olfactory lobes, cerebrum, venous sinuses, cerebellum, medulla, and spinal cord. A majority of adult *P. tenuis* are oftentimes found within the cranial cavity (Samuel et al. 1992). *Parelaphostrongylus tenuis* has been implicated in both clinical and subclinical infections in elk (Woolf et al. 1977, Corn et al. 2010).

Neurologic signs resultant from *P. tenuis* infection in elk can include depression, seclusion, reduced fear of negative stimuli, lethargy, and progressive loss of strength of the hindquarters. Repetitive circling is also often indicative of *P. tenuis* infection along with torticollis. Clinically infected elk often exhibit weight loss, excessive salivation, bulging eyes, decreased home range, and impaired coordination (Olsen and Woolf 1978). Neurologic symptoms have been observed to dissipate and reappear, potentially due to the pathways taken by the nematodes (Olsen and Woolf 1978). Symptoms occurring shortly before mortality commonly include paraplegia and a drooped head (Olsen and Woolf 1978, Samuel et al. 1992). The average time between first symptom observed and death in experimentally infected elk was reported to be 100 days by Olsen and Woolf (1978).

While elk can survive *P. tenuis* infection, the probability of survival is inversely related to parasite loads (Samuel et al. 1992). Exposure to a lower worm burden is typically non-fatal with no sign of infection, a moderate worm burden generally results in patent non-fatal infections, and larger numbers of viable larvae result in mortality (Samuel et al. 1992). Fatal *P. tenuis* infections occur more commonly in elk younger than 3 years old (Olsen and Woolf 1978, Larkin et al. 2003, Corn et al. 2010). The

increased likelihood of younger elk contracting *P. tenuis* infections has a strong negative effect on population recruitment, limiting population growth (Woolf et al. 1977). Infected elk have a higher risk of mortality not only due to fatal disease progression, but other deleterious effects from the disease. Neurologic elk experience an increased risk of death from predation and hunting, attributed to their reduced fear of negative stimuli and inclination toward areas with less cover such as fields and trails (Olsen and Woolf 1978). Clinically infected elk are often secluded, precluding learning normal behavior including calving processes, appropriate calving and bedding areas, predator avoidance, and strategic foraging. Females may not be able to become pregnant due to isolation and abnormal social behavior. If females are able to breed, normal maternal processes and care may be inhibited leading to neonatal mortality and limited population growth. Additionally, males with neurologic effects during rut are potentially excluded from obtaining and maintaining harems (Olsen and Woolf 1978).

The risk of *P. tenuis* infection and geographic spread has been used as a reason to not translocate elk (Samuel et al. 1992, Jacques and Jenks 2004, Bender et al. 2005). Varying incidence and impact of *P. tenuis* in reintroduced eastern elk has been reported. Within the first 5 years after translocation from Elk Island National Park (EINP), elk in southern Ontario had a *P. tenuis* infection prevalence rate of 59% (17/29) upon postmortem examination (McIntosh et al. 2007). Larkin (2003) attributed 23% (30/129) of non-capture related mortalities to *P. tenuis* infection in newly released elk in Kentucky monitored from 1997 to 2001. However, from 2001 to 2004, Corn et al. (2010) did not find any evidence of *P. tenuis* infection in sampled harvested Kentucky elk

(0/18). During that same monitoring period, clinical and subclinical cases of *P. tenuis* infection (14.3%, 4/28) were reported in elk reintroduced approximately 15 years prior to Arkansas. From 2001 to 2006, *P. tenuis* was implicated in 48% (12/25) of subadult and adult mortalities in the GSMNP after reintroduction from EINP and LBL in 2001 and 2002 (Murrow et al. 2009).

Parelaphostrongylus tenuis was not anticipated to be a limiting factor for the viability of the reintroduced Tennessee elk population, but some mortalities due to the parasite were expected (Wathen et al. 1997). As the Tennessee habitat was novel to elk, the impacts of *P. tenuis* were difficult to predict (Anderson 2009a). Kindall et al. (2011) found *P. tenuis* to be one of the largest causes of mortality within the NCWMA population accounting for 13.9% (5/36) of known mortalities during the 4 years following reintroduction.

Currently, the only antemortem diagnostic test publicly available for *P. tenuis* infection is the Baermann technique in which detection of L1, first stage larvae, is attempted through microscopic examination of scat. Confirmation of *P. tenuis* infection is then accomplished through polymerase chain reaction (PCR) of larvae (Ogunremi et al. 2002). However, detection of *P. tenuis* infection in elk using the Baermann technique is imperfect due to the low and intermittent occurrence of parasite excretion, and high volume of elk fecal production (Ogunremi et al. 2002). A serological test that detects antibodies is more reliable than the unreliable Baermann technique (Ogunremi et al. 2002). A novel *P. tenuis* antigen test was developed using full genome sequencing technology along with antigen antibody complex discovery. The newly developed ELISA

test (J. Richards, University of Tennessee, unpublished data) will potentially increase reliability of antemortem *P. tenuis* infection detection.

Other Pathogens of Interest

Elk were tested prior to translocation for diseases and pathogens known to infect elk with potential negative population effects (Thorne et al. 2002, Kindall 2011). All translocated elk were negative for brucellosis, bovine tuberculosis, Johne's disease, anaplasmosis, vesicular stomatitis, bluetongue, epizootic hemorrhagic disease (EHD), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and strains of leptospirosis prior to translocation (Kindall et al. 2011). Since reintroduction, limited postmortem disease testing and no antemortem testing has occurred in Tennessee. Many pathogens are cause for concern within elk populations not only for potentially affecting survival with negative population effects, but transfer risk to and from sympatric agricultural animals.

Brucellosis, caused by *Brucella* spp. can cause sickness in humans, economic losses in animal agriculture from aborted animals, and morbidity and mortality in elk (Thorne et al. 2002). *Brucella abortus* is only endemic to elk in the Greater Yellowstone Area (Thorne et al. 2002). However, the significant risk the bacteria presents to elk, agricultural animals, and public health necessitates monitoring for presence in Tennessee.

Johne's disease, or paratuberculosis, is caused by the bacteria *Mycobacterium avium* subspecies *paratuberculosis* (Davidson 2006). In cervids, infections can be subclinical or can cause progressively deteriorating body condition (i.e., poor hair quality

and weight loss), diarrhea, and death (Jessup et al. 1981, Crawford et al. 2006, Davidson 2006). Natural infections of free-ranging elk with Johne's disease are uncommon, with detection limited to 2 herds of tule elk (*C. c. nannodes*) in California (Thorne et al. 2002, Manning et al. 2003, Crawford et al. 2006, Corn et al. 2010). In the early 2000s, *Mycobacterium avium* subspecies *paratuberculosis* was isolated from a lymph node of an elk in Kentucky but conclusions about the impact of the bacteria on herd health were not drawn (Corn et al. 2010). Detection of isolated wildlife cases may be indicative of transmission from livestock (Sleeman et al. 2009, Corn et al. 2010).

Leptospirosis is caused by the zoonotic bacterium *Leptospira interrogans*. There are over 180 known serovars (serovarieties) of the bacteria (Davidson 2006). The bacterium has been implicated in various diseases and clinical signs in cervids including anemia, liver and kidney disease, abortion, and death (Davidson 2006). However, reports of clinical signs associated with leptospirosis in elk are not common (Thorne et al. 2002). States adjacent to Tennessee have detected infections in free-ranging elk not exhibiting clinical signs (Corn et al. 2010). While leptospirosis is not considered a significant cause of disease in elk, surveillance of the bacteria is considered important because of its significance to the agricultural animal industry (Thorne et al. 2002). Similarly, bovine parainfluenza virus-3 (PI-3), BVD, and IBR should be monitored due to potential transmission risk between elk and cattle. Bovine parainfluenza virus-3 is a paramyxovirus within the genus *Respirovirus*, BVD is caused by viruses within the genus *Pestivirus* (Van Campen et al. 2001), and IBR is caused by Bovine herpesvirus 1 (BHV-1), an alphaherpesvirus from the family *Herpesviridae* and genus *Varicellovirus*

(Castro et al. 2001, Van Campen and Early 2001, Van Campen et al. 2001, King et al. 2011). While elk have been seropositive for the causative agents of PI-3, BVD, and IBR in the eastern United States, only subclinical infections have been reported (Van Campen and Early 2001, Corn et al. 2010)

Vesicular stomatitis is caused by a group of viruses in the genus *Vesiculovirus* (Davidson 2006). Clinical signs of the disease for livestock and cervids include vesicles in the mouth and around the coronary bands. The disease is generally found in livestock including cattle, horses, sheep, and swine, but many other hosts have been documented. Vesicular stomatitis is zoonotic with human symptoms including influenza-like sickness (Davidson 2006). In the United States, vesicular stomatitis is a reportable animal disease and considered enzootic on Ossabaw Island, Georgia, and potentially parts of Arkansas, Florida, and Louisiana. White-tailed deer experimentally infected with vesicular stomatitis viruses developed vesicles and exhibited clinical similarities to infected livestock (Davidson 2006). While natural infections of elk have been observed (Webb et al. 1987), vesicular stomatitis is not well studied in elk.

Hemorrhagic disease is the most significant viral disease for white-tailed deer in the southeastern United States and the presence of the virus circulating through free-ranging elk populations is concerning due to potential viral spread (Thorne et al. 2002, Davidson 2006). Epizootic hemorrhagic disease virus and bluetongue virus are from the genus *Orbivirus* and cause EHD and bluetongue, respectively. Transmission of the viruses occurs through the vector biting midges from the genus *Culicoides* (Davidson 2006). Clinical signs of infection in white-tailed deer, the species most impacted by the

viruses, are variable but can include weakness, loss of coordination, excessive salivation, nasal discharge, emaciation, and death (Davidson 2006). Elk are susceptible to natural infections of the viruses and clinical neurologic disease associated with EHD virus was observed in the western United States in both free-ranging and captive elk (Thorne et al. 2002, Corn et al. 2010). However, observing clinical signs is uncommon (Thorne et al. 2002). Elk in Arkansas had antibodies to bluetongue virus detected while EHD virus was detected in both Arkansas and Kentucky. No clinical signs of disease in elk were recorded in either state (Corn et al. 2010).

The coccidian protozoa *Toxoplasma gondii* is another pathogen of interest within the NCWMA elk population. The protozoa can infect a wide spectrum of hosts and has been detected worldwide. The parasite is zoonotic and of public health importance. Humans are infected with *T. gondii* when sporulated oocysts or tissue cysts are ingested, including the fomite of improperly cooked meat (Dubey, 2010). Elk can establish *Toxoplasma* infections and present a risk for humans through consumption of hunter-harvested meat (Dubey 1980, Cox et al. 2017, Dubey et al. 2017).

