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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Matthew M. Berta

A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

REGIS UNIVERSITY
May, 2023

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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CHAPTER 1. LITERATURE REVIEW

Human Encroachment Effects on Chronic Wasting Disease: Revisiting Existing Management Strategies

Introduction

Chronic wasting disease (CWD) is a specific neurodegenerative prion disease that affects both humans and animals as part of a broader family of diseases known as transmissible spongiform encephalopathies (TSEs) (Gilch et al., 2011). CWD, observed primarily in cervid species such as deer and elk, is a highly infectious and ultimately fatal disease currently present in 30 states in the United States, four provinces in Canada, and other localities in South Korea and Norway (Pritzkow, 2022; Nemani et al., 2020). Cervids affected by CWD express symptoms including behavioral changes, excessive salivation, decreased ability to feed, and weight loss (Peters et al., 2000). Behavioral changes like stereotypy, excessive thirst, excessive urination, lack of awareness, fixed stare, and lowered head are characteristic of CWD, often being the leading diagnostic technique for the eventual culling of that animal (Williams, 2005). CWD cases have been confirmed in both captive and wild white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), moose (*Alces alces*), and elk (*Cervus elaphus*), all of which are prominent game species with economic and natural beauty value (Uehlinger et al., 2016; Manjerovic et al., 2014).

Chronic wasting disease has been researched extensively for years, including the relative effectiveness of management techniques to prevent its spread such as culling, vaccinations, and hunting (Mateus-Pinilla et al., 2013). However, there has not been extensive research on the mechanism by which encroachment of human populations into native cervid habitats may

facilitate CWD spread and consequently how its spread might be managed in human-dominated environments. This literature review aims to evaluate the mechanisms by which human encroachment affects the spread of CWD in cervids and how we might manage this problem to help the species. Human encroachment constricts populations into smaller areas, facilitating an increase in CWD spread to healthy individuals in the population and increasing the possibility of the development of zoonotic disease (Farnsworth et al., 2005; Storm et al., 2013). Additionally, the density of cervid populations has been found to increase as they come closer to human populations, with greater access to food, water, and other resources not found naturally (Farnsworth et al., 2005). Higher densities and less space will ultimately lead to an increase in CWD transmission when infected individuals encounter those susceptible to the disease (Farnsworth et al., 2005). Management of CWD with human land use in mind leads to inefficiencies in the use of culling or hunting as these wildlife-urban interfaces come closer. To curb CWD, the management of anthropogenic resources utilized by these cervids must be considered and investigation into vaccinations should be continued. This review should ultimately inform management teams across the United States on a better way to manage CWD prevalence in both wild and human-dominated cervid populations.

Chronic Wasting Disease

Since the first description of CWD in the 1970s, the disease has expanded throughout the US, Canada, South Korea, and possibly Scandinavia (Gilch et al., 2011). When research on CWD commenced in the 1980s, the disease was primarily observed in captive animals in research facilities and therefore was believed to be a nervous response to the stress of captivity (Salman, 2003). The first occurrence of a positive case in wild deer was recorded in the 1990s in Colorado and Wyoming, and since that time CWD has expanded to 28 other states in the US

(Salman, 2003; Pritzkow, 2022). Researchers are currently unsure whether the consistent increase in prevalence is due to increased and more accurate testing, improved diagnostic techniques, public knowledge, the natural spread of the disease, or some combination of these explanations (Gilch et al., 2011).

There is still much to discover about the genetic pathogenesis of CWD, such as what conditions trigger the initial misfolding and the molecular basis of the disease (Escobar et al., 2020). Unlike most cellular pathogens, prions such as those that cause CWD do not propagate genetically since they contain no nucleic acid (Robinson et al., 2012). Instead, prions are infectious misfolded proteins that can alter the conformation of a normally folded protein with which they come into contact (Robinson et al., 2012; Escobar et al., 2020). Disease progression occurs as more misfolded proteins accumulate and continue to transmit their conformational changes within individuals (Robinson et al., 2012; Haley & Richt, 2017). As mentioned before, CWD is inevitably fatal, making the accumulation of misfolded proteins extremely important. The misfolded prions accumulate amyloid-like fibers, abnormal extracellular protein deposits that begin to collect in the brain and become toxic and eventually neurodegenerative (Escobar et al., 2020).

To understand the disease better, researchers need accurate, rapid, and reliable testing to diagnose individuals. There are currently two tests available to ascertain whether an individual is positive for CWD, enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC), with the latter being more commonly referenced in the literature (Osterholm et al., 2019). The largest obstacle to overcome with testing is the lengthy delay between disease contraction and symptom onset since there have not been pre-symptomatic tests developed. Scientists have noted that while CWD is a fast-acting disease, it still could take several years before clinical

symptoms appear; by that time, it is too late for the organism (Haley & Richt, 2017). The development of pre-symptomatic testing is necessary to try and save the individual from the fatality of CWD.

Transmission Mechanisms

Understanding the transmission mechanisms of diseases is key in fighting, managing, and eventually extinguishing the disease. TSEs such as bovine spongiform encephalopathy (BSE, known colloquially as “mad cow” disease), were easily detectable and researchers had a clear understanding of how the disease was transmitted from infected to susceptible individuals (Osterholm et al., 2019). The only transmission route for cattle to contract BSE was feeding directly on scrapie-contaminated meat-and-bone meal, and the only method of zoonotic transmission was through direct consumption of BSE-infected meat (Osterholm et al., 2019). Therefore, it was easy for farmers and the US Food and Drug Administration to manage the spread of BSE and eventually eradicate it. However, researchers studying CWD have not reached a similar consensus on its main transmission routes, with studies and reviews as recent as 2022 stating that there is still no clear answer (Pritzkow, 2022).

