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A Socio-Ecological Approach to Wildlife Disease Risk

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A SOCIO-ECOLOGICAL APPROACH TO WILDLIFE DISEASE RISK

By

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An Abstract of the Thesis Presented in
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Many Eastern moose (*Alces alces,* Linnaeus; 1758) populations along the southern edge of their North American range are declining, including those in Minnesota, Vermont, and New Hampshire. More recently, in Maine, winter ticks (*Dermacentor albipictus;* Packard 1869) are suspected to also be influencing the population through periodic widespread mortality of calves. While metabolic stress from heavy winter tick parasitism has been implicated in these moose population declines, little is known about the relative effects of tick-borne diseases, which may compound metabolic stress. Tick-borne pathogens known to infect cervid species include *Anaplasma* species, a group of bacteria that cause a disease known as anaplasmosis. Furthermore, the decline of moose and emergence of ticks in Maine could influence outdoor recreation behavior, cultural practices, nature-based tourism businesses, and wildlife management. Perceived risk in regards to a decline in the moose population, the effects of winter ticks on moose, and the impacts that these may have on human systems could potentially influence people's behaviors and management decision-making. To address both biological and social concerns, I applied an interdisciplinary approach with the following three goals: (G1) determine the prevalence and distribution of *Anaplasma* species infections in Maine's moose and winter tick populations, and genetically characterized the species through sequencing and phylogenetic analyses, (G2) investigate whether fitness (in terms of calf survival through the

winter) is predicted by its *Anaplasma*-infection status, tick load, and/or related health indices, and (G3) identify which factors (e.g. the experiences a person has had with the moose/winter tick system) determine Penobscot Nation citizens' risk perceptions in regards to moose health, and the impacts of winter tick moose infestation on human systems.

In addressing G1, I tested for the presence or absence of *Anaplasma* species DNA in moose and winter ticks by amplifying a 16S rRNA gene locus, capable of genus-level taxonomic specification. These data revealed that a large proportion (~54%) of moose calves in Maine are infected with an uncharacterized *Anaplasma* species, with a significant difference in *Anaplasma* prevalence between northern and western study sites as well as between sexes. *Anaplasma* was also detected in winter ticks, but only in a single pooled sample (<1%). A Bayesian phylogenetic analysis revealed that the single *Anaplasma* strain in moose was highly divergent from the strain identified in winter ticks, and most closely related to an uncharacterized North American cervid strain. Therefore, I classified it as "*Anaplasma spp.* Cervus". For G2, a survival analysis and multiple model selection criteria demonstrated that, for moose with light, moderate and severe infestations of winter ticks, *Anaplasma* spp. Cervus significantly decreased survival. Furthermore, peripheral blood smear analysis and calculation of packed cell volume (PCV) showed moose infected with *Anaplasma* spp. Cervus had significantly increased frequency of red blood cell inclusions, and decreased red blood cell volume. My evidence suggests that *Anaplasma* spp. Cervus has sub-clinical effects on the moose in Maine.

In addition, to address G3, I administered a questionnaire to citizens of Penobscot Nation to explore which factors determine perceived risk in relation to the effects of ticks on moose and human systems. The questionnaire aimed to explore the influence of several constructs on risk perceptions, including: experiential processing, cognitive factors, socio-cultural factors, and

socio-demographics. However, for this thesis I only tested the role that participants' experience with a specific threat, and status as a hunter played in predicting the risk perceptions about the impact of (1) winter ticks, (2) all types of ticks, and (3) a decline in the moose population on people, the Penobscot Nation, and the environment. Results suggest that there is no influence of a respondent's status as a hunter on determining risk perceptions. However, I did find that an individual's level of experience with winter ticks and moose (i.e. people have seen a moose, people who have seen a dead moose, people who have seen a moose with winter tick infestations) is significantly and positively correlated with the risk perceived from a decline in moose, and presence of winter ticks towards the individual, Penobscot Nation, and the natural environment. Text analysis of open-ended responses to the question of how participants defined a "healthy" moose population showed that the majority of respondents emphasized the quality of moose (i.e. healthy weight, no missing hair) over the quantity of the moose population. The work detailed by this thesis provides valuable insight into the relationships between moose, ticks and disease, and risk perceptions; information that is key for maintaining healthy moose populations and human systems into the future. Furthermore, this study also underlines the need for future transdisciplinary research to fully understand complex wildlife conservation—disease management—human wellbeing issues.

DEDICATON

I am happy to dedicate this thesis to my awesome Dad, Gregory Elliott, my amazing grandmother Alice (“Pink”) Comeau, and my future wife, Katherine Plotner. All of you inspire me everyday to be better and I wouldn't be nearly as accomplished without your constant support.

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LIST OF ABBREVIATIONS

AIC: Akaike's Information Criterion

BIC: Bayes Information Criteria

BLAST: Basic Local Alignment Search Tool

CCRPM: Climate Change Risk Perception Model

CI: Confidence Interval

CL: Confidence Limit

DNA: Deoxyribonucleic Acid

DNR: Department of Natural Resources

EDTA: Ethylenediaminetetraacetic Acid

ESS: Effective Sample Size

GPS: Global Positioning System

HGA: Human Granulocytic Anaplasmosis

HGE: Human Granulocytic Ehrlichiosis

HKY: Hasegawa-Kishino-Yano

MCMC: Markov Chain Monte Carlo

MDIFW: Maine Department of Inland Fisheries and Wildlife

NRBC: Nucleated Red Blood Cells

mtCOI: Mitochondrial Cytochrome Oxidase I

PCR: Polymerase Chain Reaction

PCA: Principal Component Analysis

PCI: Potential of Conflict Index

PCV: Packed Cell Volume

PN: Penobscot Nation

PP: Posterior Probability

SEM: Structural Equation Modeling

SPSS: Statistical Package for the Social Sciences

RNA: Ribonucleic Acid

RISP: Risk Information Seeking and Processing

RBC: Red Blood Cell

TBD: Tick-Borne Disease

WBC: White Blood Cell

WMD: Wildlife Management District

ZDRISP: Zoonotic Disease Risk Information Seeking and Processing

CHAPTER 1

INTRODUCTION AND ORGANIZATION OF THESIS

Risk is defined as the objective probability and severity of consequences (Haimes, 2009). Risk can be measured using an array of biophysical and social sciences methods and frameworks that have been developed and tested over several decades. This thesis aims at studying risk in the moose-winter tick-human system by applying tools from the biological and social sciences. This chapter (1) introduces the research topic and study area; (2) summarizes key frameworks that allow us to measure and understand risk from both biological and social science approaches; and (3) describes how the thesis is organized.

1.1 Moose, Winter Ticks, and Disease

There are numerous pathogens, diseases, and environmental factors documented that affect Eastern moose (*Alces alces americana*; Linnaeus, 1758) (Lankester & Samuel, 2007). In New England and some parts of Maine, moose have been seriously threatened by the parasitism of the winter tick (*Dermacentor albipictus*; Packard, 1869) (Musante, Pekins, & Scarpitti, 2010). While the Maine moose population is considered to be stable at approximately 70,000 (Kantar & Cumberland, 2013), there has been evidence that winter ticks influence the population through periodic widespread mortality of calves during epizootic events (referred to hereafter as "epizootics", or a calf mortality rate greater than 50%). These epizootics are increasing in frequency in coincidence with an increase in recorded calf mortalities in western Maine (Jones et al., 2019). Increases in moose density paired with a changing climate resulting in a later onset of winter snow are considered to be the reasons for epizootics becoming more frequent in moose

(Dunfey-Ball, 2017; Musante et al., 2010). Specifically, Dunfey-Ball (2017) established that drought conditions in the late-summer and high snow in the early-fall were the most predictive variables for a winter tick epizootic due to the effect of drought on larval winter tick survival. Moose calves (<1 year of age) are especially vulnerable to mortality attributed to winter tick parasitism, so they have been prioritized in many studies, including the present one. In New Hampshire, tick-related mortality was responsible for 41% of radio-marked deaths with calves representing 88% of all deaths (Musante, 2006), but all age classes of moose have been previously associated with winter tick-related mortality in other jurisdictions in western Canada (Samuel & Barker, 1979).

Although ticks are typically considered hazardous due to the diseases they vector, the primary damage to moose is from anemia due to winter tick parasitism. It is thought that the large volume of blood loss associated with severe tick infestations further reduces nutritional status during March – April when tick feeding is greatest (Samuel, 2004). Conservative estimates indicate that blood loss associated with moderate (30,000 ticks) to severe (70,000 ticks) infestations has a substantial impact on energy and protein balance (Musante, Pekins, & Scarpitti, 2007). Winter ticks cause population decline through widespread mortality of calves and compromised adults during epizootic years, and they may also have more long-term population effects through reduction of adult cow productivity (Musante et al., 2010). Adult female moose with high annual winter tick loads experience reduced physical condition in late winter/early spring due to the compounding effects of a nutritionally deficient diet and a substantial protein shortage from blood loss (Musante, Pekins, & Scarpitti, 2007). This decreased condition has been predicted to result in reduced fertility, low yearling productivity, increased age of first reproduction, and low twinning rates (Jones et al., 2017; Musante et al., 2010). A

decrease in annual productivity of adult cows and neonatal calf survival are key parameters used to manage moose in Maine to ensure there is a healthy, stable population.

Within the last decade, there has been a substantial effort from the Maine Department of Inland Fisheries and Wildlife (MDIFW) to generate reference values for mortality rates, birth rates, disease, parasites, serum chemistry, trace nutrient, and heavy metals for the moose (Kantar, 2018). Beginning in 2014, that effort has resulted in a comprehensive data set that allows us to model what factors contribute most to moose mortality in the northeastern United States. Moose populations in Minnesota, Manitoba, Nova Scotia, Vermont, New York, and New Hampshire have all been threatened by a variety of different regional ailments (Broders, Coombs, & McCarron, 2012; Jones et al., 2019; Murray et al., 2006), however the changing climate seems to be the common thread in all of the declining populations (Dunfey-Ball, 2017; Jones, 2016). While there is currently no evidence to indicate that a decline in moose is certain in Maine, the threat of future declines generates conflict among residents in the areas of wildlife management, economic vitality, recreation opportunities, and cultural identity.

There has been ample research on the impacts of winter ticks on moose in Maine, and also a substantial effort for surveillance of some parasites and diseases like meningeal worm, lungworm (*Dictyocaulus* spp.), *Echinococcus granulosus*, *Taenia ovis krabbei*, and even a mosquito borne disease, Eastern Equine Encephalitis (Jones et al., 2019; Lichtenwalner, Adhikari, Kantar, Jenkins, & Schurer, 2014; Lubelczyk et al., 2014; Musante, 2006). By contrast, tick-borne disease in moose has been understudied, and moose could also be considered a neglected species in the tick-borne disease literature (Appendix G). In part, the reason for this oversight may be due to the fact that the winter tick is considered a “one host-tick,” spending the entirety of its life on a single host. This unique life cycle is presumed to hinder the winter tick

from transmitting disease at all because it does not have the opportunity to transmit a disease from one host to another (Samuel, 2004). However, I have not found any literature disputing or supporting either of the claims that (1) winter ticks do not play a significant role in disease transmission, or (2) moose do not act as a reservoir for disease amplification. To address the insufficient evidence that moose or winter tick play a role in tick-borne pathogen maintenance, I focused primarily on screening for an emerging group of pathogens; *Anaplasma* spp. and examined the effects it may have on the health of the moose in Maine.

1.2 The Study Area

This study took place in Maine, USA. For the biological component of the study, data were obtained on moose health and moose health-winter tick interactions in two wildlife management districts (WMDs) (Figure 2.1). Wildlife management districts are geographical areas defined by MDIFW within which similar biological, geophysical, and hunting characteristics exist. The western Maine (WMD8) study district is north and west of the town of Greenville to the Quebec border. It is ~3154 km^2 and encompasses the same study site used by Jones et al. (2019) most recently in Maine. This study area is a privately owned, managed commercial timberland where the dominant cover type is a northern hardwood forest with some conifer stands (DeGraaf, Yamasaki, Leak, & Lanier, 1992). In contrast, WMD 2 is a smaller area at approximately 1867 km^2, but has a higher density of moose due to a higher quality habitat (Kantar & Cumberland, 2013), which could be due to the abundance of forested areas where there is significant snow cover in the winter, cooler temperatures in the summer, and ample access to ponds and lakes (DeGraaf et al., 1992; Franzmann & Schwartz, 1997).

Furthermore, the social sciences component was conducted with citizens of the Penobscot Nation (PN). Penobscot Nation is one of several tribes within the Wabanaki confederacy that re-obtained ownership to land as a result of the 1980 Maine Indian Claims Settlement Act (Waldman, 2014). Penobscot tribal lands make up a total of 126,267 acres of Maine (Figure 1.1), and consist of fee lands (27,948 acres), trust lands (93,454 acres), hundreds of reservation islands (4,841 acres) within the Penobscot River, and the Matagamon reservation land (24 acres) (Personal Communication, K. Peet). According to the Bureau of Indian Affairs, a *fee* is a form of land ownership status where citizens may freely alienate and encumber title without federal approval (Bureau of Indian Affairs, 2016). Land in *trust* status or restricted status is not held in fee, and is not subjected to state law (Bureau of Indian Affairs, 2016). Hunting primarily takes part on these tribal lands where citizens enjoy wildlife harvests separate from the jurisdiction of MDIFW (Personal Communication, K. Peet).

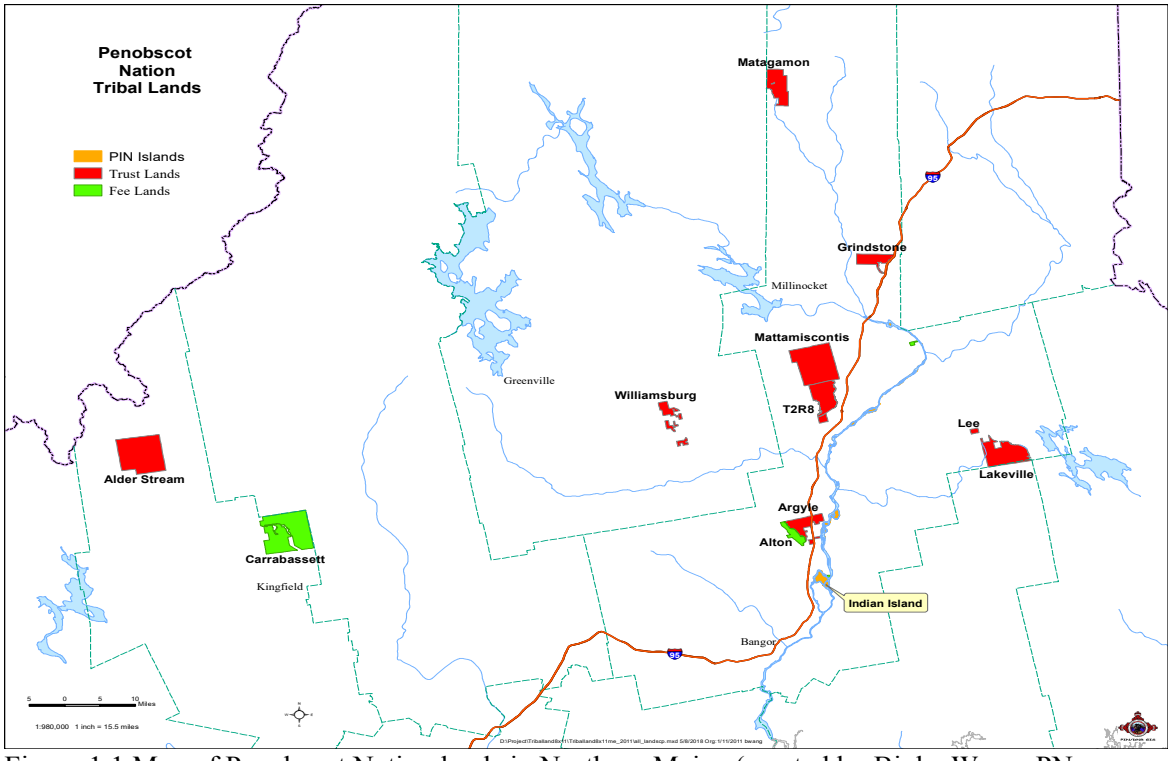

Figure 1.1 Map of Penobscot Nation lands in Northern Maine (created by Binke Wang, PN-DNR), listing the towns and unorganized territory nearby tribal lands. County borders are shown on the map.

1.3. Objective Risk—Wildlife Disease Risk

Risk, hereon referred to as objective risk, is defined as the probability and consequence of a given scenario (Kaplan & Garrick, 1981). *Objective risk* refers to biophysical measures of exposure and vulnerability to a threat (Kaplan & Garrick, 1981). Vulnerability is the extent to which one is exposed and susceptible to a threat with which they are unable to cope, the susceptibility level of exposure to a threat, or the state or fact of being likely or liable to be influenced or harmed by a particular threat (Swim et al., 2009).

Objective risk generally consists of quantifying three features: (1) the identification of some scenario, (2) how likely that scenario is to happen, and (3) the consequences of that scenario (Kaplan & Garrick, 1981). It is nearly impossible to produce accurate probabilities of disease risk (i.e. true risk), especially in systems that are difficult to measure, such as with wildlife. Further, according to Slovic (1987), "Risk in the wildlife context refers to the possibility that a wildlife event or interaction leads to negative outcomes for people of something people value" (p. 4) (Buttke, Decker, & Wild, 2015). One method to prioritize wildlife disease is an expert-based risk analysis (Ciliberti et al., 2014). In one such study, 92 experts graded various wildlife diseases with respect to their global importance for animal welfare, species conservation, trade/economic impacts, impacts to public health, pathogen variability, host specificity, potential for contagion, and speed of spread (R_0) (Ciliberti et al., 2014). The elicitation of scientific and technical judgments from experts can be a valuable addition to the calculation of objective risk, but criticism of this approach exsits due to qualitative uncertainty language and overconfidence of "experts" (Morgan, 2014). In the context of vector-borne disease, entomological risk is quantified as a proxy for disease risk by using multiple case-specific factors, such as the prevelance of some pathogen (Chapter 2), minimum infection rate (MIR) of pathogens in vectors

(Walter, Hildreth, Beaty, 1980), basic reproduction ratio (R_0) (Blackburn et al., 2019), vector competence (Bartholomay & Michel, 2018) and environmental factors (e.g., biodiversity or land cover; Johnson, Ostfeld, Keesing, 2015). However, risk factors are highly context dependent within the field of zoonotic diseases due to the complexity and the dependence on local biotic and abiotic factors that have been shown to influence host and vector populations (Braks, Mancini, de Swart, Goffredo, 2017). Therefore, studies on vector-borne disease are most effective when placed in specific context and one should avoid generalizations.

In this study, to measure *objective risk* from the impacts of winter ticks on moose, I estimated the prevalence of *Anaplasma spp.* infection in winter ticks and whole blood collected from several moose in Maine between 2016 and 2018 (Chapter 2; G1). Susceptibility to these diseases was then estimated by modeling the effects of the pathogen presence on moose health at the population and individual levels (Chapter 3; G2).

1.4 Risk Perceptions: Definitions and Theoretical Frameworks

Risk perceptions are mental constructs that refer to an individual's judgment about the severity of the risk based on their perceived vulnerability, knowledge and feelings about an issue (Siegrist, Gutscher, & Earle, 2005; Slovic, Monahan, & MacGregor, 2000). To some extent, perceived risk is a reflection of objective risk, especially when risks are well known (Sjöberg, 1995). However, a person's own estimate of risk (perceived risk) may be very different from the "objective" estimate (Boholm, 1996). Perceived risk seeks to measure attitudes, judgements, thoughts, feelings, and beliefs that an individual may have towards a particular hazard (Micic, 2016). Multiple researchers (Axelrod, Mcdaniels, & Slovic, 1999; Clarke, 2009; De Urioste-Stone, Le, Scaccia, & Wilkins, 2016; Kasperson et al., 1988; van der Linden, 2015) have studied

perceived risk to discover what people mean when they say that something is (or is not) "risky" and to determine what factors are predictive of those perceptions (Slovic, 1987). Understanding more about the factors that influence risk perceptions often improves the communication of risk information among technical experts and policy makers (Slovic, Fischhoff, & Lichtenstein, 1982). Risk perception research aims to develop a comprehensive theory that predicts how people respond to new hazards and management strategies in order to develop techniques for assessing the complex opinions that people have about risk (Slovic, Fischhoff, & Lichtenstein, 1982).

Researchers from multiple social science disciplines have sought to determine the factors that contribute to predicting risk perception (Mase, Cho, & Prokopy, 2015; Needham & Vaske, 2008), with various theoretical frameworks devised usually falling under two overarching traditions or approaches: (1) psychological models that measure cognitive factors (i.e. knowledge) and experience with a hazard (Milfont, 2012), and (2) sociocultural models that assess factors like cultural norms (Akerlof, Maibach, Fitzgerald, Cedeno, & Neuman, 2013) and values (Schwartz & Bilsky, 1987). The latter approach was influenced by tenets from cultural theory (Douglas & Wildavsky, 1983), which holds that there are four types of people: *egalitarian* (those concerned with technology and the environment), *individualistic* (those who are concerned with war and other threats to the markets), *hierarchic* (those concerned with law and order), and *fatalistic* (those concerned with none of the above) (Douglas & Wildavsky, 1983; Sjöberg, 2000). Unfortunately, multiple studies that have attempted to operationalize cultural theory constructs have not been able to explain more than 5–10% of the variance of perceived risk (Sjöberg, 2000), and other value scales have similarly failed (Triezenberg, Gore, Riley, & Lapinski, 2014). So while value scales may increase explanatory power in more comprehensive

models (Morgan, 2017; van der Linden, 2015), they provide low power on their own (Oltedal, Moen, Klempe, & Rundmo, 2004). The former approach, the psychometric model (Fischhoff, Slovic, Lichtenstein, Read, & Combs, 1978), has been used more extensively, although the explanatory value is still typically around 20% of the variance of raw data (Sjöberg, 2000). So while existing models frequently break risk perceptions into different dimensions (Douglas, 1982; Fischhoff et al., 1978), significant work still remains to generate a conceptual framework that incorporates key sociopsychological determinants that can help explain a substantial amount of the variance of risk perceptions (van der Linden, 2015).

One of the theoretical frameworks that have been used to organize different theoretical perspectives to measure risk perception is the Heuristic-Systematic Model (HSM) of information processing developed by Eagly and Chaiken (1993). The foundation for this theory is the idea that people who are asked to evaluate risk seldom have facts or scientific evidence in hand to systematically evaluate the risk (Slovic, Fischhoff, & Lichtenstein, 1981). Therefore, people must make inferences or decisions based on what they remember hearing or observing about the risk in question. There are a number of general inferential rules that people use in such situations; these rules, known as heuristics, are employed to reduce difficult mental tasks to simpler ones (Folkes, 1988). Early in the search for a comprehensive theoretical model to predict perceived risk, it was believed that heuristics were important constructs because they were thought to underlie many intuitive judgments under uncertainty (Gilovich, Griffin, & Kahneman, 2002), and uncertainty is always present with risk (Kaplan & Garrick, 1981). The HSM holds that individuals will use one or both modes of information processing--systematic or heuristic--when attempting to evaluate information in order to arrive at a judgment (Eagly & Chaiken, 1993).

Systematic processing is defined by careful analysis and comparison of information; whereas heuristic processing is defined by the use of cognitively available cues to navigate judgment quickly and easily (Eagly & Chaiken, 1993). Using the HSM model, Trumbo (2002) explained up to a third of the variance in people's risk perceptions to cancer, and similar results have been obtained in other studies (Trumbo, 1999; Kim & Paek, 2009). Thus, the HSM is considered a valuable tool for risk perception research. More recently, one study that implemented this framework found no significant relationship between the heuristic information processing and the degree of the perception of risk (Ryu & Kim, 2015), but heuristic processing has been shown to increase risk perceptions towards the likelihood of contracting an infectious disease in other instances (Choi, Yoo, Noh, & Park, 2017).

Another framework used to predict risk perceptions is the Zoonotic Disease Risk Information Seeking and Processing model (ZDRISP) (Clarke, 2009; Triezenberg et al., 2014). This framework aims at measuring risk perceptions by incorporating how people process, seek, select and process information about hazards. Risk information processing has been emphasized by some due to its potential to aid risk communicators design appropriate message interventions (Griffin, Dunwoody, & Neuwirth, 1999) that target how people process the risk of some hazard (e.g. poor diets or buckling seat belts) and what they do about it (behavior change). As described by Triezenberg et al (2014), the ZDRISP builds on the Risk Information Seeking and Processing (RISP) framework (Griffin et al., 1999) by incorporating two well-established constructs that have proven to influence risk perceptions: wildlife value orientations (Fulton, Manfredo, & Lipscomb, 1996) and personal values (Schwartz & Bilsky, 1987). Elements of the ZDRISP framework include (1) affective response (e.g., worry), (2) information sufficiency (current knowledge and information sufficiency threshold), (3) channel beliefs (affective response, trust,

and perceived similarity of ethics to mass media), information subjective norms (i.e., the pressure an individual perceived to stay informed), and (4) perceived information gathering capacity (i.e., the perceived accessibility and relevance of information about a source of risk) (Clarke, 2009). However, according to Triezenberg et al. (2014), it has been challenging to test ZDRISP empirically due to its complexity, having several constructs that require several scales. When the ZDRISP was tested in the context of bovine tuberculosis risk, Triezenberg et al. (2014) found that wildlife values (i.e., wildlife protection and wildlife use) were significantly correlated with descriptive and subjective norms about disease risks; these norms in turn were found to be negatively related to disease risk perceptions. However, Triezenberg et al. (2014) found no significant relationship between personal values and disease risk perceptions, as originally proposed for the ZDRISP (Clarke, 2009).

Recently, van der Linden (2015) developed a socio-psychological framework to study climate change risk perceptions. The Climate Change Risk Perceptions Model (CCRPM) integrated experiential processes (i.e., personal experience with climate change), cognitive factors (e.g., knowledge about climate change), socio-cultural influencers (i.e., value orientations and norms), and socio-demographic factors (e.g., political affiliation, gender, sex) as key sociocultural and psychological determinants of perceived risk towards the impacts of climate change (van der Linden, 2015). All of the aforementioned constructs were found to be significant predictors of climate change risk perception, and the full model accounted for 68% of the variance (van der Linden, 2015). In addition to climate change, multiple risk perception theoretical frameworks have been widely used to describe other social-ecological systems including wildlife diseases (Needham & Vaske, 2008) and natural hazards (Axelrod, Mcdaniels, & Slovic, 1999).

This study uses a modified version of the CCRPM (Figure 1.2) to measure risk perceptions towards the impacts from (1) winter ticks, (2) all types of ticks, and (3) a decline in moose on human-natural systems (personal, Penobscot Nation, and the environment). I selected the following constructs as determinants of risk perception: cognitive factors (i.e., cause, impact, response), experiential processing (i.e., affect and personal experience), sociocultural variables (i.e., descriptive and prescriptive norms), socio-demographic variables (i.e. ethnicity, education, age and gender).

