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MS Environmental Biology Capstone Project

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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CAPSTONE PROJECT

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CHAPTER 1. LITERATURE REVIEW: THE EFFECTS OF HEAVY METAL POLLUTANTS ON BIRDS AT DIFFERENT TROPHIC LEVELS AND THE CONSEQUENCES TO ECOSYSTEMS

Increased human population and the consequent overconsumption of natural resources has resulted in wastes being put into the environment (Durmus, 2018). One group of pollutants, which result from industrial activities, are heavy metals. Heavy metals are elements with a high atomic weight, a density at least five times greater than that of water that occur naturally in the environment (Tchounwou et al., 2012). Industries such as mining, mills, and other factories that use metal, contribute to increased concentrations of heavy metals in the environment. Heavy metal pollutants make their way into the environment through leaching from waste dumped on land or water, and through atmospheric transport (Durmus, 2018). Common heavy metal pollutants found in the environment include arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), zinc (Zn), mercury (Hg), aluminum (Al), cadmium (Cd), lead (Pb), and silver (Ag).

Although they are found on earth naturally in low concentrations, when organisms are exposed to high concentrations over long periods of time, these heavy metals can cause organ damage (Tchounwou et al., 2012). Pollutants, such as heavy metals, cannot be excreted and therefore will accumulate and remain in the tissues of organisms. Accumulated heavy metals have negative effects on morphology and bodily function which can lead to death and population declines. Consequently, the presence of these pollutants in the environment is a serious threat to the stability and quality of ecosystems (Abbasi et al., 2014).

Studies on birds show that there are negative physiological impacts from heavy metal pollutants, which contribute to population declines in different avian species (DeSorbo et al., 2018; Durmus, 2018; Perez-Lopez et al., 2008). Ecosystems rely on birds for many functions and a decline in their populations can have negative implications. Birds have key roles and relationships to other organisms, and if disrupted, can greatly change the entire dynamics of an ecosystem (Sekercioglu et al., 2016). Ecological relationships that may be affected by declining bird populations may include a decrease in seed dispersal from frugivores, reduced nutrient cycling from scavengers, a lack of rodent population control through predator-prey interactions, an increase in facultative scavenging and an increase in wildlife diseases from fewer detritivores. An abundance of literature claims that increases in heavy metals correlate to population declines, but not that these population declines cause trophic cascades. It can be difficult to determine the precise cause of trophic cascades because many factors can contribute to their occurrence.

Birds are situated at different trophic levels with a wide range of dietary habits. Insectivorous and frugivore bird species ingest heavy metals through their diet when they eat soil or plants that have high concentrations of heavy metals (Liu et al., 2015). Carnivorous and scavenger birds eat other organisms that have accumulated heavy metals in tissues by feeding on contaminated resources. Because carnivorous and scavenger birds continuously consume high concentrations of heavy metal pollutants in their diets, it remains in body tissue and bioaccumulation occurs that increase heavy metal concentrations throughout the bird’s life. Aside from bioaccumulation, biomagnification is another process in which birds can accumulate heavy metals in their tissues. Biomagnification refers to the process by which heavy metal pollutants increase through trophic levels of an ecosystem to top predators feeding on organisms lower in the food web. By consuming food sources at lower trophic levels over a lifetime, heavy metals travel from lower trophic level organisms, such as fish and rodents, and accumulate in birds of prey. These concentrations can reach lethal dosages in the bloodstream and cause death (Battaglia et al., 2003). The diet of a bird is directly related to heavy metal concentration levels found in their blood (Durmus, 2018). Carnivorous birds, in the same ecosystem as frugivore and insectivorous birds, have been found to have higher concentrations of heavy metals pollutants in their tissues (Durmus, 2018). Carnivorous birds, including owls, eagles, hawks, falcons and vultures, are found at the top of food chains and any declines in these populations can have serious impacts on an ecosystem because trophic cascades may occur across ecosystems.

The far-reaching effects that trophic cascades have on an ecosystem or the environment is cause for concern. Disruptions in an ecosystem from declining carnivorous and scavenger birds can affect vegetation or other populations of animals, such as rodents, that will have consequences for the human environment as well. Increased heavy metal concentrations have the greatest effect on carnivorous and scavenger birds and declines in their population may alter ecosystem functions and cause trophic cascades, however, the literature does not demonstrate the link between bird population declines caused from heavy metals and its negative impact on ecosystems. More studies and data collection that explicitly demonstrate a relationship between the impacts of heavy metals on carnivorous and scavenger birds and the potential trophic cascades that may occur, are recommended. Addressing this knowledge gap will preserve the quality of wildlife and prevent trophic cascades that cause malfunctioning in an ecosystem. Here I review the physiological effects of heavy metal pollutants on birds and the potential consequences for ecosystems. The aim is to determine which trophic level of birds conservation efforts should focus on.

Impacts of Heavy Metals on Birds

Assessment Methods

Heavy metal concentrations tend to be measured from breast feathers and/or blood samples from individual birds. Bird feathers may provide more long-term data, as they integrate heavy metal concentrations in blood gathered over time because feathers are connected to a blood source throughout the bird's life (Abbasi et al., 2015). Feathers better represent the heavy metal pollutants a bird has accumulated over time, instead of the concentration at one point in time, like a blood sample would portray. Testing bird feathers does not harm the birds, feathers are also easy to collect, store, and transport, as opposed to other biological samples (Abbasi et al., 2015). However, sampling a bird feather that has recently grown back will not represent heavy metals over a lifetime, but for only the short time period that the feather has been attached (Abbasi et al., 2015). In addition to using feathers and blood samples, some investigators will also collect deceased birds and measure heavy metal concentrations in liver and kidney samples to better estimate organ damage (Battaglia et al., 2003).

Effects on Growth and Development

Heavy metals travel through food chains and this biomagnification may have devastating health effects on birds, including mortality (Tchounwou et al., 2012). Certain heavy metals can cause different physiological effects in birds. Cadmium (Cd), for example, causes a depletion in calcium, leading to brittle bones that leave birds more vulnerable to injury (Goutte et al., 2014). Other metals, such as mercury (Hg) and lead (Pb), may cause developmental defects in embryos, and impair the nervous and immune systems, physiology and behavior (Goutte et al., 2014). Stunting of growth is also a result of high levels of cadmium (Cd) in birds (Liu et al., 2015). Underdevelopment can impair a bird's ability to flourish in its environment, by hindering feeding or mating processes. Stunted growth and development also result in decreased chances of survival and fecundity.

Effects on Reproductive Success

High concentrations of mercury (Hg) in the wandering albatross were found to negatively impact the breeding probability of females, and hatching rates of their eggs (Goutte et al., 2014). Physiological effects on birds that decrease breeding and hatching of eggs may decrease populations over time from the effects on recruitment. Evidence shows that heavy metals in birds can disrupt the endocrine system by reducing hormone concentrations such as prolactin, corticosterone and testosterone (Chatelain et al., 2018). The inhibition or reduction of these hormones correlates with lower parental investment on their eggs and young, thus decreasing reproductive success. Eggshells from magpies (*Pica pica*) that were nested near a brick kiln in central Iran, were thinner and smaller when compared to magpies that did not nest near it (Zarrintab et al., 2017). Close proximity to the source of heavy metal pollutants is directly relatated to the poor reproductive health of birds (Zarrintab et al., 2017). Birds will also continue to breed in urban areas despite the presence of pollution, and excess amounts of light and noise (Roux et al., 2007). Although these factors may be negatively impacting their survival and fitness, most birds will not leave the area and continue to produce young there. These impacts can magnify over future generations by causing decreases in populations and recruitment rates.