Some reports have indicated that CWD can be transmitted vertically, from the infected mother to her offspring, while others have reported horizontal transmission between infected individuals or environmental reservoirs containing prions (Pritzkow, 2022; Escobar et al., 2020). When CWD positive animals shed prion infected material such as antler velvet or bodily fluids, prions can leak into the environmental reservoirs such as water or soil (Robinson et al 2012; Pritzkow, 2022; Escobar et al., 2020). One group of researchers ultimately concluded that vertical transmission is exceedingly unlikely due to the incredibly small effect of transmission from mother to offspring found in their study (Potapov et al., 2016; Potapov et al., 2016). The

researchers argued this was due to a small likelihood of occurrence naturally, with the likelihood of a positive mother passing CWD to their offspring being very low (Potapov et al., 2016; Potapov et al., 2016). Despite this assertion, there have been documented cases of CWD-infected fetal, gestational, and reproductive tissues as well as newborns positive for CWD (Pritzkow, 2022).

The most likely and common mode of transmission is horizontal, with far higher efficiency than vertical transmission because the probability of uninfected animals encountering infected individuals is greater than the probability of an infected mother giving birth to an infected offspring (Pritzkow, 2022). Additionally, the likelihood that a mother survives the gestation period to give birth is low due to the high mortality of the disease, especially if the pregnant mother has been infected for some time (Pritzkow, 2022; Potapov et al., 2016). In addition to contact between individuals, CWD can also be transmitted through contact between an uninfected individual and an ‘environmental reservoirs’ (Robinson et al., 2012).

Environmental reservoirs are sources of indirect horizontal transmission in the soil, water or air that are established when infected tissues or fluids, such as saliva, feces, urine, blood, antler velvet, or placental tissue release prions (Escobar et al., 2020; Pritzkow, 2022). Because these released prions can remain infectious for several years, they can infect new individuals long after they are shed (Pritzkow, 2022). On the other hand, direct horizontal transmission between individuals occurs during fighting and mating, both of which are incredibly common in the polygamous mating system characteristic of cervids (Pritzkow, 2022; Bowyer et al., 2020). Because males fight for territory, female access, and resources and mate multiple times throughout the breeding season, they tend to exhibit higher prevalence of CWD (Rogers et al. 2021; Bowyer et al., 2020).

Human Encroachment

The effect of human distribution on CWD prevalence is well-established, with multiple studies across the US showing that as urbanization and land-use increases so too does CWD positivity rates (Joly et al., 2006; Farnsworth et al., 2005; Blanchong et al., 2007). Human land use and urbanization have spread to around 75% of our earth, with more being appropriated each day (Bradley & Altizer, 2006; Brearley et al., 2012). Previous studies have shown there to be a relationship between urbanization and CWD prevalence, however, they were completed many years ago (Farnsworth et al., 2005; Bradley & Altizer, 2006; Brearley et al., 2012).

With human land use increasing exponentially, and CWD prevalence increasing dramatically, these estimates are sure to under-represent the extent of CWD in cervid population's current condition. Farnsworth et al (2005) studied free-ranging mule deer in northern Colorado between 1997 and 2002. They found that land use was an important predictor of CWD prevalence, with males in more developed areas being twice as likely to be infected (Farnsworth et al., 2005). Another study investigated whether CWD transmission occurred in a density-dependent manner (Joly et al., 2006) and found that the prevalence of CWD decreased farther away from the area with overwhelmingly positive herds and was positively correlated to the number of individuals in the area (Joly et al., 2006). Most importantly, the conclusions of this study led to the assertion that the artificial congregation of deer in any one place, such as feeding areas like baiting stations for hunting purposes and supplemental feed during periods of drought, may increase disease transmission (Joly et al., 2006). Not only are cervids subsidized by anthropogenic food source, but they can also find urbanization as an asylum from hunting and predation pressure (Farnsworth et al., 2005). Therefore, increased appropriation of natural habitat

by humans can affect disease transmission by two mechanisms: 1) by pushing deer into smaller habitats through encroachment and 2) by increasing populations of deer in more urbanized areas.

As established previously, horizontal transmission is the most efficient route of disease transmission for CWD (Pritzkow, 2022). Human land use change and encroachment affect both indirect and direct methods of horizontal transmission, with environmental reservoirs with positive prions, the likelihood of encountering the reservoirs, and the likelihood of encountering a positive individual all increasing (Mathiason et al., 2009; Farnsworth et al., 2005; Blanchong et al., 2007). As humans move into native deer habitats, the wildlife-urban interface becomes less rigid, and our two ecosystems begin to converge. Physical barriers whether artificial (neighborhoods) or natural (large rivers or dense forest) have been proven to affect the rate of disease transmission and likelihood of encountering infected individuals (Mathiason et al., 2009; Blanchong et al., 2007). Whether through higher likelihood of environmental contraction (Mathiason et al., 2009) or through being physically restricted to one area (Blanchong et al., 2007), urbanization has an incredibly high influence on the mechanisms of wildlife disease.

Current Management

Wild cervids are extremely important in food webs as both a food source for large carnivores such as wolves, big cats, and bears and as a consumer of vegetation. As CWD continues to spread in wild populations and is facilitated by human encroachment, loss of wild cervids could result in both bottom-up and top-down trophic cascades that could disrupt ecosystems where CWD is present. The development of a sound management plan to prevent such ecosystem-wide effects is necessary to maintain critical ecosystem functions in the midst of human encroachment.

