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CHAPTER 1. LITERATURE REVIEW: INVESTIGATING THE ECOLOGICAL FACTORS THAT DRIVE FROG POPULATION DECLINES DUE TO *B. DENDROBATIDIS* TO DEVELOP NATURE RESERVES

In recent decades, a multitude of amphibian populations have rapidly declined and several have gone extinct due to major threats such as habitat loss, overexploitation, and diseases. A leading cause of amphibian species decline around the world is chytridiomycosis, an amphibian disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd) (Boyle, Boyle, Olsen, Morgan, & Hyatt 2004). To prevent the spread and presence of this disease, nature reserves are necessary for numerous frog species to survive. Traditional conservation efforts have been ineffective at ridding this disease from protected habitats (Berger et al. 1998). By analyzing the various environmental and biological factors that control the spread of chytridiomycosis, measures to design new conservation reserves that allow frog species to thrive without the threat of the pathogen can be successfully implemented. It is highly important that this investigation be done because chytridiomycosis in frogs has caused the greatest loss of vertebrate biodiversity due to disease ever recorded (Skerratt et al. 2007) which can have negative implications to ecosystems globally.

First reported in Panama and Australia (Boyle et al. 2004), chytridiomycosis has now locally extirpated numerous frog populations in every continent except Asia (Weldon et al. 2004). In some cases, losses have been so extreme that species have gone extinct. The disease has affected 400 species comprising two orders, Anura and Caudata (Boyle et al. 2004). The biological agent of the disease is the virulent, highly-transmissible skin pathogen of the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which originated in Africa (Weldon et al. 2004).

Bd, a member of the phylum Chytridiomycota, is a heterotrophic fungus mainly found in soil and water (Berger et al. 1998). Bd is the first member of Chytridiomycota to be recognized as a parasite of the sub-phylum Vertebrata (Barr 1990). Bd infects the mouthparts of larvae and the epidermal cells of post-metamorphic frogs causing an increase in tissue growth (Boyle et al. 2004). These growths disrupt frogs' skin functions such as respiration and the maintenance of the water balance (Van Rooij, Martel, Haesebrouck, & Pasmans 2015), which can ultimately lead to death.

The ubiquity of Bd is due to the high international and intra-national trade of frog species for pets and human consumption (Schlaepfer, Hoover, & Dodd 2005). Although Bd is globally distributed, its genetic variation is low (Morehouse et al. 2003), indicating that Bd is a fairly recent pathogen (Parris & Cornelius 2004). In some geographical areas Bd thrives quite well because it infects a wide-range of amphibian host species and quickly attacks vulnerable hosts in a new territory (Davidson et al. 2007).

Host vulnerability differences can drive the ecological occurrences of pathogen dilution or amplification within a community (Searle et al. 2011a). The dilution effect occurs when biodiversity and disease risk are inversely related, and amplification occurs where biodiversity has a positive relationship to disease risk (Searle et al. 2011a). Bd does not necessarily occupy every available amphibian species where it occurs (Skerratt et al. 2007). In some cases, certain species can act as a reservoir for the pathogen. These species allow the pathogen to persist in an area even when the density of susceptible hosts is low (Haydon, Cleaveland, Taylor, & Laurenson 2002). Understanding which hosts act as reservoirs for Bd is important for predicting its heterogenic transmission and whether amplification or dilution effects are occurring.

Davidson et al. (2007) tested yellow-legged frogs' (*Rana boylii)* susceptibility to Bd at different life stages. Their results showed that post-metamorphic juveniles survived when exposed to chytridiomycosis, but their growth rate was reduced by approximately one-half (Davidson et al. 2007). Another study examined the relationship between gray treefrogs (*Hyla chrysoscelis*) and Bd by exposing them to the fungus during development (Parris & Cornelius 2004). The researchers then recorded the survival rates, metamorphosis rates, and the body mass of individuals. Although Bd did not directly affect the frogs' survivorship, it increased the time it took individuals to metamorphose, and reduced their body mass. Thus, Bd has a notable effect on the life-history performance of certain species during development (Parris & Cornelius 2004) in aquatic habitats. Although Bd did not cause high rates of mortality in these studies, it greatly reduced their bodily growth. Post-metamorphic frogs with relatively smaller bodies were more likely to be infected and carried more intense infection levels than those with larger body sizes (Kriger & Hero 2007). Because these species survive infection, they are an ideal reservoir for the disease to remain present in environment.