Minimal Impacts

Some birds, such as the insectivorous Great Tit in Finland, are minimally impacted by heavy metal pollutants (Eeva et al., 2003). Although heavy metal pollutants were detected in their feces, no significant relationship was found between the number of young produced and heavy metal concentrations (Eeva et al., 2003). Minimal impacts from heavy metal pollutants to the Great Tit suggest that an insectivorous diet may have resulted in lower heavy metal concentration levels.

Bird Diets and Heavy Metal Pollutants

Insectivores and Frugivores

As previously mentioned, birds of varying diets do not ingest heavy metal pollutants in the same manner. Lower trophic level birds, such as herbivorous and insectivorous species, obtain heavy metal pollutants in their systems from eating producers or small consumers. Plants may be growing and retaining nutrients from soil that contains heavy metals from waste disposal or leaching (Battaglia et al., 2003). Atmospheric heavy metals can also be deposited and trapped inside soil particles, which increase heavy metal concentrations in that ecosystem (Battaglia et al., 2003). Bird species may also eat insects living in soil with heavy metals present and ingest contaminated dirt in the process of feeding on insects (Liu et al., 2015).

Predators and Scavengers

Heavy metal concentrations in birds are influenced by trophic level, taxon, age and size of bird (Abbasi et al., 2015). Forty-eight bird species had their feathers tested in Pakistan for a wide array of heavy metal pollutants, and birds at higher trophic levels, such as carnivorous birds, had the greatest concentrations of heavy metals (Abbasi et al., 2015).

Raptors that exclusively feed on fish and small animals, ingest heavy metals by consuming prey that has accumulated heavy metals. Fish living in water sources with high levels of heavy metals, such as mercury, tend to concentrate these metals over time (Perez-Lopez et al., 2008). Thus, fish of older age or at the top of the aquatic food chain, have higher concentrations of heavy metals. Heavy metal biomagnification in piscivorous birds has been well documented in eagles, leading to reduced breeding and increased mortality (Perez-Lopez et al., 2008). When comparing heavy metal concentration levels in soil, water, plants, and fish in a polluted wetland in China, fish and benthic invertebrates had the highest concentrations of heavy metals (Lie et al., 2015). Aquatic organisms accumulate heavy metals to a greater degree due to processes, such as osmosis, of surrounding polluted waters (Malaj et al., 2012), which puts birds that feed on them at a greater risk of exposure (Lie et al, 2015). These concentrations build up within a bird's body over time and increase the chances of mortality and other defects.

Dietary toxins were found to be the main cause of scavenger bird declines and mortality rates across the world (Buechley et al., 2016). Avian scavengers feed on any carcass in their environment since their food source is quite unpredictable and they cannot afford to be selective. This puts scavengers at greater risk of being exposed to animals that have died themselves from heavy metal toxicosis, or have been shot by lead bullets, which then get ingested by the scavenger (Battaglia et al., 2005). High exposure levels have been documented in the little owl and common buzzard living in northern Italy that have consumed lead shots found inside their prey (Battaglia et al., 2005).

Other factors that contribute to carnivorous and scavenger bird species having greater heavy metal concentrations could be that they spread out over larger distances to find prey (Perez-Lopez et al., 2008). This suggests that heavy metals may be transferred from a contaminated source to their nesting area. If a carnivorous bird's habitat is in an area with low pollution, they may migrate to areas with high levels of pollutants in order to hunt prey. Bald eagles in Maine ate fish from different locations in a polluted watershed, even though their nests weren't located directly near the source of pollutants (DeSorbo et al., 2018). This shows far-reaching effects for scavenger and carnivorous birds that do not feed locally. Predator and scavenger behavior and their varying diets put them at risk for heavy metal exposure. These species high concentration levels correspond with their diet of organisms that retain heavy metals over long periods.

Impacts on Ecosystems

The physiological impacts of heavy metal pollutants on birds can lead to declines in populations from decreased breeding or higher mortality rates. Birds play various key roles in ecosystems and their decline can lead to trophic cascades. These trophic cascades will alter the functions of the ecosystem and can indirectly harm other species as well (Buechley et al., 2016; Gaston et al., 2018).

The decline in obligate scavengers can result in an increase in facultative scavengers that may eat carcasses (Buechley et al., 2016). Obligate scavengers feed exclusively on carcasses, while facultative scavengers take advantage of feeding on anything deceased or living. The populations of these facultative scavengers, such as the large kelp gull, will increase from having a larger food source (Buechley et al., 2016). The large kelp gull can negatively impact whale populations by eating blubber off of live whales that breach to the water's surface to breathe (Buechley et al., 2016). This effect demonstrates how a decrease in obligate scavengers may ripple across different food webs and environments. Nest predation and lowered chick survival rates also increased in Ohio when facultative scavenger populations increased (Buechley et al., 2016). Facultative scavengers disrupt the balance of populations in an ecosystem by feeding on eggs and young of other organisms. Consequently, growing populations of facultative scavengers can impair ecosystem dynamics when they decrease populations of other species.

A growing population of facultative scavengers could also lead to an increase in wildlife diseases. With declining obligate scavenger populations, more carcasses will remain unconsumed and will result in longer decomposition times. These unconsumed carcasses can become a potential breeding ground for bacteria (Buechley et al., 2016). Longer decomposition time also poses the risk that more animals may contact the carcass and aggressively compete with other individuals over the carcass (Ogada et al., 2012). Competitive aggression may include physical conflicts that create injuries which become infected from microbes found on the carcass. This could facilitate the further spread of wildlife disease beyond those individuals involved in the matter. Some diseases whose transmission may be enhanced include Ebola, plague, anthrax, and rabies (Buechley et al., 2016). Some of these wildlife diseases are able to be transmitted to humans (Buechley et al., 2016), thus raising concerns that human health is at risk from a decrease in obligate scavenger species as well.

Although in comparison to scavengers and carnivores, frugivore bird species are not as greatly impacted by heavy metal pollutants, a decline in their population can still alter an ecosystem and its functions. A decline in frugivorous bird population can occur from eating contaminated soil and fruits and building these concentrations over time in tissues. Frugivore birds disperse seeds in their feces from the fruits they eat. Their traveling nature allows ecosystems to expand its range and give plants a better chance of survival in other locations. Decreased dispersal of seeds will alter the plant species composition of an ecosystem, thus causing other changes to occur in a plant community (Pegman et al., 2017). A disruption in genetic connectivity between fruit bearing plants and lowered survival rates are other implications from a decrease in frugivorous bird populations from seeds not being dispersed to other nearby plant populations (Pegman et al., 2017). Without dispersal from birds, plants may self-pollinate over multiple generations and lack genetic diversity.

Trophic cascades from declining bird populations negatively impact other roles of plants in an ecosystem, as well. Biomass of primary producers has shown positive responses when birds are present in the ecosystem (Mäntylä et al., 2011). Top predators, such as eagles, owls, and hawks, control the populations of herbivorous animals, relieving plants from herbivory. If a decline in top predator birds occurs from increased heavy metal concentrations, growing populations of herbivores overgraze plants and decrease primary production (Mäntylä et al., 2011). These indirect relationships that birds have with primary producers are altered through trophic cascades. The presence of birds in an ecosystem is crucial to more species than just the organisms they prey upon.

Conclusion

Heavy metal pollutants expelled into the environment are persistent and negatively affect the health of ecosystems and organisms. Heavy metal pollutants decrease the reproductive success, and growth/development of birds (Chatelain et al., 2018; Goutte et al., 2014; Liu et al., 2015). The inhibition or reduction of endocrine hormones, due to increased heavy metal concentrations, correlates with lower parental investment in birds that decreases reproductive success (Chatelain et al., 2018). Increased heavy metal concentrations also deplete calcium and other nutrients in the body that stunt the growth of birds and decrease chances of survival and fecundity (Liu et al., 2015). Consequently, the physiological effects of heavy metal pollutants on birds increase chances of mortality and other defects that cause population declines.