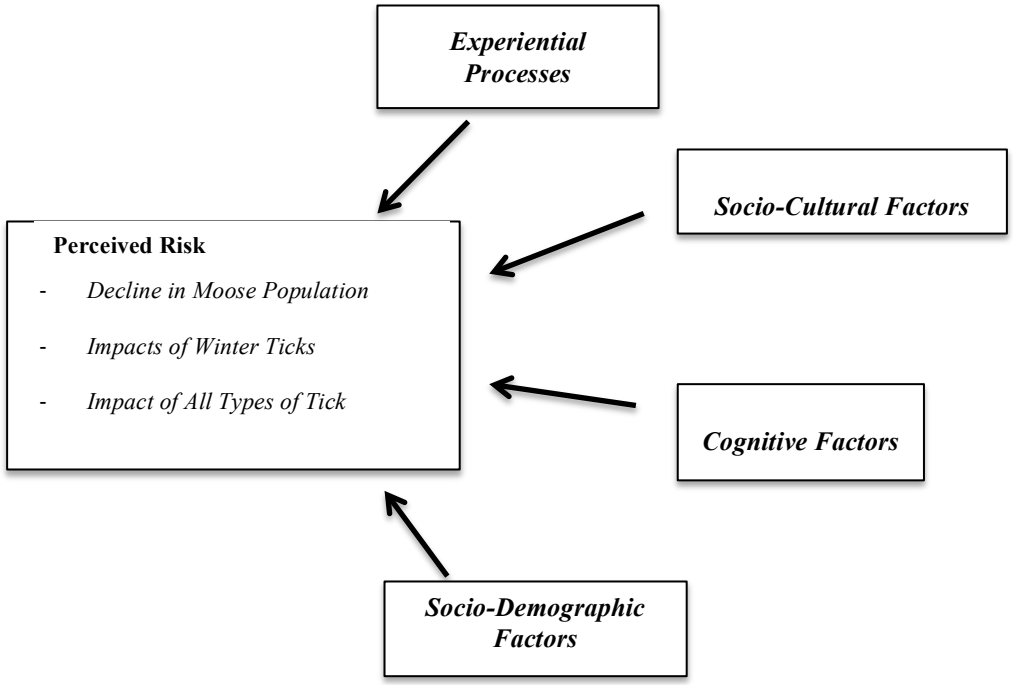

Figure 1.2 Risk perceptions model modified from van der Linden 2015 CCRPM.

1.4.1 Cognitive Factors of Risk Perception

Cognitive factors, such as general knowledge of a hazard, have been shown to significantly alter the perceptions of individuals to environmental risk (Pidgeon, 2012). Research indicates that the amount of knowledge one has correlates with risk perceptions (Helgeson, van der Linden, & Chabay, 2012; van der Linden, 2015). However, knowledge is a fairly complex construct (Charles et al., 2013) with research showing mixed results as to the influence that cognitive factors have on risk perceptions—in some cases, researchers have found a negative relationship exists between risk perceptions and knowledge (Kellstedt, Zahran, & Vedlitz, 2008; Rolison & Hanoch, 2015) whereas the relationship is positive in other studies. For example, when investigating the influence of knowledge of a deadly virus (Ebola) on people's perceived risk of the virus, respondents who were more knowledgeable of Ebola perceived less risk of contracting the virus, but also regarded the virus as more serious than less knowledgeable respondents (Rolison & Hanoch, 2015). Further, in a survey of residents in multiple districts of Connecticut, USA, individuals that responded as being knowledgeable about Lyme disease felt they had a high likelihood of contracting Lyme disease (Gould et al., 2008).

It has been argued that the varying conclusions on the influence knowledge has on risk perceptions, and the direction of the association, could be due to a lack in conceptual distinction between different types of knowledge (Kaiser & Fuhrer, 2003). To mitigate the multi-dimensionality of knowledge, a more reliable assessment of knowledge has been proposed by measuring different dimensions of knowledge: the *impact*, *causal* and *response* knowledge. For these different knowledge scales, van der Linden (2015) defined causal knowledge as the knowledge an individual has on whether or not some potential hazard is a source of risk; *impact* knowledge as the knowledge an individual has on whether or not some potential hazard would

have on an increase, decrease or no change to risk; *response* knowledge is how much a behavior (e.g., management strategy) is likely to reduce risk if used. Although it was shown that *impact*, *causal* and *response* knowledge are positively and significantly correlated with risk perceptions to climate change, *causal* knowledge contributed less to the explained variance than either *impact* and *response* knowledge. Moreover, the influence of *impact*, *causal* and *response* knowledge on *personalized* risk perceptions (as opposed to *societal* risk perceptions) was shown to be negligible (van der Linden, 2015).

1.4.2 Experiential Processes and Risk Perception

Experiential processes include personal experiences and the emotion attached to those experiences (i.e., positive and negative affect) that may play a role in influencing perceived risk (Slovic & Peters, 2006). The risk information and seeking model suggests that affective response mediates the influence of experiences on perceived hazards, arguing that how we process experiences—and the emotions associated with those experiences—is highly related to perceptions of risk (Clarke, 2009). In the literature, it has been widely recognized that human information processing is guided by emotion that result from personal experiences (van der Linden, 2014). Early studies of risk perception showed that a feeling of dread was a major determinant of public perception of risk (Fischhoff et al., 1978). It was later determined that a person's general affective evaluation of a threat was the major predictor of perceived risk (Alhakami & Slovic, 1994). More recently, multiple studies have found that operationalizing experiential processes greatly influenced risk perceptions, explaining 47-68% of the variance in perceived risk (Akerlof et al., 2013; Leiserowitz, 2005; Leiserowitz, 2006; Smith & Leiserowitz, 2012; van der Linden, 2015). There are exceptions to this relationship though, and the influence

of affect is case dependent (Jepson & Chaiken, 1990). For example, it was found that individuals that have direct experiences with flooding events did not differ significantly in their responses to the attitude statements about uncertainty and skepticism in relation to climate change (Whitmarsh, 2008).

1.4.3 Socio-Cultural Factors and Risk Perception

Socio-cultural factors include a variety of constructs to measure norms and values. Normative beliefs (norms) are defined as the "expectations of how people are supposed to act, think or feel in specific situations" (Poponoe, 1983). Norms greatly influence risk perceptions, and have even been attributed as a primary predictor of behavior (Heberlein, 2012).

Further, cultural theory has provided a foundation for capturing cultural differences in risk perception, which originally used scales measuring the four concepts illustrated by the types noted previously and variously termed "cultural biases" or "cultural worldviews" (Douglas, 1982; Sjöberg, 2000). Recent studies have shown that "cultural worldviews" and climate change risk perceptions are positively correlated (Akerlof et al., 2013). However, as with other constructs used to predict risk perceptions, cultural theory and values associated are said to be difficult to operationalize. Critics of cultural theory—who mostly come from a psychological approach to risk perception—argue that these constructs have low explanatory power (Oltedal et al., 2004; Sjöberg, 2000).

Standardized scales, like the "Personal Norms" scale, have been developed to measure participants' views regarding the environmental obligations of individuals society, based on the principals of norm activation theory (Schwartz, 1977). Norm activation theory describes the circumstances under which personal norms are likely to be *activated*, particularly in the context

of altruistic behaviors (Stern 2018). However, this theory has been applied to the measure of risk perception as well. When surveying members of stakeholder groups using the Personal Norms scale to investigate variability in ecological risk perception, a significant relationship was found between norms and ecological risk perceptions (Willis & DeKay, 2007). Even with these advances from cultural theory, there is still difficulty in measuring norms because specificity of context is of great importance (Zinn, Manfredo, Vaske, & Wittmann, 1998). Normative beliefs do not occur in isolation, rather, they are influenced by situational variables, attitudes, and values (Knight, 2008). Noting the importance of context, it is difficult to accurately predict the influence that norms might have on risk perception. Conversely, in a recent application of the ZDRISP, a significant negative relationship was identified between norms and disease management risks. Perceptions of disease management risks are low when hunters perceive that others are taking action or want hunters to take action, and because norms are significant for disease risk and management perceptions, linking norms to the disease and its management may be an essential component of effective wildlife disease management and modeling risk perceptions of zoonotic diseases (Triezenberg et al., 2014)

1.4.4 Socio-Demographic Factors and Risk Perception

Although socio-demographic factors have been used previously as a control to assess the influence of other constructs (van der Linden, 2015), having a relationship between attitudes and demographics would better inform which groups in society may sense a greater risk for wildlife health or societal wellbeing (Decker et al., 2012). While multiple studies have found no significant relationship between demographics and risk perception (Sjöberg, 2000), factors such as age (Macias, 2016), political affiliation (Leiserowitz, 2006), socio-economic status (Slimak &

Dietz, 2006), education (van der Linden, 2015), and gender (Finucane, Slovic, Mertz, Flynn, & Satterfield, 2000) have all been associated with an increase in risk perceptions. The dominant pattern for socio-demographic differences in risk perception research has been that there are greater perceived risks among non-whites than whites (Macias, 2016), particularly in females (Olofsson & Rashid, 2011). Further, income has an inverse relationship with risk perceptions (Slimak & Dietz, 2006), a finding supported by Flynn, Slovic, and Mertz (1994), but contradicted by Lazo, Kinnell, and Fisher (2000). Gender is another widely demonstrated factor related to risk perception (Slovic, 1999), with men tending to judge risks to be less problematic than women (Davidson & Freudenburg, 1996). As previously mentioned, in some cases, the socio-demographic and risk perception relationships can interact with other constructs. For example, in the context of climate change risk perceptions, Kellstedt et al. (2008) found a negative relationship between how much somebody knows about climate change and their perceived risk towards the impacts of climate change. However, when the study population is exclusive to a particular demographic (i.e., liberal political affiliation), the amount of knowledge about climate change only amplified the perceived risk from the impacts of climate change (Kellstedt et al., 2008). Therefore, most studies incorporate socio-demographic variables to control for any interaction or mediation effects (van der Linden, 2015)

1.5 Organization of Thesis

The overarching purpose of this thesis was to incorporate biological and social science data to identify possible gaps and connections between objective and perceived risk to wildlife and human systems in relation to moose-winter tick-human interactions. This information could potentially enhance future communication efforts by management agencies such as MDIFW and

the DNR of Penobscot Nation on the topic of moose and winter ticks, and its connection with human systems. Understanding risk perceptions is crucial for effective communication and outreach to close the gap between objective and perceived risk in order to maintain support for decisions surrounding moose management. Two dimensions of research will be introduced: the biological science (Chapter 2 & 3) and social science (Chapter 4). More specifically, Chapter 2 introduces a novel species of *Anaplasma* (referred to as *Anaplasma spp*. Cervus) found in the Eastern moose and presents a phylogenetic reconstruction that identifies the taxonomic placement of this bacterial species. Chapter 3 then discusses the potential implications of the novel bacteria within a more comprehensive assessment of survival and causes of winter mortality in Maine moose calves, thus describing the objective risk of disease to the moose population in Maine. Chapter 4 presents the results of a questionnaire administered to a Native American population in Maine (i.e., Penobscot Nation citizens) that uses a theoretical framework to measure risk perceptions to wildlife and human systems. The focus of this questionnaire was to measure the experience processes of participants with moose and winter ticks, cognitive factors associated with winter ticks and moose, normative beliefs about the concern for the moose population, and socio-demographic factors in order to determine participants' risk perceptions associated with a decline in the moose population, impacts of winter ticks (the primary parasite of moose) and all types of ticks on moose and human systems. The concluding chapter (Chapter 5) will provide a review that highlights the importance of integrating the natural and human dimensions in wildlife tick-borne disease research.

CHAPTER 2

MOLECULAR ANALYSIS OF A NOVEL *ANAPLASMA* SPECIES IN EASTERN MOOSE (*ALCES ALCES AMERICANA*) AND WINTER TICKS (*DERMACENTOR ALBIPICTUS*) IN MAINE, UNITED STATES

2.1 Chapter Summary

Eastern moose (*Alces alces americana,* Linnaeus, 1758) are heavily parasitized by winter ticks (*Dermacentor albipictus;* Packard 1869), the dominant cause of increased calf mortality in the northeastern United States. Blood loss from heavy infestations of winter ticks on moose is associated with anemia, reduced feeding, hair loss, and body mass depletion. It is unknown whether or not tick-borne disease also plays a significant role in the health of Maine moose. I explored the role that moose and winter may have in maintaining tick borne disease by: (1) estimating prevalence and (2) determining phylogenetic placement of *Anaplasma* spp. in moose and winter ticks with respect to *Anaplsama* spp. found in other hosts and vectors. As a part of a larger study investigating the general health of moose, 157 moose (142 calves, 15 adults; 57% female) were captured in western (n = 83) and northern (n = 74) Maine study areas between 2016 and 2018. Using whole blood samples from moose, I screened for *Anaplasma* spp. using a genus-specific PCR-based assay to amplify and sequence a region of the *Anaplasma* 16S rRNA gene. Over half (54%) of the moose tested positive for *Anaplasma*. There was a significant difference between the proportions of *Anaplasma*-positive moose in the western (67%) and northern study areas (38%). Male moose also exhibited a higher prevalence than females (63% vs. 47%). *Anaplasma* was also detected in winter ticks, but in a single pooled sample (<1%). The Bayesian

phylogenetic analysis revealed that the single *Anaplasma* strain in moose was highly divergent from the strain identified in winter ticks, and is most closely related to an uncharacterized North American cervid strain. Based on these data, I conclude that moose are carriers of a newly identified *Anaplasma* spp., but found no evidence for a significant role of winter ticks in *Anaplasma* transmission.

2.2 Introduction

Anaplasma bacteria are among several vector-borne pathogens that are emerging in the northeastern United States (Dumler *et al.* 2005). With the growing threat to public and wildlife health, there has been an increased surveillance for *Anaplasma phagocytophilum,* in particular, which is the disease causing agent of human granulocytic anaplasmosis (HGA) (formerly human granulocytic ehrlichiosis or HGE; Rikihisa 2011). Transmission of *Anaplasma* among vertebrate hosts is vector-borne and occurs in two main ways: biologically, involving replication of the bacteria within ticks (e.g., *A. phagocytophilum*), and less frequently, mechanically by biting flies or by blood-contaminated fomites (e.g., *A. marginale*). In addition, transplacental transmission of *A. marginale* to the calf fetus has been reported in beef cattle (Zaugg 1985; Rey *et al.* 2003; Grau *et al.* 2013). Multiple genetically distinct species of *Anaplasma* have been found recently with unknown pathogenicity in wildlife and humans (Lobanov *et al.* 2012; Hailemariam *et al.* 2017).

Even though *Anaplasma* spp. are known to infect wildlife and humans, knowledge regarding the epidemiology and occurrence of *Anaplasma* spp. within wildlife in the northeastern United States is remarkably scarce (Rikihisa 2011; Stuen *et al.* 2013). *Anaplasma* spp. was recently detected in Eastern moose from New Hampshire, USA where a high prevalence of *Anaplasma* serologically-positive moose (80%) was detected, but was not investigated further

due to a lack of correlation with selected health metrics and inconclusive identification (Jones 2016). Eurasian moose (*Alces alces alces)* are known to carry *A. phagocytophilum* at a prevalence up to 82%, and the bacteria has been specifically identified as a moose pathogen having implications for both humans and animal health (Jenkins, Handeland *et al.* 2001; Malmsten *et al.* 2014; Malmsten *et al.* 2018). In Europe, the primary vectors of pathogenic *Anaplasma* spp. are ticks (i.e. *Ixodes ricinus,* Linnaeus, 1758) and occasionally deer ked (*Lipoptena cervi,* Linnaeus, 1758) (Malmsten *et al.* 2018). The tick vector *I. ricinus* is not present in the northeastern United States, and it is unknown what effect *L. cervi* have on the health of populations of moose reported in Vermont, USA (C. Alexander, personal communication), however the primary ectoparasite of moose in the northeastern United States, the winter tick (*Dermacentor albipictus*), is abundant and may be capable of vectoring pathogens that are either acquired from the environment, transstadially, or transovarially. I tested the possibility for winter ticks to carry tick-borne pathogens examining the prevalence of *Anaplasma* spp. in winter ticks collected from moose in Maine.

The winter tick is a species of hard tick that has several hosts in its native range of North America and is thought unlikely to act as a disease vector due to its one-host, one-year life cycle, both of which are characteristics of a poor disease vector (Samuel 2004). Conversely, evidence suggests that transovarial transmission of *A. phagocytophilum* variants occurs in winter ticks (Baldridge *et al.* 2009), and it has been suggested winter ticks are competent vectors of *A. marginale* (Stiller *et al.* 1983). It is thought that anemia and metabolic stress from winter tick parasitism is the major cause of some declining moose populations in the northeastern United States (Jones *et al.* 2019), however, pathogens such as *Anaplasma* spp. could have an additive role in deciding the fate of compromised moose calves. These pathogens and their effects on

moose have received less attention in the northeastern United States when compared to the effects of blood loss from winter tick infestation, the primary cause of moose mortality in the northeast range (Jones *et al.* 2019). Therefore, in this project I explored the role that moose and winter may have in maintaining tick borne disease by: (1) estimating prevalence and (2) determining phylogenetic placement of *Anaplasma* spp. in moose and winter ticks with respect to *Anaplsama* spp. found in other hosts and vectors.

2.3 Materials and Methods

2.3.1 Specimen Acquisition and Study Area

Moose blood specimens (n = 157) were collected during 2017 and 2018 by the Maine Department of Inland Fisheries and Wildlife (MDIFW) as part of a study to assess adult cow and calf moose survival in Maine. Whole blood from a total of 157 moose (15 adults, 142 calves; 57% female) was used for detection of *Anaplasma* spp. Additionally, 82 winter ticks were collected from moose during MDIFW captures spanning 2014-2018. Both moose and winter tick sampling locations fell within Maine's Wildlife Management Districts 2 and 8 (Figure 2.1).

Maine Medical Center Research Institutes' Vector-Borne Disease Lab provided an additional 162 ticks from hunter-harvested moose. Winter tick specimens from the MMCRI were from several unknown locations across Maine. Thirty larval winter ticks (n = 30) and six blacklegged ticks (*Ixodes scapularis,* Say, 1821) were also donated from the University of Maine Cooperative Extension Tick Identification Lab. Blacklegged ticks are known vectors of *A. phagocytophilum*, so these were obtained to serve as comparative sequences in the phylogenetic reconstruction of *Anaplasma*. Tissue from a total of 274 (30 larvae, 154 nymphs, 88 adults, 2 unknown) winter ticks were screened for *Anaplasma* spp. infections.

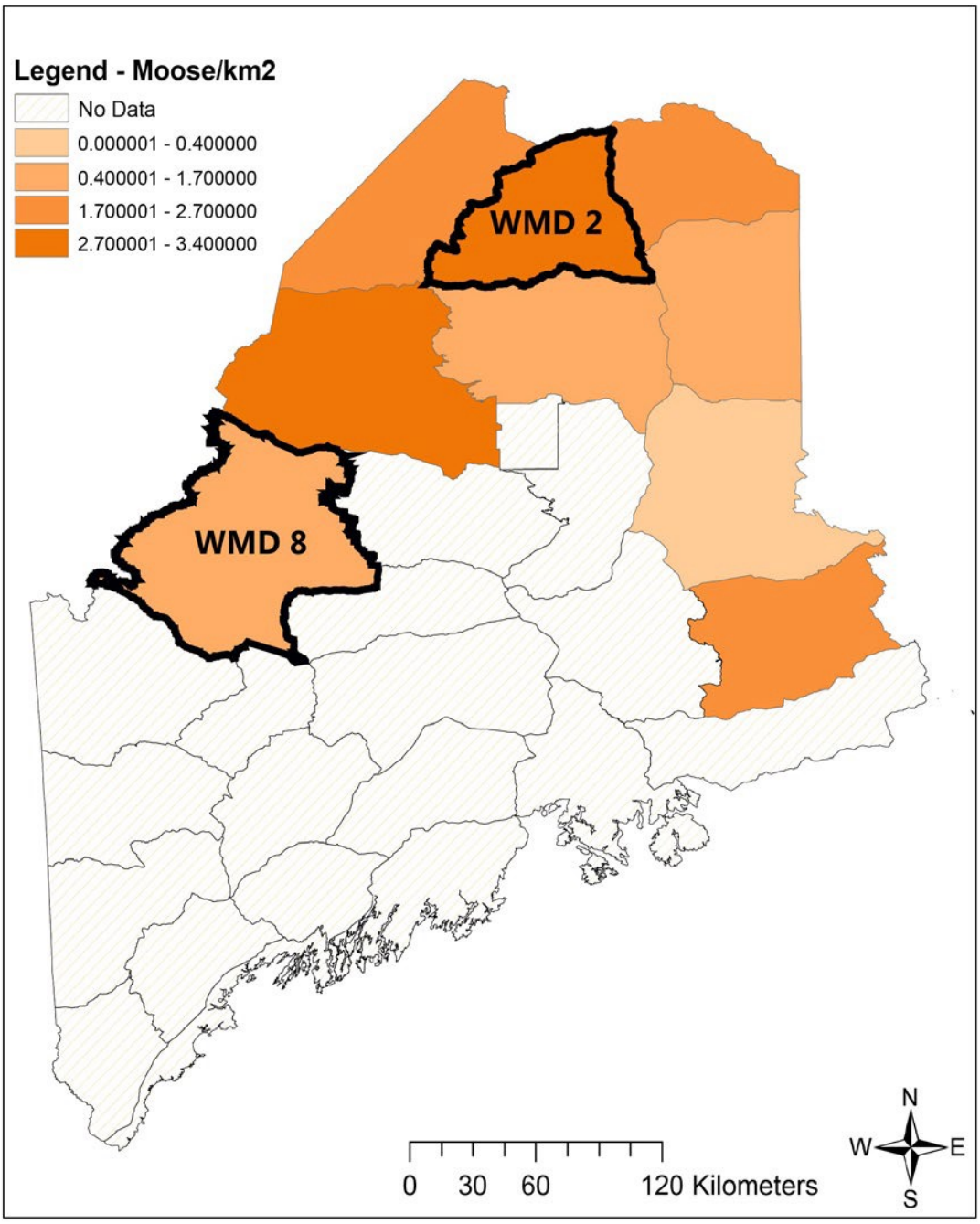

Figure 2.1 Map showing the western (WMD 8) and northern (WMD 2) moose study areas. Estimated moose population density shown by gradient and based on date from Kantar and Cumberland (2013)

2.3.2 DNA Extraction and Specimen Processing

Genomic DNA was extracted from moose whole blood and winter ticks using the Qiagen DNeasy protocol (Valencia, CA), and all extractions were checked for purity based on examination of 260/280nm ratios. It is important to note that most of the ticks from which DNA was extracted were not engorged (98%), as engorged female ticks tend to have high concentrations of DNA and inhibitors that can interfere with polymerase chain reaction (PCR) amplification (C. Lubelczyk, personal communication). All nymphal and adult tick samples originating from the same moose were pooled, with one to five ticks per extraction. Pooling of ticks was done to (1) increase total DNA concentration prior to PCR, (2) maximize the cost efficiency to allow for an increased sample size of winter ticks screened, (3) increase the probability of detecting low prevalence infections, and (4) account for correlated infections in winter ticks collected from a single moose. Winter tick larvae were also tested to assess the potential of transovarial transmission. Larval specimens all originated from the same clutch in Jackman, Maine, so were pooled into one sample for the sake of efficiency. In preparation for downstream processing, all extractions were standardized to a DNA concentration of < 25ng/uL.

2.3.3 PCR Amplification, Electrophoresis, and 16S rRNA Sequencing

Genomic DNA was extracted from tissue samples using a modified Qiagen DNeasy extraction kit protocol (Valencia, CA), with the proteinase K incubation step extended to approximately 12-24 hours. I amplified, through nested polymerase chain reaction (nested PCR), a partial sequence of the *Anaplasma* species' 16S rRNA gene, as described previously by Barlough *et al.* (1996). For ticks, the mitochondrial COI (mtCOI) region was amplified and

sequenced as described by Herbert *et al.* (2003) as a control for tick species identification (Table 2.1).

PCR amplifications for *Anaplasma* testing were carried out in a total volume of 25 μL and contained 2 uL of template DNA (standardized at < 25 ng/uL), 5 μL of 5 × PCR buffer (Promega 5X buffer), 200 μM deoxynucleotide triphosphates (dNTPs, New England BioLabs, Ipswich, MA), 0.5U Promega GoTaq DNA polymerase (Applied Biosystems, Foster City, CA), and 0.4 μM of each primer (EE-1 and EE-2). The second reaction used the same reagents as specified above, with the exception of the nested primers (EE-3 and EE-4), and used 2uL of the amplified product from the first reaction as a template. Thermocycling conditions for the first, outer reaction were: an initial denaturation at 94 °C for 4 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 74 °C for 1.5 min; final extension at 74 °C for 10 min. Thermocycling conditions for the second, inner reaction were: an initial denaturation at 95 °C for 2 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 5 min.

PCR amplifications for the mtCOI tick control region utilized the same reagents and concentrations, except using a touchdown PCR protocol for cycling conditions. Thermocycling conditions were: an initial denaturation at 94 °C for 1 min; 5 cycles of 94 °C for 1 min, 45 °C for 1.5 min, and 72 °C for 1.5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min, and 72 °C for 1 min; final extension at 72 °C for 5 min. All PCRs were performed using an Eppendorf or BioRad thermocycler.

Table 2.1 List of oligonucleotide primers in 5' to 3' orientation

Primer	*Amplicon Size*	*Sequence*	*Source*
EE-1	*~1400bp*	5'-TCCTGGCTCAGAACGAACGCTGGCGGC-3'	Barlough *et al.* (1996)
EE-2		5'-AGTCACTGACCCAACCTTAAATGGCTG-3'	
EE-3	*~900bp*	5'-GTCGAACGGAT TATTCTTTATAGCTTGC-3'	
EE-4		5'-CCCTTCCGTTAAGAAGGATCTAA TCTCC-3'	
LCO149	*~600bp*	5'-GGTCAACAAATCATAAAGATATTGG -3'	Herbert *et al.* (2003)
HC02198		5'-TAAACTTCAGGGTGACCAAAAAATCA -3'	

Each reaction was held at 4 °C until the reaction was qualified by gel electrophoresis, using a 1-2% Agarose gel in standard 0.5X TBE buffer. Upon confirmation of pathogen presence, PCR products were purified using the Illustra ExoProStar (GE) and sent to the University of Maine Sequencing Facility for sequencing on an ABI 3730 sequencer. All individual sequences were manually edited prior to alignment. Sequence data were compared and aligned against the nucleotide collection in GenBank (NCBI, https://www.ncbi.nlm.nih.gov/genbank/) using the BLAST (basic local alignment search tool) search (completed on February 28th, 2019) for taxonomic identification using the MUSCLE alignment available in the *Geneious* software, v. 11 (https://www.geneious.com) (Benson et al., 2005; see Table 2.2 for all sequences used in analysis).

Table 2.2 Associated metadata for all 16S rRNA partial gene sequences used in this study. Taxonomic identification, host species derived from, geographic origin, and NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) accession numbers. *Anaplasma* sp. denotes an uncharacterized strain type.