Several different management strategies have been implemented to curb CWD spread in wild cervids, including culling, vaccinations, and hunting (Mateus-Pinilla et al., 2013).

Management plans have yet to witness complete success in curbing the spread of CWD and therefore have little public support (Mateus-Pinilla et al., 2013; Potapov et al., 2016; Rogers et al., 2021). Rather than complete eradication of CWD, success is better gauged by the ability of a management plan to keep CWD at the lowest feasible prevalence in the long term (Mateus-Pinilla et al., 2013). Recently, there have been studies that investigated the effectiveness of cervid vaccination as a means of CWD control. Wood et al. (2018) vaccinated elk who were housed in an environment where prions were present. Instead of a successful vaccination, the onset and progression of CWD was accelerated and survival was significantly shorter than the control group by 63 days (Wood et al., 2018), highlighting that effective vaccinations are still very far from being practically useful. In comparison to other techniques, localized and selective culling of CWD-infected cervids was shown to be a useful and effective way to control the spread of the disease in densely populated areas through population reduction (Mateus-Pinilla et al., 2013; Uehlinger et al., 2016). However successful culling may be, this method is impractical for urbanized areas due to the dangers associated with using guns in areas with a high human population (Rondeau & Conrad, 2003; Kilpatrick et al., 2004). Therefore, the development of new techniques to reduce cervid populations in wildlife-human interfaces is imperative. Kilpatrick et al (2004) have suggested the development of bow-hunting as a method of control with less possibility of collateral damage. However, the killing of deer is still not widely accepted by the public whether they are infected individuals or not. Public support to control CWD is possibly the most important factor in curbing the spread.

CWD management cannot be achieved solely by governmental agencies or conservation organizations. Because they are such a highly economically valuable species, private stakeholders like hunters and landowners should be involved in management (Rubino & Serenari, 2022). Hunters are possibly the most important stakeholders to involve since many recognize that CWD threatens their economic livelihood and/or food sources (Ufer et al., 2022). One initiative to assist in management that involves the public specifically is the federal conservation stamp program that has been implemented in the past for ducks (Ufer et al., 2022). The stamp program could raise money for science to progress in its knowledge of CWD, vaccinations, and other methods that do not involve the killing of cervids. According to Ufer et al (2022), the duck stamp program has raised around \$850 million since its inception in 1934 with 98% of the funds mandated to the conservation fund. Not only do hunters have to buy these stamps in order to hunt, but avid outdoors-people, animal lovers, recreational conservationists, and others can also purchase these stamps and use them as normal mailing stamps or in collections (Ufer et al., 2022). Implementing a program similar to this one, in addition to regular hunting tags could generate funding and spread the word about CWD to the general public. With these funds, researchers could develop testing procedures to identify CWD hotspots, prion infected environmental reservoirs, and begin further research into vaccination technology. Reducing the knowledge gap is imperative to solving any query, especially one with such dire consequences as the CWD epidemic.

Zoonotic Potential

Transmission of CWD to non-human primates was first reported by Marsh et al. (2005), who infected two squirrel monkeys with CWD-infected mule deer brain tissue. 31 and 34 months post-infection both monkeys showed progressive neurodegenerative diseases, mutated prion

proteins, and spongiform degeneration on autopsy. Ultimately Marsh et al. (2005) concluded that cross-species infection was not only possible but deadly. Multiple TSEs have crossed from animals into humans, such as bovine spongiform encephalopathy (BSE), which became known as Creutzfeldt-Jakob Disease (CJD) in humans (Nemani et al., 2020; Osterholm et al., 2019). Exposure risk in humans is difficult to assess with tests both *in vitro* and *in vivo* giving their own challenges in modeling to humans (Osterholm et al., 2019). Whether CWD makes the jump over the species barrier to humans depends on the real-world exposures humans encounter in daily life (Nemani et al., 2020; Osterholm et al., 2019). There exist multiple possibilities of transmission and they are not static, with variations coming in strain diversity, the species infected, and variations in polymorphisms (Osterholm et al., 2019). Overall, the risk of human transmission has not been proven, and the chances of human contraction are not zero (Nemani et al., 2020; Osterholm et al., 2019; Pritzkow, 2022). Pairing expanding urbanization and increasing CWD prevalence in urbanized areas with the lack of a sound control method suggests we may be increasing the likelihood of an irreversible situation. A mobilization of scientific resources is necessary to test the possibility of transmission to humans, as it looms over the US and expands.

Conclusion

Chronic wasting disease is an inevitably fatal neurodegenerative disease affecting the cervid family. Since the first case in 1970, the disease has spread to multiple states and outside of the United States, leading to both national and international efforts for eradication. Management of the disease has come in many iterations, with both state and federal programs attempting to halt the spread of CWD. Thus far culling and hunting have proven to be the most successful methods of keeping the prevalence in feasible numbers, with prior vaccination trials having been unsuccessful. Although culling is successful, increases in urbanization leads to an issue of human

safety since managers utilize firearms in their culling techniques. Developing new methods of population reduction in the face of urbanization and generating funding is crucial. The combination of funding new research, increased testing, and a new technique to carry out culling such as bow-hunting may be the best answers to curbing CWD in urbanizing areas. Results from other studies have shown that the density-dependent horizontal transmission mechanisms by which CWD is transmitted between conspecifics could be directly influenced by human encroachment and land use increases. For that reason, reducing the populations of these cervids in a human dominated landscape should be the main priority, especially with the threat of zoonotic diseases developing. This threat looms over the human populations who directly interact with these species daily in wildlife-urban interfaces, which are becoming increasingly intertwined as human populations spread throughout once native cervid lands. The future of Chronic wasting disease is directly linked to a sound management plan for reducing the population in areas where once successful methods are becoming obsolete, raising funds for future research, public support, and minimization of the current rampant urbanization.