Concentrations of heavy metal pollutants found in the tissues of birds are directly influenced by trophic level and diet (Abbasi et al., 2015). Bird species that exist at the top of food chains or are detritivores, accumulate the greatest amounts of heavy metal concentrations. These concentrations contribute to population declines, but also have ramifications for other species with which they interact. The potential trophic cascades that occur from these declines will alter the quality and stability of a variety of ecosystems (Gaston et al., 2018). Scavenger bird species help prevent the spread of wildlife diseases, and their decline could have widespread negative impacts on the health of other organisms. The declines in certain species will also allow other organisms, such as facultative bird species, to dominate in ecosystems and alter food web interactions. The indirect relationship birds of prey have with primary producers by controlling herbivory populations also show the far-reaching effects the declines of bird populations will have.

Although all species of birds are imperative to ecosystems, carnivorous and scavenger species are at the greatest risk of decline from heavy metal pollution. The relationship between bird diet and heavy metal concentrations correspond with higher levels found in carnivorous and scavenger birds. Increased heavy metal concentrations have the greatest effect on carnivorous and scavenger birds and declines in their population may alter ecosystem functioning that cause trophic cascades, however, the literature does not demonstrate the link between bird population declines caused from heavy metals and its negative impact on ecosystems. Thus, future studies and conservation efforts towards birds affected by heavy metal pollutants should be focused on scavenger and carnivorous species to mitigate the risk of greater impacts on ecosystems. More research on the declines of these birds from high heavy metal concentrations will aid in providing greater knowledge about the consequences it has for ecosystems. However, addressing the threats of bird population declines with breeding additional birds will not be helpful if heavy metals continue to exist in the environment. The source of threats to birds, which are heavy metal pollutants, must be drastically reduced before this problem can be resolved.

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CHAPTER 2. GRANT PROPOSAL: ECOLOGICAL ASSESSMENT OF HEAVY METAL POLLUTANTS IN SOUTH BOULDER CREEK

Abstract

High heavy metal concentrations can alter aquatic ecosystems and benthic organism composition. Power plants contaminate aquatic ecosystems with heavy metal pollutants through burning and waste disposal. Excel's Valmont Generating Station (EVGS) in Boulder, Colorado previously used coal-firing methods and stored waste into ash retention ponds. Ash ponds at EVGS may have leached heavy metal pollutants into the soil and contaminated nearby South Boulder Creek (SBC). Heavy metals are of concern to both the human and natural environment because they negatively impact physiological functions of organisms. I propose to assess the degree to which heavy metals have leached from ash ponds and affected stream biota by comparing reaches downstream and upstream of EVGS. First, I will measure heavy metal concentrations in sediments from these reaches. Then, by sampling macroinvertebrates, I aim to assess whether the changes in ambient metal concentrations have altered macroinvertebrate community composition and biomagnified through the macroinvertebrate food web. The results from the proposed research will allow Boulder County Parks and Open Space to determine the need for pollution mitigation near EVGS at SBC, and if needed, prioritize locations and actions for management. The findings will also inform the public about current conditions of SBC and to the hazards of heavy metal pollution on water quality in aquatic ecosystems.

Introduction

Heavy metals enter the environment from a number of sources including urban road runoff, waste dumping, and by-products from factories. Coal-fired power plants also expel pollutants, such as heavy metals, into the environment (Ramya et al., 2013). The ash generated from coal-firing is commonly disposed of in ash ponds, engineered in-ground structures (Lokeshappa & Dikshit, 2012). The waste held in these ponds contain heavy metals that are harmful to humans and wildlife including arsenic (As), selenium (Se), chromium (Cr), zinc (Zn), lead (Pb) and barium (Ba) (Lokeshappa & Dikshit, 2012). These contaminants leach into groundwater supplies and make their way to surface waters, such as rivers and streams (Ramya et al., 2013). These metals not only threaten the health of local residents, but also aquatic ecosystem integrity (Reash et al., 2015).

When exposed to high heavy metal concentrations over long periods of time, organ systems, including the nervous system, become damaged (Tchounwou et al., 2012). Accumulated heavy metals negatively affect growth and physiological function which may lead to toxicosis or death (Yang et al., 2017). Organisms that live in surface waters can bioaccumulate heavy metals in their tissues through the water directly, or by feeding on other organisms that have accumulated heavy metals. Consequently, fish and other aquatic organisms at the top of food chains sustain greater negative impacts from heavy metals (Pérez-López et al., 2008). Because these organisms continuously consume high concentrations of heavy metal pollutants in their diets, bioaccumulation occurs, and heavy metal concentrations increase over an organism's life span. Humans who catch and eat contaminated fish in polluted waters are then exposed to high heavy metal concentrations, and their health is put at risk (Reash et al., 2015).

To measure the effects of heavy metal pollution in freshwater ecosystems, sediment sampling and macroinvertebrate community composition are typically examined because sediments act as sinks for pollutants, measuring heavy metals in sediment can portray the chronic effects they have on aquatic ecosystems (Skorbiłowicz et al., 2018). Some macroinvertebrate species in aquatic systems are sensitive to high concentrations of heavy metals, while others tolerate their presence (Clements et al., 2000). Water and sediment with high concentrations of heavy metals tend to reduce the diversity of sensitive macroinvertebrates (Costas et al., 2018). Because sensitive taxa tend to be absent from waters contaminated by heavy metals, the structure of the community differs strikingly from uncontaminated waters (Clements et al., 2000). But even tolerant taxa will bioaccumulate heavy metals, so, analyzing body concentrations of heavy metals in macroinvertebrates will determine the severity of pollution (Malaj et al., 2012). The consequences of heavy metal pollution on aquatic ecosystems threaten the health of organisms and humans that interact with the water source.

In response to environmental concerns from local residents, EVGS in Boulder, Colorado switched from coal-firing methods to burning natural gas in 2017. However, heavy metals are persistent pollutants that accumulate and stay within an environment (Tchounwou et al., 2012). Although coal-firing is no longer used, contaminants from former ash ponds may still be leaching into local waterways, such as SBC. No research has been conducted on the current heavy metal concentrations in SBC or their effects on aquatic life, after the improvements at EVGS. Current conditions may indicate that eliminating coal-firing methods has not reduced heavy metal concentrations in water, sediment, or organisms from decades of coal burning.

Objective

Coal-fired power plants pollute waterways with heavy metals by discharging into coal-ash ponds that contaminate groundwater and leach pollutants into surface waters (Ramya et al., 2013). I aim to assess current heavy metal concentrations in SBC after the recent conversion from coal to natural gas at EVGS in Boulder, Colorado to determine whether heavy metals persist and continue to impact the aquatic ecosystem. Specifically, I propose to characterize sediment, heavy metal concentrations in macroinvertebrates, and macroinvertebrate species composition.

Anticipated Value

Boulder County Parks and Open Space (BCPOS) is dedicated to preserving the landscape and native species found in Boulder County. Their mission to improve water quality and reduce negative environmental and public health impacts to local water sources will be aided by the proposed research. First, an assessment of heavy metal concentrations in SBC will elucidate improved conditions and/or continued impacts from EVGS discharge. This will allow BCPOS to determine the need for pollution mitigation, and if needed, prioritize locations and actions. Specific heavy metals that are of concern will also be identified, which will aid in management strategies. Second, the proposed research will inform local residents about current water quality conditions in SBC and draw attention to what clean-up still needs to be done post-EVGS conversion from coal-firing to natural gas. Finally, the findings of the proposed research will be widely used to advance and encourage studies on the effects of industrial power plants on water sources and their associated biota.

Questions and Hypotheses

Question 1: How have historic coal-firing methods at EVGS in Boulder, CO influenced sediment heavy metal concentrations in SBC downstream of the plant?