Surveillance of disease agents is important for considering possible causes of elk morbidity and mortality, especially as many diseases are understudied in the species and could have population limiting effects. In addition to potential risks to elk, brucellosis, PI-3, IBR, BVD, leptospirosis, vesicular stomatitis, and Johne's disease have serious risks of agricultural animal infections with resulting economic loss (Cook et al. 1997, Van Campen and Early 2001, Van Campen et al. 2001, Thorne et al. 2002, Davidson 2006, Ellis 2010, Neill 2013). Elk at the NCWMA are sympatric to agricultural

lands and monitoring disease agents is of interest not only for potential impacts to the elk population but also spillover risk to and from agricultural animals.

Tick-Borne Diseases

Black-legged ticks (*Ixodes scapularis*) infected with the causative agent for Lyme disease (*Borrelia burgdorferi*) are established in eastern Tennessee counties encompassing the NCWMA (Hickling et al. 2018). Evidence of increasing prevalence of *B. burgdorferi* infected *I. scapularis* in Tennessee presents greater risk of zoonotic transmission (Hickling et al. 2018). The lone star tick (*Amblyomma americanum*) is also present in Tennessee (Hickling et al. 2018) and can transmit *Theileria cervi*, the protozoa implicated in causing theileriosis in elk (Mans et al. 2015). The genus *Theileria* causes a wide range of clinical signs and diseases using multiple vectors and within multiple hosts (Mans et al. 2015). Theileriosis in elk is generally benign, although the disease is relatively unstudied in the species (Cauvin et al. 2019). Cervids, such as white-tailed deer, have shown clinical signs of loss of body condition and death (Haus et al. 2018). Infected animals have increased risk of exhibiting clinical signs if stressed, experiencing simultaneous infections with other parasites, immunocompromised, or relocated from non-endemic to endemic areas (Yabsley et al. 2005, Cauvin et al. 2019).

Babesiosis is another tick-borne disease able to infect elk and humans (Schoelkopf et al. 2005, Fritzen et al. 2014). Human infection has been documented in Tennessee, and potentially linked to close proximity to cervids (Fritzen et al. 2014). Babesiosis is caused by protozoans from the genus *Babesia* and has the potential to be spread where *I. scapularis* reside. Both the range and incidence of infections are

increasing in the United States, making babesiosis an emerging disease (Fritzen et al. 2014). Some elk herds have had reported infection rates of 100% with some elk exhibiting subclinical infections, indicating they may be capable of being reservoirs for the protozoa (Schoelkopf et al. 2005, Pattullo et al. 2013). Clinical signs in infected elk can include anemia and death (Pattullo et al. 2013).

Anaplasmosis, also transmitted by ticks, is caused primarily by *Anaplasma marginale* and is a significant cause of disease in cattle (Thorne et al. 2002). The bacteria is not considered a risk to free-ranging elk populations as significant clinical disease has not been detected (Thorne et al. 2002). However, hemolytic anemia caused by *Anaplasma ovis* was recently implicated in the death of 3 farmed elk calves, and elk should be considered potential wildlife reservoirs for *Anaplasma* spp. (Hendrix et al. 2019).

Limited research of NCWMA elk health or population viability has continued beyond the initial reintroduction study by Kindall et al. (2011), annual TWRA abundance estimates, and bull harvests. Surveilling causes of morbidity and mortality provides information on survival, which directly impacts abundance and population viability. Therefore, updated abundance estimates with high precision, survival rate estimates, and elk health and mortality surveillance can better inform management strategies and policies to support elk population viability.

Study Area

The NCWMA encompasses 793.18 km² in eastern Tennessee. The multiple-use area contains both TWRA-owned land and leased land. The area supports all-terrain vehicles (ATV), wildlife viewing, hunting, and wildlife (TWRA 2018). The landscape is composed of approximately 86% deciduous forest, 12% openings (mainly fields and reclaimed coal strip mines), and 1% agricultural land (TWRA 2000). The area is a mixed-mesophytic forest, with elevation ranging from 312.4–1,016.9 m and slope percentages of 0–229.56% (USGS 2017). The soil type imposes limitations to cultivation and is primarily limited to grazing, forest, or wildlife habitat (TWRA 2018). Weather in eastern Tennessee is temperate. From 1999 to 2020, average monthly temperatures ranged from 3.9°C to 25.8°C (NOAA 2021). Average annual precipitation was 131.9 cm with snowfall of <11.7 cm/year (NOAA 2021). The NCWMA includes 6 Elk Hunt Zones in the southeastern portion, the Sundquist Unit containing the Hatfield Knob Viewing Tower, and the Ed Carter Unit including 1 Elk Hunt Zone.

Research Objectives

Research objectives included evaluating abundance, survival, causes of mortality, and health of the NCWMA elk population. Specifically:

- 1) Estimate population abundance with increased precision using noninvasively collected scat and genetic identification of individuals
- 2) Estimate yearly survival rates and causes of elk mortality to assess changes in survival over time and identify possible threats to the population

3) Investigate pathogens present in the elk to identify potential health threats

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CHAPTER TWO

**ESTIMATING ELK ABUNDANCE USING FECAL PELLET DNA AND
CAPTURE-RECAPTURE**

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Abstract

Precise abundance estimates are needed for effective population management strategies. Estimating abundance in eastern elk populations can be challenging due to low detection in forested landscapes. Traditional mark-recapture methods involve invasive handling and possible capture-related stress. Therefore, to estimate elk abundance in the North Cumberland Wildlife Management Area in Tennessee we used DNA from non-invasively collected scat to genetically identify elk for use in a Huggins closed capture model. We collected scat searching 2 x 3,378-m transects primarily within wildlife openings ($n=65$) weekly from February to May 2019. Sixteen microsatellites were analyzed to identify 85 individuals (21 males and 64 females) from 157 successfully genotyped samples (44%). We estimated 159 elk (95% CI: 110.1–207.1; coefficient of variation: 15.6%) within the study area and identified a male to female sex ratio of 1:5 in Elk Hunt Zones and 1:1 in an area without elk hunting. In 2019 and 2020, we captured elk using chemical immobilization darts and fit them with GPS collars to assess the use of wildlife openings. Collared elk routinely frequented wildlife openings, and estimated home ranges were composed of 11% wildlife opening

landcover on average indicating we had reasonable likelihood of detecting elk from collecting scat predominately in wildlife openings. Our abundance estimation approach increased precision from previously used methods, identified previously unknown sex ratio disparities, and did not subject elk to invasive capture. Non-invasive genetic sampling using scat can be an effective strategy for estimating abundance and sex ratios of eastern elk in forested landscapes.

Introduction

The distribution of a population often reveals patterns in habitat and resource selection, while analyzing abundance estimates over time can provide insight into important population drivers including mortality, birth, emigration, and immigration (Williams et al. 2002, Fryxell et al. 2014). Elk (*Cervus canadensis*) population sizes have been estimated in numerous ways with varying degrees of success and precision (Bear et al. 1989, Skalski et al. 2005, TWRA 2018, Bristow et al. 2019). Traditional methods of estimating population density and visually detecting individuals with aerial counts are not feasible for most elk populations in the eastern United States due to the mountainous terrain, dense forest cover, and uneven distribution (Brinkman et al. 2009, TWRA 2018). However, accurate and precise abundance estimates and sex ratios are critical to establishing population management strategies and hunting quotas for game species.

Effectively estimating population sizes requires counting individuals while accounting for imperfect detection (Otis et al. 1978, White et al. 1982, Williams et al. 2002). Capture-recapture methods rely on detecting, marking or observing natural

distinctions for identification, releasing individuals within a closed population, and observing the marked individuals over multiple sampling periods to obtain an abundance estimate (Otis et al. 1978, Williams et al. 2002). Mark-resight using GPS-collared animals is a type of capture-recapture and has been employed annually at the North Cumberland Wildlife Management Area (NCWMA) in Tennessee since 2006 to estimate elk population size (Anderson 2009, TWRA 2018).

Annual Tennessee Wildlife Resources Agency (TWRA) mark-resight surveys included visually identifying and recording elk wearing active collars along with non-marked elk. Population size was then estimated using Program NOREMARK and the Bowden estimator (White 1996, Bowden and Kufeld 1995, Anderson 2009). The Bowden estimator does not assume a homogenous distribution of individuals or geographic closure; however, requires marked elk to be a representative sample of the entire population (Bowden and Kufeld 1995). Results from the 2016 TWRA mark-resight survey estimated a population of 349 elk with a 95% confidence interval of 196.9–636.1 (TWRA 2018). Large confidence intervals indicated low precision for abundance estimates (TWRA 2018). To address low precision and difficulty in visually observing a low-density species, alternate methods of estimating population abundance were needed.

Analyzing DNA from scat samples collected non-invasively on the landscape has been used to identify individuals and estimate population abundance (Kohn et al. 1999, Lukacs and Burnham 2005, Royle et al. 2013). Non-invasive techniques using scat deposited on the landscape potentially decrease stress related to invasive capture methods (Goode et al. 2014). Therefore, the aim of our study was to estimate elk

abundance by sex across a large area within the NCWMA using non-invasive sampling of DNA from scat with capture-recapture modeling. We estimated home range sizes to evaluate elk distribution at the NCWMA and assessed use of wildlife openings to investigate if sampling predominately wildlife openings is a viable strategy for efficient non-invasive scat collection.

Study Area

The NCWMA is a 793.18 km² multi-use area in Tennessee and the site of elk reintroduction into the state. The area contains both TWRA-owned and leased land and supports wildlife, all-terrain vehicles (ATVs), wildlife viewing, and hunting (TWRA 2018). The NCWMA is primarily composed of mixed-mesophytic forest interspersed with human development such as roads and trails (TWRA 2000). The landscape is composed of approximately 86% deciduous forest, 12% openings, and 1% agricultural land (TWRA 2000). Weather at the NCWMA is temperate with average monthly temperatures from 1999 to 2020 ranging from 3.9°C to 25.8°C (NOAA 2021). Annual precipitation was 131.9 cm with snowfall occurring rarely and <11.7 cm/year on average (NOAA 2021). We sampled the Sundquist Unit and all 6 Elk Hunt Zones in the southwestern portion of the NCWMA (Fig. 2.1 in Appendix). The study area comprises approximately 304.95 km² (38.5%) of the NCWMA. Managed wildlife openings constitute 1.53% (2.33 km² out of 151.95 km²) of the southwestern Elk Hunt Zones and 0.48% (0.73 km² out of 152.99 km²) of the Sundquist Unit. Elk hunting is allowed in Elk Hunt Zones but prohibited in the Sundquist Unit (TWRA 2018).