Taxa ID	**Host Species**	**Origin**	**Accession Number**
Anaplasma sp.	*Alces alces americana*	Maine, USA	TBD
Anaplasma sp.	*Dermacentor albipictus*	Maine, USA	TBD
Anaplasma sp.	*Ixodes scapularis*	Maine, USA	TBD
Anaplasma sp.	*Odocoileus heminous*	British Columbia, Canada	JN673772
Anaplasma sp.	*Odocoileus virginianus*	British Columbia, Canada	JN673768
Anaplasma sp. Saso	*Bos taurus*	Illubabor zone, Ethiopia	KY924885
Anaplasma sp. Dedessa	*Bos taurus*	Illubabor zone, Ethiopia	KY924886
Anaplasma phagocytophilum	*Homo sapiens*	Connecticut, USA	KT454992
Anaplasma phagocytophilum	*Ixodes scapularis*	Connecticut, USA	EF123258
Anaplasma phagocytophilum	*Ixodes scapularis*	Saskatoon, SK, Canada	HG916767
Anaplasma phagocytophilum	*Ixodes ricinus*	Warsaw, Poland	MH122891
Anaplasma phagocytophilum (1)	*Alces alces alces*	Norway	KT070819
Anaplasma phagocytophilum (2)	*Alces alces alces*	Norway	KT070822
Anaplasma phagocytophilum (1)	*Alces alces alces*	Sweden	KC800983
Anaplasma phagocytophilum (2)	*Alces alces alces*	Sweden	KC800985
Anaplasma bovis	*Bos taurus*	India	MH244925
Anaplasma bovis	*Lepus sylvaticus*	Massachusetts, USA	AY144729
Anaplasma centrale	*Bos taurus*	Southern Italy	EF520690
Anaplasma marginale	NA	Florida, USA	AF309867
Anaplasma ovis	*Ovis aries*	China	AY262124
Anaplasma platys	*Canis familiaris*	Southern Italy	EU439943
Anaplasma platys	*Canis familiaris*	India	KT982643
Rickettsia rickettsii	NA	NA	L36217
Neorickettsia sennetsu	NA	NA	M73225
Ehrlichia chaffeensis	NA	NA	M73222
Ehrlichia ewingii	NA	NA	L36217

2.3.4 Phylogenetic Analyses

Phylogenetic analyses were conducted on the completed aligned sequence data set using a Bayesian-based Markov Chain Monte Carlo (MCMC) approach, as implemented in MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001) via the *Geneious* software, v. 11 (https://www.geneious.com). *Rickettsia rickettsii* was used as the out-group to root the tree (Lobanov *et al.* 2012). Prior to running the model, the best-fit nucleotide substitution model was selected by examining likelihood scores calculated for 24 hierarchical substitution models, and applying the Bayes information criteria (BIC), in jModelTest v.2 (Guindon and Gascuel 2003; Darriba *et al.* 2012). Phylogenetic reconstruction was carried out by performing two independent runs, using four chains per run. Each analysis ran for 1,100,000 generations, sampling every 200 generations, and a burn-in of 110,000 generations was used. Convergence and stationarity of runs was assessed using Tracer v. 11 (https://www.geneious.com) by examining trace outputs, standard deviations of the split frequencies between runs, potential scale reduction factors and effective sample size (ESS) for the estimated parameters.

2.3.5 Statistical Analyses

Contingency analyses (Chi square test) for testing differences in infection prevalence and other binary variables (sex, study area) were performed using program R (v 3.2.2, Vienna, Austria). Prevalence by age (calf, adult) was assessed, but not tested for significance due to the much lower sample size of adult versus calf moose. The Wilson score interval (Wilson 1927) was calculated to provide confidence limits for the proportion of infected moose overall, and within each sex and district for a specified level of 95% confidence. For winter ticks, maximum likelihood methods were used to estimate the prevalence of *Anaplasma* spp. in the pooled

samples as described by Williams and Moffitt (2001). Both the Wilson score interval and the pooled prevalence for the variable winter tick pool sizes were calculated using the Epitools epidemiological calculators (http://epitools.ausvet.com.au/).

2.4 Results

2.4.1 Prevalence of Anaplasma Species in Moose

Over half (84 out of 157; 54%) of the moose tested positive for *Anaplasma* using the PCR-based assay. There was a significant difference between the proportions of *Anaplasma*-positive moose in district 8 (67%) versus district 2 (38%) ($p < 0.001$), and male moose also exhibited a higher prevalence versus females (63% vs. 47%, $p = 0.055$), (Figure 2.2). Calves exhibited a higher prevalence of infection (80/142, 56%) when compared to adults (4/15, 27%), but there was insufficient sample size to test for significance. Of the 84 *Anaplasma*-positive moose, there was only one unique bacterial sequence. A BLAST search using the single consensus sequence showed that the most similar sequence available on GenBank was only 95% identical, and all of the matches above a 91% threshold were uncharacterized *Anaplasma* spp.

All the winter ticks used in this study were identified using a BLAST search with the amplified mtCOI sequence (data not shown). Only one of 274 winter ticks (<1%) tested positive for *Anaplasma* using the same PCR-based assay. Specifically, the estimated prevalence for the variable pool size was 0.40% with a 95% CI of 0.02%-1.5%. The pooled sample that tested positive represented two adult winter ticks (1 male, 1 female) collected from the same moose. The adult winter ticks from the *Anaplasma*-positive pooled sample were obtained in January 2017 from a female moose calf in WMD 2 (northern study area) with a reported heavy winter tick load; however, that same female moose did not test positive for *Anaplasma* and survived the

following winter. The BLAST search showed the highest similarity (98.5%) to an uncharacterized *A. phagocytophilum* (Ap-variant-1) 16S rRNA sequence amplified from *Ixodes scapularis*.

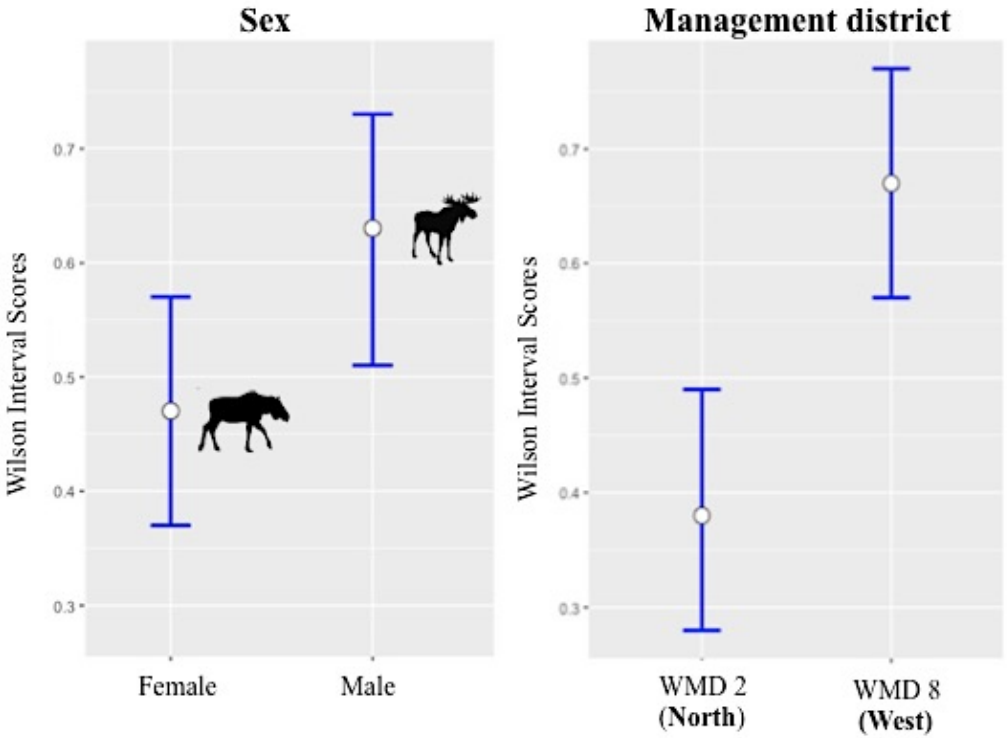

Figure 2.2 The proportion of infection and Wilson interval scores (y-axis) for each group of moose (x-axis).

2.4.2 Bayesian Phylogenetic Analysis

Based on the Bayes Information Criteria (BIC) model selection results, the Hasegawa-Kishino-Yano (HKY I+G) model was identified as the best-fit model and included as a prior for nucleotide substitution, which assumes variable base frequencies, one transition rate and one transversion rate (Hasegawa *et al.* 1985). Topology and convergence statistics were consistent across the two independent runs. The model placed the *Anaplasma* strain found in moose into a clade (posterior probability, PP = 1) with other uncharacterized *Anaplasma* spp. (Figure 2.3).

This clade shared a most recent common ancestor with an uncharacterized *Anaplasma* spp. found in cattle (*Bos taurus*) from the Illubabor zone, Ethiopia. Within the clade of uncharacterized *Anaplasma* species are sequences sourced from other ungulates, specifically *Anaplasma* sp. Saso (KY924885) found in cattle (Hailemariam *et al.* 2017), and two *Anaplasma* sp. found in white-tailed deer (*Odocoileus virginianus*; Zimmermann, 1780; JN673768) and mule deer (*Odocoileus hemionus*; Rafinesque, 1817; JN673772) in British Columbia, Canada (Lobanov *et al.* 2012). My phylogenetic model suggests that this clade of uncharacterized *Anaplasma* spp. share a more distant common ancestor with *A. marginale*, *A. centrale*, and *A. ovis*.

The high posterior probability for the *A. platys* cluster suggests that the two *A. platys* sequences are related to each other; however, the more ancestral node that represents the common ancestor to *A. platys* and *A. phagocytophilum* has weak support (PP=0.64), suggesting that the relationships within this cluster cannot be resolved and placement of the taxa within this cluster is uncertain. The unknown *Anaplasma* spp. sourced from the winter tick clustered with all *A. phagocytophilum* sequences, including strains sourced from humans (KT454992), European moose (KT070819. KT070822, KC800983, KC800985) and blacklegged tick from Maine, USA. The *Anaplasma* strains found within both winter and black-legged tick were closely related, sharing homology with the cluster of *A. phagocytophilum;* these tick strains were also highly divergent from those found in moose and other North American cervid species.

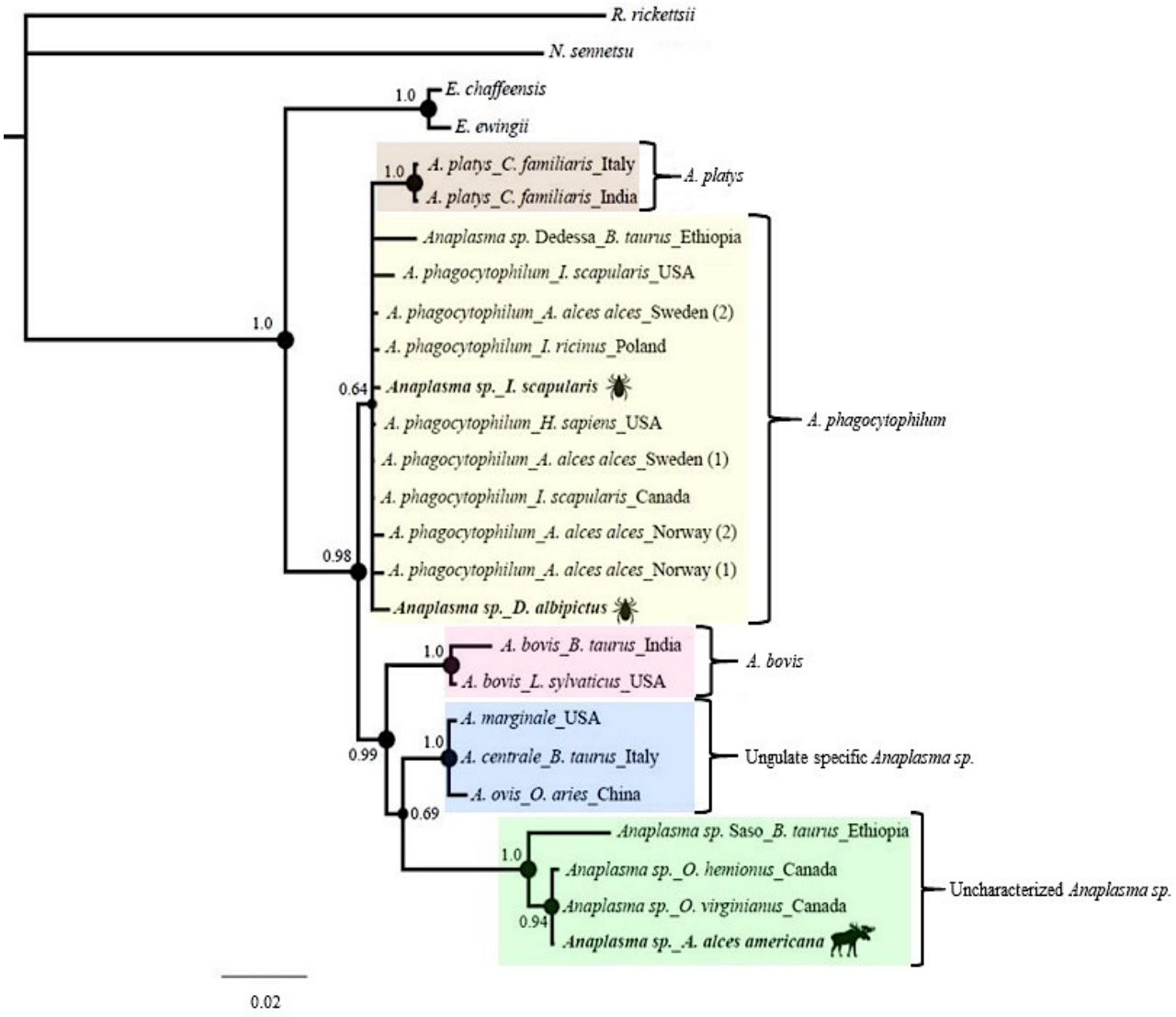

Figure 2.3 *Anaplasma* consensus tree based on 16S rRNA partial gene sequences. Additional sequences were obtained from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The naming convention used for each sequence is as follows: "taxonomic identification_host species (if applicable)_geographic origin". A taxonomic identification of *Anaplasma* sp. denotes an uncharacterized strain type.

2.5 Discussion

I found evidence for *Anaplasma* spp. infecting the majority of moose and a single pooled sample of winter tick in Maine. The *Anaplasma* phylogeny (Figure 2.3) revealed that the single strain found in moose was highly divergent from those identified in both winter and blacklegged ticks, and most closely related to an uncharacterized North American *Anaplasma* strain derived from other cervids. That strain had a surprisingly high prevalence (54%). Male moose had higher infection prevalence than females, but with questionable statistical significance. It is estimated that different sex would have the same exposure, so there is little evidence to suspect a different in infection prevalence between male and female moose. Moose in WMD 8 had a significantly higher prevalence of *Anaplasma* than WMD 2, potentially due to the differences in the density of moose or some vector, which could increase the exposure to the disease. Although there was insufficient sample size to test for a difference between adult and calf infection rate, far more calves in this study were *Anaplasma*-positive (80/142, 56%) than adults (4/15, 27%). The discrepancy in infection status between calves and adults could be due to adults having increased time to clear an infection.

In contrast to the high proportion of moose infected, prevalence of *Anaplasma* in winter tick was extremely low (<1%) and the strain most closely resembled *A. phagocytophilum,* the responsible agent for HGA. I speculate that this may have occurred as a result of a rare event in which a winter tick was groomed off of an *A. phagocytophilum*-competent reservoir host and then reattached to a moose. I conclude that the *Anaplasma* species found in both the blacklegged tick and the winter tick are *A. phagocytophilum*, and because the same species found in moose was not present in any of the ticks tested, winter and blacklegged tick are unlikely vectors for the cervid-specific *Anaplasma* spp. identified in this study. Therefore, further investigation is

necessary to identify and describe the vertebrate host range, pathogenicity, and transmission vector of the *Anaplasma* spp. found on moose in Maine.

While the findings from this study are compelling and novel, some limitations should be acknowledged. First, the pooling of ticks from the same moose affects my ability to calculate exact proportions of ticks that were positive; but, due to the low number of positive samples, this was not deemed a significant limitation. Second, the imperfect geographic scope of the samples does not allow us to generalize to the prevalence of infection in other moose populations in Maine as well as adjacent states and provinces. Many of these jurisdictions are experiencing a decline in their moose populations due to intensive harvest and high winter tick infestations (Timmermann and Rodgers 2017; Jones *et al.* 2019), so the presence or absence of an infection in these areas may be of future interest. Third, while the *Anaplasma* spp. described in this study is a highly distinct species that falls well beyond the classic threshold for classifying a novel prokaryotic species, further loci should be tested before identifying the placement of the bacteria, as the *Anaplasma* genus already has a complex lineage. Despite these limitations, these data indicate a novel species with a potentially novel life cycle suggested by the genetic dissimilarity of *Anaplasma* spp. found in moose and ticks.

The incongruence of *Anaplasma* strains found in moose and both deer tick and winter tick raises the question of how this *Anaplasma* spp. is transmitted to moose. Although transmission of *Anaplasma* spp. among vertebrate hosts can vary, and the most common transmission involves the replication of the bacteria within ticks, there are instances where blood-sucking or biting insects have acted as vectors (Scoles *et al.* 2005). For example, mosquitos and Muscid flies are potential blood-sucking flies that feed on moose (Burger and Anderson 1974; Moon 2019), and should be evaluated further to determine any role in the

transmission of *Anaplasma* bacteria. In addition, vertical transmission should not be ruled out as a possible route, because transplacental transmission of *A. marginale* to the fetus has been reported in beef cattle (Zaugg 1985; Rey *et al.* 2003; Grau *et al.* 2013).

Similarly, I found no evidence that moose act as hosts of *A. phagocytophilum* in Maine. Also, it is unlikely that *A. phagocytophilum* is a threat to moose health given that *A. phagocytophilum* was not detected in moose, and because prevalence in winter ticks was very low. Furthermore, the single female moose carrying the *Anaplasma*-positive winter tick survived the winter despite a heavy winter tick load. My results are in contrast to what has been observed in European moose populations, in which *A. phagocytophilum* was identified in a large proportion of individuals and shared a >99% identity with the pathogenic strain responsible for HGA in humans (Pūraitė *et al.* 2015; Malmsten *et al.* 2018). Because many other animals in Maine may carry *A. phagocytophilum* (Stuen *et al.* 2013), more research is warranted to determine the potential of winter ticks to transmit *A. phagocytophilum* and the subsequent risk these ticks may pose to other susceptible hosts, such as humans.

It is anticipated that the results from this study will inspire further research to (1) characterize the novel *Anaplasma* spp. detected in moose at a high prevalence (54%), (2) identify a vector for transmission of the novel *Anaplasma* spp. found in moose, (3) determine the geographic extent at which the infection persists, and (4) identify potential effects of the *Anaplasma* spp. on individual moose health and any implications for moose management in the northeastern United States.

CHAPTER 3

EVALUATING THE INFLUENCE OF A NOVEL *ANAPLASMA* SPECIES ON THE HEALTH OF THE EASTERN MOOSE POPULATION OF MAINE, USA

3.1 Introduction

The North American moose population has realized both increases and decreases in abundance over the last decade. While populations in Maine and Alaska have grown substantially since 2001 (Lichtenwalner et al., 2014; Wattles & DeStefano, 2011), moose in Vermont, New Hampshire, Nova Scotia, Newfoundland, Manitoba, Minnesota, Idaho and Wyoming are considered to be in decline (Timmermann & Rodgers, 2017). Several jurisdictions across North America have reported a decline that can be attributed to diseases and parasites, including increased incidence of brainworm (*Parelaphostrongylus tenuis*) in North Dakota (Lankester & Samuel, 2007; Maskey, 2011), the giant liver fluke (*Fascioloides magna*) in Minnesota (Murray et al., 2006), and winter tick (*Dermacentor albipictus*; Packard 1869) (Jones et al., 2019) in Vermont and New Hampshire, as well as increased predation and hunting from overabundant moose populations in specific regions like Manitoba (Timmermann & Rodgers, 2017) and Newfoundland (West, 2009). In a recent review, parasites and disease were implicated as key factors affecting population instability in 73% of the North American moose management jurisdictions reporting declines, and, of these jurisdictions plagued by parasites and disease, a changing climate appeared to be the common thread (Timmermann & Rodgers, 2017). In the Northeast, global climate change may specifically increase winter tick survival, abundance, and

attachment rates (Dunfey-Ball, 2017; Jones et al., 2019), thereby threatening the long-term viability of moose populations in the region.

Maine has a particularly well-established moose population of high sociocultural, ecological, and economic importance. It has been managed since the 1980's, and is currently considered stable, at approximately 70,000 animals (Kantar & Cumberland, 2013; Wattles & DeStefano, 2011). Population stability has been attributed to a set of management guidelines established in 2000 by the Maine Department of Inland Fisheries and Wildlife (MDIFW) which provided an integrative approach considering recreation management (viewing and hunting activities), road safety (mitigating motor vehicle collisions), and wildlife conservation (Kantar, 2018; Wattles & DeStefano, 2011). There has been evidence (Jones et al., 2019) that winter ticks may influence the population through periodic widespread mortality of calves during epizootic events (defined as an event where calf mortality is greater than 50%). These epizootics have been increasing in frequency in conjunction with an overall rise in the recorded number of calf mortalities, specifically in western Maine (Wildlife Management District 8, WMD 8; see Figure 2.1 in Chapter 2).

While other tick species are typically considered hazardous due to the diseases they vector, the damage to moose from winter ticks is believed to be from severe blood loss and associated anemia. The large volume of blood loss associated with severe winter tick infestations further reduces an already poor nutritional status during March and April, when feeding by adult female winter tick is greatest (Samuel, 2004). Conservative estimates indicate that blood loss associated with moderate (30,000 ticks) to severe (70,000 ticks) infestations on individual moose has a substantial impact on energy and protein balance, and thus, calf survival (Musante, Pekins, & Scarpitti, 2007) Severe infestations may induce an estimated blood loss of up to 149% of the

total blood volume for a 150 kg calf over the 8-week engorgement period (early March – late April) by female adult winter ticks, with up to 75% loss of blood volume during the 2 weeks of peak female winter tick engorgement (Musante, Pekins, & Scarpitti, 2007). Moose calves (<1 year of age) are especially vulnerable to mortality attributed to winter tick parasitism during this critical period, so they have been prioritized in many studies (Jones, Pekins, Kantar, O'Neil, & Ellingwood, 2017; Jones et al., 2019). In New Hampshire, winter tick related mortality was responsible for 41% of radio-marked deaths, with calves representing 88% of all deaths (Musante, 2006); nonetheless, all age classes of moose had been previously associated with winter tick-related mortality in western North America (Samuel & Barker, 1979; Jones, Pekins, Kantar, O'Neil, & Ellingwood, 2017).

It is believed that these winter tick infestations can be exacerbated by secondary parasitic infestations, disease and severe winters (Musante, Pekins, & Scarpitti, 2007); but, until recently, there has been little research to determine the extent of compounding infections in moose and their potential impact on individual and population-level health. In response to limited systematic research in this area, I conducted a study to screen for *Anaplasma* bacteria in moose using a PCR-based assay (Chapter 2) and found a relatively high (54%) prevalence of an uncharacterized *Anaplasma* spp. (hereafter referred to as *Anaplasma* spp. Cervus) in Maine moose. It remains unknown whether this recently discovered *Anaplasma* bacterium has any impact on moose health. Therefore, the aim of this study was to evaluate potential fitness impacts of *Anaplasma* spp. Cervus infections in moose, by examining relationships between winter tick load, *Anaplasma* infection status, and moose winter survival.

Anaplasma species are the most widely distributed of several important tick-borne pathogens. Members of Anaplasmataceae family are small, obligate intracellular bacteria that

typically parasitize blood cells, and are either transmitted mechanically or biologically in their hosts (Sonenshine & Roe, 2013). Known pathogens in this family include those causing emergent tick transmitted diseases such as human granulocytic anaplasmosis (*A. phagocytophilum*) and human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), as well as established diseases such as bovine anaplasmosis (*A. marginale*). Historically, species in the genus *Anaplasma* were thought to exclusively infect the red blood cells (RBCs) of ruminants, causing various levels of hemolytic anemia. Since the reclassification of several *Ehrlichia* spp. as *Anaplasma* spp. (Uilenberg, Thiaucourt, & Jongejan, 2004), it is now recognized that members of this genus may infect red blood cells (RBCs. *A. marginale*), white blood cells (WBCs, *A. phagocytophilum*), or platelets (*A. platys*), and some may not cause anemia (Weiss & Wardrop, 2011). *Anaplasma marginale* is notable within this group, because it can be transmitted, will grow and survive in a large number of domestic and wild animals (Kuttler, 1984).

The persistence of *Anaplasma* species like *A. marginale* in a wildlife reservoir host could have implications for survival of both wild and domestic animals (Worthington & Bigalke, 2001), as experimental infection has reduced pack cell volume (PCV) in white-tailed deer (*Odocoileus virginianus*) (Kuttler, 1984), and caused anemia and weight loss in domestic cattle, often leading to death (Kocan, De la Fuente, Guglielmone, & Meléndez, 2003). Although considered unlikely that the presence of *Anaplasma* species would cause disease in some wildlife species (Kuttler, 1984), an infection with *Anaplasma* spp. Cervus could result in immunosuppression or subclinical effects that may not be detectable during routine wildlife surveillance. Also, wildlife could be carriers of infection and the source of pathogen spillover into domestic species (e.g., cattle); this possible epidemiological significance requires new strategies for managing wildlife (Wobeser, 2002). Many new species of *Anaplasma* have been

discovered recently in cattle (Hailemariam et al., 2017), deer (Lobanov, Gajadhar, Al‐Adhami, & Schwantje, 2012), and Eastern moose (Chapter 2), however these novel species currently have unknown health implications for the animals they infect.

Much of the knowledge regarding moose and anaplasmosis is from European literature; however unanswered questions exist regarding the implications to moose health and the ecology of *A. phagocytophilum* (Malmsten et al., 2018). The first official case study in which a moose was found infected with *A. phagocytophilum* was in a Norwegian moose calf (Jenkins et al., 2001). Along with the detection of *A. phagocytophilum,* further examination revealed *Klebsiella pneumoniae* in pure culture from the lungs and liver, and the eventual death of the moose calf was attributed to *K. pneumoniae* septicemia, secondary to immunosuppression caused by *A. phagocytophilum* (Jenkins et al., 2001). Furthermore, immunosuppression due to *A. phagocytophilum* has been recorded in a number of mammals (Woldehiwet, 2008).

Since the first case study on *A. phagocytophilum* in moose (Jenkins et al., 2001), multiple investigations have been conducted to determine the prevalence of the bacteria in European moose (Malmsten et al., 2014; Malmsten et al., 2018; Pūraitė, Rosef, Paulauskas, & Radzijevskaja, 2015). In Sweden, all tested moose serum samples had antibodies against *A. phagocytophilum*, and the mean DNA-based prevalence was 26.3%, with high mortality rates attributed to being infected by the bacteria (Malmsten et al., 2014). Malmsten et al. (2014) detected *A. phagocytophilum* in a dead moose with severe bacterial bronchopneumonia, which was consistent with the clinical findings described by Jenkins (2001). Together, the results of these studies support the hypothesis that a primary *A. phagocytophilum* infection could cause immunosuppression, facilitating secondary bacterial infections and disease progression. Similarly, in Norway, Pūraitė, Rosef, Paulauskas, & Radzijevskaja (2015) found a moderately

high prevalence (31 - 41%, *n* = 99) of an *A. phagocytophilum* strain in moose that was genetically similar to that found by Malmsten et al. (2014). Pūraitė, Rosef, Paulauskas, & Radzijevskaja (2015) hypothesized that *A. phagocytophilum* had a possible health effect on moose calves due to the finding that carcass weights of five infected calves were considerably smaller than an uninfected calf from the same region, an observation previously made in other ruminants (Grøva, Olesen, Steinshamn, & Stuen, 2011; Stuen, Bergström, & Palmer, 2002). Most recently, a seven year study by Malmsten et al. (2018) found a much higher prevalence of bacteria (82%) in Norway, Sweden and Finland than previously described, strengthening the hypothesis that moose play a significant role in the epidemiology of *A. phagocytophilum*. From this collective evidence, it is clear that moose have the capacity to carry *A. phagocytophilum*, but further research is required to determine the role moose have on the spread and maintenance of other emerging vector-borne pathogens (Malmsten et al., 2018).