Hypothesis & Prediction 1: Coal-fired power plants leach heavy metal pollutants into groundwater from ash ponds that settle in streams sediments and accumulate over time. If this is true, sediments in SBC downstream of EVGS ash ponds will have greater heavy metal concentrations than sediments of upstream reaches.

Question 2: How does the macroinvertebrate community composition in SBC differ between reaches downstream of EVGS and those upstream, and are observed differences related to heavy metal concentrations?

Hypothesis & Prediction 3: Certain macroinvertebrate taxa are sensitive to pollutants and will not be found in waters with high heavy metal concentrations. I predict that macroinvertebrate species composition in reaches of SBC downstream of EVGS will differ from reaches upstream of the power plant. Species intolerant to heavy metals will not be found in downstream reaches where heavy metal concentrations are likely to be high but will be found in upstream reaches where heavy metal concentrations are likely to be lower.

Question 3: To what degree do macroinvertebrates bioaccumulate metals downstream versus upstream of power plants and how does feeding guild influence this bioaccumulation rate?

Hypothesis & Prediction 2: Macroinvertebrates accumulate heavy metals through their environment and diets. I predict that the heavy metal concentrations will be higher in macroinvertebrates downstream of EVGS versus upstream of SBC, and at higher trophic levels.

Methods

Eight 100-meter reaches, separated by 100-meters, will be chosen based on accessibility and relative location to EVGS. Five will be downstream starting 50-meters South of EVGS, and three will be upstream starting 50-meters North of EVGS in SBC (Figure 1).

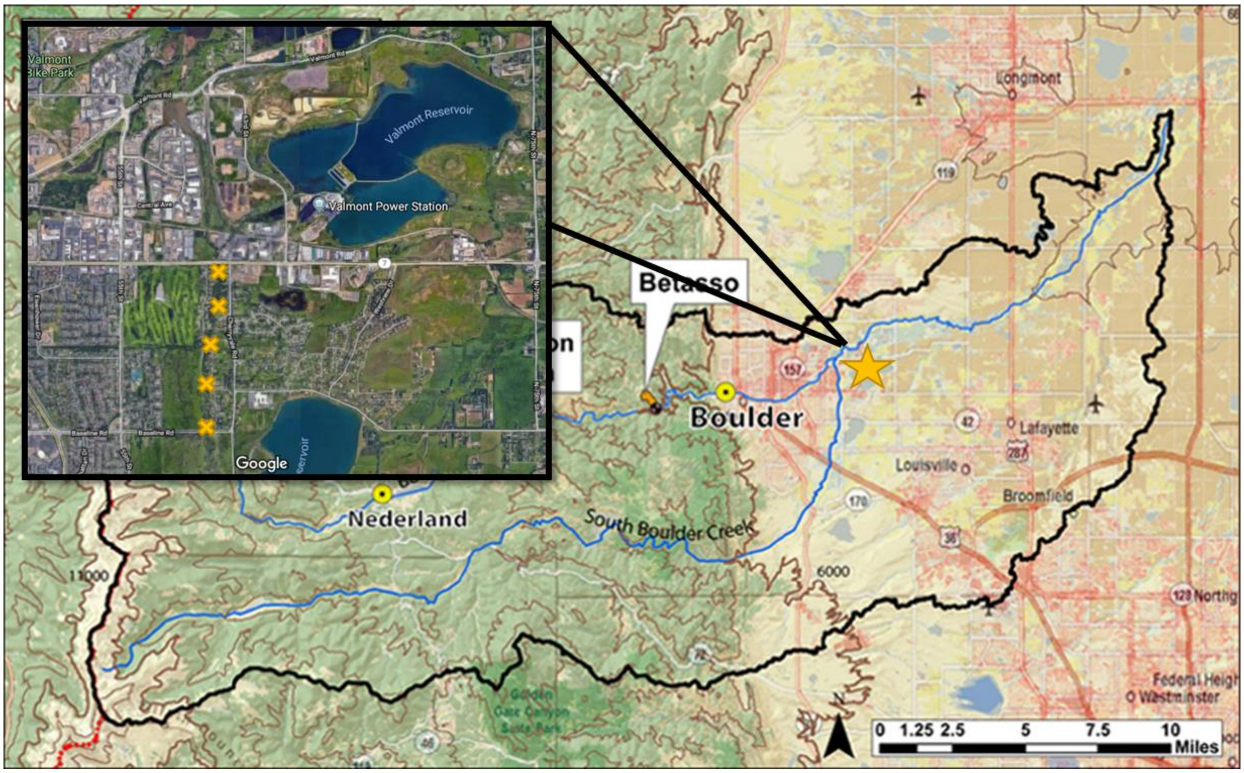

Figure 1: Map of Boulder Creek Watershed with a star indicating location of Excel's Valmont Generating Station and a Google map of approximate locations of downstream reaches 100-meters apart (Boulder Critical Zone Observatory).

Question 1

To assess heavy metal concentrations in downstream versus upstream reaches of SBC, five collections of fine-grain bottom sediment will be taken at 20-meter intervals along each 100-meter reach. Since sediment acts as a sink for pollutants in waterways, this will provide an estimate of sediment accumulation of heavy metals (Skorbiłowicz et al., 2018). Sediment from the first five cm of the creek bottom (40 total) will be extracted using a Guillotine sampler, dried to 40°C, and sent to Colorado Mountain College laboratories for an elemental analysis, by ICP-OES, on the following heavy metals: Zn, Hg, Cr, Pb, Cd and Cu. A generalized linear mixed model estimating concentrations of each heavy metal, as a function of location (i.e. upstream versus downstream reaches) and distance, with an added random effect of site.

Question 2

To compare the composition of macroinvertebrate communities upstream and downstream of EVGS, samples will be taken using a 6-1/2" deep, 500 μm nylon mesh D-net. I will disturb benthic sediment for 30 seconds at four replicate riffles to ensure consistency at each site. Whenever possible, the riffles will be approximately 20-meters apart, and the distance between each sampling riffle will be recorded, considering some may be clumped while others are well spaced. The contents of the net will be rinsed through a 0.5 mm sieve, and specimens will be stored in 70% ethanol to be sorted in the lab (Clements et al., 2000). At Regis University, macroinvertebrates will be sorted under a stereoscope, and then identified to family, enumerated and categorized into four functional feeding groups: scraper, shredder, collectors, and predators. (Clements et al., 2000). A non-metric multidimensional scaling ordination of community structure will be conducted and will indicate whether pollution levels at each reach correlate with community structure. The results will also identify dominant taxon of each functional feeding group (FFG) needed for Question 3.

Question 3

To compare heavy metal body concentrations of macroinvertebrates between upstream and downstream reaches of EVGS, sampling will be done as described in Question 2; however, in this case the contents of the net will be rinsed through a 0.2 mm sieve, and 10 individuals from the dominant taxa of each FFG and will be kept alive and stored in creek water (Clements et al., 2000). 40 macroinvertebrate samples will be sent to Colorado Mountain College laboratories for digestion and to test the heavy metal body concentrations by ICP-OES of the following metals: Zn, Hg, Cr, Pb, Cd and Cu. The effect of feeding guild, and site and location on heavy metal concentrations will be assessed using generalized linear mixed models in R. I expect greater heavy metal concentrations in higher trophic organisms, and in downstream reaches as predicted.

Potential Negative Impacts

Disturbance to aquatic habitats in SBC will be short term, and only during sample collections. No significant damage will occur to the creek or any of BCPOS resources. Wildlife will only be briefly disturbed during sampling times. This will pose no long-term threat to local ecosystems.