In addition to yellow-legged frogs and gray treefrogs' potential to be a reservoir for Bd, multiple experiments demonstrate that bullfrogs (*Rana catesbeiana*) can also efficiently carry Bd within an area, but are resistant to chytridiomycosis (Daszak et al. 2004). Researchers analyzed the occurrence of Bd in populations of anurans in Oregon and northern California (Adams et al. 2010). They found that Bd is highly dependent on the population being introduced to the habitat. Bd was more prevalent in nonnative populations of American bullfrogs (*Rana catesbeiana*) than in native populations (Adams et al. 2010).

Contrarily, southern toads (*Anaxyrus terrestris*) and wood frogs (*Lithobates sylvaticus*) have higher Bd-related mortality rates although their Bd infection levels are similar to species that can survive the pathogen (Searle et al. 2011b). Searle et al. (2011b) also found that Western chorus frogs (*Pseudaris triseriata*) that were inoculated with Bd multiple times resisted infection by Bd, which indicates that they may have a genotype that can quickly recover from or is resistant to Bd. Comparative studies show that Bd influences the survival rates inconsistently among species. Bd's effect on different species changes the biodiversity of amphibians which can ultimately lead to dilution or amplification effects of the disease in a community (Searle et al. 2011a). Searle et al. (2011a) experimentally manipulated host biodiversity and found that a dilution effect occurs. Incorporating species richness into natural reserves can dilute the spread of Bd if it possibly gets introduced to the habitat.

Frog species highly associated with aquatic life-stages are most susceptible to Bd (Bielby, Cooper, Cunningham, Garner, & Purvis 2008). Not only are bodies of water ideal habitats for the pathogenic fungus (Boyle et al. 2004), but they also serve as habitat for the pre-metamorphic developmental stages of exclusively aquatic tadpoles. Even more susceptible species are aquatic ones with small clutch sizes (Bielby et al. 2008) because escaping infection is less likely when population sizes are small. This ultimately leads to a local extinction in that species (de Castro & Bolker 2005). Increasing species richness in a protected area can reduce the risk of Bd infecting vulnerable aquatic pre-metamorphic amphibians, because highly diverse communities may contain fewer infected individuals with reduced pathogen loads compared with communities with lower diversity (Searle et al. 2011a).

Temperature, elevation, and geographical landscape also have an influence on species susceptibility to infection. The susceptibility of the host significantly depends on the strength of its immune response and the growth rate of the pathogen, especially in amphibians whose immune responses are temperature-dependent (Raffel, Rohr, Kiesecker, & Hudson 2006). During winter in temperate regions, the pathogen grows more slowly at low temperatures (Ratkowsky, Olley, McMeekin, & Ball 1982). Furthermore, when temperatures vary seasonally, maintaining the "optimal immunity level" is not possible in some amphibians, thus making them more vulnerable to the disease (Raffel et al. 2006). In addition to temperature's effect on hosts' immunity, it also impacts Bd's existence for Bd does not survive at temperatures above 29°C (84°F) (Piotrowski, Annis, & Longcore 2004). Focusing on those areas with climatic temperatures that are on average higher than 29°C and without much between-season fluctuation would be ideal for the design of future natural reserves.

The geographical distribution of frog species plays an extremely important role in Bd transmission. In Central and South America, most endemic amphibian species reside on mountain tops (Lips, Reeve, & Witters, 2003). High-elevation species are highly vulnerable to contracting chytridiomycosis from Bd because of the lower air temperatures (Stuart et al. 2004). Consequently, numerous documented population declines have occurred at sites above 500 meters in elevation (Lips et al. 2003). At high elevations, Bd thrives better in stream-breeding amphibians as opposed to pond-breeders because streams efficiently spread Bd to various areas and frog populations (Kriger & Hero, 2007). Locations with low temperature variation or low annual rainfall have exhibited rapid declines in frog species due to Bd (Lips et al. 2003). Overall, species with restricted ranges had a higher chance of population decline because they could not recruit conspecifics from other populations that are a bit out of their range (Bielby et al. 2008). Together these studies agree that high elevations seem to be ideal for Bd, therefore, reserves should be implemented at lower elevations that could be a secure habitat for multiple frog species and not for Bd. Furthermore, reserves at lower elevations should not connect to higher elevations by water flow; the stream or river could easily transport the fungal pathogen to lower areas (Sapsford, Alford, & Schwarzkopf 2013).