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CHAPTER 2. GRANT PROPOSAL

Top-Down Predation Effects on Chronic Wasting Disease: Reintroduction of the gray wolf (*Canis Lupus*) to Colorado

Abstract

Predators exert strong top-down pressure on the size of the prey populations, thereby controlling the spread of disease in those populations. Chronic wasting disease (CWD) is a neurodegenerative prion disease that affects both humans and animals. CWD affects both captive and wild white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), moose (*Alces alces*), and elk (*Cervus elaphus spp. canadensis*), all of which are prominent game species with economic and natural value. We aimed to evaluate whether top-down predatory pressure reduces the number of individuals in the population and therefore reduces the likelihood of a susceptible individual encountering CWD-positive individuals. Selective predation has been shown to modestly reduce the overall population but sharply decline the prevalence of CWD, which when combined makes a favorable environment for cervid survival. This study aims to evaluate the effect of top-down predation from gray wolves (*Canis lupus*) on CWD prevalence in cervids. We will take historic CWD data from the region in Colorado where these wolves will be introduced, in addition to our own collection of data immediately preceding the reintroduction. From then on, we will collect data at 1-, 3-, 6-, and 12-months post reintroduction. With CWD steadily increasing and spilling over into states where it was previously not found, we believe investigating disease control methods is increasingly important.

Question

How does restoration of top-down control by gray wolf (*Canis lupus*) reintroduction affect the prevalence and transmission of chronic wasting disease Colorado populations of elk (*Cervus elaphus spp. canadensis*) and white-tailed deer (*Odocoileus virginianus*)?

Objectives

To evaluate whether reintroduction of gray wolves to their historic natural range in Colorado will lower CWD prevalence in elk and white-tailed deer.

Hypotheses

Reintroduction of gray wolves will restore top-down predation pressure on elk and white-tailed deer reducing their population sizes. Since CWD is transmitted in a density-dependent manner and gray wolves reduce prion concentrations during digestion, wolf reintroduction will reduce the transmission and prevalence of CWD. gray wolf predation will reduce the prevalence of prion environmental reservoirs.

Anticipated Value

CWD has been steadily increasing for years in natural populations of Cervids in Colorado, causing managers to seek new methods of control. Hunting and culling works well to keep CWD to a manageable level, but the goal is to eradicate this disease. Unfortunately, vaccinations have not proven to be effective to prevent disease spread. Therefore, it is necessary to investigate new methods to control for the spread of the disease to susceptible individuals. wolves are set to be reintroduced to their native range in Colorado, giving us the opportunity to see whether their presence influences the prevalence of the disease. We hope to establish an understanding of the relationship between gray wolf populations and CWD prevalence as a management strategy for CWD across the natural historic range of the gray wolf.

Literature Review

Introduction

Chronic wasting disease (CWD) is a specific neurodegenerative prion disease that affects both humans and animals (Gilch et al., 2011). CWD is a highly infectious and ultimately fatal disease currently present in wild and captive cervid (deer and elk) populations in North America, South Korea and Norway (Pritzkow, 2022; Nemani et al., 2020). Cervids affected by CWD express symptoms including behavioral changes, excessive salivation, decreased ability to feed, and weight loss (Peters et al., 2000). Behavioral changes like stereotypy, excessive thirst, excessive urination, lack of awareness, fixed stare, and lowered head are characteristic of CWD; often being the leading diagnostic technique for the eventual culling of that animal (Williams, 2005).

Chronic wasting disease has been researched extensively for years, including the relative effectiveness of management techniques to prevent its spread such as culling, vaccinations, and hunting (Mateus-Pinilla et al., 2013). This literature review aims to evaluate the mechanisms by which top-down predation control populations of wild cervids and therefore reduce the prevalence of CWD. This review should ultimately inform management teams across the United States on whether the reintroduction of gray wolves (*lupus*) has a positive effect in the control of CWD.

Transmission Mechanisms

Understanding the transmission mechanisms of diseases is key in fighting, managing, and eventually extinguishing the disease. Researchers have defined two methods of CWD transmission; either vertically, from the infected mother to her offspring, or horizontally, between infected individuals or environmental reservoirs like bodily fluid and antler velvet

(Pritzkow, 2022; Escobar et al., 2020). The most likely and common mode of CWD transmission is horizontal, with far higher efficiency than vertical transmission due to the probability of encountering an infected individual being greater than the probability of an infected mother giving birth (Pritzkow, 2022; Potapov et al., 2016). Not only could CWD be transmitted through contact between infected and uninfected individuals, but it could also be transmitted through ‘environmental reservoirs’ so coined by Robinson et al (2012). These reservoirs are sources of indirect horizontal transmission and are established when infected tissues or fluids, such as saliva, feces, urine, blood, antler velvet, or placental tissue release prions (Escobar et al., 2020; Pritzkow, 2022). Because these released prions can remain infectious for several years, they can infect new individuals long after they are shed (Pritzkow, 2022). Direct horizontal transmission between individuals occurs during fighting and mating, both of which are incredibly common in the polygamous mating system characteristic of cervids (Pritzkow, 2022; Bowyer et al., 2020). Because males fight for territory, female access, and resources and mate multiple times throughout the breeding season, they tend to exhibit higher prevalence of CWD (Rogers et al. 2021; Bowyer et al., 2020).