Budget

Purchased

Item	Justification	Cost/Unit (Source)	Quantity	Total Cost
Gas	Four round trips to Boulder, CO using the federal mileage reimbursement rate	$0.535/mile (IRS)	272	$145.52
Containers	Containers to hold macroinvertebrates and sediment samples	$0.40/container (Amazon)	50	$19.89
Heavy metal analysis	Metal analysis of 40 sediment samples and body concentrations of 40 macroinvertebrates	$40.00/sample (Colorado Mountain College) $10.00/digestion per sample	80	$4,000.00
Ethanol	To preserve specimens for sorting	$53.99.00/gallon (Amazon)	1	$53.99
Stipend	To complete field and laboratory work.	$11.30/hour (Regis)	50	$565.00
Total Amount Requested				**$4,784.40**

Regis University

Item	Justification	Cost/Unit (Source)	Quantity	Total Cost
D-net	To conduct macroinvertebrate sampling	$192.89/net (Wildco)	2	$0.00
Microscope	To observe community composition of macroinvertebrates	$849.00/microscope (Microscope World)	1	$0.00
Total Amount Requested				**$0.00**

Regis will donate necessary equipment and I will donate additional time beyond than what is awarded to complete this study.

Timeline

Date	**Activities**	**Deliverables**
May 2019	Visit sites and randomly select reaches for study	List of chosen sites/reaches
June 1st-15th, 2019	Conduct study and collect sediment samples and macroinvertebrates for community composition	Raw data and collected samples
June 15th-30th, 2019	Collect live macroinvertebrate samples for heavy metal analysis	Raw data and collected samples
July 2019	Send sediment and macroinvertebrate samples to lab for heavy metal analysis	Samples submitted for analyses
July-November 2019	Analyze and summarize all data	Data summaries and figures completed
November 2019	Complete project report draft	Submit draft by November 31, 2019
December 2019	Complete final project report draft	Submit final draft by December 31, 2019

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CHAPTER 3. JOURNAL MANUSCRIPT: PARASITE PREVALENCE IN THE LEAST CHIPMUNK ACROSS COLORADO

Abstract

Rising temperatures and more frequent extreme weather events due to climate change will potentially alter parasite-host relationships. These changes in climate may cause parasite ranges to expand due to increasing habitat suitability, thus facilitating the spread of infectious diseases. Parasites play an important role in ecosystems by controlling host populations, and any changes in their ranges can alter the stability of ecosystems. In order to study how climate change will affect parasite ranges and their hosts, elevation is used as a proxy for climate change due to the gradient in temperature associated with changes in elevation. The Least chipmunk (*Tamias minimus*) is a widely distributed rodent in the Sciuridae family that is found at many elevations and host to many internal and external parasites. I examined the influence of elevation and other host characteristics on various types of parasite prevalence on the Least chipmunk in Colorado. Specifically, I predicted that Least chipmunks at lower elevations in Colorado will exhibit higher parasite prevalence of all types than those found at higher elevations. Contrary to my prediction, elevation was not a good proxy for climate change. Elevation significantly increased the odds for parasite prevalence in ectoparasite and overall parasite prevalence for this species. However, longitude was a consistent predictor across all models and suggested that the farther west a chipmunk was, the greater the odds of having a parasite. These results suggest that habitat characteristics or geographic region of a host may be a better predictor for parasite prevalence than elevation.

Introduction

Anthropogenic climate change will potentially alter the relationships between parasites and their hosts (Auld & Brand, 2017; Mysterud et al., 2016). Warmer temperatures and more frequent extreme weather events allow parasite ranges to expand due to habitat suitability, thus facilitating the spread of diseases carried by parasites (Auld & Brand, 2017; Short el al., 2017). These changes in climate allow for more contact between hosts and parasites to occur and increase the overall survival of vectors and parasites themselves (Short el al., 2017). Elevation can be used as a proxy for some of these abiotic factors, such as temperature, to investigate the interaction between hosts, parasites, and the changes in their environment (Maher & Timm, 2014). Because a gradient in temperature and climate typically exists with changes in elevation, inferences can be made about parasite prevalence on hosts found at various elevations. Increasing temperatures correspond to higher survival rates of parasites; therefore, parasite prevalence is expected to be lower at higher elevations where temperatures are much lower. For example, red deer in Norway that migrate seasonally from lower to higher elevations have a smaller parasite load during the portion of their migration that is at higher elevations (Mysterud et al., 2016). Other studies have also observed that populations breeding or inhabiting higher elevations tend to have fewer parasites than host populations found at lower elevations (Bergstrom, 1992; Mumladze et al., 2015). Therefore, environmental conditions may be a limiting factor for parasite survival and range.

Although increased parasitic ranges and their influence on rates of disease have been studied, the relationship between hosts, parasites and climate change is not clear. Biotic factors, such as the host's behavior and biology, also play a role in parasite prevalence (Han et al., 2015). Host body size, sex, age, and intensity of social interactions could also influence parasite prevalence. In order to investigate how climate change affects parasite ranges, both host characteristics and environmental variables must be incorporated into studies to get a more complete understanding of the effects of climate change on parasites.

Although parasites can spread disease, they also play a very important role in the environment and in controlling host populations (Mysterud et al., 2016; Poulin & Morand, 1998). Changes in parasite populations, whether an increase or decrease, would significantly alter the environment and their associated hosts. Discovering trends about parasites, hosts and their environment will inform predictions about the future conditions of ecosystems. These predictions may assist in maintaining organism populations and prevent trophic cascades. Studying the trends of parasites along an elevational gradient may also aid in decisions regarding the spread of infectious diseases by parasites with future global climate change (Krasnov et al., 2010). As parasite ranges expand, there is a fear that contact between humans and parasites will increase, as well as increased exposure to the diseases that they carry (Short et al., 2017).

Hosts that live along an elevational gradient typically show how changes in environmental conditions influence variation in populations. Examining the relationships between the environment and host traits at different elevations make organisms that live along a gradient ideal study organisms. The Least chipmunk (*Tamias minimus*) thrives in numerous habitats and can be found at almost all elevations in North America. Additionally, various internal and external parasites are often reported on this species (Bergstrom, 1992; Verts & Carraway, 2001). This species provides the opportunity to study how parasites respond to environmental gradients, such as elevation. The Least chipmunk is also widely distributed across the North American continent, making it more accessible than other species for research (Verts & Carraway, 2001).

The Zoology Department at the Denver Museum of Nature and Science (DMNS) maintains a parasite collection from all mammalian specimens brought into the museum. In this study, I investigate the parasite prevalence on Least chipmunks collected in Colorado and donated to DMNS. Using data such as host body size, gender, latitude, longitude and elevation, I demonstrate the relationship between parasites and changes in the environment, using elevation as a proxy for those abiotic factors. I hypothesize that parasites prefer hosts found at lower elevations due to less harsh environmental conditions, so I expect to find a higher prevalence of parasites on Least chipmunks at lower elevations in Colorado after accounting for various host features.

Methods

Species Background

The Least chipmunk (*Tamias minimus)* is a widely distributed rodent in the Sciuridae family (Verts, 2001). Its distribution ranges north from the Yukon territory, south towards New Mexico, and as far east as the Hudson Bay (Verts, 2001). The Least chipmunk is the smallest species of chipmunk found in North America. Much of their diet consists of seeds; however, when seeds are not readily available, the chipmunk also feeds on leaves, arthropods, flowers, fruit, and fungi (Verts, 2001). Due to their adaptability and large distribution, the Least chipmunk has been found at many elevations, including those above 11,500 feet (Verts, 2001). Because of this elevational gradient, their habitat can range anywhere from arid sagebrush to dense coniferous forests.

These widely dispersed chipmunks are often hosts to many internal and external parasites. Fleas, ticks, mites, lice and flies have all been documented on the Least chipmunk (Verts, 2001). A variety of species of ectoparasites have been found on the Least chipmunk, including tick species that transmit Colorado tick fever and Lyme disease (Verts, 2001). Internal parasites, such as nematodes, also commonly infest the gastrointestinal tract of the chipmunk.