Methods

Scat Sampling

We assumed elk habitually use NCWMA wildlife openings on a near daily basis, and scat would be difficult to find outside of the openings. We targeted wildlife openings for sample collection to increase feasibility and efficiency while surveying a geographically dispersed population. Precision of capture-recapture density estimates is highly dependent on sample sizes of individuals and numbers of recaptures (Robson and Regier 1964, Pollock et al. 1990); therefore, covering a larger area should increase accuracy and precision of estimates. Prior to initiating sampling, we assessed approximate scat density of the study area during preliminary investigations by considering TWRA's previous sampling efforts and counting scat throughout the NCWMA. We estimated we would need to search an average 2,252.3-m² area to find 1 fresh sample. Therefore, we used 2 x 3,378-m belt transects to find an estimated 3 fresh samples at each sampling area.

We selected 65 sampling areas across the study area representing over 99.1 ha and based selection on knowledge of the population including locations where elk had and had not been observed by TWRA personnel. Designated sampling areas were grouped by geographic region: Elk Hunt Zones and the Sundquist Unit (Fig. 2.1 in Appendix). We concentrated sampling within the Elk Hunt Zones as the area had heightened importance to herd management. Using ATVs, we conducted transects with no standardized starting point or path within each scat collection area. We recorded starting points and transect paths in GPS units (Garmin eTrex GPS, Garmin, Olathe, Kansas, USA) to monitor the random paths surveyed. By utilizing variable and non-

linear transects, collectors targeted areas elk were recently seen and sampled a variety of areas within larger scat collection areas. Transects ended when the standardized transect length was met, as indicated by the GPS unit. If the area was not large enough to complete the standardized transect length, we searched the entire scat collection area.

To approximate a demographically closed population, we collected scat in weekly intervals prior to calving during the winter and spring months from February to May 2019. We waited to sample an area at least 1 day after heavy precipitation to increase the likelihood of successful genetic analysis and 7 days to resample an area to allow for scat reaccumulation (Brinkman et al. 2009, Lupardus et al. 2011). We only collected fresh samples indicated by a deep color and moist appearance and consistency (Lupardus et al. 2011, Goode et al. 2014). We recognized waiting to sample after precipitation and only collecting fresh samples might reduce overall detection; however, selecting samples most likely to genetically amplify maximized potential for genotyping success and reduced lab costs. We recorded a GPS point where each sample was collected and individually stored samples in plastic bags in a -20° C freezer until analysis.

DNA Analysis

We removed scat samples from the freezer and allowed them to thaw at room temperature for 10-15 minutes. We gently moved 1 flat wooden toothpick along the outside surface of 4 fecal pellets to collect epithelial cells. For each elk, we sampled 12 pellets for a total of 3 toothpicks. We dried toothpicks at room temperature and placed

them in individually labeled coin envelopes. Toothpicks were sent to Wildlife Genetics International (Nelson, British Columbia, Canada) for genetic analysis.

Wildlife Genetics International performed DNA extraction using Qiagen's DNeasy Tissue kits (Qiagen, Valencia, CA, USA) using the manufacturer protocol. They used DNA purification methods described by Woods et al. (1999) and Paetkau (2003). They followed methods described by Paetkau et al. (1998) for standard cycling and buffer conditions for polymerase chain reaction (PCR). The PCR products were visualized using Biosystems 310 or 3130 xl DNA sequencers (Applied Biosystems, Grand Island, NY, USA) and capillary electrophoresis. Wildlife Genetics International analyzed each sample for 16 microsatellites routinely utilized in game-farmed elk analyses (Muller et al. 2018) and an amelogenin sex marker to determine individual identities. Microsatellite loci included BL42, BM203, BM3507, BM4028, BM4107, BM4513, BM6506, BM888, BMC1009, CSSM041, FCB193, INRA107, OvH, Rt1, Rt13, and Rt7. Each marker was amplified in its own tube rather than using a multiplex approach. Samples that produced high-confidence genotypes at ≤ 9 markers were culled. Errors can lead to pairs of genotypes matching at all-but-one or all-but-two markers. Wildlife Genetics International conducted genotyping error-checks following the protocol of Paetkau (2003) involving inspecting and reanalyzing genotype pairs with suspiciously high similarity to prevent false identification of individuals (Kendall et al. 2009). Samples with exact matches at each unique multilocus genotype were considered to be from the same individual.

Population Abundance Models

We used Huggins closed capture models using ver. 2.2.7 of the package RMark in R to obtain elk abundance estimates (Huggins 1989, White and Burnham 1999,

Laake 2013, R Core Team 2019). Huggins closed capture models use a conditional likelihood inference framework to allow for individual heterogeneity in capture probabilities and individual covariates. Models allowing individual covariates are advantageous as sources of heterogeneity can be described, covariate effects can be evaluated, and relevant covariates often result in models with lower bias and greater precision (Chao and Huggins 2010). Two parameters are estimated within a Huggins closed capture model: probability an individual within the population will be detected and marked (p) and probability of recapture conditional on the individual being previously captured (c). The parameter c is primarily used to model behavioral changes after initial capture.

We included individual covariates for sex and site (Elk Hunt Zones or Sundquist Unit) and an occasion covariate on detection. We tested 3 models including the null model; model including sex and occasion on p; and a global model (sex, occasion, and site on p; Table 2.1 in Appendix). Sex and occasion were included in all models aside from the null as we hypothesized they were important sources of heterogeneity. We used Akaike's Information Criteria adjusted for small sample sizes (AICc) to evaluate all candidate models, and those with the lowest AICc and greatest Akaike weights were considered among the best approximating models (Burnham and Anderson 2002). Abundance estimates for each sex and site were combined for total abundance. We calculated standard errors and confidence intervals for combined abundance estimates using the Delta method and the package emdbook (Bolker 2020). The coefficient of variation (CV; $CV = \frac{\text{Standard Error } (\hat{\theta})}{\hat{\theta}} * 100$) was used to assess precision. A CV of <20%

is a common target for acceptable precision of abundance estimates (Pollock et al. 1990).

Elk Capture and Movement Analysis

We captured elk within the NCWMA from 2019 to 2020 using free darting methods and corral traps. We chemically immobilized elk using CO₂ dart projectors (Dan-Inject, Kolding, Denmark) and darts (PneuDart Inc., Williamsport, Pennsylvania) containing a mixture of butorphanol (27.3 mg/ml), azaperone (9.1 mg/ml), and medetomidine (10.9 mg/ml; BAM TM, ZooPharm, Windsor, Colorado). Female elk and small males received 2.0 ml of BAM and large males received 3.0 ml. We monitored rectal temperature, heart rate, respiration rate, and oxygen saturation levels during immobilization. We administered supportive oxygen intranasally if oxygen saturation levels were <90%. If body temperatures were >40° C, we injected banamine (50 mg/ml, Merck Animal Health) intramuscularly at a volume of 2.25 ml for females and small males and 3 ml.

We fitted elk with Global Positioning System (GPS) collars (Lifecycle Pro 500 or LiteTrack Iridium 500 TL; Lotek Wireless, Inc., Newmarket, Ontario, Canada). Collars were programmed to record a GPS location every 5 hours for the Lifecycle Pro 500 and every 150 minutes for the LiteTrack Iridium 500 TL collars. We reversed the effects of anesthesia using intramuscular injections of naltrexone (50 mg/ml, ZooPharm, Windsor, Colorado; 0.5 ml per elk) and atipamazole (25 mg/ml, ZooPharm, Windsor, Colorado; 2 times the volume of BAM administered per elk).

Elk collars recorded locations until mortality, collar failure, or data collection was stopped on 20 September 2021. We obtained GPS data remotely unless direct

download from the collar was possible in the case of mortalities, dropped collars, and removed collars. We estimated individual home range sizes with a 95% contour by calculating the area used by collared elk through a bivariate normal utilization-kernel in the package `adehabitatHR` (Calenge 2006). The kernel approach is sensitive to bandwidth selection methods (smoothing parameters; Kernohan et al. 2001, Gitzen et al. 2006). Large bandwidths can result in over-smoothing the utilization distribution and overestimate home range while smaller bandwidths can result in fragmented distributions and underestimate home range (Kernohan et al. 2001, Gitzen et al. 2006). We used the plug-in bandwidth selection method as a smoothing parameter as it can analyze clustered data without resulting in highly fragmented or oversmoothed utilization distributions and performs as effectively or better than other methods for elk (Gitzen et al. 2006).

Locations were censored to exclude any GPS point with a 2-dimensional fix status or dilution of precision >4 (Uno et al. 2010, Jung et al. 2018). We estimated home range sizes for elk with ≥ 9 months of data collection and >500 GPS points (Morrow 2007). We calculated the percentage of home range landcover composed of wildlife openings and determined the percentage of days collared elk had GPS fixes within wildlife openings during the scat collection period. Using the package `maptools` (Bivand and Lewin-Koh 2020), we determined if GPS fixes occurred during day or night to assess if elk frequented wildlife openings more often at night.

Results

We searched 689 km of transects and collected 357 scat samples on the landscape. Following poor performance during initial genotyping, Wildlife Genetics

International removed microsatellite *Rt13* from analysis. The remaining microsatellites were used to successfully genotype 44% of samples ($n=157$). Of the 157 successfully genotyped samples, only 12 were incomplete and no sample was missing >2 markers. From analysis, 85 individual elk were identified (21 males and 64 females). Most individuals were only identified once ($n=46$), but 39 individuals were identified at least twice, and 16 individuals were identified 3 or more times on the landscape. Our empirical data was collected during 9 sampling occasions, but we constructed capture histories using 3 occasions to increase probability of capture within each occasion.

The maximized log likelihoods between model 1 and model 2 were nearly identical and the second ranked model only differed by the addition of site ($\beta = -0.59$, 95% CI: -1.93–0.75) indicating the parameter was uninformative for probability of detection (Table 2.1 in Appendix; Burnham and Anderson 2002, Arnold 2010). Therefore, model 2 was not considered a supported or competitive model and the model including sex and occasion with a model weight of 0.987 was selected to estimate abundance. Occasion had the greatest effect and probability of detection decreased with each subsequent occasion (occasion 2: $\beta = -0.42$, 95% CI: -0.93– -0.08; occasion 3: $\beta = -1.07$, 95% CI: -1.65– -0.50), while sex had less of an effect ($\beta = 0.22$, 95% CI: -0.78–1.23; Table 2.2 in Appendix). Estimated abundance was 123.0 females (95% CI: 82.5–163.5; CV: 16.8%) and 35.6 males (95% CI: 20.5–50.7; CV: 21.6%) for a total of 158.6 elk (95% CI: 110.1–207.1; CV: 15.6%; Table 2.3 in Appendix). The male to female sex ratio was 1:5.3 (20.4 males to 107.6 females) in the Elk Hunt Zones and 1:1 (15.3 males to 15.4 females) in the Sundquist Unit.