Management

Several different management strategies have been implemented to curb CWD spread in wild cervids, including culling, vaccinations, and hunting (Mateus-Pinilla et al., 2013), but these strategies have yet to witness complete success in curbing the spread of CWD and therefore have little public support (Mateus-Pinilla et al., 2013; Potapov et al., 2016; Rogers et al., 2021). Rather than complete eradication of CWD, success is better gauged by the ability of a management plan to keep CWD at the lowest feasible prevalence in the long term (Mateus-Pinilla et al., 2013). In comparison to other techniques, localized and selective culling of CWD-

infected cervids was shown to be a useful and effective way to control the spread of the disease in densely populated areas (Mateus-Pinilla et al., 2013; Uehlinger et al., 2016). Although culling and hunting are established methods of CWD control, public support for these activities is extremely low with the idea of killing deer to any extent being questioned as an ethical dilemma. In addition, vaccination trials have proven to be unreliable and can even harm the animal further (Wood et al., 2018). Therefore, other methods of control are important to research with public support in mind.

Rather than relying on hunting and culling by the public or government agencies, reestablishing natural predation may result in decreased spread and prevalence of CWD. Top-down predatory pressure should reduce the number of individuals in the population and therefore reduce the likelihood of a susceptible individual encountering CWD-positive individuals (Wild et al., 2011). In addition to reducing the population, these carnivores engage in selective predation (Wild et al., 2011; Brandell et al., 2022; Krumm et al., 2010), whereby predators selectively hunt and kill individuals based on their physical condition (i.e., diseased animals) (Brandell et al., 2022). Selective predation may only result in modest declines in deer and elk populations but cause dramatic decreases in CWD prevalence (Wild et al., 2011). Therefore, wolf reintroduction is less likely to impact population sizes of key game species but have strong control over CWD transmission.

Methods

Description of Research

Study Area and Design

This study will take place in both Larimer county and Gunnison county in Colorado. Larimer county will be used as a control with no wolves expected to be reintroduced in this area

in the current plans, while Gunnison county expects wolves to be reintroduced (Figure 1). This will be a yearlong study with sampling being completed before reintroduction, then again in monthly intervals at 1-, 3-, 6-, and 12-months. This study should be longitudinal to assess the full picture of reintroduction of top-down pressure on the amount of CWD in Colorado's mountain range.

Survey and Sampling Methods

We will collect historical CWD data, as well as using testing on deer and elk carcasses both reported (accidental collisions and public reporting) and through samples taken from deer and elk killed during hunting season. These data will be collected before the reintroduction of wolves and analyzed to give us a baseline of the prevalence of CWD in Colorado at that time. After the wolves are introduced, we will collect samples at 1-, 3-, 6-, and 12-months post introduction to assess whether there is immediate change or if it is prolonged. We will also collect scat samples of deer, elk, and wolf and assess whether these are sources of prions as well. We will also use camera traps to collect population density data for both elk and deer in both counties to assess whether the wolves are drastically reducing their numbers. There will be no collection of live deer or elk throughout the study as this would produce considerable and unnecessary stress to the animal and poses risks to research staff.

Data Analysis

Data will be analyzed after compilation of previous historical data, data collected before, and after reintroduction. We will use statistical packages in R to assess whether the prevalence of CWD increased or decreased, by how much, and if that information is of statistical significance. We will likely use multiple t-test analyses to assess these differences.

Logistical Requirements

To complete field studies in Colorado, we will need permits from both state and counties to access the lands in which we hope to install camera traps and collect scat. Because we are not working with any animal listed as endangered, we will not need to comply with the ESA's standards of practice for experimental studies. However, we will need to comply with IACUC standards approved by Regis University. We will also be in communication with state and federal Parks and Wildlife in order to obtain data on CWD not publicly available.

Project Schedule

Dates	Activities	Deliverables
January 2023-February 2023	<ul style="list-style-type: none"> Collect information and submit permits 	<ul style="list-style-type: none"> Accepted permits Dataset of historical data obtained
March 2023-April 2023	<ul style="list-style-type: none"> Purchase and calibrate trail cameras Visit location and plan camera placements 	<ul style="list-style-type: none"> 40 cameras ready to be placed Idea of land and where to place
May 2023-May 2024	<ul style="list-style-type: none"> Place trail cameras Conduct surveys 	<ul style="list-style-type: none"> 40 cameras placed Raw data collection
July 2024-September 2024	<ul style="list-style-type: none"> Analyze data 	<ul style="list-style-type: none"> Draft report
September 2024-November 2024	<ul style="list-style-type: none"> Write report 	<ul style="list-style-type: none"> Final report