In New Hampshire, USA, Jones (2016) found that 80% of moose tested serologically positive for *Anaplasma* spp., and most notably, identified an active infection of *Anaplasma* spp. in one calf and one winter tick sample. However, these results were deemed inconclusive due to the inability to genetically characterize the bacteria and questions regarding the validity of the serological assay, which was specific to *A. marginale* (Jones, 2016). Because *Anaplasma* spp. Cervus is genetically distinct from the *A. phagocytophilum* described in European moose (Chapter 2), the clinical implications of this bacterium are completely unknown.

While previous work has shown that a large proportion of the moose in Maine harbor *Anaplasma* infections (Chapter 2), it is unknown whether these infections have an impact on individual fitness (in terms of mortality). In order to address this gap in knowledge, I integrated data on presence and absence of the bacteria (*Anaplasma* spp. Cervus) in moose with data on

winter tick loads and *Anaplasma* serology to estimate population level effects of *Anaplasma* spp. Cervus. The primary goal of this study was to investigate the potential effects of *Anaplasma* infection on moose health, which was accomplished by: (1) examining peripheral blood smears and packed cell volume (PCV) for evidence of a hematologic disorder in moose infected with *Anaplasma* spp. Cervus; (2) using survival analysis to model the probability of calf survival given varying tick loads and infection status; and (3) assessing the correlations between moose calf winter survival and potential predictor variables (calf weight, tick load, *Anaplasma* infection status, wildlife management district, sex). As we are confronted with a changing climate, there is insecurity about the future of the Maine moose population, particularly as shifting environmental conditions may favor increased tick populations and a subsequent rise in tick-borne diseases. In this Chapter, I address this need by estimating the effect (if any) that *Anaplasma* spp. Cervus has on moose health, as compared to known predictive factors of moose calf survival over the winter.

3.2 Methods

3.2.1 Moose Capture and Study Areas

Moose were captured and monitored from two separate areas in western and northern Maine, as part of a long-term population monitoring study by MDIFW. The two study areas are both predefined wildlife management districts (WMD) from MDIFW (Figure 2.1, Chapter 2). Although the western study area (WMD 8) is slightly larger than the northern study area (WMD 2), it has a lower estimated density of moose than the northern site per km^2 (WMD 2= 3 moose/km2, WMD 8 =1.7 moose/km2) (L. E. Kantar & Cumberland, 2013). Specifically, WMD 2 extends over 1,160 m^2 (1867 km^2) from Ashland west of State Highway 11, to the Allagash

River and north from the Reality Road to the New Brunswick Border along the St. John River. The western study area, WMD 8, extends over 1,960 m^2 (3154 km^2) from the west side of Moosehead Lake to the Quebec border and from the Golden Rd south to Pleasant Ridge Plantation over to Flagstaff Lake and up State Highway 27 to the Canadian Border.

This study analyzes the results of three capture years, taking place from 2016 - 2018, during the months of December and January. All captures were facilitated by MDIFW, and utilized the services of the Native Range Capture Services. During the 2016-17 season, 73 calves (<1 years of age) were captured by net-gun out of a Robinson 44 rotary aircraft. During the 2017-18 winter capture, 68 calves (~7-8 months) and 15 adult cows (unknown ages) were captured by net-gun (Table 3.1). All moose captured between 2016 and 2018 were included in the survival analysis. At each capture, whole blood (6 ml) and serum (24 ml) were collected from all moose. Only calves were weighed, but all adults and calves were ear-tagged and fitted with GPS/VHF radio collars (Vectronics Aerospace GmbH), which enabled personnel to respond to transmit signals elicited by lack of movement for a predetermined time, and interpreted as mortality. Upon mortality signal, MDIFW personnel and other research personnel collected samples of blood (methods in Chapter 2). Tick loads were estimated based on a standardized MDIFW protocol, where tick abundance was measured by four repeated transects of the shoulder and rump and the total number of ticks was then categorized into three ordinal variables; “light” (0-9 ticks), “moderate” (10-45 ticks), and “heavy” (45-100+). This scale was created *post hoc* based on the distribution of ticks counted.

Table 3.1 Distribution of moose captured by capture year, sex, district, and age group.

	Capture Year	Sex		District		Age		
		M	F	WMD 2	WMD 8	Calf	Adult	Total
Survival Analysis (calves only)	2016-2017	37	36	38	35	73	0	73
Anaplasma-Seroprevalence Packed Cell Volume	2017-2018	30	54	36	48	69	15	84
	Total	67	90	72	83	142	15	157
Peripheral Blood Smear Analysis	2018-2019	13	27	14	26	25	15	40

3.2.2 Packed Cell Volume

Packed cell volume (PCV) of each moose blood sample was estimated to test for an association with *Anaplasma* spp. Cervus infection status using a Wilcoxon rank sum test. Methods were adapted from the standardized Mayo Clinic protocol for measuring PCV (Van Assendelft et al., 2001). After gentle inversion to mix EDTA blood tubes, each blood sample was loaded into micro-capillary tubes about 2/3 full and loaded into a hematocrit centrifuge and sealed with clay. The micro-capillary tubes were loaded with the clay-sealed end pointed towards the outside of the rotor. All samples were centrifuged for 10 minutes at 5.6x g, then, each micro-capillary tube was removed and PCV was read using a standard microhematocrit reader. The PCV was then read at the separation point between the red blood cells and the plasma.

3.2.3 Testing for Seroprevalence and Anaplasma Infection

Seroprevalence of *Anaplasma* spp. Cervus was assessed using a cELISA, performed at the University of Minnesota Veterinary Diagnostic Laboratory (St. Paul, MN). The cELISA is sensitive to the MSP5 protein, which is conserved through many *Anaplasma* species and has been validated to detect *A. marginale* (Strik et al., 2007), which may share a common ancestor with *Anaplasma* spp. Cervus (Figure 2.3, Chapter 2).

While seroprevalence indicates a response to an infection with some *Anaplasma* species, an active *Anaplasma* spp. Cervus infection was detected using a PCR-based genetic assay. All PCRs were performed using an Eppendorf or BioRad thermocycler. PCR amplifications for *Anaplasma* testing were carried out in a total volume of 25 μL and contained 2 uL of template DNA (standardized at < 25 ng/uL), 5 μL of 5 × PCR buffer (Promega 5X buffer), 200 μM deoxynucleotide triphosphates (dNTPs, New England BioLabs, Ipswich, MA), 0.5U Promega GoTaq DNA polymerase (Applied Biosystems, Foster City, CA), and 0.4 μM of each primer (EE-1 and EE-2). The second reaction used the same reagents as specified above, with the exception of the nested primers (EE-3 and EE-4), and used 2uL of the amplified product from the first reaction as a template. The thermocycling conditions for the first, outer reaction were: an initial denaturation at 94 °C for 4 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 74 °C for 1.5 min; final extension at 74 °C for 10 min. The thermocycling conditions for the second, inner reaction were: an initial denaturation at 95 °C for 2 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 5 min. Each reaction was held at 4 °C until the reaction was qualified by gel electrophoresis, using a 1-2% Agarose gel in standard 0.5X TBE buffer.

3.2.4 Examination of Peripheral Blood Smears

I determined whether a given moose had an *Anaplasma*-like infection based on the pairing of peripheral blood smear analysis with a DNA and/or serological test (Sonenshine & Roe, 2013). Possible features that could manifest in ruminant blood cytology from an *Anaplasma* spp. infection could include: round, 0.5–1μm, basophilic bodies frequently present on the periphery (*A. marginale*) or center (*A. centrale*) of RBCs, cytoplasmic inclusions (morulae) in WBCs (*A. phagocytophilum*), as well as other occurrences of abnormal RBC morphology (i.e., RBC inclusions, basophilic stippling, nucleated RBCs, polychromasia, etc.) (Sonenshine & Roe, 2013; Weiss & Wardrop, 2011). These features could be seen with other infections however (Weiss & Wardrop, 2011), so it is acknowledged this is very subjective and exploratory in nature.

Out of the 40 moose that were randomly selected for examination of peripheral blood smears, there were more females than males randomly selected (male= 13, female= 27), more moose from WMD 8 (WMD 8= 26, WMD 2= 14), and more calves than adults (adult= 15, calf= 25). No adult males were included in the analysis. Any clinically significant morphologic abnormalities in blood cytology were recorded microscopically from Wright-stained slides of EDTA-anticoagulated blood collected from moose. Approximately 50-100 high power fields (hpf) at a 100X oil objective were examined for a maximum of 10 minutes before the sample was declared free of abnormalities in blood cytology. In the absence of standardized criteria for examining moose blood cytology, guidance for blood smear examination was completed as described by Gulati, Song, Dulau Florea, and Gong (2013) and Weiss and Wardrop (2011). The morphological assessment of the peripheral blood smear remains a valued diagnostic tool (Bain, 2005), despite the considerable inter-observer variation in interpretation (van der Meer, van

Gelder, de Keijzer, & Willems, 2007). Still, I developed a systematic process for quantification of abnormal blood cytology. RBC inclusions were recorded as "0" (no presence of RBC inclusions), "1" (mild amount of RBC inclusions, or ≤ 1/hpf), or "2" (high amount of RBC inclusions, or > 1 RBC inclusions/hpf). The relative abundance of polychromasia and reactive lymphocytes were also recorded using the same scale as RBC inclusions. These scales were developed *post hoc* after the distribution of occurrences was assessed. Only the presence ("1") or absence ("0") was noted of nucleated red blood cells (NRBC), white blood cell (WBC) inclusions, basophilic stippling, hypersegmented neutrophils, and giant platelets (those larger than surrounding RBCs). RBC inclusions were noted if I saw a very dark purple spot within the center of the cytoplasm or at the periphery of RBCs, while polychromasia is defined as discolored, blue-gray RBCs that are often larger and lack the characteristic central pallor of surrounding mature RBCs (Weiss & Wardrop, 2011). If the inclusions were particularly large (>50% of RBC), it was marked as a NRBC. Basophilic stippling was marked as present if RBC had variably sized basophilic 'granular' discolorations across its entire cytoplasm (Ford, 2013).

For the WBCs, there are no standardized definitions regarding the morphology of the various cells, and interpretation is based on individual experience and dependent on the availability of additional clinical information (van der Meer et al., 2007). I marked presence of a neutrophil inclusion if neutrophils exhibited a Howell-Jolly body-like inclusion, a solitary round mass approximately 10–20% of the diameter of WBC (Ford, 2013). I classified lymphocytes as reactive if they were large with indented or irregular nucleus, abundant cytoplasm, and contained dark stain on the periphery of the cell and/or were particularly large in comparison to other lymphocytes encountered on the same slide. Hypersegmented neutrophils were marked as present only if there were > 2 to 5 lobes joined by a thin filament (Adewoyin, 2014).

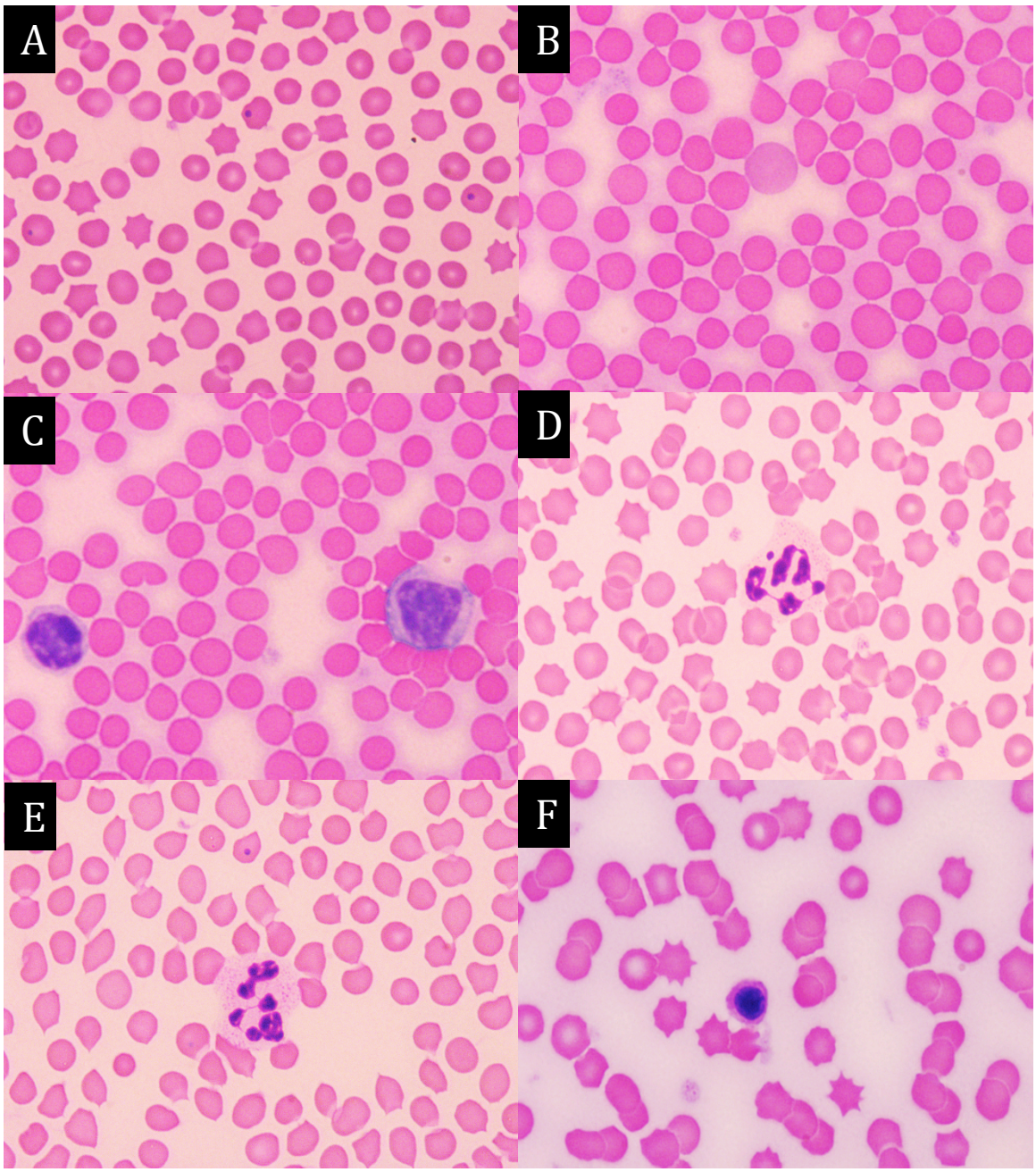

Figure 3.1 Reference pictures of the abnormalities recorded from moose blood smear analysis: (A) Three examples of RBC inclusions; (B) Polychromasia of a RBC (C) A normal lymphocyte (left) compared to a reactive lymphocyte (right); (E) A hypersegmented neutrophil with a single RBC inclusion above it; (F) A nucleated red blood cell (NRBC).

3.2.5 Survival Analyses

Unless stated otherwise, statistical analyses were performed using the program R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). All parameters were tested for normality using histograms, quantile-quantile plots, and the Shapiro-Wilk test for normality. Collinearity and interactions prior to generating survival plots were diagnosed by the use of contingency analysis and generalized linear models (*glm*) for parametric data, or the Mann–Whitney U test or Wilcoxon test for nonparametric comparisons. Contingency analyses (Fisher Exact test for dichotomous variables, Likelihood Ratio Test for non-dichotomous) were used for testing differences in seroprevalence between binary variables (sex, study area). As in Chapter 2 seroprevalence by age (calf, adult) was estimated, but not tested for significance due to the much lower sample size of adults versus calves. The Wilson score interval (Wilson, 1927) was calculated to provide 95% confidence intervals (CI) for the proportion of infected moose using the Epitools epidemiological calculators (http://epitools.ausvet.com.au/).

While calves are in a continuous decline of energy and protein balance as a result of winter tick infestations, moose calf mortality is not constant because it is concentrated around the time of peak female winter tick engorgement (Jones et al., 2019); therefore, I applied a nonparametric approach using the Kaplan-Meier estimator, followed by a log-rank test to compare survival curves of different strata (i.e. *Anaplasma*-uninfected versus *Anaplasma*-infected). For the log-rank test, a $p < 0.05$ indicates that strata are significantly different in terms of the probability of survival. To generate the Kaplan-Meier estimator values, we utilized the `survival` and `survminer` packages in Program R, using the `ggsurvplot` function to visualize the survival model objects generated. “Days” were used as the dependent variable in each model, and therefore survival plots were visualized on this timescale. Data from both

capture years were combined to increase sample size in each model. Shaded 95% confidence intervals were included in each curve when it was visually appropriate. Survival plots were generated for: *Anaplasma*-uninfected versus *Anaplasma*-infected moose calves based on active infection data from the PCR-based assay (1 = uninfected, 2 = infected), winter tick loads of moose at capture (see capture methods), sex ("Male", "Female"), study area ("District 8", "District 2"), as well as the combinations of these independent variables to account for interactions among covariates.

For model selection, the `stats` package was used to fit generalized linear models (*glm*) with a binomial error distribution and link function using the covariates: *Anaplasma*-infection status (1 = uninfected, 2 = infected), winter tick loads at capture ("light", "moderate", "heavy"), sex ("male", "female"), study area ("District 8", "District 2"), as well as the combinations of these independent variables to account for interactions among tick load, *Anaplasma*-infection status, study district, and sex. Interaction terms were included, where collinearity was identified using the survival analysis and preliminary data exploration prior to analysis. The variance explained by each model was also presented by calculating the r^2 using the *rsq* function in the `rsq` package. Both the *AIC* and *BIC* functions were used to report and calculate Akaike's Information Criterion (AIC) and Bayes Information Criterion (BIC) for the fitted models. The major difference between the model selection criteria is the weight of the penalty for added degrees of freedom. Both model selection criteria were used to exploit the advantages of the two criteria. While BIC values parsimony over fit (an attractive output for practical use in wildlife management), AIC-type criteria value fit over parsimony. Burnham and Anderson (2004) demonstrated that BIC could select a model that is in fact a poor fit to the data, whereas AIC virtually never does so. Therefore, we used both criteria to identify parameters that best predict

moose mortality. Model selection results were then interpreted by the percent variance explained (r^2 value) as well as the ΔAIC/ΔBIC values, where a model with difference <2 received similar supports by the data. Models with a difference of 2-7 likely differ in their support, and models with a difference >7 have strong evidence that they differ (Burnham and Anderson 2002).

3.3 Results

3.3.1 PCV, Seroprevalence, and Peripheral Blood Smear Analysis

All moose infected with *Anaplasma* spp. Cervus had an average PCV of 47.5% ± 0.45%, which was significantly lower than the average PCV moose uninfected with *Anaplasma* spp. Cervus at 49.0 ± 0.49% ($p = 0.036$). Adult moose (54.9% ± 1.29%) had a significantly higher PCV than calves (47.7% ± 0.34%, $p<0.001$). There was no significant difference in PCV between districts, sex, or tick loads.

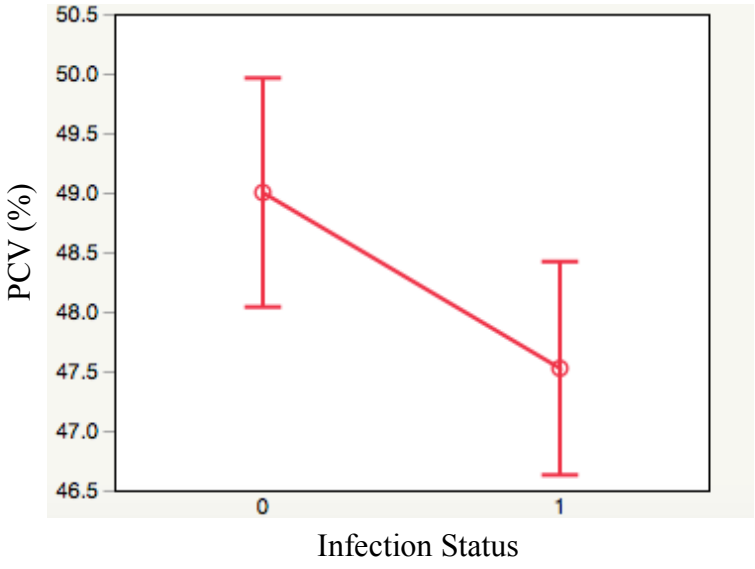

Figure 3.2 The PCV volume difference between moose infected with *Anaplasma* spp. Cervus (1) and moose uninfected with *Anaplasma* spp. Cervus (0). Wilcoxon test indicated a significant difference at $p<0.001$

Anaplasma seroprevalence was 61% in moose with a 95% CI of 53%-68% (Wilson score interval, http://epitools.ausvet.com.au/) and was only slightly higher in male moose (63% versus 59%), but not statistically significant. The seroprevalence in WMD 8 was 76%, which was significantly higher than the 43% seroprevalence seen in WMD 2 ($p < 0.001$). All but one of the adult moose (93%) was seropositive in the *Anaplasma* cELISA, whereas 57% of the calves tested positive. The seroprevalence in moose with heavy (66%), moderate (60%), and light (59%) tick loads was not significantly different ($X^2 = 0.449$, $p = 0.799$). There was not a significant difference in the tick load between moose of different sex ($X^2 = 1.818$, $p = 0.403$) and district ($X^2 = 4.846$, $p = 0.089$). Although the sample size for adults was too low for significance testing ($n = 15$), there were no adults observed with "heavy" tick loads, whereas 20% of calves were found with heavy tick loads. Out of the moose that were considered to be carriers of *Anaplasma* spp. Cervus from PCR (Chapter 2), 86.9% were seropositive.

Results for the abundance of RBC inclusions, polychromasia and reactive lymphocytes are provided in Table 3.2, and the presence or absence basophilic stippling, NRBCs, giant platelets and WBC inclusions are shown in Table 3.3. *Anaplasma*-infection status was not significantly correlated with presence of basophilic stippling ($X^2 = 1.018$, $p = 0.313$), NRBCs ($X^2 = 0.133$, $p = 0.715$), giant platelets ($X^2 = 3.455$, $p = 0.063$) or WBC inclusions ($X^2 = 3.285$, $p = 0.194$), and all of these anomalies were in less than 20% of all 40 moose examined through peripheral blood smear analysis. In all moose examined, there were more frequent occurrences of polychromasia and reactive lymphocytes (32.5%), however there was no significant relationship with *Anaplasma*-infection status (polychromasia, $X^2 = 3.285$, $p = 0.194$; reactive lymphocytes, $X^2 = 5.298$, $p = 0.071$). RBC inclusions were the most commonly recorded, as they were present in 78% of moose analyzed, and 15% of moose were recorded to have many RBC inclusions

(index value = 2). All of the moose that were noted with a RBC inclusions index value = 2 were female, and all of them tested positive for *Anaplasma* spp. Cervus. Further analysis indicated there were a significantly higher number of RBC inclusions in *Anaplasma*-infected moose (X^2 = 6.86, p = 0.03).

Table 3.2 Frequencies of blood cell abnormalities by PCR infection status, sex, study district and age. Data based on peripheral blood smears from 40 randomly selected moose, sampled during the winter of 2018-2019. Index values were recorded as "0" (e.g. no presence), "1" (e.g. mild amount, or ≤ 1/hpf), or "2" (e.g. high amount, or > 1 RBC inclusions/hpf).

	RBC Inclusions*			**Polychromasia**			**Reactive Lymphocytes**		
	0	1	2	0	1	2	0	1	2
	n = 9	*n* = 25	*n* = 6	*n* = 27	*n* = 8	*n* = 5	*n* = 27	*n* = 10	*n* = 3
Infection Status*									
Negative (*n* = 16)	4 (44.4%)	12 (48.0%)	0 (0.0%)	8 (29.6%)	6 (75.0%)	2 (40.0%)	12 (44.4%)	4 (40.0%)	0 (0.00%)
Positive (*n* = 24)	5 (55.6%)	13 (52.0%)	6 (100.0%)	19 (65.2%)	2 (25.0%)	3 (60.0%)	15 (55.6%)	6 (60.0%)	3 (100.0%)
Sex									
Female (*n* = 27)	5 (55.6%)	16 (64.0%)	6 (100.0%)	16 (59.3%)	6 (75.0%)	5 (100.0%)	18 (66.7%)	7 (70.0%)	2 (66.7%)
Male (*n* = 13))	4 (44.4%)	9 (36.0%)	0 (0.0%)	11 (40.7%)	2 (25%)	0 (0.0%)	9 (33.3%)	3 (30.0%)	1 (33.3%)
Study District									
WMD 2 (*n* = 14)	1 (11.1%)	9 (36.0%)	4 (66.7%)	8 (29.6%)	3 (37.5%)	3 (60.0%)	9 (33.3%)	4 (40.0%)	1 (33.3%)
WMD 8 (*n* = 26)	8 (88.9%)	16 (64.0%)	2 (33.3%)	19 (70.4%)	5 (62.5%)	2 (40.0%)	18 (66.7%)	6 (60.0%)	2 (66.6%)
Age									
Adult (*n* = 15)	4 (44.4%)	9 (36.0%)	2 (33.3%)	4 (14.8%)	6 (75.0%)	5 (100.0%)	9 (33.3%)	5 (50.0%)	1 (33.3%)
Calf (*n* = 25)	5 (55.6%)	16 (64.0%)	4 (66.7%)	23 (85.2%)	2 (25.0%)	0 (0.0%)	18 (66.7%)	5 (50.0%)	2 (66.7%)
Total									
	9	25	6	27	8	5	27	10	3

*Chi-squared likelihood ratio test indicated that there were more inclusions in *Anaplasma*-infected moose.

Table 3.3 Frequencies for peripheral blood smears, group sample sizes, and PCR-infection results of 40 randomly selected moose blood samples collected during the winter of 2018-2019. Only the presence ("1") or absence ("0") was noted of nucleated red blood cells (NRBC), white blood cell (WBC) inclusions, basophilic stippling, and giant platelets (those larger than surrounding RBCs).