We captured 29 elk in 2019 and 2020 (9 males and 21 females). Twenty elk (4 males and 16 females) had data collected for ≥ 9 months and >500 points retrieved directly from collars ($n=3$), the Lotek website ($n=15$), or a combination of the 2 if the elk was recollared and we were only able to retrieve 1 collar ($n=2$). Mean elk home range size was estimated to be 6.20 km^2 . Females ($n=16$) had estimated home ranges from 0.09 km^2 to 16.69 km^2 with a mean of 5.29 km^2 . Home ranges for males ($n=4$) ranged from 0.31 km^2 to 27.05 km^2 with a mean of 7.52 km^2 (Table 2.4 in Appendix). Five (25%) estimated home ranges encompassed portions of both the Elk Hunt Zones and Sundquist Unit. Every elk had wildlife openings within their estimated home ranges. Estimated home ranges were comprised of 11% wildlife opening landcover on average, ranging from 1.7% to 55.8%. Movement data from all GPS collared elk during the scat collection period indicated elk used wildlife openings an average of 70.8% of days with similar use between females (70.6%) and males (71.4%). Elk entered wildlife openings more frequently at night (66.8% of total night GPS points were in wildlife openings) than during the day (39.1% of total day GPS points were in wildlife openings). A higher proportion of landcover was comprised of wildlife openings in the Elk Hunt Zones than Sundquist Unit. However, we observed less frequent wildlife opening use by elk in the Elk Hunt Zones (48.8% of total points were in wildlife openings) compared to elk in the Sundquist Unit (64.3% of total points were in wildlife openings).

Discussion

Our approach using DNA from non-invasively collected scat and closed capture-recapture modeling successfully estimated abundance over a large area with minimal disruption to elk. We obtained sex ratios and estimated abundance with greater

precision than previous methods. Our estimates were lower than the 349 elk estimated in 2016. However, direct comparisons are difficult as we estimated abundance within defined areas while the previous study included both public and private lands around the NCWMA using different methodology (TWRA 2018).

Wildlife openings were present within each estimated home range. Availability in conjunction with frequent daily use of wildlife openings suggested we had reasonable likelihood of detecting elk from scat primarily collected in designated sampling areas. However, collared elk were primarily captured in wildlife openings presenting the possibility of collecting field use data only from elk already known to frequent wildlife openings. It is possible that elk not using wildlife openings went undetected.

Additionally, sampling across a wider variety of landcover types and integrating spatial information including heterogeneity in capture probability related to trap location could further improve accuracy and precision (Royle et al. 2013). Wildlife openings are not consistently spaced across the NCWMA presenting challenges for extending our approach to the entire area. For example, the Ed Carter Unit of the NCWMA (184.95 km²) only had 2 adjacent TWRA managed wildlife openings totaling 0.55 km². Sampling solely wildlife openings may be more appropriate where there are multiple wildlife openings per average elk home range size spaced across the landscape (Otis et al. 1978). In this way, high detection probability is more likely (Lukacs and Burnham 2005). Using spatial capture-recapture across multiple landcover types would combat the challenges of small and irregularly spaced wildlife openings but would require increased sampling effort. Using ATVs in denser landcover may not be possible but would increase the survey effort. Simulations could be used to examine the trade-offs between

effort and precision using different methods and sampling protocols (Efford and Boulanger 2019).

Fresh scat became noticeably scarcer as the scat collection season progressed and probability of detection decreased with each subsequent occasion as indicated by the beta values for occasion in the most supported model. We collected 170 samples (43.53% amplification success) during occasion 1, 142 samples (37.32% amplification success) during occasion 2, and 45 samples (66.67% amplification success) during occasion 3. Amplification success did not consistently decrease, indicating less favorable weather for scat preservation was not the driver of decreasing probability of detection. The percentages of elk GPS points taken in wildlife openings were 59.7% and 60.4% during occasions 1 and 2, respectively. The percentage decreased to 46.7% during occasion 3. Less frequent field use as the scat collection period progressed seemingly resulted in a reduction of available samples. Food habits analysis of NCWMA elk during the scat collection period indicated shifting food habits with an increase in forb consumption as time progressed (Metts 2021), potentially reflecting less wildlife opening use. Initiating scat collection a few weeks earlier would maximize when elk are using wildlife openings and potentially increase probability of detection.

Elk movement and selection of landcover types are influenced by individual, sex, age, and body condition and typically indicates risk-reward trade-offs related to predator avoidance, foraging, herd dynamics, and thermal regulation (Anderson et al. 2005, Murrow 2007). We observed large variation in home range estimates for NCWMA elk; however, our average estimated home range sizes were smaller than home ranges of geographically nearby Great Smoky Mountains National Park elk (Murrow 2007). Great

Smoky Mountains National Park females and males have reported average home range sizes of 10.4 km² and 22.4 km², but estimations took place shortly after reintroduction when there was greater frequency of elk dispersal movements potentially resulting in larger home ranges (Murrow 2007). Direct comparisons of home range sizes are often difficult to accurately achieve due to differences in methodology, geographic location, landscape heterogeneity, and individual animal variation (Cassirer 1992, Skovlin et al. 2002, Anderson et al. 2005, Laver and Kelly 2008). Despite challenges, home range size is directly related to resource distribution and smaller home ranges can indicate the presence of a higher proportion of adequate resources per unit area (Macdonald and Johnson 2015).

Resource dispersion hypothesis suggests where resources are patchily distributed spatially and temporally, individuals can have overlapping home ranges and share resources (Johnson et al. 2002; Macdonald and Johnson 2015). It is possible that dense patches of resources at the NCWMA resulted in smaller home range sizes. However, animal density is thought to be independent of home range sizes, and instead increases with greater resource richness, renewal, and/or heterogeneity (Macdonald and Johnson 2015). More research is needed to assess if richness and pattern of resource availability spatially and temporally are impacting elk density at the NCWMA.

We detected a large disparity in sex ratios between the Elk Hunt Zones and Sundquist Unit. The sex ratio was skewed toward females in the Elk Hunt Zones compared to a more even sex ratio observed in the Sundquist Unit. One notable difference between the areas is the occurrence of hunting in the Elk Hunt Zones. In 2019, 6 males were harvested in the southwestern Elk Hunt Zones (TWRA 2021)

comprising 29% of the estimated male population in the area. There is predominant landscape connectivity between the 2 sites and 25% ($n=5$) of estimated home ranges included portions of both areas as elk had GPS fixes near site boundaries. However, we did not observe any collared elk moving between the 2 sites. Genetic differentiations have been observed between the 2 sites indicating some level of population segregation (E. Watson, University of Tennessee, unpublished data). More research is needed to determine the level of population segregation between sites and if harvest is contributing to the skewed sex ratio within the Elk Hunt Zones and potentially affecting population growth. We recommend rigorous estimation methods with appropriate study designs to ensure scientifically defensible harvest quotas and further investigation of the impacts of a female skewed sex ratio.

Elk sex ratios can vary widely and sex ratios optimized for population sustainability are lacking (White et al. 2001). Negative impacts from skewed sex and age ratios have been observed in ungulates including elk (Noyes et al. 1996, White et al. 2001, Mysterud et al. 2002, Milner et al. 2007). Age cannot be determined from non-invasively collected elk scat limiting our analysis of age class structure, but future work estimating age class structure for NCWMA elk is warranted.

Management Implications

Non-invasive scat sampling using DNA as an individual identifier and closed capture-recapture modeling was a useful approach for obtaining precise elk abundance estimates which are imperative for effective population management strategies. We successfully obtained abundance estimates non-invasively across a large area and detected differences in sex ratios between hunted and non-hunted areas warranting

more research into potential influencing factors. Our approach minimized stress of monitored individuals, eliminated the need to visually sight collared animals in difficult terrain, and increased precision.

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Ethical Approval

We handled elk according to protocols approved by the University of Tennessee Institutional Animal Care and Use Committee (UT-IACUC #2671-0211).