Map

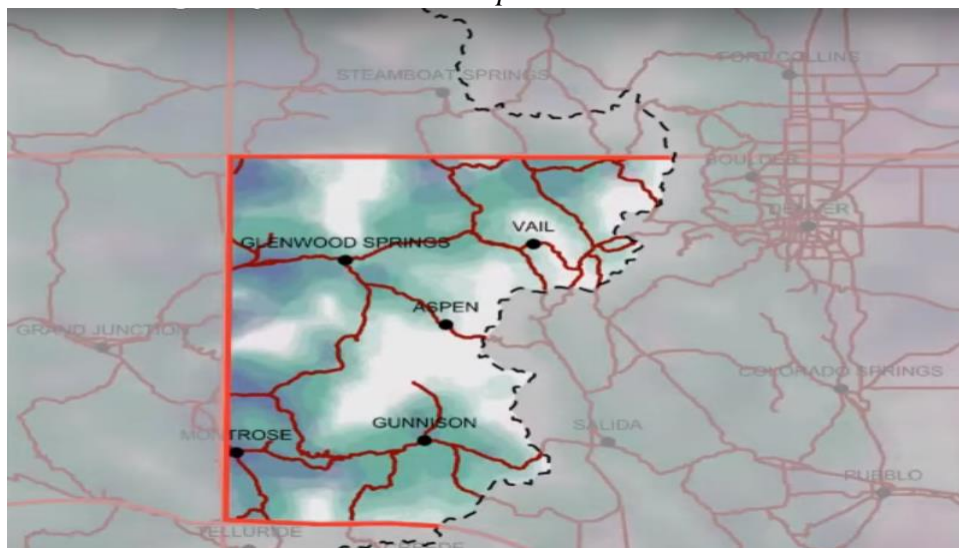


Figure 1: Map of area in which the wolves will be reintroduced to Colorado.

Negative Impact

There should be zero negative impact.

Budget

Item	Justification	Cost, unit (Source)	Quantity	Total Cost
Gas	40 round-trip travels from Regis to Larimer & Gunnison	\$0.535/mile (IRS)	3,900	\$2,086.5
Trail Camera	Used to capture pictures and video of cervids	\$100/ camera	40	\$400
Stipend	Research assistant stipend	\$500/ week	8	\$4,000
CWD testing	Determination of CWD positive organisms	\$142/ test (Fish & Wildlife)	100	\$1,420
Researcher salary	Principle researcher salary	\$1,000/ week	16	\$16,000
Total				\$23906.50

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CHAPTER 3. MANUSCRIPT

Summary of Embargo

Chapter 3 of this Masters Project has been embargoed on behalf of the United States Department of Agriculture for use of data which cannot be published. Please contact mberta@regis.edu or kvoss@regis.edu with any questions

CHAPTER 4. STAKEHOLDER ANALYSIS

Expanding Colorado Parks & Wildlife Stakeholder Analysis for Gray Wolf (*Canis lupus*) Reintroduction to Include Further Native Representation and Long-term Monitoring.

Introduction

Gray wolf (*Canis lupus*) reintroduction to the Colorado Western Slope was narrowly passed by Colorado voters through proposition 114, passing by only 0.2% of the vote. This contentious proposition met with support based on the ecological benefits of past wolf reintroductions (Smith & Peterson, 2021; Smith et al., 2003; Ripple & Beschta, 2003; Ripple & Beschta, 2012), yet it also met with strong opposition based on well-founded concerns for safety, financial impact to livestock farmers, and taxpayer burdens (Hoag et al., 2022; Switalski et al., 2002; Muhly & Musiani, 2009; Niemiec et al., 2020). Because wolf reintroduction to Colorado is now law, Colorado must now reintroduce wolves when almost half of voters opposed it. How does Colorado mitigate the impacts of wolf reintroduction on residents of the Western Slope, farmers, and recreationists? Colorado Parks & Wildlife (CPW) has plans to mitigate risks by considering the values of multiple stakeholder groups, but I argue these plans can be improved. In this analysis I recommend that CPW consider a wider range of stakeholder values by including native tribe members from the Arapahoe, Apache, Cheyenne, and Pueblo tribes in the SAG group and by implementing strategic plans to periodically monitor the effects of wolf reintroduction on vegetation and wildlife in areas where reintroduced.

Rewilding is an increasingly expanding field within conservation and restoration ecology that looks to restore an ecosystem to its natural condition by reintroducing extirpated species and even introducing new species with similar ecological functions (Larimer et al., 2015; Nogués-Bravo et al., 2016). Yellowstone National Park, the first national park established in the world, is considered by many to be one of the world's most pristine ecosystems that has benefited from continuous protection and management since 1872 (Smith & Peterson, 2021). Even with 150 years of protection, Yellowstone's ecosystem was not free from anthropogenic influence (Smith & Peterson, 2021). By 1995, key ecosystem processes had been lost after complete extirpation of grey wolves, and Yellowstone managers sought to restore them by reintroducing this top-predator species (Smith & Peterson, 2021). Since the reintroduction, multiple studies have shown both expected and unexpected changes to Yellowstone's ecosystem. These include the expected increases in native vegetation and the unexpected correlation between beaver and wolf populations (Smith & Peterson, 2021; Smith et al., 2003; Ripple & Beschta, 2003; Ripple & Beschta, 2012). However, those expected increases in native vegetation did not come through the expected mechanism of elk population decline; elk populations remained steady but their foraging behavior changed drastically, ultimately decreasing the elk browsing pressure in Yellowstone Valley (Smith & Peterson, 2021). Yet, due to the innate stochasticity of natural systems and inherent ecological differences between Colorado's Western Slope and Yellowstone, it is incredibly difficult to predict what the specific effects of wolf reintroduction will be in Colorado. What we know from Yellowstone can assist in these predictions, but we will not observe the full range of effects until these wolves are established in Colorado's Western Slope. Using assumptions based on the effects observed at Yellowstone and the natural history of gray wolves, CPW has created a wolf management plan which addresses potential economic

impacts, livestock depredation, ungulate impacts, and the agency's overall philosophy of management (Colorado Parks and Wildlife, 2022). In addition to its continued effort to make this reintroduction as equitable as possible for many stakeholders, CPW has also established a stakeholder advisory group (SAG) which includes members who represent multiple different perspectives and values.