Anthropogenic stressors, such as populated areas, roadways, and agriculture increase the presence of Bd in native species (Adams et al. 2010) because they can negatively impact frog species' immune systems. Pesticides in urban and agricultural areas inhibit natural immune responses in yellow-legged frogs (*Rana boylii)* in California, making them more susceptible to Bd (Davidson et al. 2007). When designing protected areas, anthropogenic impacts should be absent from the habitat so that toxic effects of pesticides do not influence the prosperity of the sheltered species.

Currently, no techniques guarantee that frog species will survive Bd infection in the wild (Stuart et al. 2004). To control Bd in 2006, Australia finalized a Threat Abatement Plan (Skerratt et al. 2007) which includes quarantine strategies, projects on recovering endangered species, and goals for future research. Australia's work is on-going; it is only a start to what must be applied world-wide due to Bd's abundance. Because of the long list of factors that determine Bd's success rate, it is necessary that there be an all-inclusive conservation plan proposed. It is urgent that more countries dedicate major funding, like Australia, to develop protected areas and to better understand the distribution of the pathogen (Skerratt et al. 2007).

Conservation agencies must direct their attention and resources to the management and eradication of chytridiomycosis. To quantify all of the factors involved with the transmission of Bd is challenging, but this thorough investigation is necessary as a foundation to understanding its spread and designing conservation areas. Lower elevations, higher ambient temperatures with low variation, the absence of pesticides, and the presence of non-continuous waterways together can conceivably sustain frog populations and evade the penetration of Bd into protected areas.

These habitats should also feature high amphibian biodiversity due to its negative effect on Bd transmission. By implementing reserves with these specific characteristics, we can prevent further declination of species populations, and ensure the longevity of amphibian biodiversity.

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CHAPTER 2. GRANT PROPOSAL: INVESTIGATING ECOLOGICAL FACTORS THAT INHIBIT *B. DENDROBATIDIS* TO DESIGN NATURE RESERVES

Abstract

Due to the worldwide decline of biodiversity, protected nature reserves are necessary for species facing extinction. Many frog species have been experiencing rapid population decline and extinction due to the threat of the fungus Batrachochytrium dendrobatidis (Bd), which inflicts the highly transmittable and deadly disease chytridiomycosis. Chytridiomycosis causes increased skin tissue growth which disrupts respiration and the maintenance of water balance, which can ultimately lead to death. Certain ecological and geographical characteristics have been studied in order to assess their influence on Bd's prevalence. Specifically, it is hypothesized that high elevations, lotic habitats, and low species richness have a positive effect on Bd's success in certain areas. I plan to survey multiple sites of lentic and lotic habitats in California at both high and low elevations. I will collect data on the species richness and prevalence of Bd at these sites. I predict that sites at low elevation with lentic habitats and high species diversity will be the best fit for a protected area against Bd. After assessing any relationships between Bd prevalence and the three factors that seem to influence its presence, I will make a recommendation as to which location is the most ideal for a nature reserve to protect frog species from Bd. This will not only contribute to the current literature on this disease, but it could potentially play a key role in the conservation of the amphibian biodiversity globally.

Background/Rationale/Significance

In recent decades, an astounding number of amphibian populations have rapidly declined and several have gone extinct due to threats such as habitat loss, overexploitation, and diseases (Kriger et al. 2007). A leading cause of this decline around the world is chytridiomycosis, an amphibian disease caused by the fungus Batrachochytrium dendrobatidis (Bd) (Boyle, Boyle, Olsen, Morgan, & Hyatt 2004). This significant decrease in frog biodiversity can consequently impact many ecosystem functions globally due to frogs' importance in ecosystem dynamics (Kupferberg 1997). Traditional conservation efforts such as captive breeding have been ineffective at ridding this disease from protected habitats (Berger et al. 1998). Investigating the factors that contribute to the pathogen's success may inform nature reserve strategies to protect amphibians from the threat of Bd. Designing reserves safe from Bd is essential to protect frogs from chytridiomycosis and the ecosystem functions they support because the disease is responsible for the greatest loss of vertebrate biodiversity (Skerratt et al. 2007).