Identification and Data Collection

DMNS obtains Least chipmunk specimens through a variety of means. Some are donated by locals that find them at their residences and others are provided to the museum by researchers that are funded by the museum. All specimens given to DMNS are deceased upon arrival. Animals found by other organizations, such as the Denver Zoo, are donated to DMNS as well. Consequently, the study sites vary greatly across regions and climates. The specimens that come to DMNS are then stored in freezers and tagged according to location, biological data, and the circumstances that brought them in. Over time, the chipmunks are brought into the zoology prep lab to be dissected and examined for ectoparasites and endoparasites by trained staff. Any parasites collected from an organism are placed into two separate vials filled with ethanol. The vials are categorized according to location of parasite (ecto/endo) and then labeled by host ID number and type (ticks, fleas, mites, worms, etc.). The dissections and collections are performed by a large number of staff members that are kept on record. A list of preparators can be found under each observation listing.

After the parasites are placed into vials and stored in freezers, they are further identified using taxonomic keys and other identification resources under a dissecting microscope. The level of taxonomic identification achieved is recorded into the database, such as phylum, order, family, genus or species. The results from the identification process are put into the ARCTOS database that is then used by DMNS. Each mammalian observation has its official ID number, species name, country of origin, state/province, coordinates, date, specific locality, sex, weight and total length.

All observations for this study were obtained from the online ARCTOS database and filtered using the species name "*Tamias minimus*". The results were further filtered by only selecting observations found in the state of Colorado. There was a total of 530 observations of the Least chipmunk in Colorado, with or without parasites found during the initial examination. Other observations were eliminated from the search results that did not have a proper dissection performed or had no clear record of parasite examination. Two hundred and twenty observations were used for this study dating from 2003 to 2018, with elevations ranging from 5,062 to 12,864 feet.

Data Analysis

There is a total of nine chipmunk observations that did not have weight recorded in the data set. I used an allometric equation ($y = ax^b$) of all complete observations with weight and total length recorded to create a linear regression in R version 3.3.2 (R Core Team, 2016). I inputted the total length for those nine missing observations to estimate their weight in grams.

To investigate parasite prevalence, three different observations of parasites were coded as 0 or 1 (absent or present). If I found that an endoparasite was detected on a chipmunk observation, I coded a 1 for endoparasite presence, no matter the type or number of internal parasites. Ectoparasites and overall parasite prevalence (either endoparasite or ectoparasites present) were coded similarly. To compare parasite prevalence between chipmunks and various predictors, I fit three generalized linear mixed models (GLMM), one for each parasite type observation recorded. I used the lme4 package (Bates et al., 2015) in R version 3.3.2 to fit all models (R Core Team, 2016). I also calculated percentages of findings, such as the percent of chipmunk observations with endoparasites, ectoparasites, or either, to assess if the Least chipmunk in Colorado commonly has parasites, and if so, what type (endo/ecto).

Results

Contrary to my predictions, elevation did not lower parasite prevalence for all three models: endoparasites, ectoparasites, and overall prevalence (p = .707, p = .049, p = .034). I found a positive relationship between parasite prevalence and elevation in the Least chipmunk for ectoparasites and overall parasites (Table 1). However, longitude showed a consistent negative relationship to the odds of having a parasite for all binomial GLMMs (Table 1). I found that for every decimal degree a chipmunk is located west in longitude, the odds of it having a parasite significantly increase (p = .032, p = .051, p = .016) (Table 1). Larger body size also corresponded to having a parasite (Table 1). However, this was only statistically significant for endoparasites (p = .057). Interestingly, the interaction between elevation and weight of a Least chipmunk has a significant role in parasite prevalence for overall parasites (p = .037). This shows that the effect of weight is lower at higher elevations, therefore, having a smaller body size at higher elevations increases the chance of having a parasite.

The percentages of "present" endoparasites, ectoparasites, or any parasite was calculated from the total number of observations in the data frame. Seventy one percent of all Least chipmunks collected had a parasite; of those 71%, 29% had endoparasites and 66% with ectoparasites. This was out of 219 total observations of the Least chipmunk in Colorado (Figures 1 & 2).

Table 1: Results of all three binomial GLMM's with 90% confidence intervals.

	Dependent variable:		
	Endoparasites	Ectoparasites	Overall Parasites
	(1)	(2)	(3)
Elevation	0.001	0.004**	0.005**
	(−0.003, 0.005)	(0.001, 0.007)	(0.001, 0.008)
Weight	0.114	0.217	0.281*
	(−0.169, 0.397)	(−0.001, 0.434)	(0.037, 0.524)
Longitude	−0.353**	−0.334*	−0.449**
	(−0.623, −0.082)	(−0.616, −0.051)	(−0.757, −0.141)
ELEVATION:WEIGHT	−0.00002	−0.0001*	−0.0001**
	(−0.0001, 0.0001)	(−0.0002, −0.00001)	(−0.0002, −0.00002)
Constant	−43.007**	−44.108**	−58.722***
	(−73.845, −12.168)	(−74.538, −13.678)	(−92.185, −25.259)
Observations	219	219	218
Log Likelihood	−127.891	−133.502	−123.821
Akaike Inf. Crit.	265.781	277.005	257.642
Note:			*p<0.1; **p<0.05; ***p<0.01

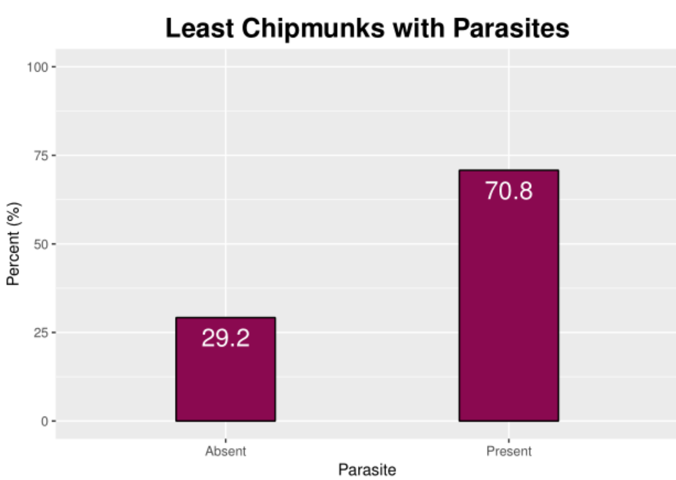

Figure 1: The majority of Least chipmunks in Colorado exhibit parasite prevalence.

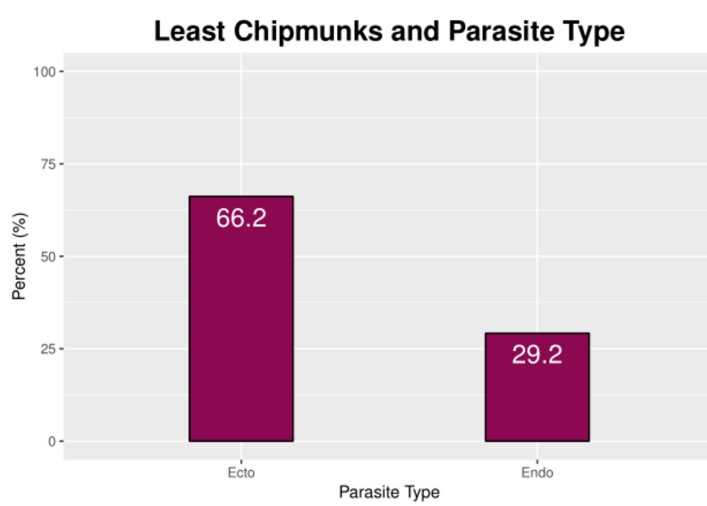

Figure 2: Of the chipmunks that had parasites, external parasites were more common than internal.