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APPENDIX

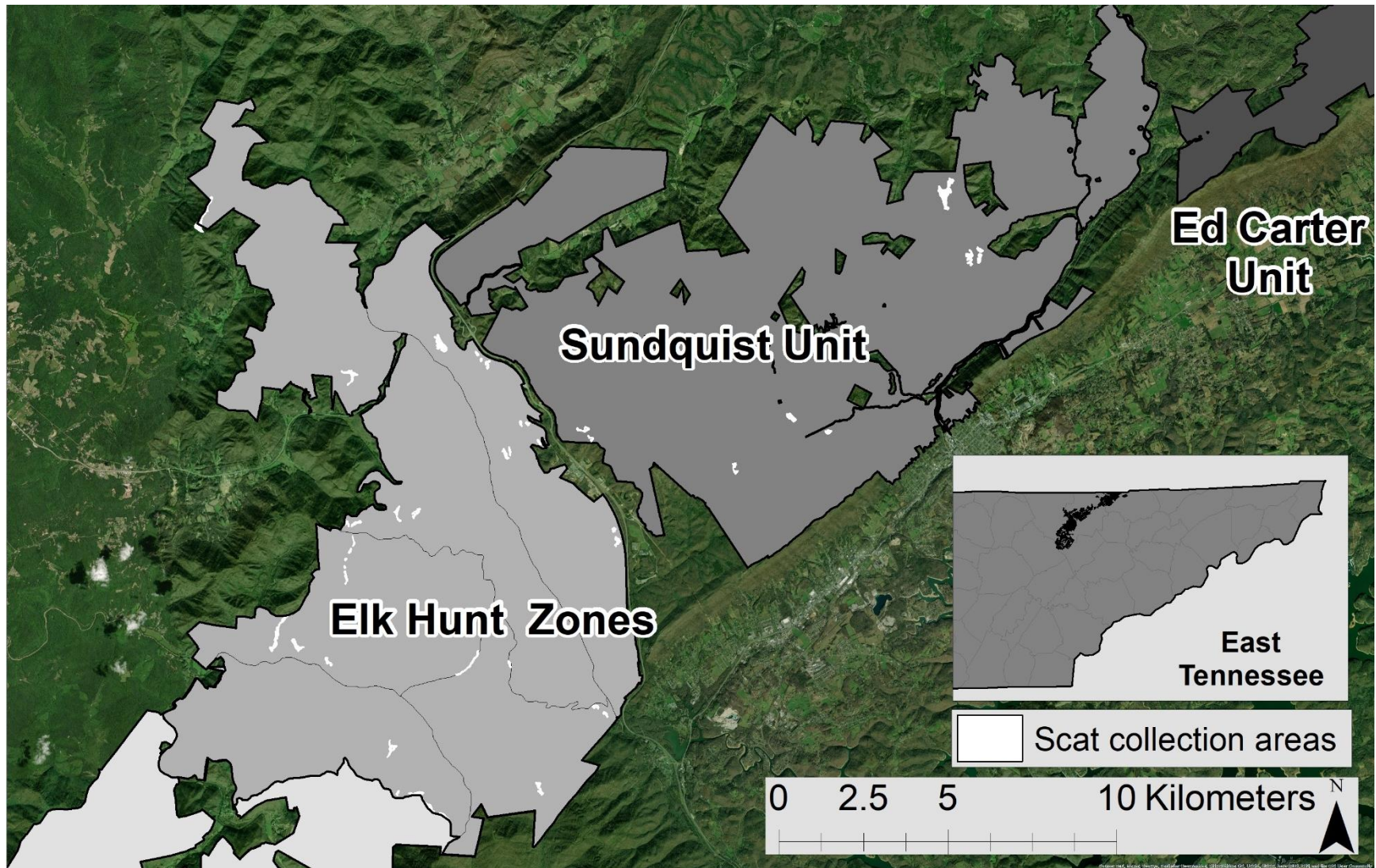


Figure 2.1. Locations of scat collection areas within the study area comprised of the southwestern Elk Hunt Zones and Sundquist Unit at the North Cumberland Wildlife Management Area in eastern Tennessee. Transects were conducted within the scat collection areas during 2019 to estimate elk abundance.

Table 2.1. Huggins closed capture model selection for estimating elk abundance within the study area located at the North Cumberland Wildlife Management Area in 2019. Probability of detection is represented by p and probability of recapture conditional on first capture is represented by c . Site is either the Elk Hunt Zones or Sundquist Unit. Occasion is a categorical variable for the 3 scat sampling periods. Model selection was based on Akaike's Information Criteria adjusted for small sample sizes (AICc), differences in AICc compared to the top model ($\Delta AICc$), and Akaike model weights (w_i ; recalculated after removing second-ranked model with uninformed parameter). The number of parameters in the model is represented by K .

Model	K	Log likelihood	AICc	$\Delta AICc$	w_i
$p(\sim \text{sex} + \text{occasion}) c()$	4	270.783	278.943	0	0.987
$p(\sim \text{sex} + \text{occasion} + \text{site}) c()$	5	269.967	280.208	1.265	-
$p() c()$	1	285.611	287.627	8.683	0.013

Table 2.2. Beta estimates for parameters of the selected model to estimate abundance of elk within the North Cumberland Wildlife Management Area in 2019. Probability of detection is represented by p. Occasion is a covariate representing the 3 scat collection periods.

Parameter	Beta	Standard error	95% Confidence interval
Intercept	-0.857	0.316	-1.477– -0.237
p: males	0.223	0.514	-0.784–1.230
p: occasion 2	-0.421	0.257	-0.925–0.083
p: occasion 3	-1.076	0.294	-1.653– -0.500

Table 2.3. Abundance estimates for male and female elk within the Elk Hunt Zones and Sundquist Unit at the North Cumberland Wildlife Management Area in eastern Tennessee during 2019 determined from Huggins closed capture modelling using DNA from scat samples ($n=157$) collected non-invasively on the landscape.

	NCWMA elk estimate	Standard error	95% Confidence interval	Coefficient of variation %
Elk Hunt Zone females	107.6	20.2	80.6–164.1	18.75
Elk Hunt Zone males	20.4	6.0	14.4–41.7	29.60
Sundquist females	15.4	4.5	10.4–30.3	29.42
Sundquist males	15.3	4.8	10.7–32.8	31.47
Total	158.6	24.8	110.1–207.1	15.61

Table 2.4. Mean, standard errors (SE), minimum, and maximum 95% home range sizes (km²) using kernel density estimation methods for elk within the North Cumberland Wildlife Management Area in eastern Tennessee. Data was collected from GPS collared elk ($n=20$) from 2019-2021.

Sex	Home ranges km ²			
	Mean	SE	Minimum	Maximum
Male ($n=4$)	7.52	6.53	0.314	27.054
Female ($n=16$)	5.87	1.06	0.088	16.693
Total ($n=20$)	6.20	1.44	0.088	27.054

CHAPTER THREE

**ELK MORTALITY, SURVIVAL, AND HEALTH IN THE CUMBERLAND
MOUNTAINS OF TENNESSEE**

A version of this chapter was written for journal submission by:

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Katherine Kurth conducted the data collection, analysis, and writing of this chapter with assistance from coauthors.

Abstract

Comprehensive disease surveillance has not been conducted in Tennessee elk (*Cervus canadensis*) since reintroduction 20 years ago. We identified causes of death, estimated annual survival, and identified pathogens of concern in elk at the North Cumberland Wildlife Management Area (NCWMA). In 2019 and 2020, we captured 29 elk (21 females, 8 males) using chemical immobilization darts and fit individuals with GPS collars with mortality sensors. Elk that died between February 2019 and February 2022 were necropsied, and necropsy reports were used to identify causes of death which included meningeal worm (*Parelaphostrongylus tenuis*) associated disease ($n=3$), poaching ($n=1$), vehicular collision ($n=1$), legal hunter harvest ($n=1$), and unknown due to carcass degradation ($n=3$). Using data from GPS collars and known-fate survival models, we estimated an average yearly survival rate of 80.2% indicating elk survival has not significantly increased from estimates soon after elk reintroduction (79.9%). We collected blood, tissue, feces, and ectoparasites from anesthetized elk for health surveillance. We identified lone star ticks (*Amblyomma americanum*; $n=53$, 85.5%, 95% CI: 73.72–92.75%), American dog ticks (*Dermacentor variabilis*; $n=8$, 12.9%, 95% CI:

6.13–24.40%), and black-legged ticks (*Ixodes scapularis*; $n=1$, 1.6%, 95% CI: 0.08–9.83%). We detected evidence of exposure to *Anaplasma marginale* (100%, 95% CI: 84.50–100.00%), *Leptospira interrogans* (70.4%, 95% CI: 49.66–85.50%), *Toxoplasma gondii* (55.6%, 95% CI: 35.64–73.96%), epizootic hemorrhagic disease virus (51.9%, 95% CI: 32.35–70.84), and *Theileria cervi* (25.9%, 95% CI: 11.78–46.59%). Results indicated Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*, MAP) is potentially established within the population which has not been previously documented in eastern elk populations. *Parelaphostrongylus tenuis* associated disease was a primary cause of death and more research is needed to understand *P. tenuis* ecology and epidemiology. Similarly, research to determine NCWMA elk population implications of pathogens including MAP is warranted.

Introduction

The necessity of wildlife health surveillance for population sustainability and the health of humans and non-human animals is well established. Elk reintroduced to the North Cumberland Wildlife Management Area (NCWMA) in Tennessee had extensive disease testing conducted prior to release in 2000 in an attempt to translocate healthy individuals and reduce transport of pathogens to the state (Kindall et al. 2011). Population monitoring after release indicated a mean adult annual survival rate of 0.79 (Total SE=0.02), with primary causes of mortality including poaching, vehicular collisions, environmental hazards, and infection caused by the parasitic nematode *Parelaphostrongylus tenuis* (meningeal worm; Kindall et al. 2011). Both subadult and adult survival has been shown to be more influential for extinction and growth rates than

other elk population parameters (Nelson and Peek 1982, Murrow 2009, Kindall et al. 2011), and mortality reduction was emphasized as necessary for a sustainable NCWMA elk population (Kindall et al. 2011).

Conducting health surveillance is needed to assess population status and develop mortality management strategies. Postmortem surveillance has been conducted by TWRA since reintroduction but was limited in scope in pathogens tested. Continued postmortem surveillance in conjunction with antemortem disease surveillance can increase knowledge of the health status of the population. Our objectives were to evaluate causes of NCWMA elk mortality, estimate survival rates, and detect pathogens in tested elk to aid in identifying potential population limiting factors.

Methods

Study Area

The NCWMA is a 793.18 km² multi-use area with temperate weather. Average monthly temperatures from 1999 to 2020 ranged from 3.9°C to 25.8°C (NOAA 2021). Average annual precipitation was 131.9 cm with <11.7 cm/year of snowfall (NOAA 2021). The NCWMA supports human recreation including wildlife viewing and hunting. Human development such as roads and trails are dispersed throughout the area (TWRA 2000, TWRA 2018). There is consistent human presence at the NCWMA, and elk are adjacent to private agricultural lands (TWRA 2018).

Elk Capture

During 2019 and 2020, we captured elk across the NCWMA using free darting methods and corral traps. We handled elk according to procedures approved by the University of Tennessee Institutional Animal Care and Use Committee (UT-IACUC #2671-0211). We immobilized elk using CO₂ dart projectors (Dan-Inject, Kolding, Denmark) and darts (PneuDart Inc., Williamsport, Pennsylvania) containing a combination of butorphanol (27.3 mg/ml), azaperone (9.1 mg/ml), and medetomidine (10.9 mg/ml; BAM TM, ZooPharm, Windsor, Colorado). The BAM volume for female and small male elk was 2.0 ml and 3.0 ml for large males. We monitored vitals including rectal temperature, heart rate, respiration rate, and oxygen saturation levels throughout anesthesia. For elk with oxygen saturation levels <90%, we administered supportive oxygen intranasally. From elk, we collected blood, fecal pellets, pinna tissue, and ticks. We collected up to 30 ml of blood from the jugular or cephalic veins and stored samples in vacutainers (BD, Franklin Lakes, New Jersey) which were centrifuged for 5 min at 1,792 g. We separated blood serum and stored samples at -20°C until thawed at room temperature for disease diagnostics. We collected feces directly from elk and stored samples individually at approximately 4°C.

We fitted elk with Global Positioning System (GPS) collars (Lifecycle Pro 500 globalstar or LiteTrack Iridium 500 TL; Lotek Wireless, Inc., Newmarket, Ontario, Canada) and ear tags. We reversed anesthesia using intramuscular injections of Naltrexone (50 mg/ml, ZooPharm, Windsor, Colorado; 0.5 ml per elk) and Atipamazole (25 mg/ml, ZooPharm, Windsor, Colorado; 2 times the volume of BAM administered).