First reported in Panama and Australia (Boyle et al. 2004), chytridiomycosis has now locally extirpated or caused extinction in numerous frog populations globally (Weldon et al. 2004). Most commonly, 400 species in the two orders Anura and Caudata are highly affected by the disease (Boyle et al. 2004). Bd is a heterotrophic fungus that is mainly found in soil and water and causes chytridiomycosis (Berger et al. 1998). Bd infects the areas around the mouth of larvae and the epidermal cells of post-metamorphic frogs causing an increase in tissue growth (Boyle et al. 2004). These growths disrupt frogs' skin functions such as respiration and the maintenance of water balance (Van Rooij, Martel, Haesebrouck, & Pasmans 2015), which can lead to death.

Bd has decreased frog populations in almost every continent (Boyle et al. 2004). The ubiquity of Bd is due to the high international and intra-national trade of frog species for pets and human consumption (Schlaepfer, Hoover, & Dodd 2005). Although Bd is globally distributed, its genetic variation is low (Morehouse et al. 2003), which indicates that Bd is a fairly recent pathogen (Parris & Cornelius 2004). In some geographical areas Bd thrives because it infects a wide range of amphibian host species and quickly attacks vulnerable hosts in a recently colonized territory (Davidson et al. 2007).

Host vulnerability differences can drive the ecological mechanisms of pathogen dilution or amplification within a community (Searle et al. 2011). The dilution effect arises when biodiversity and disease risk are inversely related, as in the case of Bd presence in a community (Searle et al. 2011). Bd does not necessarily infect every available amphibian species where it occurs (Skerratt et al. 2007) and in some cases, certain species can act as a reservoir for the pathogen. These species allow the pathogen to persist in an area even when the density of susceptible hosts is low (Haydon, Cleaveland, Taylor, & Laurenson 2002). By incorporating numerous frog species in a protected area, Bd is not transmitted efficiently due to the dilution effect brought about by the high biodiversity in the area.

The biogeography of frog species plays an important role in Bd transmission. In Central and South America, most endemic amphibian species reside on mountain tops (Lips, Reeve, & Witters 2003). Frog species residing at high elevations are especially vulnerable to contracting chytridiomycosis because Bd thrives in lower temperatures common in montane regions (Stuart et al. 2004). Consequently, many population declines have occurred at locations above 500 meters in elevation (Lips et al. 2003). Additionally, Bd thrives better in lotic habitats, which are characterized by rapidly moving water such as streams, rather than lentic habitats, which have still bodies of water such as ponds. Bd is able to infect stream-breeding amphibians more efficiently as opposed to pond-breeders because streams easily spread Bd throughout watersheds (Kriger, Pereoglou, & Hero 2007).

The only certain way to allow frog species to thrive in the wild is to create protected areas where Bd cannot persist. Previous studies agree that high elevations seem to be ideal for Bd (Haydon et al. 2002; Kriger et al. 2007); therefore, reserves should be implemented at lower elevations that could be a secure habitat for multiple frog species and not for Bd. Furthermore, the reserve should not connect to higher elevations by water flow because the stream or river could easily transport the fungal pathogen to sites at lower elevations (Sapsford, Alford, & Schwarzkopf 2013). Quantifying all factors that relate to the transmission of Bd is challenging, but investigating them is necessary to predict Bd's spread and design effective reserves. Lower elevations and the presence of non-continuous waterways together can conceivably sustain frog populations and biodiversity by preventing the penetration of Bd into protected areas (Bielby et al. 2008; Lips et al. 2003; Searle et al. 2011). These habitats should also feature high amphibian biodiversity due to its dilution effect on Bd transmission. Previous studies suggest that these factors can be beneficial in the preservation of frog species (Searle et al. 2011; Haydon et al. 2002), however, the application of the habitat elevation and hydrology have not been applied. By implementing reserves with these specific traits, we can prevent future decline of frog populations.