Table 2: List of parasite species identified during study.

Type	**Species**
Flea	*Catallagia decipiens*
Flea	*Eumolpianus eumolpi*
Worm	*Heteroxynema cucullatum*
Louse	*Hoplopleura arboricola*
Flea	*Hystrichopsylla dippiei*
Louse	*Neohaematopinus pacificus*
Flea	*Orchopeas leucopus*
Flea	*Oropsylla idahoensis*
Worm	*Rauschtineria eutamii*
Worm	*Syphacia eutamii*

Discussion

My hypothesis that there would be lower parasite prevalence at higher elevations was not supported. On the contrary, my results suggest that there is a positive effect of elevation on overall parasite occurrence in the Least chipmunk in Colorado. I was testing the premise that elevation would be a suitable proxy for climate change because of the natural climate gradient that occurs with increases in elevation, but this was not the case. Instead, the higher in elevation that a chipmunk was found, the more likely it was to be infected with a parasite. My results that indicate a positive relationship between elevation and parasitism are inconsistent with other published studies (Bergstrom, 1992; Mumladze et al., 2015; Mysterud et al., 2016). It is assumed that parasites would not survive on hosts at higher elevations due to changes in the environment.

For the Least chipmunk, parasites were not limited by the environmental conditions associated with elevation.

The interaction between elevation and weight also requires additional explanation. Mammals are typically smaller at higher elevations, which may provide some context as to why there are more small chipmunks at higher elevations that were infested with parasites. This also raises the following question: does smaller body size correspond to higher susceptibility to parasites in the Least chipmunk? Smaller body size may decrease the overall fitness of an organism, leading to increased vulnerability to parasites and disease. It is difficult to differentiate whether this interaction is influenced by smaller body size at higher elevations or increased susceptibility. Future research should account for the natural variation in body size across regions to account for any interactions associated with location.

Although elevation was not an adequate predictor for parasite prevalence, the effect of host body size produced significant results as predicted. Recent research suggests that a host's body size influences parasitism, resulting in an intensification of parasites on larger hosts (Han et al., 2015). My findings supported this idea for all models; however, it was only statistically significant for overall parasite prevalence on the Least chipmunk. This may have been due to the greater number of data points in overall parasite prevalence, in comparison to ectoparasites and endoparasites.

Interestingly, longitude was the most consistent and significant predicator of parasite prevalence for all three models in this study. I did not expect that moving farther west would increase parasite prevalence. The effect of longitude on parasite prevalence indicates that environmental factors in the western region of Colorado influence parasite-host relationships on the Least chipmunk. The differences in parasite prevalence due to geographic location may be better explained by other factors that were not measured in this study. Since elevation was not an adequate proxy for environmental gradients, more specific environmental data, such as precipitation and land cover, should be used to determine their effect on parasite prevalence. Elevation accounts for changes in temperature, but precipitation along elevation may vary due to region and direction of the slope. Western Colorado has higher precipitation but may not hold true for the entire distribution of the Least chipmunk. Therefore, elevation cannot accurately describe environmental characteristics of every study site. A positive relationship between endoparasite prevalence and mean annual precipitation was demonstrated in the striped mouse (*Rhabdomys pumilio*) of South Africa, furthering the idea that environmental factors influence host-parasite relationships (Froeschke et al., 2010). Because annual precipitation is greater in western Colorado than the east, my study complements the connection made between greater parasite prevalence and increased precipitation. Including additional environmental variables, such as precipitation, in the data analysis will give a more accurate picture of a species habitat than elevation alone.

It's likely that my lack of positive results stem from using elevation as a proxy, however, my study also had some inconsistencies in the methods that should be addressed. Not all chipmunks were collected using the same technique, and some may have been found dead before collection, which would certainly increase the parasite load and generate a higher rate of parasite prevalence within the data. However, given the manner in which DMNS receives specimens from researchers and donations, this cannot be avoided. Other issues that could be addressed with future data collection to avoid bias is to incorporate more random sampling by DMNS researchers. The data set had more observations at higher elevations, rather than lower, which may have skewed the results. To avoid bias, traps can be placed systematically along transects so that a true random sample of a host population is collected. Implementing new methods for species collection may be difficult for the museum to regulate but will improve the distribution of the data points. The majority of statistical significance also came from overall parasite prevalence, which may be because it had a greater number of observations than ecto- and endoparasites. Insuring a larger number of data points in the future may also reduce bias.

My findings indicated that although elevation may not be a reliable proxy to determine the effects of climate change on parasite prevalence, host traits and geographic location may provide some insight to these relationships. Elevation may be too broad and other variables too focused to certain geographic areas to make accurate predictions. A simple model that predicts the effects of climate change on parasites is needed but difficult to achieve because of the variation between study sites and host/parasite species. The results of this study may not hold true for similar research, such as the effect of longitude, because of this variation. Climate change may also have varying effects across regions. Where some regions may exhibit increases in annual temperature, others may not, making it even more difficult to predict the effects of climate change on parasites using a constructed model. Changes in environmental conditions may also be advantageous to some parasite types, while detrimental to others. Further knowledge on environmental requirements for parasites is needed in order to produce more well-informed findings.

The threat of anthropogenic climate change and its effect on parasite ranges are a significant threat to human and ecosystem health. If environmental factors limit parasite ranges, the changing climate may expand these ranges further into densely populated areas, posing a risk of spreading the diseases carried by these vectors. If we have a sense of what factors influence parasite ranges and disease, there is an opportunity to manage it. The expansion of parasite ranges will also negatively affect their ecological function of maintaining population sizes by infecting a larger number of hosts. More environmental research with multivariate factors is vital to preventing these future alterations to human health and the environment. The endeavor of finding a simple proxy for climate change should be taken on a case by case basis, according to region. The unpredictability of microclimates and weather complicates the ability to produce a model that predicts the effects of climate change on parasite prevalence. Parasites facilitate the spread of infectious diseases to humans and animals; therefore, it is critical to continue to investigate what factors influence parasites' ability to thrive.

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CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: CONTROLLING INVASIVE SPECIES AT RIO MORA NATIONAL WILDLIFE REFUGE

Invasive, nonnative species are a threat to native biodiversity and ecosystem integrity around the world (Lopez et al., 2019). Common invasives that alter ecosystems in the United States include various bullfrog and crayfish species. Species, such as bullfrogs and crayfish, are aggressive predators that reduce native populations, including fish and bird species (Degerman et al., 2007; Ficetola et al., 2007). The Rio Mora National Wildlife Refuge (RMNWR) is located in Watrous, New Mexico and run by the Denver Zoo and United States Fish and Wildlife Service (Figure 1). Like other ecosystems, RMNWR suffers from invasive bullfrogs and crayfish in the Mora River, that runs about five miles through the refuge (Figure 2). The refuge is currently not open to the public, as its main goal is to focus on conservation of the area and its native species.

Recent efforts to trap and reduce nonnative and invasive populations to better protect the biodiversity at RMNWR have not been successful. The reintroduction of the North American river otter to the area is proposed as a way to restore natural top-down controls that would alleviate the problem. The river otter is known to eat crayfish and bullfrogs found in and near their habitat (New Mexico Department of Game and Fish, 2017). The North American river otter is a native predator that has been effectively reintroduced to other rivers in the United States, and therefore is a practical solution for RMNWR that aligns with their goals. The reintroduction of the river otter will restore natural ecosystem processes in the Mora river and reduce invasive bullfrog and crayfish populations that threaten the native species found there.