Elk collars remained active until mortality, collar failure, or data collection was stopped at the end of January 2022. We viewed GPS data remotely on the Lotek website to determine if elk were alive and moving each month. Collars also had mortality sensors designed to issue alerts when individuals were immobile for 10 hrs. After receiving a mortality alert, we used the last reported GPS location and very high frequency (VHF) telemetry to confirm mortality. Carcasses suitable for necropsy were transported to the University of Tennessee College of Veterinary Medicine to determine cause of death. Necropsy findings leading to a *P. tenuis* infection diagnosis included observing hemosiderin laden macrophages surrounding numerous small vessels within the brain, linear cavitations suggestive of migration tracks, and/or identifying adult worms. The obex and pharyngeal lymph nodes of necropsied elk were sent to The Colorado State University Diagnostic Laboratories (from 2019 to fall 2021) and the C. E. Kord Animal Health Diagnostic Lab (from fall 2021 through 2022) to be tested for chronic wasting disease using an enzyme-linked immunoassay (ELISA; Bio-Rad Laboratories, Inc., Hercules, CA).

Survival Rate Estimate

To evaluate elk survival rates, we used the RMark package (Laake 2013) in R (R Core Team 2019) to construct known-fate models for program MARK (White and Burnham 1999). Known-fate models estimate survival probabilities, typically with high precision, and have a staggered entry design to account for capture taking place over the study period (White and Burnham 1999, Lopez et al. 2003). The models allow removal of elk from the study and right censoring. We constructed encounter histories

for collared elk, categorizing each as alive, dead, or censored in monthly intervals from February 2019 through January 2022. We tested 7 models based on *a priori* hypotheses using combinations of the covariates sex, year, and season to better estimate survival (Table 3.1 in Appendix). Year 1 included data collected from February 2019 through January 2020, year 2 included February 2020 through January 2021, and year 3 included February 2021 to January 2022. December, January, and February were considered winter, March, April and May were considered spring, June, July, and August were considered summer, and September, October, and November were considered fall. To consider the best approximating models, we created a confidence set including models with a delta Akaike's Information Criterion corrected for small sample sizes ($\Delta AIC_c \leq 4$) (Burnham and Anderson 2002). In this way, the relative likelihoods of the considered models were small and the majority of the total weight was accounted for while minimizing the inclusion of models with spurious effects (Burnham and Anderson 2002). Models in the confidence set with the lowest AIC_c and greatest Akaike weights were considered those with the highest support and used to estimate survival. We used the Delta method to calculate standard errors and confidence intervals for all survival estimates using the package emdbook (Bolker 2020).

Pathogen Testing

We conducted surveillance for pathogens based upon causative agent presence in the southeastern United States, potential for pathogens to cause elk morbidity and mortality, agricultural animal risk, and/or zoonotic risk (Table 3.2 in Appendix). We calculated the percentage of positive samples for each diagnostic test and calculated a

95% confidence interval of the population proportion. As we only tested a portion of NCWMA elk, the confidence interval indicated the statistical probability of the true proportion of NCWMA elk with the pathogen or antibodies to the pathogen. We tested serum for exposure to *Toxoplasma gondii* using a modified agglutination test (MAT) using two-fold dilutions from 1:25 to 1:3,200 according to Desmonts and Remington (1980). Briefly, serum is serially diluted to determine the concentration cutoff of antibodies reacting to *T. gondii* antigen. Individuals with seropositivity at higher cutoff values are more likely to have clinical disease and higher parasite loads in tissues (Gerhold et al. 2017). A cutoff value of $\geq 1:25$ was considered seropositive (Su and Dubey 2020). While MAT has not been validated for elk diagnostics, it has been extensively tested in cervids and is considered specific regardless of the species (Dubey 2010, Cox et al. 2017). Athens Veterinary Diagnostic Lab tested remaining serum for evidence of exposure to *Brucella abortus* (brucellosis, buffered acidified plate antigen), epizootic hemorrhagic disease virus (EHD, *Orbivirus*, agar gel immunodiffusion), bluetongue virus (*Orbivirus*, ELISA), *Pestivirus* spp. (bovine viral diarrhea, BVD, serum neutralization), *Varicellovirus* sp. (infectious bovine rhinotracheitis, IBR, serum neutralization), *Respirovirus* sp. (bovine parainfluenza virus-3, serum neutralization), *Mycobacterium avium* subspecies *paratuberculosis* (MAP, Johne's disease, ELISA), *Anaplasma marginale* (anaplasmosis, ELISA), *Leptospira interrogans* (leptospirosis, microscopic agglutination test), and *Vesiculovirus* spp. (vesicular stomatitis Indiana and New Jersey strains, serum neutralization). Additional testing for MAP exposure was done at the University of Tennessee using

electrochemical detection (Hatate et al. 2021). The National Veterinary Services Lab within the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) conducted vesicular stomatitis testing on any suspect samples using a competitive ELISA.

We searched anesthetized elk primarily around the ears and tail for ectoparasites and collected a random portion of adult ticks (up to 7) which were stored in 70% ethanol. We identified ticks to species by morphological characteristics to investigate species in the region and potential disease risk. We tested collected black-legged ticks (*Ixodes scapularis*) and pinna tissue samples for the presence of *Borrelia burgdorferi*. We used a DNeasy tissue extraction kit (Qiagen, Germantown, MD) to extract DNA from approximately 4mm diameter pinna tissue samples and whole collected *I. scapularis* cut in half. We eluted the extracted DNA from each sample in 100 µl buffer AE (10 mM Tris–HCl, 0.5 M EDTA, pH 9) followed by storage at -20°C prior to quantitative PCR analysis (qPCR). To perform qPCR on the reaction mixture, we used a StepOne Real-Time PCR system thermocycler (Applied Biosystems, Waltham, MA; Xu et al. 2016; Supplementary Table 3.1 in Appendix; Attachment 1, attachment1.pdf). We included a positive control, negative PCR control using sterile water, and negative DNA extraction control.

We tested for *T. cervi*, *Babesia* spp., and *A. marginale* using whole blood. We performed DNA extraction on 200 µl of whole blood from each sample using a DNeasy blood extraction kit (Qiagen, Germantown, MD) per the manufacturer's instructions. We eluted the extracted DNA in 100 µl buffer AE and then stored it at -20°C. We used

qPCR analysis to amplify the extracted DNA to detect the presence of *A. marginale* using a StepOne Real-Time PCR system thermocycler (Applied Biosystems, Waltham, MA; Carelli et al. 2007; Supplementary Table 3.1 in Appendix; Attachment 1, attachment1.pdf). We performed nested PCR on extracted DNA for the detection of *Babesia* spp. and *T. cervi* using the Veriti Thermal Cycler (Applied Biosystems, Waltham, MA; Gubbels et al. 1999; Yabsley et al. 2005a; Supplementary Table 3.1 in Appendix; Attachment 1, attachment1.pdf). We performed gel electrophoresis on a 1.5% agarose gel to separate and visualize amplicons after staining with ethidium bromide and ultraviolet transillumination. In each PCR run, we included a negative DNA extraction control, negative PCR control, and a positive control for all PCR testing. We purified samples that had electrophoretic gel bands corresponding with the positive control using a QIAquick PCR purification kit (Qiagen, Valencia, CA). Purified nested PCR products were submitted to the University of Tennessee Genomics Core (Knoxville, TN). We visualized resulting sequences (Sequencher 5.1 software, Gene Codes, Ann Arbor, MI) and used a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) in GenBank to identify species.

We examined fecal samples for parasites using centrifugal fecal flotation techniques with Sheather's sugar solution (Zajac and Conboy 2012). We used Sheather's sugar solution as the higher specific gravity allowed for recovery of a wider variety of parasites (Zajac and Conboy 2012). Slides were examined microscopically on a 10x objective lens. We recorded observed parasites and applied the Baermann

technique to fecal samples with larvae present to identify any dorsal-spined larvae, if present (Zajac and Conboy 2012).

Results

Cause of Death Analysis

We captured a total of 29 elk (21 females and 8 males) between 2019 and 2020. Three elk were recaptured to replace collars for a total of 32 captures, but only samples collected during initial capture were used for health surveillance. During the study, 50% (16/32) of collars permanently stopped transmitting data including 68% (13/19) of globalstar collars and 23% (3/13) of Iridium collars. Those collars transmitted for an average of ≤ 1.25 years (globalstar: mean 1.4, range 0.4–2.5; Iridium: mean 0.8, range 0.2–1.7) which limited mortality data. Between February 2019 and February 2022, there were 9 known mortalities (6 females and 3 males). For 67% (6/9) of our known mortalities, we did not receive mortality signals or signals were not received for days or weeks after the elk had died resulting in severely scavenged and decomposed bodies. In these cases, elk were wearing globalstar collars and carcasses or skeletons were noticed by TWRA personnel or members of the public. Causes of death included *P. tenuis* associated mortality ($n=3$, 33%), vehicular collision ($n=1$, 11%), poaching ($n=1$, 11%), hunter harvest ($n=1$, 11%), and unknown causes of death due to carcass degradation ($n=3$, 33%). We did not detect any mortalities during year 2.

Survival Rate Estimate

The number of elk monitored monthly ranged from 6 to 22 elk due to additional captures, recaptures, mortalities, and collar failures. Two survival models were within $\Delta AICc \leq 4$ (Table 3.1 in Appendix). Models 1 (Year) and 2 (Year + Sex) had a $\Delta AICc < 2$, similar maximized log likelihoods, and differed by 1 parameter (sex; $\beta=0.76$, 95% CI: -0.71–2.23) indicating sex was an uninformative parameter for survival (Burnham and Anderson 2002; Arnold 2010). Therefore, we did not consider model 2 to be a competitive model and selected the model including year ($w_i=0.833$) as the best approximating model to estimate survival. Estimated survival for years 1, 2, and 3 of the study were 68.2% (95% CI: 45.25%–91.06%), 100% (95% CI: 100%–100%), and 75.6% (95% CI: 51.61%–99.52%), respectively. Average yearly estimated survival was 80.2% (95% CI: 68.00%–92.49%).

Pathogen Testing

Chronic wasting disease was not detected in any necropsied elk ($n=6$, 0%, 95% CI: 0.00–48.32%). We collected and tested blood from 27 captured elk. Nineteen elk had antibodies detected for *L. interrogans* serovars (70.4%, 95% CI: 49.66–85.50%; Attachment 2, attachment2.pdf). Elk were most commonly positive for the serovar icterohaemorrhagiae ($n=18$), followed by pomona ($n=5$), and bratislava ($n=3$). Fifteen elk had antibodies to *T. gondii* (55.6%, 95% CI: 35.64–73.96%; Table 3.3 in Appendix) and 14 had evidence of EHD virus (51.9%, 95% CI: 32.35–70.84). Four elk had evidence of antibodies for MAP using the Zoetis ELISA (14.8%, 95% CI: 4.86–34.61%; SERELISA™ Para TB Mono Indirect, Zoetis, Kalamazoo, MI) while 6 elk and 2 suspects

had evidence of antibodies for MAP using electrochemical detection ($n=6$, 22.2%, 95% CI: 9.38–42.73%). No elk were deemed positive for antibodies to the causative agents of vesicular stomatitis, bluetongue, BVD, IBR, PI-3, or brucellosis (0%, 95% CI: 0.00–15.50%).