I will explore the relationship between Bd prevalence and biogeographic characteristics of certain frog habitats. My study's results can be beneficial for the bigger issue of the loss of amphibian biodiversity that is occurring globally. The community of students, faculty, and administration at Regis University prides itself in its active role in caring for all aspects of our diverse world. My own principles are in sync with the university's mission; I believe that I have a responsibility to contribute to the conservation of our world's valuable assets, especially biodiversity. By investigating how biogeography influences the health of frog populations, solutions can be found and expanded upon in order to sustain biodiversity in different ecosystems in the near future.

Purpose and Specific Aims

The specific aim of my proposed work is to analyze which habitat type is most suitable for frog species to thrive without the threat of the fungal pathogen Batrachochytrium dendrobatidis (Bd). The answers to the following two questions will shape what type of location can be implemented as a frog reserve.

1. Does elevation and the presence of waterways have an effect on Bd?

I predict that sites at low elevation (lower than 500m) with lentic habitats will be the best suited areas for frog species to live in absence of Bd. Numerous frog species populations have declined or gone extinct from infection of Bd at elevations above 500 meters because Bd thrives at lower temperatures, which are more common at high elevation (Stuart et al. 2004). Additionally, lotic habitats are excellent carriers of Bd and can transmit the pathogen to multiple areas (Kriger et al. 2007).

2. Does frog biodiversity affect Bd's presence?

I predict that the site with the greatest number of frog species will have the lowest Bd prevalence due to the dilution effect. Bd does not necessarily infect every available amphibian species where it occurs (Skerratt et al. 2007); therefore, by having high species richness, Bd will not be easily transmissible in a community.

After concluding which biogeographic area is ideal for a nature reserve, I will propose the most suitable area to be a reserve for multiple frog species. This will ultimately save numerous frog species from population declines and extinction, and preserve the biodiversity of amphibians around the world. By increasing and maintaining the current state of frog biodiversity, the protected habitats can in turn positively impact the overall health of many ecosystems and their functions.

Methods

Field Collection & Laboratory Analysis

Prior to the field surveys, I will obtain Institutional Animal Care and Use Committee (IACUC) approval in order to handle the frog species in the wild. I will collect data at twelve different locations in California, USA, three for each combination of the geographic characteristics. The factors I will be testing are high elevation (>500m), low elevation (<500m), lotic and lentic habitats. My six high elevation sites will consist of multiple areas within Shasta-Trinity National Forest (STNF) (40.741822, -123.248939) which has an elevation of approximately 4,300 m (USDA n.d.). Within STNF, I will have multiple 1 hectare sampling plots. In order to pinpoint three lotic sites and three lentic sites, I will evaluate drainage density and still-water density of STNF by using GIS mapping. Drainage density is determined by the length of streams divided by meter squared within a 1 hectare site. Still-water density will be calculated by the area of ponds in a 1 hectare site. Sites with high drainage densities and no still-water densities will be considered lotic sites, and ones with high still-water densities and no drainage densities will be considered lentic sites. Jackson State Forest (JSF) (39.373919, -123.679018), which has an elevation of approximately 50m (National Park Service 2017), will be used for the six low elevation sites. GIS mapping will again be used in this forest to determine three lentic and three lotic habitats.

After I establish the site locations, I will conduct an area-based survey modeled after guidelines set by Dodd (2010). Within the 1 hectare plots in lotic habitats, I will randomly set up six 4 m^2 quadrats along the edge of the stream and record the stream characteristic (i.e. pool, run, riffle) of each quadrat. For the lentic habitats, six 4 m^2 quadrats will be randomly selected around the edge of the pond. Each quadrat will be thoroughly surveyed for frogs by searching within vegetation and underneath leaf litter and rocks. I will record the species, sex, and body length of each individual. Following Adams et al. (2010), in each quadrat I will test frogs that are found for Bd by rubbing a rayon swab (Puritan Supply, Guilford, ME) over the body surface and in the mouth of each frog found. Ideally, I would hope to obtain 30 swabs per site. Therefore, I will swab the first 5 frogs in each quadrat. If there are more present, I will continue to record the species, sex, and body length for each individual. I will replace the gloves I am wearing after handling each individual in order to avoid potentially spreading Bd. The swabs will be air dried for 30 minutes out of direct sunlight and stored in a pre-labeled sterile microcentrifuge tube (Fisher Scientific International, Pittsburgh, PA, USA). Vials will be kept cool and shipped to Regis University Biology laboratory for analysis. The swabs will be analyzed for the presence of Bd by using a real-time Taqman PCR assay (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