	NRBC		**WBC Inclusions**		**Basophilic Stippling**		**Giant Platelets**	
	N	Y	N	Y	N	Y	N	Y
	n = 34	*n* = 6	*n* = 33	*n* = 7	*n* = 33	*n* = 7	*n* = 33	*n* = 7
Infection Status								
Negative	14	2	13	3	12	4	11	5
(*n* = 16)	(41.1%)	(33.3%)	(39.4%)	(42.9%)	(36.4%)	(57.1%)	(33.3%)	(71.4%)
Positive	20	4	20	4	21	3	22	2
(*n* = 24)	(59.8%)	(66.7%)	(60.6%)	(57.1%)	(63.6%)	(42.9%)	(66.7%)	(28.6%)
Sex								
Female	22	5	22	5	24	3	22	5
(*n* = 27)	(65.7%)	(83.3%)	(66.7%)	(71.4%)	(72.7%)	(42.9%)	(66.7%)	(71.4%)
Male	12	1	11	2	9	4	11	2
(*n* = 13)	(35.3%)	(16.7%)	(33.3%)	(28.6%)	(27.3%)	(57.1%)	(33.3%)	(28.6%)
Study District								
WMD 2	12	2	10	4	13	1	11	3
(*n* = 14)	(35.3%)	(33.3%)	(30.3%)	(57.1%)	(39.4%)	(14.3%)	(33.3%)	(42.9%)
WMD 8	22	4	23	3	20	6	22	4
(*n* = 26)	(65.7%)	(66.7%)	(69.7%)	(42.9%)	(60.6%)	(85.7%)	(66.7%)	(57.1%)
Age								
Adult	10	5	10	5	12	3	12	3
(*n* = 15)	(29.4%)	(83.3%)	(30.3%)	(71.4%)	(36.4%)	(42.9%)	(36.4%)	(42.9%)
Calf	24	1	23	2	21	4	21	4
(*n* = 25)	(70.6%)	(16.7%)	(69.7%)	(28.6%)	(63.6%)	(57.1%)	(63.6%)	(57.1%)

3.3.2 Survival Analysis

Kaplan-Meier plots showing modeled survival probability over time (days since capture) and results of the log-rank test comparing survival curves of different strata are shown in Figures 3.3-3.5. The survival probability for *Anaplasma*-uninfected versus *Anaplasma*-infected calves was based on active infection data from a PCR-based assay. *Anaplasma*-infected calves had a significantly lower modeled survival probability over time ($p < 0.01$, Log-Rank test, Figure 3.3), but differences in survival were not apparent before 100 days after capture. Moose calves with heavy tick loads had a significantly lower survival probability than calves with moderate and light tick loads ($p < 0.01$, Log-Rank test, Figure 3.4), particularly 125 days after capture (March-

April). *Anaplasma*-infected calves with heavy tick loads also showed a significantly lower survival probability than all other combinations of *Anaplasma*-infection status and tick load ($p < 0.01$, Log-Rank test, Figure 3.5). Moose calves with heavy tick loads in WMD 8 exhibited significantly lower survival probability when compared to both calves with heavy tick loads from WMD 2 and all lighter tick loads from both districts ($p < 0.01$, Log-Rank test). Likewise, there was a significantly lower survival probability for *Anaplasma*-infected calves in WMD 8 when compared to *Anaplasma*-infected calves in WMD 2 as well as *Anaplasma*-uninfected calves from both districts ($p < 0.05$, Log-Rank test).

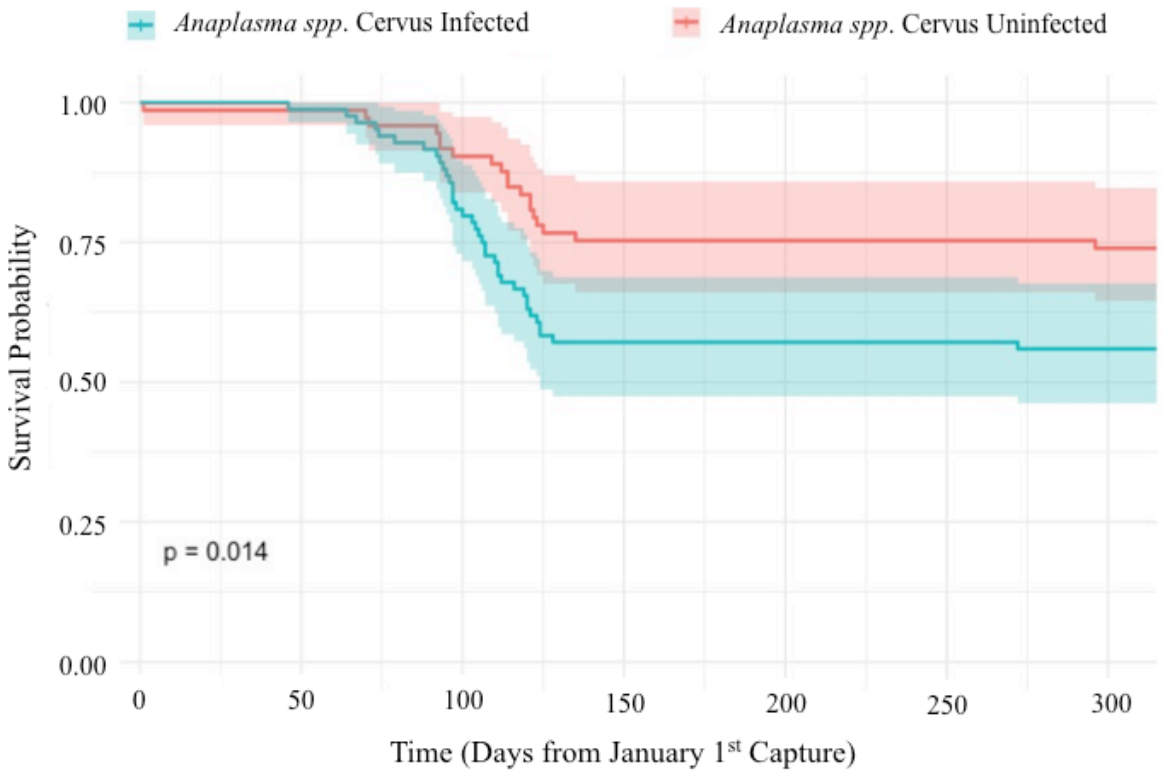

Figure 3.3 The survival probability for *Anaplasma*-uninfected versus *Anaplasma*-infected moose (y-axis) over days after capture (x-axis) based on active infection data from a PCR-based assay previously reported (see Chapter 2).

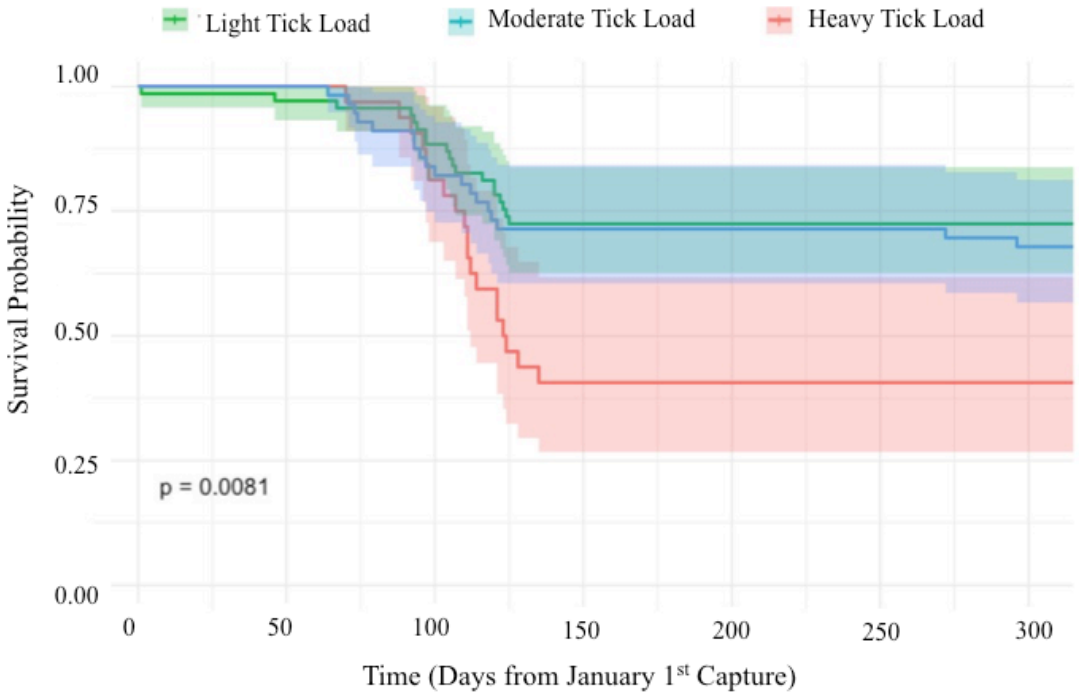

Figure 3.4 The survival probability for tick load (“light”, “moderate”, “heavy”) measured at capture by transects on the shoulder and rump (y-axis) over days after capture (x-axis).

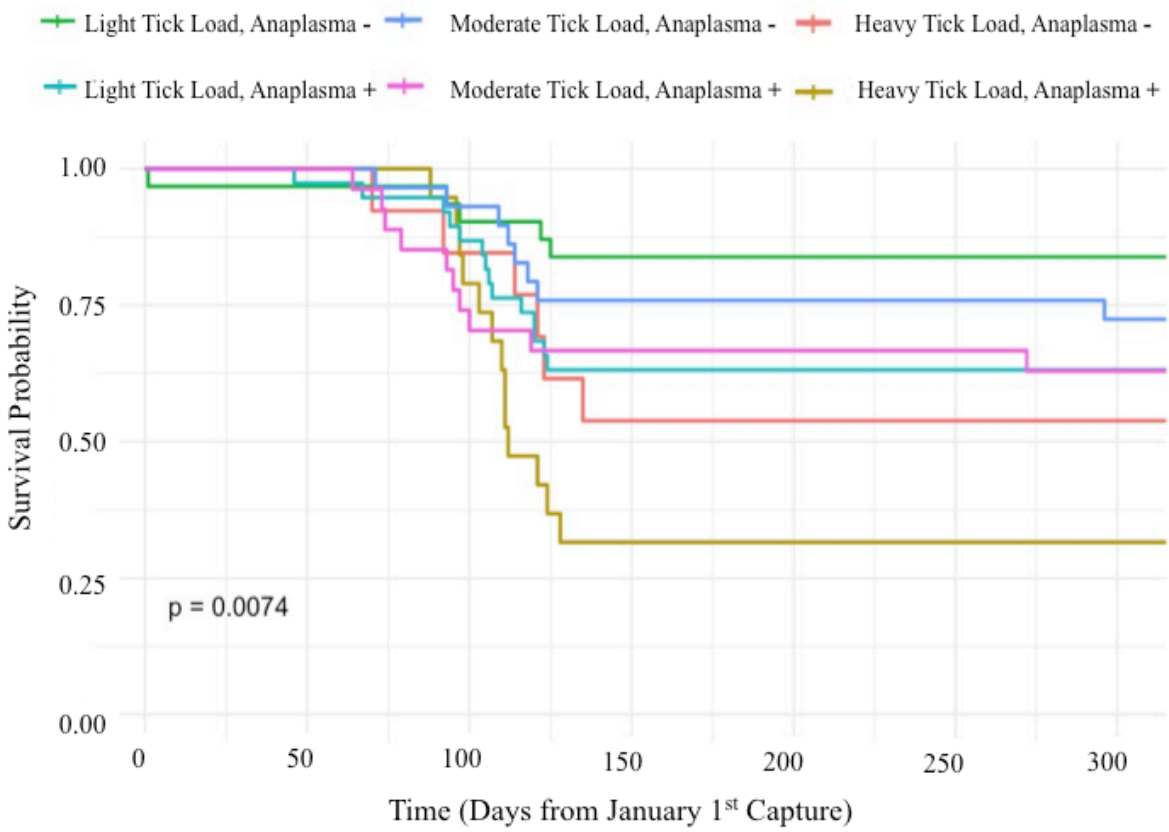

Figure 3.5 The survival probability for tick load ("light", "moderate", "heavy") and for *Anaplasma*-uninfected versus *Anaplasma*-infected moose (y-axis) over days after capture (x-axis). Tick loads were measured at capture by transects on the shoulder and rump and active infection data from a PCR-based assay previously reported (see Chapter 2).

3.3.3 Model Selection

Pairwise comparisons from Chapter 2 and the Kaplan-Meier survival analysis indicated where interactions terms were required to specify non-additive covariates. Table 3.4 shows AIC results for the 25 different generalize linear models (*glm*) that were fit to mortality ("Dead", "Alive") as the dependent variable, and Table 3.5 shows the BIC results for the same models. The models with the highest support from two separate model selection criteria and total variance explained (r^2) all included capture weight as an independent variable. When considering all model selection criteria, the most supported model included tick load and weight at capture as independent variables. Including *Anaplasma*-infection status in this model had similar support based on AIC ($\Delta AIC < 2$) and explained a greater proportion of the variance, whereas a model excluding *Anaplasma*-infection status had moderately higher BIC support ($\Delta BIC = 2\text{-}7$). In terms of goodness of fit, all models are poor, but the model with the highest variance explained included sex, *Anaplasma*-infection, tick load and weight at capture as predictive variables ($r^2 = 0.203$) and received slightly more support ($\Delta AIC = 2$) over the model only including tick load and weight at capture. None of the models with a single independent variable (df = 2) received high support from any of the three criteria.

Table 3.6 displays effects sizes for each of the top two performing models selected using BIC (M14) and AIC (M22), respectively. According to these coefficients, capture weight was weakly and negatively correlated with mortality ($\beta = -0.015$), revealing moose with lower weight have a higher probability of mortality. A positive *Anaplasma*-infection status and sex had a relatively high effect on mortality ($\beta = 1.78$; $p = 0.002$), and indicates infection with *Anaplasma* spp. Cervus is correlated with a higher probability of mortality. Similarly, high tick loads

appeared to be weakly and positively correlated with a higher mortality rate ($\beta = 0.023$; $p = 0.006$), indicating that higher tick loads at capture increases the probability of mortality.

Table 3.4 Model selection results, showing the degrees of freedom, AIC support values, AIC weight, and variance explained by the model (r^2).

Model #	Model	df	AIC	wt	r^2
M1	Mortality ~ Sex	2	204	0.000	0.027
M2	Mortality ~ District	2	207	0.000	0.008
M3	Mortality ~ Tick Load	2	197	0.000	0.069
M4	Mortality ~ Capture Weight	2	175	0.003	0.044
M5	Mortality ~ *Anaplasma*-Infection	2	203	0.000	0.035
M6	Mortality ~ District + Sex	3	205	0.000	0.036
M7	Mortality ~ District + Capture Weight	3	175	0.004	0.063
M8	Mortality ~ District : Tick Load	4	198	0.000	0.092
M9	Mortality ~ District : *Anaplasma*-Infection	4	205	0.000	0.045
M10	Mortality ~ *Anaplasma*-Infection : Sex	4	198	0.000	0.087
M11	Mortality ~ *Anaplasma*-Infection : Tick Load	4	196	0.000	0.100
M12	Mortality ~ *Anaplasma*-Infection : Capture Weight	3	172	0.013	0.080
M13	Mortality ~ Tick Load + Sex	3	197	0.000	0.079
M14	Mortality ~ Tick Load + Capture Weight	3	168	0.103	0.103
M15	Mortality ~ Capture Weight : Sex	3	172	0.009	0.071
M16	Mortality ~ District : *Anaplasma*-Infection : Sex	8	200	0.000	0.119
M17	Mortality ~ District : *Anaplasma*-Infection + Capture Weight	8	172	0.015	0.146
M18	Mortality ~ District : *Anaplasma*-Infection : Tick Load	8	200	0.000	0.120
M19	Mortality ~ District : Tick Load + Capture Weight	5	169	0.058	0.127
M20	Mortality ~ *Anaplasma*-Infection : Tick Load + Sex	5	197	0.000	0.103
M21	Mortality ~ *Anaplasma*-Infection : Tick Load + Capture Weight	5	167	0.149	0.142
M22	Mortality ~ *Anaplasma*-Infection + Sex + Capture Weight	5	166	0.341	0.152
M23	Mortality ~ Sex : *Anaplasma*-Infection : Tick Load + Capture Weight	9	166	0.293	0.203
M24	Mortality ~ District : *Anaplasma*-Infection : Tick Load + Capture Weight	9	174	0.005	0.151
M25	Mortality ~ District : *Anaplasma*-Infection : Tick Load + Capture Weight + Sex	10	174	0.005	0.162

Table 3.5 Model selection results, showing the degrees of freedom, BIC support values, BIC weight, and variance explained by the model (r^2).

Model #	Model	df	BIC	wt	r^2
M1	Mortality ~ Sex	2	210	0.000	0.027
M2	Mortality ~ District	2	213	0.000	0.008
M3	Mortality ~ Tick Load	2	203	0.000	0.069
M4	Mortality ~ Capture Weight	2	181	0.081	0.044
M5	Mortality ~ *Anaplasma*-Infection	2	209	0.000	0.035
M6	Mortality ~ District + Sex	3	214	0.000	0.036
M7	Mortality ~ District + Capture Weight	3	183	0.023	0.063
M8	Mortality ~ District : Tick Load	4	210	0.000	0.092
M9	Mortality ~ District : *Anaplasma*-Infection	4	218	0.000	0.045
M10	Mortality ~ *Anaplasma*-Infection : Sex	4	210	0.000	0.087
M11	Mortality ~ *Anaplasma*-Infection : Tick Load	4	208	0.000	0.100
M12	Mortality ~ *Anaplasma*-Infection : Capture Weight	3	181	0.076	0.080
M13	Mortality ~ Tick Load + Sex	3	206	0.000	0.079
M14	Mortality ~ Tick Load + Capture Weight	3	176	0.590	0.103
M15	Mortality ~ Capture Weight : Sex	3	182	0.053	0.071
M16	Mortality ~ District : *Anaplasma*-Infection : Sex	8	224	0.000	0.119
M17	Mortality ~ District : *Anaplasma*-Infection + Capture Weight	8	194	0.015	0.146
M18	Mortality ~ District : *Anaplasma*-Infection : Tick Load	8	224	0.000	0.120
M19	Mortality ~ District : Tick Load + Capture Weight	5	184	0.019	0.127
M20	Mortality ~ *Anaplasma*-Infection : Tick Load + Sex	5	212	0.000	0.103
M21	Mortality ~ *Anaplasma*-Infection : Tick Load + Capture Weight	5	182	0.049	0.142
M22	Mortality ~ *Anaplasma*-Infection + Sex + Capture Weight	5	180	0.109	0.152
M23	Mortality ~ Sex : *Anaplasma*-Infection : Tick Load + Capture Weight	9	192	0.000	0.203
M24	Mortality ~ District : *Anaplasma*-Infection : Tick Load + Capture Weight	9	200	0.000	0.151
M25	Mortality ~ District : *Anaplasma*-Infection : Tick Load + Capture Weight + Sex	10	203	0.000	0.162

Table 3.6 The top two performing *glm* models, based on BIC (M14) and AIC (M22) model selection criteria. Model coefficients, significance values, and variance explained by the model (r^2) are reported.

Model	Model Variables	Model Coefficient (β)	*p*-value	r^2
(M14) Mortality ~ Tick Load + Capture Weight	Tick Load	0.02	0.006	0.103
	Capture Weight	-0.01	0.006	
(M22) Mortality ~ *Anaplasma*-Infection : Capture Weight + Sex	*Anaplasma*-Infection : Sex	1.78	0.002	0.152
	Anaplasma-Infection	2.00	0.002	
	Capture Weight	-0.02	0.003	
	Sex	-2.08	0.009	

3.4 Discussion

Several studies have investigated the major causes of mortality in moose in the northeastern United States, together accumulating overwhelming evidence that winter tick epizootics are a primary driver of moose calf mortality (Lankester & Samuel, 2007; Jones et al., 2017; Jones et al., 2019). This study adds to those efforts by investigating the relationships between *Anaplasma* spp. Cervus infection status, blood cytology, and calf winter survival. In agreement with literature regarding the influence of winter tick parasitism on moose survival, I found that moose calves with higher tick loads at capture exhibited a significantly lower survival after 100 days post- capture, approximately the same critical period (March-April) that has been previously identified (Jones et al., 2019). The scale used to categorize the level of tick infestations in this study was created *post hoc*, however, my scale is mostly in agreement with Dunfey-Ball (2017), who found a threshold of 36.9 ticks (using same methods) indicated a high likelihood of winter tick related mortality. Most significantly, my survival analysis, model selection, comparison of PCV and peripheral blood smear results suggest that *Anaplasma* spp.

Cervus infection status could be contributory with the effects of winter tick related mortality of moose calves in Maine.

Members of the *Anaplasma* genus vary greatly in how they infect their hosts. A phylogenetic analysis suggested *Anaplasma* spp. Cervus shares a common ancestor with a clade containing *A. marginale, A. centrale* and *A. ovis* (Chapter 2), and all three of these variants are said to infect RBCs in their hosts (Kuttler, 1984). Interestingly, of the blood abnormalities assessed, only the number of RBC inclusions varied significantly between *Anaplasma*- infected and -uninfected moose (Table 3.2). The six moose that had a high number of RBC inclusions (index value = 2, or > 1 RBC inclusions/hpf) were female, but varied considerably in age and district. In two of these six female moose, I also observed NRBCs and WBC inclusions, one of which had a high occurrence of polychromasia (index value = 2). The presence of these abnormal WBCs is a possibly indicates that the immune system of these moose are stressed and that immature reticulocytes of the bone marrow are released in response to a blood infection, as has been noted in other *Anaplasma*-infections (Weiss & Wardrop, 2011). Due to the association between *Anaplasma*-infection and occurrence of RBC inclusions, and under the observation that moose with a high index value of RBC inclusions (index value = 2, or > 1 RBC inclusions/hpf) also harbored an *Anaplasma* spp. Cervus infection, I suggest that these infections may have a subclinical effect on moose that is additive to the known effects of winter ticks on fitness.

These results highlight the need for continued investigation of the possible immunogenetic, physiological, and behavioral factors driving variation in individual response to the presence of *Anaplasma* spp. Cervus. While peripheral blood smear analysis is a well-established veterinary practice, there is a fair amount of subjectivity in the method, especially when no references exist for moose. For example, while examining a blood smear of cattle

infected with *A. marginale*, it is easy to mistake RBC inclusions caused by the bacterial infection for benign inclusions (e.g. Howell-Jolly bodies) in lower abundances (Weiss & Wardrop, 2011). Further, the inclusions are considered to be normal for horses (Weiss & Wardrop, 2011). Also, the abundance of RBC inclusions in typical *Anaplasma*-type infections (up to 50% of RBCs infected) far exceeds the abundance seen from any moose in this small sample (Kocan et al, 2004). Therefore, I have exercised caution in interpreting these data by not drawing conclusions on the blood smear results alone given the challenges of using comparative hematology to assess the health of a species for which a reference is not fully defined.

In order to provide a reference for moose blood smear analysis, I provided the prevalence of several different potentially abnormal findings in blood cytology (Table 3.2 and Table 3.3). It should be noted that in a small sample size (n = 40). Few moose (< 20%) had basophilic stippling, NRBCs, giant platelets or WBC inclusions. The biological significance of these in moose is unknown, but polychromasia, basophilic stippling and NRBCs have been seen in cattle and other ruminants as a part of a regenerative response to anemia (Weiss & Wardrop, 2011). Polychromasia and reactive lymphocytes were more common, but because there was not relationship between bacteria infection status and other abnormal blood cytology, and there is no evidence that either are characteristic of an *Anaplasma*-like infection.

I found that PCV increases with age and decreases with *Anaplasma* spp. Cervus infection status. These results should be interpreted cautiously in combination with my other results, as even a small amount of stress during the moose capture can have significant effects on blood parameters (Wesson, Scanlon, Kirkpatrick, Mosby, & Butcher, 1979). For example, it has been suggested that tame, captive moose that are relaxed during blood draw have much lower PCV values (Addison, McLaughlin, & Broadfoot, 1998). Still, because all animals were handled

similarly, I have assumed that increased handling or stress was not biased towards infected or uninfected moose. Not surprisingly, PCV values of Maine moose were similar to what was found by Jones (2016) in New Hampshire, USA (NH, $\bar{x}$ = 46.0% ± 6%; ME, $\bar{x}$ = 48.0% ± 4%). Further, adult moose in this study had PCV values close to that of a moose in average or better than average condition (PCV = 50%) according to Franzmann and Leresche (1978). The average PCV of all moose in Maine was also much higher that values reported by Dieterich, Morton, and Zamke (1991) in Alaska (35% to 40%). This could mean that the moose in Maine are of average or better than average condition compared to Alaskan moose (Franzmann, LeResche, Arneson, & Davis, 1976), which could be a function of life history or habitat. Similar to Addison, McLaughlin, & Broadfoot (1998), my results suggest winter ticks have a minimal effect on PCV values.

While PCV was significantly lower in moose infected with *Anaplasma* spp. Cervus, the mean value of infected moose (47.5%) was within normal ranges, according to findings in New Hampshire (Jones, 2016). While it is important to note the current lacking in biological significance of the differences in PCV, it is very possible that the reference values produced in New Hampshire included moose infected with *Anaplasma* spp. Cervus, so special caution should be taken when interpreting these values and efforts to develop new reference values for moose are warranted in the future. This small, but significant difference ($PCV_{uninfected}$ = 49%; $PCV_{infected}$ = 47.5%) is in disagreement with the dramatic effects seen in deer infected with *A. marginale*, which experience a 10-24% decrease in PCV value (Kuttler, 1984). Despite the small difference in PCV observed between *Anaplasma* infected and uninfected moose, there is some evidence that a decrease in red blood cell volume could result from an infection with *Anaplasma* spp. Cervus, as experimental infection with closely related *Anaplasma* spp. (Chapter 2) has been previously

shown to reduce pack cell volume (PCV) in white-tailed deer (*Odocoileus virginianus*) (Kuttler, 1984), and cause anemia and weight loss in domestic cattle (Kocan, De la Fuente, Guglielmone, & Meléndez, 2003). A positive relationship between PCV and hemoglobin content has been shown in white-tailed deer (Rawson, DelGiudice, Dziuk, & Mech, 1992) and cattle (Turkson & Ganyo, 2015), and a decrease in PCV can minimize the O_2-carrying capacity of blood in growing juveniles, (Rawson et al. 1992). Therefore, as may be reflected in my survival analysis, even a mild drop in PCV could have negative implications for moose coping with high winter tick infestations. However, the opposite conclusion could be that the difference in PCV is biologically insignificant and there is no obvious effect from *Anaplasma* spp. Cervus infection.

The conclusion that *Anaplasma* spp. Cervus infections have an effect on fitness is supported by the observed interaction between *Anaplasma* spp. Cervus infection and tick load, indicating that together the ailments may have a compounding effect on moose survival during the winter months. However, at the scale of an individual moose, there is no identified effect known at this time because the PCV and peripheral blood smear analyses are inconclusive. It was not surprising to see that tick load decreased the predicted survival (Figure 3.4), but it was a novel observation that survival probability was the lowest for individuals with both heavy tick loads and *Anaplasma* spp. Cervus infections (Figure 3.3). Model selection results were not as clear, yet still informative. While capture weight was a common factor in all models, BIC and AIC disagreed in their relative support for difference models. AIC provided higher support for an effect of *Anaplasma*-infection status, which is likely due to AIC affinity to higher complexity (Burnham & Anderson, 2004); complexity was inevitable given the sex and district specificity of *Anaplasma* spp. Cervus (Chapter 2). BIC provided moderately more support for the model including tick load and capture weight as covariates (Table 3.5), but AIC provided equal support

between three models (Table 3.4). An arguable compromise between the highest performing models is M21 (Mortality ~ *Anaplasma*-Infection : Tick Load + Capture Weight), because it has a higher explained variance than a model (M14) excluding *Anaplasma*-infection status as a covariate. M21 was also indistinguishable between the top performing models ($\Delta AIC < 2$). Even though M14 (Mortality ~ Tick Load + Capture Weight) was most supported under BIC, the presence or absence of *Anaplasma* spp. Cervus should not be ignored given my data suggesting that an infection may have sub-clinical to minimal clinical effects on some moose that could compound other stressors; primarily low weight and high tick loads going into the winter months. I also recommend that surveillance for *Anaplasma* spp. Cervus in other domestic and wild animals should be done with DNA-based methods rather than serology because I demonstrated that there could be a high cross-reactivity between antigens for *Anaplasma* spp. Cervus, *A. marginale* and *A. centrale*, as identified in previous studies (Kuttler, 1984).