Consequences of Invasives

Invasive bullfrog (*Rana catesbeiana)* and crayfish (*Orconectes virilis, O. rusticus*) species are disrupting the populations of native and non-invasive species at RMNWR. They alter community dynamics through predation of many river species of native fish, invertebrates, and amphibians. Bullfrogs are known as voracious, opportunistic predators that can decimate populations of local fish, invertebrates, amphibians, reptiles, and small mammals (Ficetola et al., 2007). Bullfrog tadpoles are much larger than most other species, and easily outcompete the larvae of native frog species in aquatic ecosystems (Ficetola et al., 2007). In addition to their large build, these organisms are also considered to be generalists and will eat other amphibians, reducing native populations that cannot contend. Invasive crayfish are omnivores that feed on the juveniles and eggs of native fish, greatly reducing their survival and populations (Degerman et al., 2007). Aside from distressing communities through predation, bullfrogs and crayfish may also outcompete other local predators for food and other resources (Degerman et al., 2007). With populations of invasives increasing, they will consume the majority of resources that native species need in order to survive. Invasive populations may also decrease vegetation and affect other vital parts of an aquatic ecosystem (Degerman et al., 2007). Furthermore, once an introduced species has established a population in an area, extirpation of that species is very difficult to accomplish.

Recommendations

In order to control the populations of invasive bullfrogs and crayfish at RMNWR, there are three proposed actions. The first is to rid the river of invasive species with active methods, such as the physical removal of them. Although this method may work, it will require a lot of human power and money to maintain the project (Beaty & Salice, 2013). The effort to provide staff to complete the tasks is not feasible when the refuge is already understaffed. The refuge would also have to allocate money from their budget to this project in order to complete the physical removal of the species. The potential for invasives to be accidentally missed and left in the river to repopulate is another concern with this method, which would result in a waste of money and time. This recommendation will take a lot of effort and may be an ongoing project for many years.

Another proposed recommendation is to increase predator crane or heron populations that will potentially consume and control bullfrog and crayfish populations in the river. This recommendation is less costly than the physical removal of the invasive species and would not require constant human intervention to achieve. Increasing heron or crane populations at the refuge also aligns with their goals of conservation, since native herons and cranes already migrate to RMNWR. Great blue herons, Green herons, Black-crowned night herons and the Sandhill Crane are all native species that can be found at the refuge throughout the year. However, birds travel over long distances and migrate during the winter breeding season. This could cause the potential recovery of invasive populations during the time predator birds are not at the refuge. The risk of not alleviating the problem of invasives with this recommendation makes it a possibly ineffective solution.

The third recommendation is to reintroduce the North American river otter (*Lontra canadensis*) to the Mora River to restore natural top-down controls within the ecosystem. Because the river otter was historically present at the refuge, it has the potential to be the most successful method. The species was originally eradicated from the area due to trapping and hunting for its fur but is no longer threatened in the now nationally protected area. The river otter also naturally consumes bullfrogs and crayfish in its diet, which would reduce their populations at the refuge if otters are reintroduced. Initially, the reintroduction of the river otters would require a lot of resources and money, but once they are reintroduced, the ecosystem will be self-sustaining. The natural predation of the invasives will occur on its own from the otters (New Mexico Department of Game and Fish, 2017). Methods to reintroduce river otters to over 22 states across the United States have been proven successful and provide implementation strategies for RMNWR (Erb et al., 2018). There is the possibility that the otters may leave the area for suitability reasons, much as the heron or cranes would during migration. However, if the Mora River is confirmed as a suitable habitat for the river otters by thorough examination, this should no longer be a concern.

Stakeholders

There are many parties that are involved with RMNWR that are affected by the various proposals to control invasive bullfrog and crayfish populations. The Pojoaque tribe, in particular, are a group of Native Americans that own the bison herd at RMNWR. They also share the goal of conservation of the grassland and its native species with the refuge staff. However, their cultural connection to the refuge is strong and any changes to the land and wildlife there can affect the aesthetic value of the land, and the economic value from the bison herd. The river otters will naturally create latrine sites along the river of the refuge if reintroduced and they may alter the riparian vegetation that the bison use as a food source. It is possible that they will be against the proposals in order to protect their livelihood via the bison herd and the vegetation they require as food. Other groups, such as local ranchers, may be conflicted with allowing river otters to navigate the river that runs not only through the refuge, but also through their property. Various privately-owned ranches are located just outside the fences of RMNWR. They may have no desire to deal with any alterations to their property from the otters, since their cattle and horses graze throughout their property and possibly near the river. Just as the Pojoaque tribe, they may find the reintroduction as a threat to their livelihood and oppose these potential changes to the land.

Animal rights groups, such as PETA, must also be considered stakeholders for the potential recommendations. PETA is very forward about being against the killing of any animals, and all three recommendations involve the predation or removal of two different organisms from the river. Any chosen proposal may face opposition from such animal rights groups that deem it unethical. Local fisherman may also be negatively impacted by the two introduction proposals. Recreational fishing may be impacted by the introduction of otters or avian species that also feed on fish, not just bullfrogs and crayfish. These recommendations may impact the amount of fish available, and this would cause local fishermen to lose recreational value in the Mora River. Additionally, the possible cost and manpower needed to complete the proposals is a likely issue for the Denver Zoo and United States Fish and Wildlife Service. They are given a certain budget each year, and it would likely be impacted if any of the recommendations were to be chosen. However, the Denver Zoo and United States Fish and Wildlife Service are stakeholders that want to see the refuge be restored to its natural state, and protect the native species found there. Although it would requires various resources, the threat that the invasives pose to the future of the refuge is far greater than the cost of any of the proposals. No action to control the populations would make matters worse for native biodiversity in the future.

Final recommendation

After considering resources, time, and money, the clear option for controlling invasive bullfrog and crayfish species at RMNWR is to reintroduce the North American river otter to the Mora River. This recommendation will require the least amount of staff involvement, be in line with conservation goals of the refuge, and is the solution with the least amount of risk. Since this proposed method for controlling invasives is a process that occurs naturally, there is no need to maintain the project except for evaluating its success in the near future. The potential conflicts with stakeholders and the reintroduction are not severe or detrimental in any way and can be mitigated through open discussions and meetings. This option is the only way to safely restore regular ecosystem functions and protect the native biodiversity at RMNWR in a feasible way.

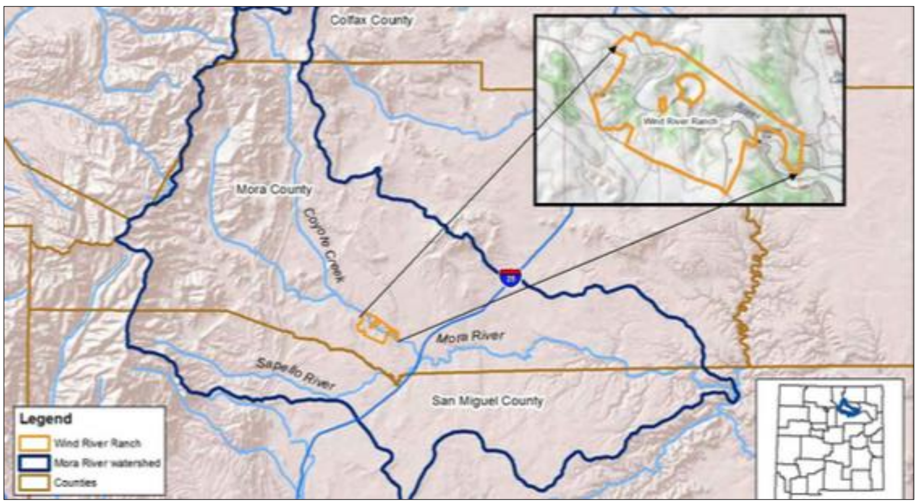

Figure 1: Map of Mora river watershed, with a map of RMNWR included on the Mora river (USFWS).

Figure 2: Photo of Mora River in RMNWR (Jenna Slabe).

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