Stakeholder Analysis

The SAG is made up of 17 voting members and 3 non-voting members who were selected through an open application process held by CPW. CPW's SAG does not claim to be a true representation of the general population, rather it is meant to involve critical stakeholder groups. The individuals who comprise the SAG represent stakeholder groups of sportspeople, scientists, ranchers, outfitters, wolf advocates, businesspeople, conservation organization leaders, and a Native American tribe. Therefore, I argue this SAG may not fully represent critical stakeholder groups with important perspectives. Indigenous representation is insufficient and should be expanded. The sole representative from Indigenous Peoples is the Southern Ute tribe's Wildlife Resource Manager who is not an active tribe member (Great Seal of the Southern Ute Indian Tribe, n.d.). At least five other native tribes inhabit Colorado's Western Slope, including the Arapahoe, Apache, Cheyenne, and Pueblo tribes (Colorado Department of Education, n.d.). In addition to those currently inhabiting the Ute reservations in the Southwestern corner of Colorado, the indigenous population of Colorado is a diverse one, that is continuing to rise each year (Colorado Commission of Indian Affairs, n.d.). Differences in values and traditions of Indigenous Peoples (Verbos et al., 2010) which should be considered when making decisions that could directly impact their ways of life, and actual members of each tribe should be represented, rather than representation via nontribal member proxy.

Those stakeholders who oppose wolf reintroduction include sportspeople, ranchers, outfitters, and some businesspeople. Many sportspeople are opposed to the reintroduction of wolves in Colorado (Smith & Peterson, 2021) because of the potential to lose prized hunting opportunities. However, since the proposition has passed, the focus now turns to how to mitigate potential consequences to this stakeholder group. Hunters consider the reintroduction of wolves to be in direct competition with their ability to hunt game because it may reduce the numbers of elk, deer, and moose (Smith & Peterson, 2021). While the introduction of a top predator may reduce the overall population of these game animals, they also indirectly increase the health of these populations by exerting top-down population control (Nilsen et al., 2007; Hetherington & Gorman, 2007; Kuijper et al., 2016). In addition, the top-down effect of wolf reintroduction in Yellowstone on elk was primarily behavioral rather than numerical; elk browsed for less time and avoided open geographic areas completely (Ripple et al., 2001). Given this information, the population size may not decrease substantially enough for the effect to be consequential to hunters, yet the changes in behavior may alter certain hunting practices, making it more difficult to take these elk. Based on previous results in Yellowstone the reintroduction of wolves could directly benefit ungulate populations by reducing overpopulation, disease, and die-offs (Smith & Peterson, 2021; Smith et al., 2003; Ripple & Beschta, 2003; Ripple & Beschta, 2012). These tradeoffs would lead to healthier populations of ungulates that hunters seek and therefore, the need to mitigate the consequences to hunters is not necessary.

Ranchers and outfitters, who guide hunts for profit, value their livelihoods which may be negatively impacted by reintroduction of wolves. Western Slope ranchers, who often moonlight as outfitters, value their livestock and the likelihood of depredation events increases dramatically with the reintroduction of a large carnivore (Muhly & Musiani, 2009). The economics of

depredation events will directly impact these ranchers, however CPW has a fund to compensate ranchers whose livestock are taken by wolves (Colorado Parks and Wildlife, 2022; Muhly & Musiani, 2009). The reintroduction of wolves in Colorado has the potential to negatively affect ranchers and outfitters primarily through depredation events and decreased frequency of commissioned hunts (Muhly & Musiani, 2009). However, the reimbursement fund created by CPW sufficiently mitigates the risk to ranchers and is not currently of concern to the CPW SAG (Colorado Parks and Wildlife, 2022). While the wolf reintroduction is not going to be favored by the ranchers and outfitters directly impacted by these decisions, the current mitigation plan is considered the best action possible by the SAG and those Colorado voters who passed the wolf legislation allowing wolf reintroduction by the end of 2023.

Businesspeople value the economic costs and benefits of reintroduction rather than the ecological. Economic impacts can be either positive or negative for a specific business or the government. Positive economic impacts come through increased ecotourism, job creation, and new research opportunities (Hoag et al., 2022; Switalski et al., 2004). A meta-analysis of the economic consequences of wolf reintroduction in Colorado identified the primary economic benefits as hunting, viewing, research, and habitat restoration, with each being either a direct or indirect impact to the respective trade (Hoag et al., 2022). In addition to the benefits, there will be costs of reintroduction such as loss of personal property, loss of commercial production such as livestock, and governmental management costs like the continued monitoring suggested below (Hoag et al., 2022). Overall, businesspeople differ in opinion depending on whether the industry reaps net benefit or cost from wolf reintroduction. While there stands to be costs associated with wolf reintroduction, the overall net benefit is many times higher and therefore does not warrant mitigation to the economy (Hoag et al., 2022).

Those stakeholder groups that support or are neutral toward wolf reintroduction include wolf advocates, some businesspeople, scientists, and Colorado's Native Tribal representation. Using scientific studies and modeling based on previous studies, the ecologists working with this project can study the potential ecological impacts of wolf reintroduction to Colorado's Western Slope. These scientists would value the scientific process that provides answers to questions about the ecosystem-level effects of wolf reintroduction. Data from such investigations would provide evidence to support decision-making, guided by current ecological and political circumstances. Ecological models of the effects of top predator reintroduction on the surrounding communities are based on the information gained from previous reintroductions. This work demonstrated that notable reductions in prey populations occur after reintroduction which cascade to release native vegetation from intense herbivory (Beschta & Ripple, 2008; Hetherington & Gorman, 2007). However, the specific results may depend on the specific environmental context, such as when reintroduction occurs in a new ecoregion or as climate change reshapes ecosystems (Coulson et al., 2011). Information from prior studies will guide ecologists who study the effects of wolf reintroduction in Colorado and provide valuable guiding principles when looking at the effects of wolves.