It is anticipated that these results will specifically support the ongoing, long-term study led by MDIFW, which is chiefly interested in quantifying survival, productivity, and establishing baseline values of several health metrics for moose in the northeastern United States. Before 2005, the majority of baseline values for moose were from Norwegian (*Alces alces alces;* (Rostal, Evans, Solberg, & Arnemo, 2012), Alaskan (*Alces alces gigas*) (Franzmann & Leresche, 1978) or Wyoming (*Alces alces shirasi*) moose (Kreeger et al., 2005), all different subspecies that occupy different habitats than the Eastern moose (*Alces alces americana*). Reference values available from MDIFW for Eastern moose include, birth rates, disease, parasites, serum chemistry, trace nutrient, heavy metals, and most importantly, winter tick (*Dermacentor albipictus*) loads (Kantar, 2018). However, these baseline values may be compromised by the presence of *Anaplasma* spp. Cervus infection. Therefore, I emphasize that special consideration

of *Anaplasma* spp. Cervus infection status when studying moose in the northeastern United States.

Although in Chapter 1, I provided the evidence that *Anaplasma* spp. Cervus was at high prevalence in Maine moose, it was not known previously that the infection could have sub-clinical effects that greatly impact the survival probability of calves with high winter tick loads. While the evidence is clear for *Anaplasma* spp. Cervus having a relationship with moose mortality, winter tick parasitism still seems to be the driver of winter mortality for moose calves in Maine.

CHAPTER 4

THE INFLUENCE OF EXPERIENTIAL PROCESSES ON PERCEIVED RISK FROM TICKS AND A DECLINE IN MOOSE AMONG PENOBSCOT NATION CITIZENS, MAINE, UNITED STATES

4.1 Introduction

Recent attention has been given to infectious disease, especially tick-borne disease (TBD) as it relates to human health. With the increase in tick abundance and distribution comes more frequent cases of Lyme disease, anaplasmosis, babesiosis, Powassan virus, and other TBDs. As these diseases and their vectors emerge, so does the importance of research and management that "focuses on beliefs, values, attitudes, behaviors, and demographic characteristics of wildlife user groups, also termed "human dimensions" (Gigliotti & Decker, 1992). Human dimensions research has been shown to facilitate an understanding for the societal consequences of wildlife disease risk perceptions (Decker et al., 2006), but in spite of the growing research on resident risk perceptions towards global and local issues that affect human health and well-being (Chapter 1), few studies have specifically measured risk perceptions related to the impacts of ticks and disease (Vaske & Miller, 2019). The northeastern United States has been exposed to emerging vector-borne disease threats that have influenced personal protective behaviors to avoid tick-borne infections (Brewer, Weinstein, Cuite, & Herrington, 2004), such as changing the frequency of recreation or culturally important activities (LeBreton et al., 2006). Much of the research that does relate risk perceptions to ticks is in the context of a particular disease, such as Lyme disease (Valente, Wemple, Ramos, Cashman, & Savageau, 2015). While the ticks themselves certainly can pose a threat like the diseases they vector, not all ticks are directly

harmful to humans, and not all ticks vector disease (Sonenshine & Roe, 2013). Rather, some are much more of a direct threat to wildlife. The winter tick is among those ticks not particularly harmful to human health, but has been identified as the primary driver of moose mortality, particularly in moose calves through heavy infestations leading to lethal blood losses (Samuel, 2004). There has been little research thus far that can provide guidance to wildlife managers and communicators for anticipating how people would react to the tick-associated decline in wildlife (Decker et al., 2012), and related effects on human systems.

Multiple theoretical frameworks have been developed and used to determine which factors help predict risk perceptions associated with wildlife disease or human wellbeing. Results from previous studies suggest that risk perceptions associated with ticks and disease is a function of a range of explanatory factors, including (1) having been bitten by or exposed to a tick—direct experience; (2) knowing somebody who has been affected by ticks—indirect experiences; and (3) knowledge of the disease—cognition (Herrington, 2004). In this study, the experiences that elicit such affect could be with moose or other wildlife. For example, based on prior research, I hypothesize that those who have seen a sick moose, or a moose that is heavily infested with winter ticks are likely to perceive a higher risk of wildlife disease and higher threat to the moose population in Maine. This study operationalized the construct of experiential processes via two scales; a personal experience scale for direct and indirect experience with moose and winter ticks, and a personal experience scale for direct and indirect experience with all types of ticks. According to Sjoberg (2000), both direct and indirect experiences increase perceived risk. The relationship between risk perception and the extent of an individuals' experience with moose and winter ticks can be tested through this construct.

Data presented in Chapters 2 & 3, paired with passive surveillance of human and winter tick encounters (Rand et al., 2007), provide little to no evidence for substantial objective risk that winter tick actually transmit pathogens to humans. Also, while winter ticks are unlikely to play a role in the transmission of a novel bacterium, *Anaplasma* spp. Cervus (see Chapter 2) to Maine moose, there is evidence that winter ticks greatly impact the survival of Maine moose (Jones et al., 2019). Although, the objective risk to human health and wildlife disease is low to nonexistent, other threats to the human system might result from winter tick infestation on moose. Further, to my knowledge, no research has been conducted to date to measure risk perceptions with regard to the impacts of winter ticks on moose populations on PN tribal lands, and likely effects on human systems. This chapter explores the relationship between experiences and risk perceptions. Furthermore, winter ticks have been known as a driver of moose mortality for some time now (Webb, 1959), but to my knowledge, there has been little effort to study people's attitudes in response to winter tick parasitism, despite knowing the value of the human dimension in wildlife management (Decker & Chase, 1997).

This study used a questionnaire that measured multiple constructs to predict risk perceptions as illustrated in Chapter 1 (Figure 1.2). However for the purpose of this thesis, Chapter 4 will only report on the results from the analysis of how experience and status as hunter/non-hunter might influence risk perceptions among Penobscot Nation citizens. The risk perception dimensions to measure are (1) the threats of winter tick presence on moose, the Penobscot Nation and to the respondent; (2) the effects that a decline of moose might have on the natural environment, PN, and the respondents; and (3) the hazard that ticks in general might present to the natural environment, PN, and the respondents.

Further, this study sought to address a concern expressed by personnel from a regional wildlife management agency in response to the uncertainty with how multiple individuals in Maine define a "healthy" population of moose (personal communication, L. Kantar, Maine Department of Inland Fisheries and Wildlife; personal communication, K. Peet, Penobscot Nation Department of Natural Resources; January 3rd, 2018). The World Health Organization defines human health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (Callahan, 1973). The definition for wildlife health has recently been defined by Stephen (2014) using three features: (1) an interaction between biologic, social, and environmental determinants that promotes and maintains health as a capacity to cope with change over time; (2) not merely what is absent (i.e., parasites or disease) but rather the characteristics of the animal that affect their vulnerability and resilience (i.e., nutrition and diet) to disease; and (3) the understanding that wildlife health is a dynamic human construct based on social expectations and scientific knowledge. On a related topic, the CDC defines One Health as a "collaborative, multisectoral, and transdisciplinary approach — working at the local, regional, national, and global levels — with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment" (Center of Disease Contol and Prevention, 2017). The One Health approach has exacerbated this focus by identifying wildlife as a major source of emerging infections of public health concern (Stephen, 2014). Thus, the questionnaire included one open-ended question to understand a range of definitions of moose health provided by respondents.

4.1.1 Study Population

This study was conducted in collaboration with members of one of four Wabanaki tribes in Maine: The Penobscot Nation (PN). The Wabanaki (meaning "People of Dawn") are composed of four Native American groups: Penobscot Nation, Passamaquoddy Tribe, the Houlton Band of Maliseet Indians, and the Aroostook Band of Micmacs (Speck, 1915). The Penobscot Nation is an indigenous Native American tribe of Eastern Maine with 2,367 citizens as of 2010, and according to the PN website, 450 tribal members live on the reservation in Orono, ME, along with some 1,399 tribal members living in Maine (penobscotnation.org).

It has been argued that a decline in moose could have cultural implications for the local Wabanaki tribes (Jacobson, Fernandez, Mayewski, & Schmitt, 2009). Moose are a primary form of sustenance for Native tribes, and have been for many generations (Fallon & Enig, 2001). For the PN specifically, the moose represents a "dominant form of sustenance for many tribal members" (K. Peet, personal communication, November 8, 2017). Beyond sustenance, PN see the moose as a cultural keystone species, as many of their legends and teachings involve the moose (Penobscot Nation Department of Natural Resources, 2011; Speck, 1940). Citizens enjoy a separate and distinct moose-hunting season on tribal lands, which fostered tradition and relationship building between and within families (K. Peet, personal communication, November 8, 2017). Upon a successful hunt, the whole animal can used for "sustenance" (blood, liver and heart), "medicinal purposes" (e.g., broth of crushed bones to ease spasms of childbirth) and "material for cultural uses" (e.g., skin used for drums, containers and occasionally a covering for wigwams) (Penobscot Nation Department of Natural Resources, 2011). Therefore, this component of my thesis sought to understand values and risk perceptions that Penobscot Nation citizens have regarding moose-winter tick-human interactions.

4.1.2 Research Questions (RQ) and Hypotheses (RH)

The primary research questions and hypotheses guiding this component of my thesis are:

RQ_1 - Do individuals with lower experience with moose and winter ticks perceive less risk to the impacts of winter ticks, all types of ticks, and a decline in moose?

RH_1 - Hunters perceive a higher risk of decline in moose population compared to non-hunters.

RH_2 - Individuals who perceive a high risk from all ticks also perceive a high risk from winter ticks.

RQ_2 – How do participants from the Penobscot Nation define a "healthy" moose population?

4.2 Materials and Methods

4.2.1 Questionnaire Design and Measurements

An online questionnaire was created using Qualtrics and was distributed among Penobscot Nation citizens. The questionnaire included eight sections measuring: (1) recreation habits, (2) experiences with moose and winter ticks, (3) knowledge of moose and winter ticks, (4) information sources that are frequently used, (5) experiences with all types of ticks, (6) normative beliefs regarding the moose population, (7) perceived risk associated with the impacts from winter ticks on the moose population in Maine (including decline in the population), impacts of all types of ticks, and impacts of moose-winter ticks interactions on human systems, and (8) socio-demographics. The questionnaire (Appendix A) utilized mostly close-ended questions with ordered response choices, five open-ended questions, and partially close-ended questions with ordered response choices to take advantage of the different strengths of multiple

types of questions (Salant & Dillman, 1994; Vaske, 2008). The questionnaire included primarily continuous scales (7-point Likert scale), and nominal dichotomous selection (YES/NO). A pre-test (n = 25) was used to assess the quality of the instrument and reduce measurement error before implementation; however, one limitation of the pre-test process used was that the pre-test was done mostly with non-Wabanaki citizens, hence a potential source of error (Visser, Krosnick, & Lavrakas, 2000). To reduce this type of error, the instrument was reviewed and approved by multiple Penobscot Nation citizens prior to implementation. I used built-in "logic" in Qualtrics software to make sure questions were relevant to the respondent. Also, I made questions as concise as possible for readability (Dillman, Smyth, & Melani, 2009).

A consent form was included at the beginning of the questionnaire to ensure that participants would understand that their privacy would be protected, the risks associated with completing the questionnaire, and that participation was voluntary (Appendix B). Recruitment and questionnaire materials were approved by the Institutional Review Board of the University of Maine, the Wabanaki Center of the University of Maine, and by Cultural Affairs within the PN (Appendix F).

4.2.2 Measurement Scales

Several items were included in the questionnaire to determine the level of respondents' perceived risk that winter tick prevalence could have to them personally, to the Penobscot Nation, and the natural environment or moose (to measure risk to wildlife). The items for risk scales were adapted from a previously tested instrument developed by Needham, Vaske, and Petit (2017) that studied perceived risks of the impacts of CWD to wild animal populations.

In section 1, respondents indicated their recreational activities from a list of 27 options, including Arts or Cultural Activity, Backpacking, Canoeing, Fiddle heading, Gathering Plants, Hunting, Ice Fishing, Sightseeing/Driving for Pleasure, Viewing Wildlife, among others.

To measure risk perception, respondents reported their level of concern or seriousness of the perceived risk from a decline in moose on a 7-point Likert scale (1= not concerned at all to 7= very concerned). Next, respondents were asked to rate on a 7-point Likert-scale how serious (1= "Not Serious at All" to 7= "Very Serious") of a threat they believed a decline in moose would pose to PN. Lastly, respondents indicated how likely (1= very unlikely to 7= very likely) they believe ticks or an increased mortality in moose would affect them personally, the Penobscot Nation, and the moose population (Table 4.2, Appendix A). Experience with moose and winter ticks (e.g., witnessing certain conditions described as part of the scale-items such as, "I have seen a live moose", or "I have seen a dead moose"…) was measured by using multiple items that asked about an individual's direct exposure to moose, winter ticks and all tick species in Maine (Table 4.3, Appendix A). Respondents were finally asked about their ethnicity (e.g., Native American/ Alaskan Native, White…), gender (Male, Female, Other) and education (e.g., Associates degree, Bachelors degree, Masters Degree…).

4.2.3 Participant Selection and Questionnaire Implementation Procedure

Multiple channels were used to invite PN citizens to participate in the questionnaire. I used five different outlets for recruitment, including the Department of Natural Resources (DNR) E-Newsletter, the DNR Website, the DNR Facebook page, the PN Community Flyer, and the PN Website. On October 30th, 2018 the community flyer was released by the DNR (Appendix E) and the online link to the questionnaire was made available on the DNR website. The community

flyer was distributed by email to Penobscot Nation Citizens, mailed to a few Penobscot Citizens on Indian Island (see Figure 1.1), and posted on the PN home page. Two reminders using the community flyer were subsequently sent using the same format on November 26th, 2018 and January 6th, 2019. Further, on November 6th, 2018 the invitation to participate was included on the PN website homepage in a section separate from the community flyer to enhance visibility (Appendix D). For most of the modes of recruitment, additional reminders were sent out to increase response rates (Dillman, Smyth, & Christian, 2014). Additional reminders were sent through the DNR E-Newsletter (Appendix C) in the following monthly newsletters, on December 3rd, 2018 and January 2nd, 2019. Those reminders in January greatly increased the response rate (Figure 4.1). Finally, the invitation to participate was offered on two different PN private Facebook pages on November 13th, 2018. An additional post was made on those Facebook pages on January 3rd, 2019. The questionnaire closed on January 22nd, 2019. Only two respondents indicated "other" recruitment modes without specifying the actual source where they learned about the questionnaire. The strategies of using multiple channels to invite participants and multiple reminders resulted in 126 people responding to the anonymous online questionnaire.

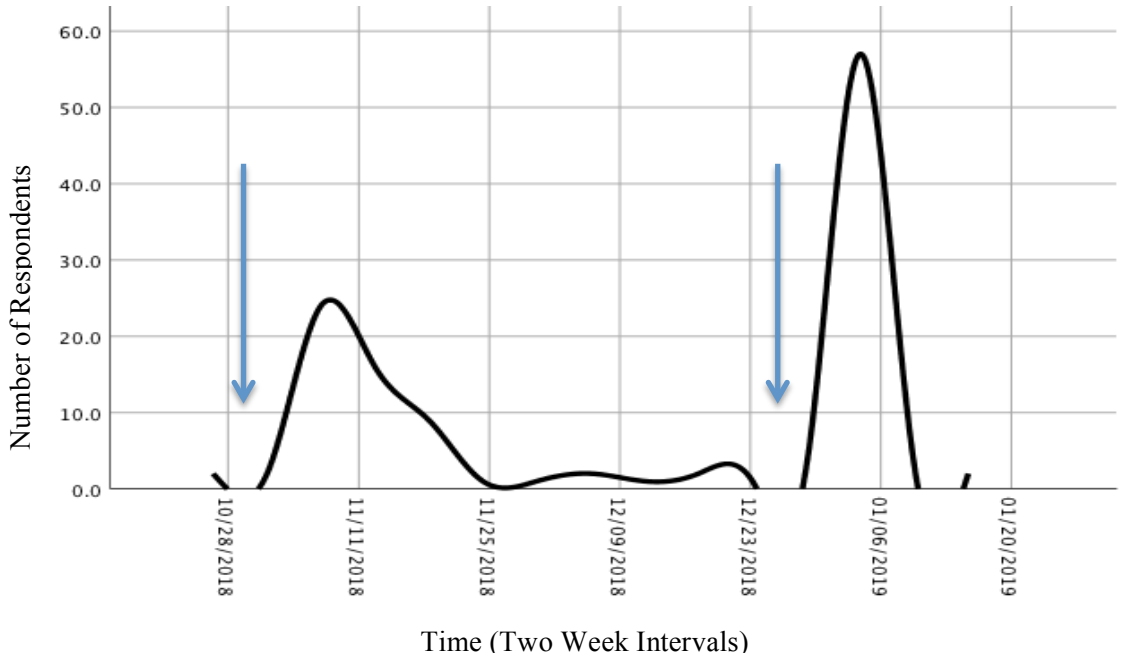

Figure 4.1 Number of responses after distribution of recruitment materials. Reminders sent using multiple modes of recruitment resulted in higher response rates. The first arrow (left) indicates the date at which the first invitation was sent out, and the second arrow (right) indicates where the reminders were sent to individuals through nearly all modes of recruitment.

4.2.4 Database Management, Indices and Statistical Analysis

All of the following was done in SPSS statistical software (IBM). Database management involved coding for no response and not applicable responses and removing respondents that did not respond to more than five questions. I recoded the item regarding the respondent's recreation activities into a dichotomous variable depending on whether they indicated they were a hunter (1) or not a hunter (0). Experiential index values were calculated by computing a new variable in which all the items seen in Table 4.3 were added together if they were answered "yes". Risk perception values were averaged for the perceived likelihood/risk to each hazard (Needham, Vaske & Petit, 2017). The independent variables were experiential index value (RQ_1) and whether or not the respondent was a hunter (RH_1). The three dependent variables were perceptions of risk associated with a decline in moose, presence of winter ticks, and presence of

all species of ticks. Regression analysis was used to test if individuals who perceive a high risk from all ticks also perceive a high risk from winter ticks (RH_2).

Cronbach's alpha (α) was calculated independently for each scale to ensure reliability of risk measurement (Cronbach, 1951). Scales were considered to have a sufficient internal consistency when $\alpha > 0.75$ (Bland & Altman, 1997). Descriptive statistics were calculated for all variables and the Shapiro-Wilk test was used to confirm normality, and where data were not normally distributed (Ghasemi & Zahediasl, 2012). Primarily nonparametric tests were used because they require minimal distribution assumptions (Higgins, 2003). Mann-Whitney Wilcoxon test (hereafter referred to as Wilcoxon test) and Kruskal-Wallis ANOVA (if there were more than two levels being compared) were used for each pair in which non-normal distribution was ranked for several sample groups (Higgins, 2003). To increase statistical power, a Student's t-test was used for several analyses where normality assumptions were met and/or parametric and non-parametric results were equal (Anderson, 1961). Spearman's ρ nonparametric regression was used to test the relationship between non-normal continuous data (Higgins, 2003), such as the relationship between the experiential index values with the three scales of perceived risk.

Responses provided to the open-ended questions about defining a "healthy" moose population (RQ_2) were analyzed using the NVivo 12 Plus © software in order to identify the most commonly used words included in respondent descriptions of a healthy moose population, and to visually explore connections between major ideas. To do so, word frequency queries were ran and code reference hierarchy charts generated (Jackson & Bazeley, 2019).

4.3 Results

Overall, 55 (75%) of the respondents exclusively identified as "Native American or Alaskan Native", and 5 (7%) respondents exclusively identified as "White". A total of 11 (16%) individuals identified as both "Native American or Alaskan Native" and "White". The most frequent education levels selected by participants were "Some college, no degree" and a "Bachelor's degree". The majority of participants live in Maine (86.8%) and of those who responded with their gender (n = 62), 56% where female and 35% were male. Frequencies of different demographic variables are in Table 4.1. Of 88 participants who reported their favored types of recreation, 26 (30%) indicated they were hunters. Of the 26 hunters, 24 (92%) reported to have hunted moose, but only 10 (42%) indicated their last moose hunt was successful.

In response to RH_1, hunters' risk perceptions towards the impacts of winter ticks and all types of ticks were lower than those that indicated they did not hunt, except in terms of the perceived risk from a decline in moose. However, even though significance was questionable, there was no significant difference between the average risk perceptions from a decline in moose, all ticks, or winter ticks when hunters were compared to non-hunters ($\alpha = 0.95$, Wilcoxon test), accepting the null hypothesis of no difference across groups. There was a significant difference between the responses to individual questionnaire items pertaining to an individual's personal risk to a decline in moose and the risk to the natural environment as a result of all tick species, with non-hunters having higher perceived risk than hunters in each case (RH_1, Table 4.2). Hunters had a significantly higher experience index value than non-hunters ($\bar{X} = 5.60$ versus $\bar{X} = 4.42$, $p = 0.018$, Student's t-test).

Table 4.1 Summary of the frequencies for gender, ethnicity and education variables for the respondents

Demographic Variable	***n***	**Percent**
Gender[a] (*n* = 63)		
Male	24	38.1%
Female	38	60.3%
Other	1	1.5%
Ethnicity[b] (*n* = 68)		
Native American/Alaskan Native	51	75.0%
Native American/ Alaskan Native and White	11	16.2%
White	5	7.4%
Education (*n* = 68)		
Some high school, no diploma	2	2.9%
High school or equivalent	6	8.8%
Some college, no degree	21	30.9%
Associates degree	10	14.7%
Bachelors degree	15	22.1%
Masters degree	11	16.2%
Doctorate degree	3	4.4%

[a] 5 individuals indicated they did not want to respond, and they were coded as missing data

[b] A single respondent identified as all of the ethnicities, but was still included in other analyses

Table 4.2 Summary of variable/item means, ranges, normality tests and concept reliabilities for risk scales. A *p*-value <0.05 for the Wilcoxon test for independent samples = difference between hunters and non-hunters. All items had a range of 5 or 6 on a 7-point scale. Means were calculated only for individuals that responded to 2 or more of the items in each scale.

Risk concepts and variables	Mean Responses		*n*	Wilcoxon Test
	Hunter	Non-Hunter		
	n = 26	*n* = 62		
Risk to Decline of Moose (α = 0.81)	$\bar{X}$ = 5.88	$\bar{X}$ = 5.65	71	*p* = 1.000
How serious of a threat a decline in moose would pose to…				
You personally	5.77	4.39	73	*p* = 0.001*
Penobscot Nation	6.05	6.22	72	*p* = 0.857
The natural environment	6.14	6.14	73	*p* = 0.781
What is your concern for the potential of a decline in moose to result in:				
Lost family traditions	5.95	5.73	71	*p* = 0.186
Reduced food/sustenance	6.00	6.02	71	*p* = 0.519
Reduced cultural material (clothing, bedding, art, etc.)	5.36	5.45	71	*p* = 0.828
Risk of All Types of Ticks to…(α = 0.83)	$\bar{X}$ = 5.35	$\bar{X}$ = 5.89	69	*p* = 0.119
How serious of a threat do you believe ticks are to:				
You personally	5.38	5.61	70	*p* = 0.479
Penobscot Nation	5.35	6.02	69	*p* = 0.110
The natural environment	5.30	6.04	69	*p* = 0.025*
Risk of Winter Ticks to.. (α = 0.92)	$\bar{X}$ = 5.40	$\bar{X}$ = 5.74	36	*p* = 0.125
How likely is it that winter ticks will have negative impact on:				
You personally	4.75	4.55	36	*p* = 0.694
Penobscot Nation	5.31	6.10	36	*p* = 0.219
The moose population	5.69	6.30	36	*p* = 0.347
How concerned are you for the potential that:				
The health of the moose population is due to winter ticks	5.25	5.75	36	*p* = 0.645
Winter ticks will threaten future moose hunting	5.56	5.80	36	*p* = 0.895
Winter ticks will dramatically reduce the moose population	5.81	5.95	36	*p* = 0.420

The relative frequencies for the items informing the experience index value for each respondent are provided in Table 3.2. Experience index values ranged from 1 to 9, and the average index value was 4.77. In response to RQ_1, "Do individuals with lower experience with moose and winter ticks perceive less risk to the impacts of winter ticks, all types of ticks, and a decline in moose?", experiential index values had a significant and moderate correlation with perceived risk from winter ticks ($\rho = 0.367$, $p = 0.028$) and perceived risk to a decline in moose ($\rho = .309$, $p = 0.028$), but there was no significant correlation between experience and perceived risk from all types of ticks. Similarly, in response to RH_2, "Individuals who perceive a high risk from all ticks also perceive a high risk from winter ticks", there was a moderate positive correlation between risk perceived from winter ticks and perceived risk from all types of ticks ($\rho = 0.320$, $p = 0.061$).

Table 4.3 Summary of variable/item frequencies used to compute the experience index values.

Items Used for Additive Experience Index	**Yes (1)**	**No (0)**
Experience with Moose		
I have witnessed a:		
Live moose in the wild	72 (94%)	5 (6%)
Dead moose in the wild	31 (43%)	41 (57%)
Dead moose with little to no fur in the wild	10 (14%)	61 (86%)
Experience with Winter Ticks		
I have witnessed a:		
Dead moose infested with winter ticks in the wild	9 (25%)	27 (75%)
Experience with All Ticks		
I have witnessed:		
Family members finding ticks on their body	53 (78%)	15 (22%)
Friends finding ticks on their body	51 (76%)	16 (24%)
I have:		
Known family members that have contracted a disease as a result of a tick bite	28 (42%)	38 (58%)
Known friends that have contracted a disease as a result of a tick bite	53 (79%)	14 (21%)
Heard of animals contracting a disease as a result of a tick bite	49 (80%)	12 (20%)

When asked about what it means for a moose population to be healthy (RQ_2), an exploration of text in the open-ended responses showed there were diverse interpretations shared by participants. The three most common conceptions about a healthy population were that (1) there was no disease in moose, (2) there was not an overpopulation of moose in Maine, and (3) moose are not competing too much for resources (Figure 4.2). For example:

"*To be in balance with the other living organisms of its ecosystem. Enough food to sustain itself, and enough moose to sustain predators. Not diseased, not affected by climate change. Neurological functions within normal range…"* - Anonymous (White female, 62 years old, non-hunter).

Some responses included description related to moose and the paticipants' culture:

"*An Elder told me a story about a dream he had, and of a time when a female moose kept him from shooting her mate. In the dream, the moose asked him to stop hunting because there wasn't enough of them. He hasn't hunted since. They'll tell us when…"*
-Anonymous (Native American, 43 years old, non-hunter).