I will organize my data by geographical locations, combining the data of the three sites of each category. The sites will be ranked by prevalence of Bd from highest to lowest, and a logistic regression will be used to quantify the relationship between Bd prevalence and biodiversity, elevation category, and type of water body in each site. By doing so, I can conclude whether there is a significant relationship between Bd and the corresponding factor. I will then be able to determine which geographical region within my study to propose as a potential reserve.

Work Plan

Frog survey and sampling will take place at the 12 identified sites during May-July 2018. The laboratory and statistical analysis of Bd presence will take place at Regis University's Biology Laboratory in August-September 2018. A final project report will be tentatively completed by November 2018.

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URSC Project Budget

Items ***(Please itemize amounts below)***	**Description**	**Funds requested from URSC**	**Funds requested from other sources**	**Source of other funds**
Supplies (Consumables)				
Rayon Swabs	Puritan Medical Products - Sterile Rayon Swab, Aluminum Shaft (50/box)	**$199.92** (8x $24.99)		
Disposable Latex Gloves	Great Glove – Latex Fit Powder Free Disposable Gloves (100/bx)	**$19.96** (4x$4.99)		
Microcentrifuge Tubes	Fisher Scientific - Nonstick, Rnase-free Microfuge Tubes, 1.5 mL (250/bx)	**$56.25**		
PCR Kit	ThermoFisher Scientific – Phusion High-Fidelity PCR Kit (200/bx)		**$488.00** (2x $244.00)	NSF Grant
Gas	For traveling from STNF to JSF (191 mi, $2.50/gal, 24mpg, 12 gal)	**$19.90**		
Hotel and flight to CA	For additional costs of hotel and initial travel to CA.	**$149.83**		
Equipment (Non-consumables)				
Cooler	Stanley 16-Quarts Adventure Leak-Proof Cooler	**$48.96**		
Field Notebook	Rite in the Rain 374-M All Weather Universal Field-Flex Memo Book, 3.5"x 5"	**$5.18**		
Total URSC Request		**$500.00**		

URSC Project Budget Justification

I am requesting $500.00 for the purchase of rayon swabs, disposable latex gloves, microcentrifuge tubes, a cooler, PCR kit, gas, travel costs, and a field notebook. Regis University's Biology Department will allow me to use their GIS device and Taqman PCR assay. The swabs, tubes, and cooler will be used to collect and store biological samples from the frogs. The gloves will be used to protect myself while exposed to the wildlife. In order to record the species that I encounter, a field notebook will be necessary. The GIS will aid me in recording the exact location where I take the samples, and a PCR kit includes reagents necessary to run the real-time Taqman PCR assay. The gas expense will cover the cost of driving from STNF to JSF. I am requesting $149.83 to contribute to my flight to California and hotel arrangements, and any additional costs will be paid for out of pocket. These supplies are essential in the collection of the sampling of frogs and for recording the species that are found at the multiple locations.

Application to Current Coursework

My proposed study will contribute to my Master's Degree in Environmental Biology at Regis University. This research aligns with the curriculum of my graduate classes such as Environmental Biostatistics and Research Design, Advanced Behavioral Ecology, and Advanced Field Ecology. Additionally, the field work and lab experience will provide me with valuable knowledge and skills for my future undertakings in the environmental biology field. My project will contribute to the scientific community's understanding of Bd and help inform conservation managers to make informed decisions about where to locate nature reserves to protect frog species.