We collected ectoparasites ($n=62$) on 21 out of 29 elk. Three species of ticks were collected including the lone star tick (*Amblyomma americanum*; $n=53$, 85.5%, 95% CI: 73.72–92.75%), American dog tick (*Dermacentor variabilis*; $n=8$, 12.9%, 95% CI: 6.13–24.40%), and *I. scapularis* ($n=1$, 1.6%, 95% CI: 0.08–9.83%). No qPCR evidence of *B. burgdorferi* was found in the *I. scapularis* recovered (0%, 95% CI: 0.00–94.54%) or from the 28 tested pinna tissue samples (0%, 95% CI: 0.00–15.02%).

All 27 elk tested for *A. marginale* antibodies using ELISA methods were seropositive (100%, 95% CI: 84.50–100.00%). When testing DNA extracted from whole blood and qPCR, no *A. marginale* DNA was identified (0%, 95% CI: 0.00–15.50%). While *Babesia* spp. was not detected (0%, 95% CI: 0.00–15.50%), 7 elk had DNA sequences consistent with *T. cervi* sequences in GenBank (25.9%, 95% CI: 11.78–46.59%; Table 3.4 in Appendix).

Fecal samples were collected from 28 elk during capture. Twenty-two elk had evidence of parasites including *Capillaria* spp. ($n=9$, 32.1%, 95% CI: 16.58–52.43%), *Eimeria* spp. ($n=7$, 25.0%, 95% CI: 11.43–45.22%), *Gongylonema* spp. ($n=2$, 7.1%, 95% CI: 1.25–24.96%), *Moniezia* spp. ($n=2$, 7.1%, 95% CI: 1.25–24.96%), *Nematodirus* spp. ($n=3$, 10.7%, 95% CI: 2.81–29.37%), *Oesophagostomum* spp. ($n=1$, 3.6%, 95% CI: 0.19–20.24%), *Trichuris* spp. ($n=1$, 3.6%, 95% CI: 0.19–20.24%), and *Trichostrongylus*

spp. ($n=15$, 53.6%, 95% CI: 34.21–71.99%; Attachment 3, attachment3.pdf). Four samples included unidentified larvae and we conducted Baermann tests on 3 of the samples (1 had insufficient sample remaining). We did not detect larvae from the Baermann tests. The larvae observed during fecal flotation lacked dorsal spines characteristic of *P. tenuis*; therefore, they were presumed to be free-living and not *P. tenuis*.

Discussion

Of known causes of death (6 out of 9 mortalities), half were attributed to non-infectious causes and half to *P. tenuis* infection. While 3 elk were categorized as *P. tenuis* infection related mortality, the nematode could have ultimately been responsible for at least 5/9 mortalities. The elk categorized as a vehicular collision mortality had indications of *P. tenuis* infection identified during necropsy. *Parelaphostrongylus tenuis* infection causes neurologic issues including reduced fear to negative stimuli (Olsen and Woolf 1978) and may have increased likelihood of vehicular collision. One elk with an unknown cause of death due to carcass degradation was also a *P. tenuis* infection suspect based upon behavior prior to death. High proportions of elk mortality caused by *P. tenuis* is consistent with previous NCWMA reports and nearby populations (Morrow 2009, Kindall et al. 2011). We did not identify any *P. tenuis* or other identifiable larvae shed in the feces, and only observed fecal parasites previously observed in cervids (Prestwood et al. 1970, Thorne et al. 2002). While elk are capable of shedding *P. tenuis*

larvae (Samuel et al. 1992), more research is needed to establish if this is occurring at the WMA in addition to determining baseline levels of other fecal parasites.

Our estimated annual survival of 0.802 is similar to survival estimated shortly after reintroduction (0.799; Kindall et al. 2011). Estimated survival in our study was higher than reports of heavily harvested Kentucky elk with mean annual survival of 0.57 for males and 0.67 for females (Slabach et al. 2018). In Kentucky, hunter harvest of both sexes is legal and the leading cause of mortality (85.2%; Slabach et al. 2018). Comparatively, our survival estimate was lower than those estimated in unhunted 2–9-year-old elk in the Great Smoky Mountains National Park with mean annual survival of 0.911 for males and 0.933 for females (Murrow et al. 2009). The proportion of diagnosed disease related mortalities out of all monitored elk were similar between the original NCWMA study (4%, 6/157; Kindall et al. 2011), Kentucky (5%; 12/237; Slabach et al. 2018), our study (10%; 3/29), and the National Park (15%; 12/81; Murrow et al. 2009). *Parelaphostrongylus tenuis* was the primary cause of disease related mortality in each study.

Health surveillance indicated NCWMA elk could have coinfections potentially increasing mortality risk. The detection of antibodies to the causative agent of Johne's disease in 4 elk using the Zoetis ELISA and 6 elk and 2 suspects using the electrochemical detection ELISA is of interest as there is limited evidence of Johne's disease agent establishment within an eastern elk population (Corn et al. 2010). Interestingly, clinical signs of Johne's disease and *P. tenuis* infections are similar requiring additional testing during necropsy. In cervids, Johne's disease infections can

be subclinical or cause deteriorating body condition (i.e., emaciation and poor hair quality), diarrhea, and death (Jessup et al. 1981, Cook et al. 1997, Crawford et al. 2006, Davidson 2006). Multiple study elk ($n=3$) had necropsy reports indicating poor hair loss, history of poor body condition, and/or diagnosis of emaciation. One such elk was positive at capture for MAP antibodies using both the Zoetis ELISA and electrochemical detection ELISA and had primary diagnoses of emaciation with a presumed *P. tenuis* infection diagnosis based on brain histopathology. Unfortunately, no histopathology testing for Johne's disease was performed during any elk necropsy to investigate other potential causes for emaciation.

Since clinical signs of *P. tenuis* infection and Johne's disease overlap, histopathology for both diseases should be performed during necropsies on elk with relevant clinical signs to further investigate natural infections of Johne's disease in Tennessee and ensure complete diagnoses. While the Zoetis ELISA used to detect MAP antibodies in NCWMA elk is 100% specific, it has not been validated for elk. The electrochemical detection ELISA has also not been validated for elk. However, our results likely indicate MAP has been or is present in the population. Surveillance of MAP should continue in elk due to clinical disease risks. Cattle surveillance is also important as MAP infections can cause potential significant economic losses from decreased milk production and mortality (Cook et al. 1997, Thorne et al. 2002).

Seven elk were PCR positive for *T. cervi*. Theileriosis in cervids is generally benign, but little is known about the disease in elk (Chae et al. 1999, Cauvin et al. 2019). Infected cervids, such as white-tailed deer, have shown to have increased

likelihood of exhibiting clinical signs including loss of body condition and death if stressed, experiencing coinfections, immunocompromised, or transported from non-endemic to endemic areas (Yabsley et al. 2005b, Cauvin et al. 2019). Every elk positive for *T. cervi* was also positive on at least 1 other diagnostic test. It is unknown if elk were experiencing coinfections as many of our diagnostic tests detected antibodies rather than active infections. However, coinfections are possible and further research is needed to understand possible clinical signs and resulting implications.

Our study aligned with previous reports of high *T. gondii* seroprevalence in elk in the eastern United States. Cox et al. (2017) found nearly identical prevalence rates of *T. gondii* in Kentucky elk (56.3%). The overlapping geographic areas between elk, bobcats (*Lynx rufus*), and domestic cats (*Felis catus*) in addition to the environmental persistence of shed oocysts in the wet and humid soil of Tennessee and Kentucky likely contribute to the observed prevalence rates (Cox et al. 2017). Elk were consistently seropositive at high titers increasing the risk of parasites in their tissue (Gerhold et al. 2017). Over half of our tested elk had evidence of *T. gondii*, increasing the need for hunter awareness of human infection risk. Wildlife agencies should provide elk meat preparation safety training within hunter education programs to reduce human infection risk (Cox et al. 2017).

The majority of elk had antibodies to *L. interrogans* serovars, although seropositivity for more than one serovar could have potentially occurred from cross-reactivity. Clinical disease from the bacteria has been reported in red deer (*Cervus elaphus*; Thorne et al. 2002). While it is conceivable for elk to exhibit similar clinical

signs and increased vulnerability to predation while infected, only serologic evidence of *L. interrogans* has been reported (Thorne et al. 2002, Barber-Mayer et al. 2007). More research is needed to establish the impact of *L. interrogans* to elk. Leptospirosis causes significant economic losses to the cattle industry. Multiple serovars, especially *pomona* detected in 5 NCWMA elk, are implicated in causing abortion and systemic disease in cattle (Thorne et al. 2002). Awareness of *L. interrogans* in free-ranging elk sympatric with agricultural animals is important for spillover purposes. Similarly, half of tested elk were EHD antibody positive and more research is needed to understand population impacts, if any.

There was no evidence of active *A. marginale* infections using qPCR. However, every elk was seropositive using ELISA testing with 99.7% sensitivity indicating elk had previously been exposed to the bacteria and maintained antibodies. While the utilized ELISA (VMRD Inc., Pullman, WA) is primarily used for bovine testing, Scoles et al. (2008) found the VMRD ELISA to be acceptable for use in wild ungulates including elk. Clinical disease from *A. marginale* has yet to be detected in free-ranging elk (Thorne et al. 2002). However, elk are considered potential reservoirs for the bacteria necessitating continued monitoring as anaplasmosis is a disease of concern in cattle (Thorne et al. 2002, Hendrix et al. 2019).

There were no indications of borreliosis in tested elk tissue or *I. scapularis*. We only recovered 1 *I. scapularis* out of 62 ticks, likely because we sampled when adult *I. scapularis* are less active (Hickling et al. 2018). Our sampling was limited to a portion of ticks feeding on captured elk and representative sampling across the NCWMA

throughout the year would be needed to assess true arthropod species composition. While we did not detect *B. burgdorferi*, targeted surveillance of *I. scapularis* in East Tennessee has indicated increasing Lyme borreliosis risk (Hickling et al. 2018). Further pathogen detection should be performed on collected ticks in future research as all tick species identified on NCWMA elk are primary vectors for various zoonotic pathogens (Stromdahl and Hickling 2012). Continued surveillance could improve human health outcomes and better detect tick species and tick-borne pathogens of concern to wildlife and agricultural animals. Increased surveillance could also help elucidate the role of wildlife as reservoirs for ticks and pathogens at the NCWMA.