These conceptions about an emphasis on quality of individual moose to define moose health are also reflected in the most frequently used words displayed in the word cloud in Figure 4.3, like the association of "ticks", "meat" and "disease". Other emerging thoughts included a healthy environment, continued breeding, and fewer ticks, though winter ticks were not specified. One benchmark for a healthy population was being able to hunt moose. When asked about the

impacts of moose decline, the ideas seemed more homogeneous. The most frequently presented idea was that moose decline would result in the loss of an important food source for PN citizens due to reduced hunting opportunities. This was supported by concerns about a decline in the ability to hunt and a loss of traditional culture, as well as negative impact to ecosystems from loss of such an iconic species as moose. For example:

"*I shoot a moose every year to provide for me and my family members. Any threat to the health or decline in population would potentially prevent us from consuming healthy meat, causing a change to a less healthy diet and culture*" - Anonymous (Hunter)

Finally, a somewhat prevalent but important idea was the decline in the ability to see moose and the detrimental impact this could have on tourism.

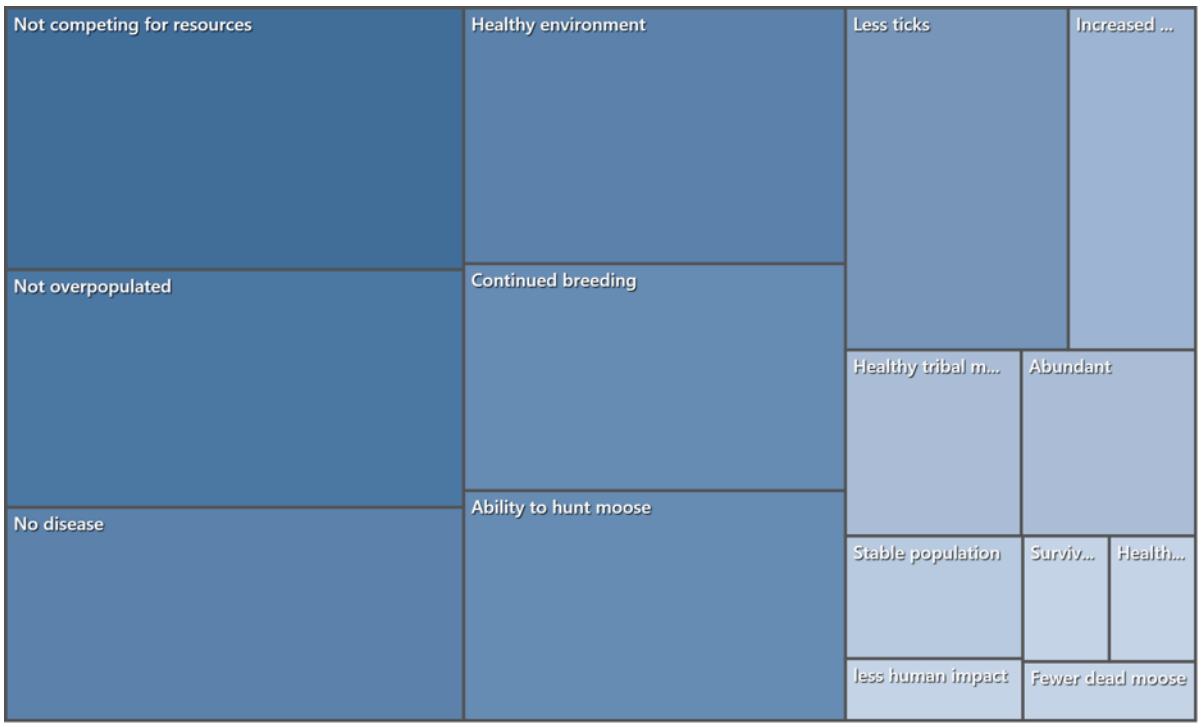

Figure 4.2 Hierarchy chart displaying the most frequently encountered ideas when individuals were asked how they define a healthy moose population.

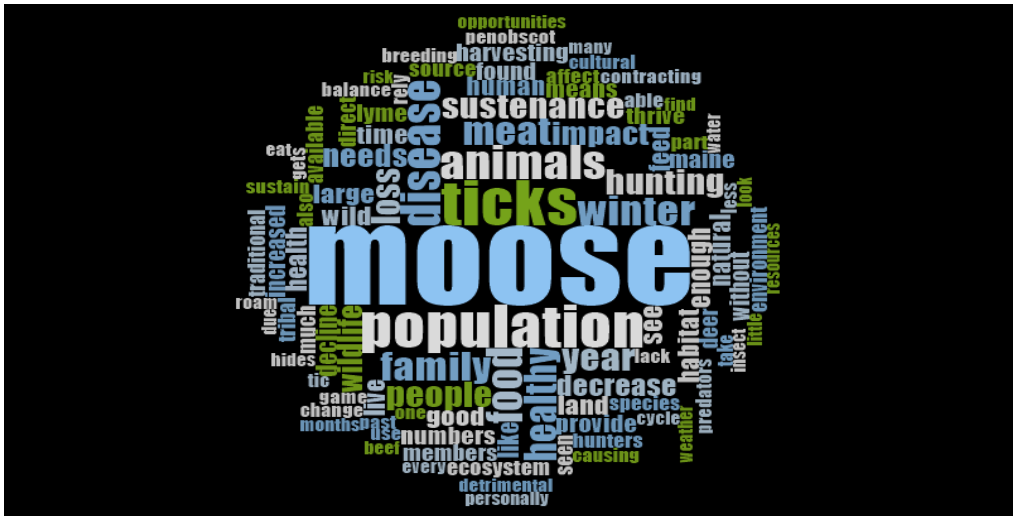

Figure 4.3 Word cloud capturing the common ideas brought up through the open-ended responses offered to respondents.

4.4 Discussion

The results help us better understand perceptions of risk by PN citizens as related to winter tick presence, prevalence of all types of ticks, and a decline in the moose population. In response to RQ_1, a greater amount of experience with winter ticks and moose seemed to increase the amount of risk perceived from both presence of winter ticks and a decline in the moose population, and likely effects on respondents, the PN, and the natural environment. In contrast, the amount of experience with winter ticks and moose had no significant relationship with perceived risk to all types of ticks, which could indicate that participants are processing winter ticks differently than other types of ticks. This is likely due to the enhanced communication from the PN DNR separating tick-borne disease (e.g., Lyme disease) and the association of winter ticks and moose (www.penobscotnation.org, Accessed April 29th, 2019).

Upon developing the climate change risk perception model (CCRPM), van der Linden (2015) also found that personal experience correlated significantly with climate change risk perceptions. However, affect was a much stronger predictor of risk perceptions in that study, and personal experience was weaker in comparison. So while the finding that all types of ticks and winter ticks are processed differently from personal experience could indicate a success in risk communication from the DNR, it is also possible that emotional reactions to risks are partially contingent on the vividness with which negative consequences can be imagined or experienced (Leiserowitz, 2006), and personal experience alone does not capture the vividness required to elicit an emotional response about all types of ticks. Therefore, the inclusion of a scale to measure the affective response from personal experience with moose and winter ticks may benefit the framework used in this study.

In response to RH_1, there was no significant difference between hunters and non-hunters when comparing their perceived risk from winter ticks, all types of ticks, or a decline in the moose population. First, it is possible there is no difference in perceived risk measured in this study between those that hunt, and those who do not hunt. However, I may have misrepresented the population of moose hunters in PN specifically, as only 10 of the 26 hunters reported a successful harvest in their last hunt. If there is indeed no difference in perceived risk between hunters, this could be a function of a coping mechanism. People sometimes believe that they are at less risk than others (e.g., smoking, wearing seatbelts), or have "risk denial" (Sjöberg, 2000), which is a possible coping mechanism for some hunters given the observed importance of moose as a form of cultural pride and sustenance. If this was the case, we could not capture a difference of perceived risk potentially due to the perceived control that hunters feel they have given their knowledge. However, I believe PN citizens simply hold a high value of moose regardless of their status as hunter or non-hunter because nearly all citizens benefit from the moose provided the high availability of the meat for sustenance, and the use of moose for cultural material and teachings (K. Peet, April 23rd, 2019).

For RH_2, I did not find strong evidence that individuals who perceive a high risk from all ticks also perceive a high risk from winter ticks. This was of questionable significance however, and could have been operationalized better because heuristic information processing was a latent variable that was inferred by the correlation coefficient between the perceived risk towards all types of ticks and perceived risk specifically towards winter ticks. However, even when operationalized well, heuristic processing is far less predictive of risk than systematic processing, which is cognitively expensive (Ryu & Kim, 2015).

I predicted that the association of winter ticks with other types of tick (e.g. deer tick or *Ixodes scapularis*, known to vector harmful diseases) would influence risk perceptions due to unfamiliarity with winter ticks (Griffin, Dunwoody, Neuwirth, 1999). I found that average risk perceptions from winter ticks and all types of ticks did not significantly influence each other, despite a moderate correlation. So while PN seems to distinguish the known impacts from all types of ticks from the known association between winter ticks and moose, I recommend further research using heuristic processing to determine risk perceptions towards winter ticks because communication efforts could be enhanced if the threat from all types of ticks was generalized toward ticks not harmful to humans, such as winter ticks.

The finding that a greater amount of experience with winter ticks and moose increased the amount of risk perceived from both winter ticks and a decline in the moose population, but not in all types of ticks, is an interesting find. For PN citizens who participated in this study, perceived risk from winter ticks and a decline in moose elicited the strongest attitudes when compared to perceived risk from all types of ticks. This result suggests that the consequences of winter ticks on moose as perceived by participants seem to elicit stronger feelings than the threat of disease transmission from all types of ticks, a topic that the DNR has included as part of its risk communication efforts (Personal Communication, K. Peet). The apparently stronger attitudes towards the risk from winter ticks and a decline in moose, as opposed to disease transmission from all types of ticks, likely stems from the reliance on moose to sustain the livelihood of the Penobscot community. Moose harvests are often needed for subsistence and are also important in social activities that help define cultural identity and provide links to their history, ancestors, land, art, and environmental philosophy; a common relationship for

indigenous people with wildlife (Kirikiri & Nugent, 1995; Menzies, 2006; Moller, Berkes, Lyver, & Kislalioglu, 2004).

Finally, I addressed RQ_2 by summarizing the common ideas by respondents when asked how they define a "healthy" moose population by sharing the emphasis of "disease" and "ticks" as a function of moose health, in addition to a common idea of reliance on the moose for sustenance and culture. The PN reliance on moose is reflected well from exploring the open-responses to what defines a "healthy" moose population. Most frequently, I found that 1) low competition for resources among moose and 2) the absence of disease defined a healthy population for many, although the mention of ticks was much less frequent. Certainly, more research is warranted to gain a deeper understanding of the diverse meanings of PN citizens regarding "health", but from limited data, it appears that at least some participants identify more with the ecosystem-based approach (Stephen, 2014), in which health is considered an interaction between biologic, social, and environmental determinants that promotes and maintains quality as a capacity to cope with change over time.

Open-ended responses aided in the interpretation of close-ended questions by providing insight into the minutiae of why individuals may value moose and what influences risk perceptions towards their decline. I anticipate that this study will encourage further research on the influence of other constructs deemed predictive of risk perceptions, especially wildlife values. Although rather intangible and often overlooked due to its minimal predictive power in multiple studies (Ajzen, 1991), values should provide further insight to why certain individuals have higher perceived risk, as previous studies have shown (van der Linden, 2015). Even in a wildlife context, wildlife values have been shown to be a sociopsychological determinant with high explanatory power (Triezenberg et al., 2014). Given the connective role of moose in the

culture and livelihoods of PN citizens, PN could be an ideal population to assess the predictive power of values. Furthermore, it is anticipated that these findings regarding the perceived risk of Penobscot Nation citizens from a decline in moose and the presence of winter ticks will help enhance future risk communication efforts by the DNR.

CHAPTER 5

CONCLUDING THOUGHTS, FUTURE DIRECTIONS AND THE USE OF SOCIAL AND BIOLOGICAL DATA TO STUDY VECTOR-BORNE DISEASE

5.1 Reshaping the Criteria for what is Studied in Disease Ecology

5.1.1 Importance of Human Risk Perceptions to Wildlife Disease

Early associations between ticks and certain wildlife have increased the incentive for more detailed research over the years to assess objective risk to wildlife populations. Although significant research has been conducted to measure *objective risk*, this approach leads to an incomplete view of the total impact of TBD. Further research is needed to understand and assess people's *risk perceptions* regarding wildlife disease management (Decker and Chase 1997). Objective and perceived risks tend to interact, as the physical health of wildlife are linked to humans' emotional well-being as a result of high risk perceptions of wildlife health (Decker et al. 2010), especially where a culture is defined by and reliant on the animal. This interaction between the objective and perceived risk is among the founding ideas for the study of One Health. Even if the animal is not directly affected by carrying the vector borne pathogen, such as with white-tailed deer and *Borrelia* species (Telford III et al. 1988), the association with disease risk can elicit a higher perceived risk (Clarke 2009). Similarly, where there is no evidence that there is a health risk to humans, the idea of disease can still influence perceived risk and impact behavior (Brown et al. 2006). This view of the interdependence of human and wildlife health emphasizes that healthy wildlife populations benefit human health and well-being, and vice versa, but this view also implies that unhealthy wildlife may pose a potential hazard to human

health and well-being (Decker et al. 2010, Decker et al. 2012). I propose that this relationship between human and wildlife health is strongest when the wildlife species is charismatic and valued for multiple purposes—ecological, economic and sociocultural—and hence, should be incorporated into the theoretical frameworks and methodological instruments for establishing a population's *holistic* risk, or the sum of the perceived and objective risk.

An accumulation of negative perceptions from associating wildlife with disease may contribute to widespread change in public perspectives about animals (Decker et al. 2012). If public attitudes are of importance, then that association will greatly influence future management practices and societal value of wildlife. However, it is evident that most wildlife management practices assume that scientific understanding of TBDs is solely the result of our application of biological sciences (Endter-Wada et al. 1998). While the evolving principles of ecosystem management recognize that people play an integral role, social considerations are usually restricted to political and policy decision-making processes, and to development of environmental outreach including workshops on preventive behavior towards TBDs (Endter-Wada et al. 1998). This approach is often a hindrance to effective management because ecosystem management decisions based primarily on biophysical factors can polarize people when socioeconomic risk factors are perceived as an afterthought (Endter-Wada et al. 1998, Decker et al. 2012). Further, numerous studies indicate that decisions and actions occur as a result of how multiple people perceive risk, and not necessarily on objective risk measures based solely on natural science techniques.

In this thesis, I suggest that *objective risk* represents the exposure or vulnerability to acquiring a disease, while *perceived risk* is the subjective and interpretive vulnerability to those consequences. There are several ways to model risk perception, however theoretical frameworks

often employed are specific to the hazard, region and population (Vaske 2008). Several of these models have been proposed, but no single theory fully explains risk perceptions (Pidgeon et al. 2003). The history of risk perception theories applied to wildlife disease risk is outlined well by Clarke (2009), and some of the constructs used to measure perceived risk in the context of wildlife disease have been tested empirically by Triezenberg et al. (2014) (see Chapters 1 and 4). I have used the case of moose and winter tick as an example of how holistic risk can be measured, as moose have been largely neglected in the TBD literature.

Furthermore, to understand objective risk or vulnerability, we can use methods to detect the disease exposure by identifying infections and screening for disease prevalence ("what can happen?"), calculating the likelihood of a disease being present as a result of infection ("how likely is it that it will cause disease?"), and the consequences if disease is caused ("if it does happen, what are the consequences?") (Kaplan & Garrick, 1981). Objective risk assessment would be of particular importance when dealing with "neglected" wildlife reservoirs. "Neglected" wildlife reservoirs are defined here as reservoirs of disease with an unknown competence, characterized by a general low level of public awareness and research focus (Tomassone et al., 2018). As with human neglected diseases, lack of awareness is specific to geographical areas, and while some diseases like Lyme disease may be reasonably highly funded, knowledge gaps remain as long as there are unknown host species (Tomassone et al. 2018). Cervids, and deer in particular, are known to play a central role in the ecology of tick-borne diseases (Duh et al. 2005, Mircean et al. 2014) by influencing the abundance and range expansion of ticks (Piesman et al. 1979, Paddock and Yabsley 2007), as well as the prevalence of pathogenic infections (Lane et al. 1991). Conversely, there is currently no evidence demonstrating that moose are different in their capacity to both maintain a tick population and

act as a reservoir for disease transmission. However, the literature is heavily skewed towards white-tailed deer and small mammal research in North America, especially when compared with European literature where moose are known to maintain certain diseases (e.g., HGA; Stuen, 2007). This is likely due to the lack of overlap between areas of high TBD occurrences and areas where moose are abundant, but this is changing with the emergence of TBDs in Maine (Rand et al., 2007). For this reason, I have found that moose are neglected as wildlife reservoirs in North America (Appendix G). Although the potential for moose to maintain human disease is unknown in North America, the risk to humans would be negligible without a vector to transmit the pathogen. I have identified this as one of the primary questions from this thesis (Chapter 2), as it remains uncertain to where the *Anaplasma spp.* Cervus originates. From my data, there is no evidence of any transmission of pathogens between ticks and moose that would cause disease in humans or other animal species.

5.1.2 One Health

The goal of integrating social and biophysical data requires a highly interdisciplinary approach. The emerging popularity of "One Health" is an idea that promotes interdisciplinary study and action, across all animals, plants and the physical environment. The initiative is founded on the understanding that, in order to measure social and ecological risk factors, new ideas are needed in all aspects of the scientific method, from conceiving experiments through analysis and interpretation. In fact, the One Health initiative was born from perceived risk from an avian-derived disease (i.e. H5N1) spilling over and causing a pandemic in the human population (Gibbs, 2005). The One Health literature could then be used as a model to reevaluate the criteria for which wildlife are to be emphasized in TBD research.

It is acknowledged that there are challenges with an interdisciplinary approach. Even in the near 20-year-old One Health initiative there have been concerns over the effective implementation of the goals to unite multiple professions. There is little argument over the utility of interdisciplinary work, but implementation has remained a challenge given institutional, funding, paradigmatic, and individual barriers to collaboration and integration (Morzillo et al., 2013). Although the One Health approach has championed interdisciplinarity, the initiative has been largely focused on integrating data across several disciplines in the biophysical sciences, such as genetics, agriculture, and veterinary science. One Health rarely incorporates a human dimension component (MacMynowski, 2007), possibly because of real or perceived difficulty in using the separate dimensions to complement each other; I propose that this thesis represents just such an example.

5.1.3 Integrating Biological and Social Sciences

The integration of socio-ecological research is difficult in a univariate framework. In any case, the first step of determining risk from a vector or a disease in a given area is to establish presence and absence of a disease at the spatial scale previously defined by the researcher. The ecological risk must be qualified based on the abundance and contact of any competent reservoir hosts, spillover host, vector, and target host of interest (i.e., humans) (Jones, Garman, LaFleur, Stephan, & Schaffner, 2002). In this step, vector niche modeling of reservoirs and suitable habitats for ticks can be used to interpolate a relative risk map that estimates the objective risk in different regions (Randolph, 2004). Land cover maps are one way to compare and effectively visualize factors that are known to enhance ecological risk, and can be overlaid with perceived

risk at some scale in order to focus communication efforts where the largest gaps in objective and perceived risk align (Bourne et al., 2016).

In both ecological and social frameworks, Principal Component Analysis (PCA) techniques and related multivariate methods have been used as a means of creating information-rich spatially explicit aggregate indices of socio-ecological vulnerability (Abson et al. 2012). For example, when Brooker et al. (2004) measured economic status of households on the basis of asset ownership without access to direct income or expenditure information, they used scores from a PCA to determine the weights for an index of asset variables in order to calculate the "wealth index". Such a technique has proved reliable (Filmer and Pritchett 1998), and with recent advances, may be used to distill the complexity of elements associated with both perceived and objective risk (Jolliffe & Cadima, 2016). When comparing questionnaire data on risk perceptions (Chapter 4) with ecological risk to livelihood (Chapters 2 and 3), a PCA can be used to reduce multiple social and biological results into a single holistic measure of risk.

Although PCA is a powerful tool, it is limited in its ability to compare across studies. In contrast, indices have the benefit to be developed for reproducibility and simplicity. The Potential of Conflict Index (PCI) has proved itself as a powerful index to enhance communications by conveying information about a distribution' s central tendency, dispersion, and form simultaneously in the context of managerial concerns (Manfredo, Vaske, & Teel, 2003; Vaske, Beaman, Barreto, & Shelby, 2010). While this is developed for the social sciences, there could be instances where biological indices are developed to meet the criteria for PCI; for example, a response scale in which there is a neutral center point with an equal number of response options on either side.

The last technique that I will propose is the use of structural equation modeling (SEM) to measure the additive effects of biophysical and social factors on both objective and perceived risk. In general, SEM uses various types of models to depict relationships among observed variables, with the same basic goal of providing a quantitative test of a theoretical model hypothesized by a researcher (Bagozzi & Yi, 1988). The goal of emphasizing SEM here is not to argue statistical techniques of implementing SEM, but rather to argue for the inclusion of social and biophysical variables in hypothesis testing where SEM is used. For example, the entomological risk could be used as a measure of objective risk, providing a direct comparison with the perceived risk that is estimated using the theoretical framework offered by this thesis (Chapter 1).

5.2 Summary of Major Findings

In response to G1, I found a large proportion (~54%) of moose calves in Maine are infected with an uncharacterized *Anaplasma* species, with a significant difference in *Anaplasma* prevalence between northern and western study sites and different sexes. *Anaplasma* was also detected in winter ticks, but only in a single pooled sample taken from one moose (<1%). I conducted a Bayesian phylogenetic analysis using these sequence data, which revealed that the single *Anaplasma* strain in moose was highly divergent from the strain identified in winter ticks, and most closely related to an uncharacterized North American cervid strain, so I classified it as "*Anaplasma spp.* Cervus". While this result is novel and interesting, several outstanding questions remain from my findings in Chapter 2. I am uncertain to what could be the vector that transmits *Anaplasma* spp. Cervus to moose, but because the majority of moose calves are born the previous year around the middle of May (personal communication, L. Kantar), and it is

unlikely that vector borne transmission occurs after November (given the Maine climate) (Sonenshine & Roe, 2013), the transmission of *Anaplasma* spp. Cervus likely occurs between May and November. Possible candidates that could act as a vector for *Anaplasma* spp. Cervus include deer ked (*L. cervi*) or the moose fly (*Haematobosca alcis*).

I addressed G2 by using a survival analysis and multiple model selection criteria, and found that in moose with severe infestations of winter ticks, *Anaplasma* spp. Cervus significantly decreased the probability of survival. Furthermore, peripheral blood smear analysis and calculation of packed cell volume (PCV) suggested moose infected with *Anaplasma* spp. Cervus may have increased frequency of red blood cell inclusions, and decreased red blood cell volume. The evidence I presented here suggests that *Anaplasma* spp. Cervus might have sub-clinical effects on the moose in Maine. I highly recommend that presence or absence of *Anaplasma* spp. Cervus be considered when estimating reference levels for blood parameters and studying other factors influencing moose survival in Maine, such as winter tick parasite load. Also, immunogenetic risk factors should be considered in the future, as my data show that not all moose react negatively to *Anaplasma* spp. Cervus infection.

In response to G3, there was no influence of a respondent's status as a hunter on risk perceptions, and although perceived risk from all ticks did not seem to influence the perceived risk from winter ticks, I did find that an individual's level of experience is significantly and positively correlated with the risk perceived from a decline in moose and winter ticks, but not perceived risk to all types of ticks. In line with an additional goal of the questionnaire, based on exploration of text from an open-ended question regarding the definition of a "healthy" moose population, several Penobscot Nation citizens used terms related to quality characteristics of moose and likelihood of cultural activities to be pursued given the status of the population.

I believe the work detailed by this thesis provides valuable insights into the relationships between moose, ticks and disease, and human systems; information that is key for maintaining healthy moose populations into the future. Furthermore, this study also underlines the need for transdisciplinary research to gain a better understanding of complex conservation and disease management issues, and will hopefully inspire further research on the interaction between vector-borne diseases, the Maine moose population, and human systems.

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APPENDIX A: QUESTIONNAIRE FOR PENOBSCOT NATION CITIZENS

How did you hear about this survey?

- ○ Department of Natural Resources E-Newsletter (1)
- ○ Department of Natural Resources Website (2)
- ○ PIN Community Flyer
- ○ Other (please specify) _____

Consent form shown here (Appendix B)

Section 1. This section will ask questions about your outdoor recreation activity.

Outdoor recreation activity is defined here as including: outdoor adventure pursuits (e.g. camping, backpacking, canoeing), motorized activities (e.g. snowmobiling, sightseeing), nature study (e.g. bird or other wildlife watching), hunting, and natural interpretation (e.g. walking a nature trail). Please answer the following questions about your outdoor recreation.

At which time of year do you recreate outdoors in Maine? *(Please select ALL that apply)*

- ☐ Spring (March - May)
- ☐ Summer (June - August)
- ☐ Fall (September - November)
- ☐ Winter (December - February)

At which time of year do you recreate most?

- ○ Spring (March - May)
- ○ Summer (June - August)
- ○ Fall (September - November)
- ○ Winter (December - February)

When you recreate outdoors, how often do you encounter ticks in Maine for each season?

	Never	Very Rarely	Rarely	Somewhat Rarely	Occasionally	Frequently	Very Frequently
Spring (March - May)	○	○	○	○	○	○	○
Summer (June - August)	○	○	○	○	○	○	○
Fall (September-November)	○	○	○	○	○	○	○
Winter (December - February)	○	○	○	○	○	○	○

Which outdoor recreation activities do you participate in? *(Please select ALL that apply)*

- ☐ Arts of Cultural Activity
- ☐ ATV Riding
- ☐ Backpacking
- ☐ Biking
- ☐ Boating
- ☐ Camping
- ☐ Canoeing
- ☐ Cross Country Skiing
- ☐ Fiddle heading
- ☐ Fishing
- ☐ Gathering Plants
- ☐ Going to the beach
- ☐ Hiking
- ☐ Hunting
- ☐ Ice Fishing
- ☐ Ice Skating

- ☐ Kayaking
- ☐ Non-technical mountain climbing
- ☐ Picking berries
- ☐ Picnicking
- ☐ Sightseeing/driving for pleasure
- ☐ Skiing
- ☐ Snowboarding
- ☐ Snowmobiling
- ☐ Snowshoeing
- ☐ Swimming
- ☐ Trail running
- ☐ Viewing wildlife
- ☐ Other (*Please specify*)

_____

IF respondents indicate they are a hunter…

How long have you been hunting in Maine?

- ○ less than 5 years
- ○ 6-10 years
- ○ 11-20 years
- ○ 21-30 years
- ○ 31-40 years
- ○ 41 years or more

Have you hunted in any of the following Penobscot Nation land trust regions? (*Please select ALL that apply*)

- [ ] Alder Stream
- [ ] Argyle
- [ ] Grindstone
- [ ] Matagamon
- [ ] Matamiscontis / South Branch
- [ ] Lee / Lakeville
- [ ] Williamsburg
- [ ] Islands on the River
- [ ] Other area in Maine _____

At which time of year do you most frequently hunt in Maine?

- ○ Spring (March - May)
- ○ Summer (June - August)
- ○ Fall (September - November)
- ○ Winter (December - February)

Have you ever hunted moose in the state of Maine?

- ○ Yes
- ○ No

IF respondent has hunted moose…

Was the last year that you hunted moose a successful hunt?

- ○ Yes
- ○ No

Have you hunted **moose** in any of the following Penobscot Nation land trust regions? (*Please select ALL that apply*)

- ☐ Alder Stream
- ☐ Argyle
- ☐ Grindstone
- ☐ Matagamon
- ☐ Matamiscontis / South Branch
- ☐ Lee / Lakeville
- ☐ Williamsburg
- ☐ Islands on the River
- ☐ Other area in Maine _____

Section 2. This section asks questions regarding your experience with moose and winter tick.