However, prior models need to be updated with data collected in Colorado, and a specific protocol for longitudinal monitoring of the Western Slope after reintroduction is required. Such a monitoring plan should be developed jointly by CPW and other agencies such as U.S. Fish and Wildlife and U.S. Department of Agriculture among others. Wolf reintroduction was useful in restoring Yellowstone's ecosystem (Ripple & Beschta, 2012; Smith & Peterson, 2021) but the same results are not guaranteed in Colorado. Wolves have incredibly large and variable home ranges (Mech et al., 2017), meaning they are not restricted to one area such as the Western

Slope. The idea that reintroduction of a large predator will be able to reverse the loss of biodiversity that occurred after its extirpation is a fallacy (Kuijper et al., 2016). This relates to the concept of alternative stable states in the ecological literature where ecosystems can be pushed into alternative configurations which are self-reinforcing (Beisner et al., 2001). There are innumerable confounding variables that could affect the success of wolf reintroduction in any place but particularly in places that have been extensively anthropogenically altered (Kuijper et al., 2016). Consequently, the use of ecological modelling results from other ecosystems cannot give Colorado ecologists the best information needed to understand the successes and failures of a project as high profile and sensitive as this one. For these reasons, I suggest CPW draft an adaptive monitoring plan to periodically assess whether the wolf reintroduction project is meeting its intended ecological goals and that mitigation strategies (i.e., payments for livestock loss, effects on elk population size) can be improved as needs arise. Adaptive monitoring should include periodic vegetative and wildlife surveys to understand the degree to which wolves have altered ecosystem services of the Western Slope.

Wolf advocates recommended that wolf welfare should be more strongly considered when capturing, transporting, and releasing these animals in Colorado (Colorado Parks and Wildlife, 2022). CPW's SAG voted on multiple best practices to maintain wolf health during reintroduction, such as what food to feed the wild animals, the time from capture to release, and the amount of human interaction (Colorado Parks and Wildlife, 2022). Due to the extensive work by the SAG, these mitigation strategies here are sufficient and do not require further recommendations. However, while the wolf advocates are satisfied by current mitigation plans (Colorado Parks and Wildlife, 2022), the future growth of Colorado wolf populations may warrant the need to hunt wolves, especially if their populations increase to a point where they are

disrupting ecosystem services (Epstein, 2017; Lute et al., 2014). If CPW allows wolf hunting in such a scenario, the wolf advocacy groups would be staunchly opposed to it (Lute et al., 2014). The inclusion of wolf population surveys in the adaptive and longitudinal monitoring plan could proactively provide information about whether hunts should be considered by CPW and would inform the wolf advocacy groups of the potential necessity.

Although United States lawmakers recognize that historical colonialism marginalized Native American tribes, such marginalization still occurs. (United States Library of Congress, n.d.). As recently as January 2023, two tribal member representatives from the Southern and Mountain Ute tribes called upon the Colorado legislature to advocate for more equal tribal representation in decision-making, specifically as it concerns wolf reintroduction (KUNC, 2023). Today, the involvement of Native Americans and their greater tribal networks are incredibly important for co-management of the lands that were once wholly their own. Each tribe's values are unique to its traditions and ethical standards (Verbos et al., 2010). For example, the Southern Ute tribe supports return of the apex wolf predator (Great Seal of the Southern Ute Indian Tribe, n.d.) because of their members' strong spiritual and traditional connections to the wolves that once shared their lands. They have a great respect for the animal and therefore believe it should be returned to its native range (Great Seal of the Southern Ute Indian Tribe, n.d.). However, the Global Indigenous Council mentions it supports the reintroduction, yet acknowledge that some tribes favor leaving the land to its own devices by implementing a minimalistic "hands-off" intervention plan that minimalizes the influence of humans (Pagosa Daily Post, 2020). The involvement of the Ute tribes in the SAG is a very important step toward including native perspectives on a complex issue that directly affects indigenous lives. However, the inclusion of

other native tribes as well as native tribal members is a critical missing step in the draft propositions for the wolf reintroduction plan.

Conclusion

After the consideration of each group's values, the SAG voted on a series of draft recommendations for the reintroduction plans proposed by CPW. These recommendations range from logistics of wolf capture and release to livestock depredation compensation for ranchers. The recommendations promulgated by the CPW SAG adequately address the values and concerns of many important and necessary stakeholder groups that would be directly affected by wolf reintroduction, whether positively or negatively. Despite these notable strengths, several shortcomings should be remedied. First, Native American representation on the SAG should be increased. I recommend the SAG reconstitute itself to represent native interests more fully by including members from different tribal groups. Secondly, in order to assess whether current mitigation plans are sufficient and whether ecological benefits of wolf reintroduction are being realized, I recommend that an interagency longitudinal monitoring plan be drafted to investigate vegetation and wildlife in areas where reintroduced. After the reconstitution of the SAG and the drafting of future monitoring plans occur, not only will the majority of stakeholder values will be adequately represented, but the long-term success of the plan at reaping ecological benefits and minimizing costs can be assessed.

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