Our study provided updated survival estimates and comprehensive health surveillance providing evidence of multiple pathogens of concern to the NCWMA elk population. Infectious disease, specifically *P. tenuis*, was the greatest cause of mortality. More research is required to establish the role of morbidities, such as Johne's disease, on elk survival. Continued surveillance is necessary to determine potential population limiting factors of NCWMA elk.

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APPENDIX

Table 3.1. Known-fate survival model selection for estimating elk survival within the North Cumberland Wildlife Management Area from 2019 to 2022. Data was collected from GPS collared individuals ($n=29$). The number of elk monitored each month varied from 6 to 22 elk. Model selection was based on Akaike's Information Criteria adjusted for small sample sizes (AICc), differences in AICc compared to the top model (ΔAICc), and model weights (w_i ; recalculated after removing second-ranked model with uninformed parameter). The number of parameters in the model is represented by K.

Model	K	Log likelihood	AICc	ΔAICc	w_i
Year	3	72.980	79.027	0.000	0.833
Year + Sex	4	72.018	80.097	1.070	-
Year + Sex + Season	7	69.748	83.969	4.943	0.070
Null	1	82.479	84.487	5.461	0.054
Sex	2	81.559	85.582	6.556	0.031
Season	4	80.462	88.540	9.514	0.007
Sex + Season	5	79.555	89.673	10.646	0.004

Table 3.2. Relevant diseases of elk and their causative agents, presence in the Southeastern United States, and mortality potential.

Disease	Causative Agent	Causative Agent Species	Disease Testing	
			Causative Agent Species Detected in the Southeastern USA	Known to Cause Elk Mortality
Anaplasmosis	Bacteria ⁸	<i>Anaplasma marginale</i> ⁸	Yes ¹⁵	Not reported
Babesiosis	Protozoa ¹⁴	<i>Babesia</i> spp. ¹⁴	Yes ⁹	Yes ^{14a}
Bluetongue	Virus ⁸	<i>Orbivirus</i> spp. ⁸	Yes ⁶	No ¹⁰
Bovine Viral Diarrhea (BVD)	Virus ¹⁷	<i>Pestivirus</i> spp. ¹⁷	Yes ⁶	Not reported
Brucellosis	Bacteria ¹⁶	<i>Brucella abortus</i> ¹⁶	No ¹⁶	Yes ¹⁶
Epizootic Hemorrhagic Disease (EHD)	Virus ⁸	<i>Orbivirus</i> spp. ⁸	Yes ⁶	No ¹⁰
Infectious Bovine Rhinotracheitis (IBR)	Virus ³	<i>Varicellovirus</i> sp. (BHV-1) ^{3, 11}	Yes ⁶	Not reported
Johne's Disease	Bacteria ⁸	<i>Mycobacterium avium</i> ⁸	Yes ⁶	Yes ^{5c}
Leptospirosis	Bacteria ⁸	<i>Leptospira interrogans</i> ⁸	Yes ⁶	No ¹⁶
Lyme Borreliosis	Bacteria ⁸	<i>Borrelia burgdorferi</i> ⁸	Yes ⁸	No ¹
Meningeal Worm	Nematode ¹²	<i>Parelaphostrongylus tenuis</i> ¹²	Yes ¹²	Yes ¹²
Parainfluenza (PI-3)	Virus ¹¹	<i>Respirovirus</i> sp. ¹¹	Yes ⁶	Not reported
Theileriosis	Protozoa ¹³	<i>Theileria cervi</i> ¹³	Yes ¹³	No ⁴
Toxoplasmosis	Protozoa ⁷	<i>Toxoplasma gondii</i> ⁷	Yes ⁷	No ⁷
Vesicular Stomatitis	Virus ⁸	<i>Vesiculovirus</i> spp. ⁸	Yes ^{8b}	Not reported

^aSuspect Mortality

^bSporadic outbreaks in cattle

^cEmaciation and possible mortality

¹(Allan 2001)

²(Barber-Mayer et al. 2007)

³(Castro 2001)

⁴(Cauvin et al. 2019)

⁵(Cook et al. 1997)

⁶(Corn et al. 2010)

⁷(Cox et al. 2017)

⁸(Davidson 2006)

⁹(Homer et al. 2000)

¹⁰(Howerth et al. 2001)

¹¹(King et al. 2011)

¹²(Larkin et al. 2003)

¹³(Mans et al. 2015)

¹⁴(Pattullo et al. 2013)

¹⁵(Pompo et al. 2016)

¹⁶(reviewed by Thorne et al. 2002)

¹⁷(Van Campen et al. 2001)

Table 3.3. *Toxoplasma gondii* seroprevalence from captured North Cumberland Wildlife Management Area elk (2019–2020). A cutoff value of 1:25 was considered seropositive.

Titer	Frequency
1:25	4
1:50	1
1:100	1
1:200	0
1:400	1
1:800	1
1:1,600	2
1:3,200	5
Total Seropositive	15 (55.56%, 95% CI: 35.64–73.96%)
Total Seronegative	12 (44.44%, 95% CI: 26.04–64.36%)

Table 3.4. Tick-borne pathogens or antibodies detected from captured North Cumberland Wildlife Management Area elk samples collected in 2019 and 2020.

Disease agent (diagnostic test)	Positive/total	% (95% Confidence interval)	Sample
<i>Anaplasma marginale</i> (ELISA)	27/27	100% (84.50–100.00%)	Serum
<i>Anaplasma marginale</i> (qPCR)	0/27	0% (0.00–15.50%)	Whole blood
<i>Babesia</i> spp. (Nested PCR)	0/27	0% (0.00–15.50%)	Whole blood
<i>Borrelia burgdorferi</i> (qPCR)	0/28	0% (0.00–15.02%)	Pinna tissue
<i>Theileria cervi</i> (Nested PCR)	7/27	25.93% (11.78–46.59%)	Whole blood

Supplementary Table 3.1. Master mix components for polymerase chain reaction (PCR) protocols.

Reaction	Component	Volume (μl)
<i>B. burgdorferi</i> qPCR	primer and probe mix (Integrated DNA Technologies, Coralville, IA)	1
	TaqMan Master Mix (Applied Biosystems, Waltham, MA),	10
	Rox reference dye	0.4
	nuclease-free water	6
	DNA template	2
<i>A. marginale</i> qPCR	primer and probe mix (Integrated DNA Technologies, Coralville, IA)	1
	TaqMan Master Mix (Applied Biosystems, Waltham, MA),	10
	nuclease-free water	7
	DNA template	2
<i>T. cervi</i> and <i>Babesia</i> spp. nested PCR	primers	2
	TaqMan Master Mix (Applied Biosystems, Waltham, MA),	10
	nuclease-free water	8.5
	DNA template	2

CHAPTER FOUR

CONCLUSIONS

Elk are an important Tennessee resource and there is strong public support for a healthy population in the region (Chapagain and Poudyal 2020, Chapagain et al. 2020, Watkins et al. 2021). We conducted research to better understand the status of North Cumberland Wildlife Management Area (NCWMA) elk. We estimated elk abundance and sex ratios to elucidate population status in addition to evaluating important parameters for population growth including survival and health.

We estimated 159 elk in the southwestern Elk Hunt Zones and Sundquist Unit. The total abundance estimate was more precise than annual TWRA estimates (TWRA 2018) with a coefficient of variation within the target for acceptable precision to be useful for wildlife management objectives (<0.20 ; Pollock et al. 1990). Our method eliminated possible capture related stress by using non-invasive fecal pellet DNA analysis to identify individuals and did not require sighting elk in heavily forested terrain. We analyzed collared elk movement to assess the validity of sampling primarily wildlife openings to collect scat for our abundance estimates. Data from GPS collars indicated that elk frequently used wildlife openings, providing support for our targeted sampling selection strategy. However, wildlife openings are not present in all areas of the NCWMA and sampling multiple areas per typical home range size is recommended to achieve high detection probability and precision (Otis et al. 1978, Lukacs and Burnham 2005). Therefore, exclusively sampling wildlife openings may be more appropriate in study sites with a suitable density of consistently spaced wildlife openings.

We identified a female skewed sex ratio in the Elk Hunt Zones while an even sex ratio was observed in the non-hunted Sundquist Unit during 2019. Hunting may have

contributed to the larger number of females compared to males as approximately 1/3 ($n=6$; TWRA 2021) of the estimated male population was harvested in the southwestern Elk Hunt Zones in 2019 (TWRA 2021). Some estimated home ranges ($n=5$, 25%) encompassed portions of the Elk Hunt Zones and Sundquist Unit due to elk GPS fixes near the unit boundaries. However, movement of collared elk between the sites was not observed suggesting there may be some level of population segregation because of a major highway between the 2 sites. Discovering a female skewed sex ratio in the Elk Hunt Zones provides a starting point for further investigation into causes and potential population effects. Rigorous estimation methods with evaluated study designs should be used to ensure scientifically defensible harvest quotas, and further investigation of the impacts of a female skewed sex ratio is recommended.

To better understand population parameters contributing to elk abundance and population viability, we estimated elk survival. Using data from GPS collars and known-fate survival models, our estimated average yearly survival rate was 80.2% indicating elk survival has not significantly changed since survival was estimated soon after elk reintroduction 20 years ago (79.9%; Kindall et al. 2011). Cause of death analysis indicated half of all known mortalities were caused by infectious disease, namely *P. tenuis* associated disease. A high proportion of elk mortality caused by *P. tenuis* aligns with previous NCWMA and nearby population research (Murrow 2009, Kindall et al. 2011), and the parasite remains a concern for NCWMA population health.

To further explore pathogens present within the population, we conducted antemortem disease surveillance. Our work provided comprehensive health surveillance

of elk at the NCWMA and established baseline information critical for understanding population health. We detected multiple pathogens present within the population including evidence of exposure to the causative agent of Johne's disease, a disease previously unknown to be established in an eastern elk population. Similarity in clinical signs between Johne's disease and *P. tenuis* associated disease in conjunction with the lack of previous surveillance for Johne's disease raises questions into the role each contributes to NCWMA elk morbidity and mortality. At the NCWMA, further investigation into Johne's disease and other pathogens detected in the elk population including *Leptospira interrogans*, *Anaplasma marginale*, *Theileria cervi*, and *Toxoplasma gondii* is warranted as they have potential implications for elk, sympatric agricultural animals, and/or humans.

We improved understanding of elk abundance at the NCWMA and contributing parameters for population growth: survival and health. Our research provides the scientific foundation for other studies and can aid in developing management strategies. Increased research into the impacts of detected pathogens will support elk population health and persistence.

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