Have you ever heard of winter ticks?

- ○ Yes
- ○ No

IF respondent says yes…

In your opinion, what is a **winter tick**?

_____

_____

_____

_____

_____

Winter ticks are different than other more well-known types of ticks, like dog and deer ticks. To the best of your ability, please answer the questions below about your experiences with moose

	Please select one		
	Yes	No	I Don't Know
I have witnessed a live moose in the wild	○	○	○
I have witnessed a dead moose in the wild	○	○	○
I have witnessed a dead moose with little to no fur in the wild	○	○	○
I have witnessed a moose infested with winter ticks	○	○	○

Please rate the following statements to the best of your ability

	Very Valuable	Valuable	Neutral	Somewhat Valuable	Not Valuable
I believe that moose are …	○	○	○	○	○
I believe that ticks, in general, are…	○	○	○	○	○

To what extent do you agree or disagree with the following statements regarding winter ticks?

	Strongly Disagree	Disagree	Somewhat Disagree	Neither Disagree nor Agree	Somewhat Agree	Agree	Strongly Agree
Winter ticks can be found in Maine	○	○	○	○	○	○	○
I am confident in my knowledge of winter ticks	○	○	○	○	○	○	○
I can tell the difference between winter ticks and other types of ticks	○	○	○	○	○	○	○
Winter ticks are becoming more prevalent in Maine	○	○	○	○	○	○	○
Winter ticks, specifically, can transmit disease to humans	○	○	○	○	○	○	○

To what extent do you agree or disagree with the following statements regarding the Maine moose population?

	Strongly Disagree	Disagree	Somewhat Disagree	Neither Disagree nor Agree	Somewhat Agree	Agree	Strongly Agree	I Don't Know
The moose are becoming harder to find each year in Maine	○	○	○	○	○	○	○	○
The moose are becoming harder to find on trust lands	○	○	○	○	○	○	○	○
The moose are at risk of decline as a result of winter ticks	○	○	○	○	○	○	○	○
Winter ticks can be found on moose in Maine	○	○	○	○	○	○	○	○

Please indicate, to the best of your knowledge, how you believe that each of the following items impact moose population health in Maine?

	Major Impact to Moose Health	Minor Impact to Moose Health	No Impact to Moose Health
Hunting…	○	○	○
Vehicle collisions leading to death or injury…	○	○	○
Winter Ticks…	○	○	○
Infectious disease leading to injury …	○	○	○
Low availability of food in the winter…	○	○	○
Human land use disrupting moose habitat…	○	○	○
The consumption of road salt leading to injury…	○	○	○
Excessive snow in the winter leading to injury…	○	○	○
Other (*please specify*)	○	○	○

How much do you think each of the following actions would directly affect the health of the moose population in Maine?

	Likely to increase moose health a lot	Likely to increase moose health a little	Not likely to increase moose health
A decrease in moose hunting…	○	○	○
An increase in moose hunting…	○	○	○
Increasing signs on roads near areas with a high density of moose…	○	○	○
Decreasing the use of road salt …	○	○	○
Deforestation...	○	○	○
Increase conservation lands...	○	○	○
Other (*please specify*)	○	○	○

Section 3: This section asks you about where you most frequently obtain information about moose. By knowing which sources of information are used most, new information gained about wildlife health can be better communicated.

Please indicate how often you use the following sources to find information about moose population health.

	Never	1 or 2 times in a year	3 or 4 times in a year	5 or more times in a year
Read about moose health in a print document from the Penobscot Nation Department of Natural Resources	○	○	○	○
Read about moose health in the Penobscot Nation Department of Natural Resources internet website	○	○	○	○
Read about moose health in an online document from another internet website	○	○	○	○
Read about moose health on a social media website (Facebook, Twitter, etc.)	○	○	○	○
Discussed moose health with friends	○	○	○	○
Discussed moose health with family members	○	○	○	○
Read about moose health in magazines or books	○	○	○	○
Read about moose health in hunting / sportsmen's club newsletter.	○	○	○	○
Learned about moose health from conservation groups	○	○	○	○
Attended a live presentation about moose health	○	○	○	○
Listened to radio news / radio programs about moose health	○	○	○	○
Other (*Please specify*)	○	○	○	○

In your opinion, to what extent do you agree with the following statements regarding moose population health?

	Strongly Disagree	Disagree	Somewhat Disagree	Neither Disagree nor Agree	Somewhat Agree	Agree	Strongly Agree
People whose opinion I value, think that I should care about moose population health	○	○	○	○	○	○	○
It is generally expected of me to know the status of moose health	○	○	○	○	○	○	○
People that are important to me would support me if I decided to help improve the health of the moose population	○	○	○	○	○	○	○

Please answer the following questions regarding your perceptions of risk to the potential of a decline in the moose population. How serious of a threat do you believe a decline in moose would pose to:

	Not Serious At All	Not Serious	Not Really Serious	Neutral	Somewhat Serious	Serious	Very Serious
You personally?	○	○	○	○	○	○	○
Penobscot Nation?	○	○	○	○	○	○	○
Maine's economy?	○	○	○	○	○	○	○
The natural environment?	○	○	○	○	○	○	○

What would some of the impacts to you personally in response to a decline in the moose population?

_____

_____

_____

_____

_____

To what extent are you concerned with the following statements?

	Not Concerned At All	Not Concerned	Not Really Concerned	Neutral	Somewhat Concerned	Concerned	Very Concerned
The health of the moose population in Maine is due to winter ticks	○	○	○	○	○	○	○
The threat that winter ticks pose to the future of moose hunting	○	○	○	○	○	○	○
The potential for winter ticks to dramatically reduce the moose population	○	○	○	○	○	○	○
The potential for winter ticks to affect human health	○	○	○	○	○	○	○
The potential for a decline in moose to result in lost family traditions	○	○	○	○	○	○	○
The potential for a decline in moose to result in reduced sustenance	○	○	○	○	○	○	○
The potential for a decline in moose to result in reduced cultural material (clothing, bedding, art, etc.)	○	○	○	○	○	○	○

Please answer the following questions about the likelihood of different consequences in response to an increased winter tick population. In your judgement, how likely is it that winter ticks will have a negative impact on:

	Very Unlikely	Unlikely	Somewhat Unlikely	Undecided	Somewhat Likely	Likely	Very Likely
Penobscot Nation?	○	○	○	○	○	○	○
Penobscot Nation culture?	○	○	○	○	○	○	○
Your health or overall well-being?	○	○	○	○	○	○	○
The moose population?	○	○	○	○	○	○	○

In your opinion, what does it mean for a moose population to be healthy?

_____

_____

_____

_____

_____

Section 4: This section asks you about your experience with **ALL types of ticks**.

To the best of your ability, please answer the questions below about your experiences with ALL types of ticks

	Please select one		
	Yes	No	I Don't Know
I have witnessed family members finding ticks on their body	○	○	○
I have witnessed friends finding ticks on their body	○	○	○
I have known family members that contracted a disease as a result of a tick bite	○	○	○
I have known friends that contracted a disease as a result of a tick bite	○	○	○
I have heard about animals contracting a disease as a result of a tick bite	○	○	○
I have heard about moose contracting a disease as a result of a tick bite	○	○	○

To the best of your ability, please answer the questions below about your experiences with **ALL types of ticks**. How serious of a threat do you believe ticks are to:

	Not Serious At All	Not Serious	Not Really Serious	Neutral	Somewhat Serious	Serious	Very Serious
You personally?	○	○	○	○	○	○	○
Penobscot Nation?	○	○	○	○	○	○	○
Maine's economy?	○	○	○	○	○	○	○
The natural environment?	○	○	○	○	○	○	○

Please answer the following questions about **ALL types of ticks** to the best of your ability.

	Strongly Disagree	Disagree	Somewhat Disagree	Neither Disagree nor Agree	Somewhat Agree	Agree	Strongly Agree
Ticks are becoming more prevalent in Maine	○	○	○	○	○	○	○
Ticks can transmit disease to humans	○	○	○	○	○	○	○
Some types of ticks are not harmful to humans	○	○	○	○	○	○	○
Wildlife diseases can always be transmitted to humans	○	○	○	○	○	○	○

In your opinion, what are the greatest threats to an increase in the tick population on tribal lands?

_____

_____

_____

_____

_____

Section 5: This final section will ask questions about yourself. Please do not feel obligated to provide information where you are uncomfortable.

Do you live in Maine?

- ○ Yes
- ○ No

What is your zip code?

_____

What is your age? (*in years*)

_____

What is your gender?

- ○ Male
- ○ Female
- ○ Other
- ○ I do not want to respond

What ethnicity to you identify with? *(Please select ALL that apply)*

- ☐ Native American or Alaskan Native
- ☐ Asian
- ☐ Black or African American
- ☐ Hispanic
- ☐ Native Hawaiian or other Pacific Islander
- ☐ White

What is the highest level of education you have completed?

- ○ 8th grade or lower
- ○ Some high school, no diploma
- ○ High school or equivalent
- ○ Some college, no degree
- ○ Associates degree
- ○ Bachelor's degree
- ○ Master's degree
- ○ Professional degree
- ○ Doctorate degree

Please feel free to add any additional comments regarding the topics in this survey:

_____

_____

_____

_____

_____

Please enter your address in order to be entered into the Cabela's Card raffle. The raffle is not connected to your responses. A winner will be chosen at the end of the study period.

_____

_____

_____

_____

_____

THANK YOU for your participation!
Your responses are greatly appreciated!

APPENDIX B: INFORMED CONSENT FORM

Dear Penobscot Citizen,

You are invited to participate in a research project being conducted by the Penobscot Nation Department of Natural Resources and James Elliott, a graduate student in the School of Forest Resources at the University of Maine. His faculty sponsor is Dr. Sandra De Urioste-Stone from the School of Forest Resources at the University of Maine. The purpose of the research is to better understand your attitudes and views surrounding wildlife and wildlife disease management so the Penobscot Nation Department of Natural Resources can be better informed for effective decision making. You must be at least 18 years of age to participate.

What will you be asked to do?

If you decide to participate, you will be asked to fill out the following questionnaire inquiring about your experience with ticks and moose. The whole process will take approximately 10-15 minutes. If you leave the survey early your responses will be saved and you may continue the survey later from the point where you left.

Risks

Except for your time and inconvenience, there are no risks to you from participating in this study.

Benefits

There are no direct benefits to you. However, this will be the first study to investigate attitudes towards wildlife disease and wildlife health. This survey will also assess what the preferred medium is for communication of wildlife related information so the Penobscot Nation Department of Natural Resources can optimize community outreach.

Compensation

At the end of the study, you will have the option of entering your address into a raffle to win one of three $50 Cabela's gift cards. You will need to reach the end of the survey for your address to be entered. The raffle will not be connected to your survey responses.

Confidentiality

Survey data will be anonymous. The investigators will not have access to your contact information because it is being administered by the Penobscot Nation Department of Natural Resources. All data will be stored in an encrypted, password-protected computer using software that provides additional security, only to be accessed by the investigators listed in this form. All data in possession of the investigators at the University of Maine will be destroyed by August 2023. Additionally, data will be shared with and housed indefinitely by the Penobscot Nation Cultural and Historic Preservation Department.

Voluntary

Participation is voluntary. If you choose to take part in this study, you may stop at any time, but you must reach the end of the survey to enter the raffle. Return/submission of the survey implies consent to participate.

Contact Information

If you have any questions about this study, please contact me at (941)504-2048; james.a.elliott1@maine.edu

You may also reach the faculty advisor on this study at (207)581-2885; sandra.de@maine.edu, or John Banks, the Director of the Department of Natural Resources, at (207)817-7330; John.Banks@penobscotnation.org.

If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, (207)581-1498 or (207)581-2657 (or e-mail umric@maine.edu).

Thank you for taking the time to complete this questionnaire!

APPENDIX C: PENOBSCOT NATION E-NEWSLETTER NOTICE OF QUESTIONNAIRE

November 2018 newsletter - Read about:
Wildlife Survey - with a chance to win a gift card

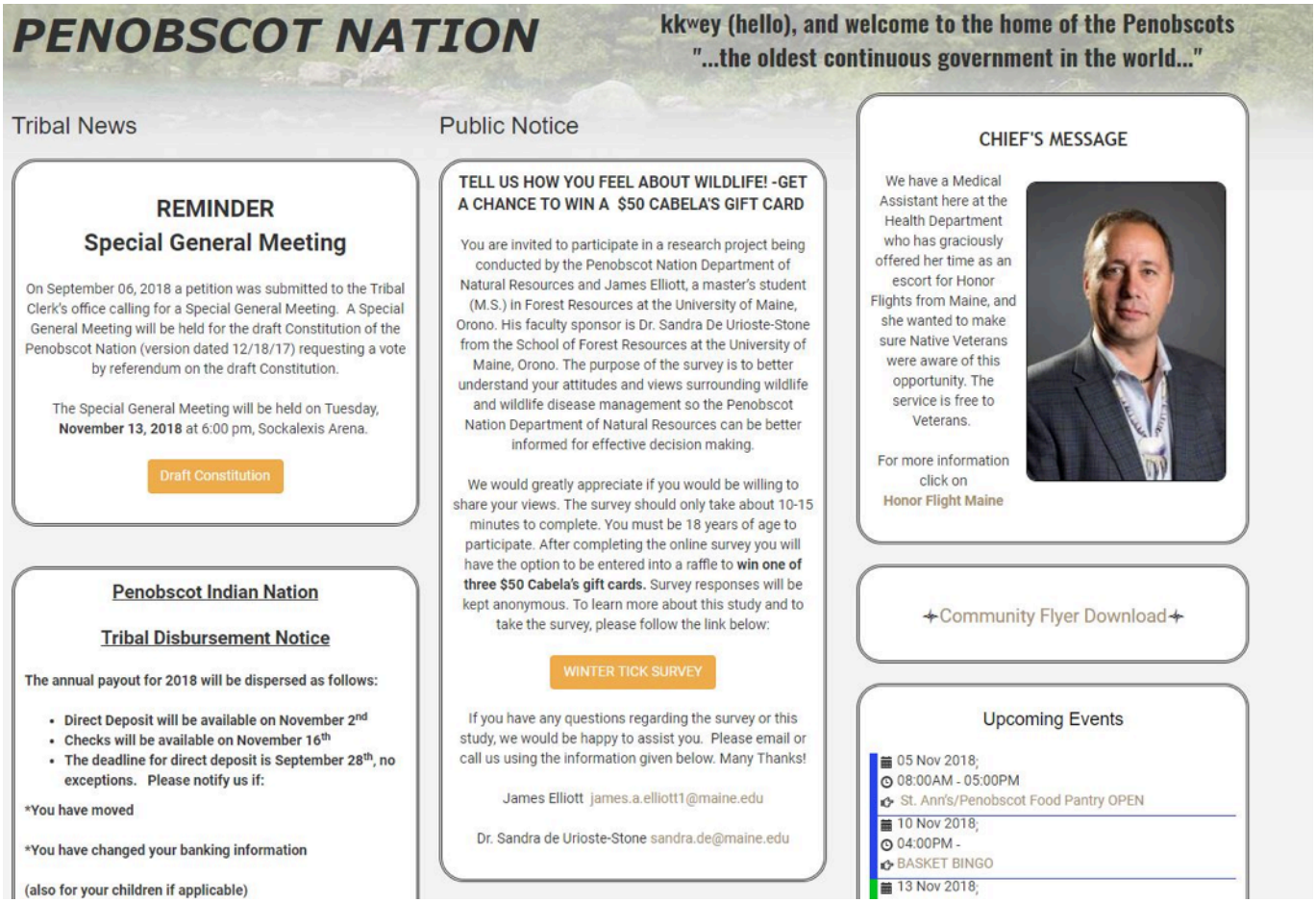
View this email in your browser

TELL US HOW YOU FEEL ABOUT WILDLIFE! – GET A CHANCE TO WIN A $50 CABELA'S GIFT CARD

You are invited to participate in a research project being conducted by the Penobscot Nation Department of Natural Resources and James Elliott, a master's student (M.S.) in Forest Resources at the University of Maine, Orono. His faculty sponsor is Dr. Sandra De Urioste-Stone from the School of Forest Resources at the University of Maine, Orono. The purpose of the survey is to better understand your attitudes and views surrounding wildlife and wildlife disease management so the Penobscot Nation Department of Natural Resources can be better informed for effective decision making.

We would greatly appreciate if you would be willing to share your views. The survey should only take about 10-15 minutes to complete. You must be 18 years of age to participate. After completing the online survey you will have the option to be entered into a raffle to **win one of three $50 Cabela's gift cards**. Survey responses will be kept anonymous. To learn more about this study and to take the survey, please follow the link below:

APPENDIX D: PENOBSCOT NATION WEBSITE NOTICE OF QUESTIONNAIRE



PENOBSCOT NATION

kkʷey (hello), and welcome to the home of the Penobscots
"...the oldest continuous government in the world..."

Tribal News

REMINDER
Special General Meeting

On September 06, 2018 a petition was submitted to the Tribal Clerk's office calling for a Special General Meeting. A Special General Meeting will be held for the draft Constitution of the Penobscot Nation (version dated 12/18/17) requesting a vote by referendum on the draft Constitution.

The Special General Meeting will be held on Tuesday, **November 13, 2018** at 6:00 pm, Sockalexis Arena.

Draft Constitution

Penobscot Indian Nation

Tribal Disbursement Notice

The annual payout for 2018 will be dispersed as follows:

- **Direct Deposit will be available on November 2nd**
- **Checks will be available on November 16th**
- **The deadline for direct deposit is September 28th, no exceptions. Please notify us if:**

***You have moved**

***You have changed your banking information**

(also for your children if applicable)

Public Notice

TELL US HOW YOU FEEL ABOUT WILDLIFE! -GET A CHANCE TO WIN A $50 CABELA'S GIFT CARD

You are invited to participate in a research project being conducted by the Penobscot Nation Department of Natural Resources and James Elliott, a master's student (M.S.) in Forest Resources at the University of Maine, Orono. His faculty sponsor is Dr. Sandra De Urioste-Stone from the School of Forest Resources at the University of Maine, Orono. The purpose of the survey is to better understand your attitudes and views surrounding wildlife and wildlife disease management so the Penobscot Nation Department of Natural Resources can be better informed for effective decision making.

We would greatly appreciate if you would be willing to share your views. The survey should only take about 10-15 minutes to complete. You must be 18 years of age to participate. After completing the online survey you will have the option to be entered into a raffle to **win one of three $50 Cabela's gift cards.** Survey responses will be kept anonymous. To learn more about this study and to take the survey, please follow the link below:

WINTER TICK SURVEY

If you have any questions regarding the survey or this study, we would be happy to assist you. Please email or call us using the information given below. Many Thanks!

James Elliott james.a.elliott1@maine.edu

Dr. Sandra de Urioste-Stone sandra.de@maine.edu

CHIEF'S MESSAGE

We have a Medical Assistant here at the Health Department who has graciously offered her time as an escort for Honor Flights from Maine, and she wanted to make sure Native Veterans were aware of this opportunity. The service is free to Veterans.

For more information click on **Honor Flight Maine**

Community Flyer Download

Upcoming Events

05 Nov 2018;
08:00AM - 05:00PM
St. Ann's/Penobscot Food Pantry OPEN

10 Nov 2018;
04:00PM -
BASKET BINGO

13 Nov 2018;

APPENDIX E:

PENOBSCOT NATION COMMUNITY FLYER NOTICE OF QUESTIONNAIRE

TELL US HOW YOU FEEL ABOUT WILDLIFE! - GET A CHANCE TO WIN A $50 CABELA'S GIFT CARD

You are invited to participate in a research project being conducted by the Penobscot Nation Department of Natural Resources and James Elliott, a master's student (M.S.) in Forest Resources at the University of Maine, Orono. His faculty sponsor is Dr. Sandra De Urioste-Stone from the School of Forest Resources at the University of Maine, Orono. The purpose of the survey is to better understand your attitudes and views surrounding wildlife and wildlife disease management so the Penobscot Nation Department of Natural Resources can be better informed for effective decision making.

We would greatly appreciate if you would be willing to share your views. The survey should only take about 10-15 minutes to complete. You must be 18 years of age to participate. After completing the online survey you will have the option to be entered into a raffle to **win one of three $50 Cabela's gift cards.** Survey responses will be kept anonymous. To learn more about this study and to take the survey, please follow the link below:

https://umaine.qualtrics.com/jfe/form/SV_0CEwabSeRMEJEvX

If you have any questions regarding the survey or this study, we would be happy to assist you. Please email or call us using the information given below. Many Thanks!

James Elliott
M.S. Candidate
School of Forest Resources
University of Maine
251 Nutting Hall
University of Maine
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james.a.elliott1@maine.edu

Dr. Sandra de Urioste-Stone
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John Banks
Director of DNR
Penobscot Nation
207-817-7330
John.Banks@penobscotnation.org

APPENDIX F: IRB APPROVAL FORM

APPLICATION COVER PAGE

- ***KEEP THIS PAGE AS ONE PAGE – DO NOT CHANGE MARGINS/FONTS!!!!!!!!!***
- ***PLEASE SUBMIT THIS PAGE AS WORD DOCUMENT***

APPLICATION FOR APPROVAL OF RESEARCH WITH HUMAN SUBJECTS
Protection of Human Subjects Review Board, 400 Corbett Hall

(Type inside gray areas)

PRINCIPAL INVESTIGATOR:	**James Elliott**	**EMAIL:**	**james.a.elliott1@maine.edu**
CO-INVESTIGATOR:		**EMAIL:**	
CO-INVESTIGATOR:		**EMAIL:**	
FACULTY SPONSOR: (Required if PI is a student):	**Dr. Sandra De Urioste-Stone**	**EMAIL:**	**sandra.de@maine.edu**
TITLE OF PROJECT:	**A Socio-Ecological Approach to Wildlife Disease Risk**		
START DATE:	**August, 15th, 2018**	**PI DEPARTMENT:**	**Forest Resources**
FUNDING AGENCY (if any):			

STATUS OF PI: FACULTY/STAFF/GRADUATE/UNDERGRADUATE G (F,S,G,U)

1. If PI is a student, is this research to be performed:

- ☐ **for an honors thesis/senior thesis/capstone?**
- ☐ **for a doctoral dissertation?**
- ☐ **other (specify)**
- ☒ **for a master's thesis?**
- ☐ **for a course project?**

2. Does this application modify a previously approved project? N (Y/N). If yes, please give assigned number (if known) of previously approved project: N/A

3. Is an expedited review requested? Y (Y/N).

Submitting the application indicates the principal investigator's agreement to abide by the responsibilities outlined in Section I.E. of the Policies and Procedures for the Protection of Human Subjects.

Faculty Sponsors are responsible for oversight of research conducted by their students. The Faculty Sponsor ensures that he/she has read the application and that the conduct of such research will be in accordance with the University of Maine's Policies and Procedures for the Protection of Human Subjects of Research. REMINDER: if the principal investigator is an undergraduate student, the Faculty Sponsor MUST submit the application to the IRB.

Email this cover page and complete application to UMRIC@maine.edu

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FOR IRB USE ONLY Application # 2018-07-03 Review (F/E): E Expedited Category:
ACTION TAKEN:

- X☐ **Judged Exempt; category 2 Modifications required? Yes Accepted (date) 7/23/2018**
- ☐ **Approved as submitted. Date of next review: by Degree of Risk:**
- ☐ **Approved pending modifications. Date of next review: by Degree of Risk:**
  Modifications accepted (date):
- ☐ **Not approved (see attached statement)**
- ☐ **Judged not research with human subjects**

FINAL APPROVAL TO BEGIN 7/23/2018
Date

01/2017

APPENDIX G:

METHODS AND RESULTS FOR SYSTEMATIC LITERATURE SEARCH

The hypothesis going into this systematic literature review was that moose and TBD is a neglected topic in North America, especially when compared to Europe. In order to obtain the results to show the disparity in North American and European literature, a total of five databases (BIOSIS, Zoological Records, Web of Science, Ecology Abstracts, and CAB Direct) were searched with a variety of key words that were guided by a database-specific thesaurus to optimize search terms. The syntax used as a consensus for all databases was: ("tickborne diseases" OR "babesiosis" OR "Lyme disease" OR "tickborne fever" OR anaplasmos*) AND (moose OR "Alces alces" OR "Eurasian Elk") AND yr:[1900 TO 2018]. In total, 30 articles were returned. The returned articles were then manually sorted through and it was determined whether or not it was a goal of the study was to directly address TBD in moose specifically. Papers that did not meet this qualification, or were from regions outside of the United States or Europe, were discarded. From this search, only 23.3% of the articles pertained to the United States, while 76.7% of the articles were of European origin, indicating North American moose populations are “neglected” in comparison.

BIOGRAPHY OF THE AUTHOR

James Elliott was born in Beverly, MA and grew up on the Gulf and Atlantic coasts of Sarasota, FL and Danvers, MA, respectively. He graduated from Danvers High School in 2012 and went on to earn a Bachelors of Science degree from Salem State University (SSU), graduating magna cum laude in 2016. James majored in Biology with two minors in Psychology and Chemistry, and was inducted into the Sigma Xi National Honors Society, the National Society for Leadership and Success, the American Chemical Society, the International Psychology Honor Society, and was a founding member of the Chemistry National Honor Society (SSU Chapter). Additionally, James was an active member of the New England Estuarine Research Society and the Crustacean Society. James began his research experience at Salem State University as a freshman by receiving a scholarship from Volunteer Morocco to study drinking water in southwest rural Morocco. While at Salem State University, James primarily worked with Dr. Alan Young studying European Green Crab (*Carcinus maenas*) population dynamics and molting cycle endocrinology, and with Dr. Lynn Atkinson investigating Common Wood Nymph (*Cercyonis pegala*) phylogenetics. As a junior at Salem State University, James worked as a research associate at the Marine Biological Laboratory in Woods Hole, MA studying the developmental biology of American Lobsters (*Homarus americanus*) in the presence of alkylphenolic compounds. James also assisted in research involving marine plastic pollution by helping to characterize microplastic and mesoplastic debris in sediments from Kamilo Beach and Kahuku Beach, Hawai'i.

While attending Salem State University, James worked at the Cat Cove Marine Lab as a research technician, where he gained experience in methods of soft-shell clam aquaculture and developed over three years of experience in husbandry of several marine vertebrates. Simultaneously, James worked for the Massachusetts Division of Marine Fisheries for 3 years on a long-term project focused on the restoration and enhancement of soft-shell clam (*Mya arenaria*) populations in Boston Harbor, Massachusetts. During his last year of Salem State University, James worked as a supplementary instructor for Cell Biology and was a registered tutor for Comparative Vertebrate Anatomy and Entomology. After graduating James worked part time for The Princeton Review as a biology instructor for the Medical College Admission Test where he taught several topics, including biochemistry, genetics, molecular biology, anatomy and physiology. Shortly after, James join another job in Beverly, MA as an Operations Apprentice at Qiagen, a biotechnology company that provides sample and assay technologies for molecular diagnostics, applied testing, academic and pharmaceutical research.

James's interests and varied experiences have led him to pursue integrative research advised by both Dr. Sandra De Urioste-Stone and Dr. Pauline Kamath. James is a candidate for the Master of Science degree in Forest Resources from the University of Maine in May 2019.