CHAPTER 3. INVESTIGATING THE DISTRIBUTION OF TREES IN DENVER AND THEIR TOLERANCE TO SOIL SALT

Abstract

Urban trees are planted to counteract the detrimental effects of poor air quality, rising temperatures, and elevated levels of CO_2. Certain tree species are more suitable for particular goals; however, they also may be restricted by damaging environmental stressors. De-icing salt permeates into adjacent soils of roads, and can have harmful consequences, such as reduced growth, chlorosis, and mortality, to road-side vegetation. This stressor particularly effects silver maple (*A. saccharinum*) and little leaf linden (*T. cordata*). By analyzing the locations of these species along with the salt-tolerant trees: green ash (*F. pennsylvanica*), white ash (*F. americana*) and honey locust (*G. triacanthos*), I assessed whether the salt sensitivity of these plants is being considered when managers and homeowners place trees in the city and county of Denver. Contrary to my predictions, the salt-intolerant species were planted closer to roads, and private homeowners tended to plant intolerant trees closer to road edges than public managers. This finding indicates that the managers and homeowners may be overlooking a main stressor to trees, and instead may be solely focusing on the benefits (shade, carbon sequestration, aesthetics) that trees provide. However, the high salinity in the soil may hamper the trees' ability to achieve these goals to their full extent. More informed practices on the health, benefits, and costs of certain tree species in urban environments is necessary in order to maximize the benefits urban trees provide while minimizing their costs and stressors. By doing so, urban trees can mitigate the impacts of urbanization to their greatest potential.

Introduction

Within the past two decades, urban forestry has shifted its focus from one of aesthetic value to one that encompasses environmental, economic, conservation, and social values of trees (McPherson, 2006). Carbon sequestration, storm-water runoff reduction, energy conservation, and increased psychological and physical health of humans are only a handful of the important benefits of urban forests (Dwyer et al., 1992). Planting urban forests is becoming increasingly crucial because 75-80% of the North American population lives in urban areas, and urbanization continues to rise (Pataki et al., 2006). However, selection of trees is not a simple task; in-depth city planning that accounts for both environmental benefits and costs of particular tree species is necessary to ensure that the choices of tree species match both the geography and the intended purposes of urban forestry initiatives (Nowak & Dwyer, 2007).

Urban trees are often selected for traits that help maximize their benefits (i.e. intended goal) and minimize the environmental and financial costs. For example, elevated summertime temperatures create urban 'heat islands.' The lack of vegetation and high solar radiation absorption by urban surfaces cause urban centers to be significantly warmer than the surrounding rural areas. This in turn leads to an increase in energy used by cooling units during the summer (Akbari, Pomerantz, & Taha, 2001). During winter, deciduous trees allow buildings to absorb light when they lack leaves, but block sunlight during summer when they have a full canopy (Akbari, 2002). Because of these characteristics, deciduous trees are a better option than evergreens which would not aid in solar heat absorption during the winter months. Some examples of deciduous trees are Norway maple (*Acer platanoides*), green ash (*Fraxinus pennsylvanica*), red maple (*Acer rubrum*), and little-leaved linden (*Tilia cordata*). These shade trees help buildings stay cooler and therefore reduce the energy required to cool buildings in summer. Consequently, over an individual deciduous tree's lifetime, the energy savings associated with the tree are estimated to be $200 (Akbari et al., 2001). In this case, deciduous trees fulfill the desired goals of mitigating heat islands during summer and taking advantage of them during winter.

Urban trees are also planted to counteract climate change and enhance nature-based aesthetics of heavily urban areas where there is little to no vegetation. Trees sequester carbon by fixing carbon dioxide during photosynthesis and storing the excess carbon as biomass (Nowak & Crane, 2002). CO_2 is the dominant greenhouse gas that contributes to global climate change (Montagnini & Nair, 2004) and by sequestering it trees reduce the impacts that CO_2 contributes to climate warming. Trees also remove air pollutants (e.g. O_3, SO_2, NO_2, CO) by absorbing them through leaf stomata and by retaining airborne particulate matter on their surfaces (Morani et al., 2011). Thus, by sequestering carbon dioxide and air pollutants, trees can impact human health by improving air quality. Certain species can maximize these specific benefits. Fast-growing species, such as quaking aspen (*Populus tremuloides*), are able to sequester atmospheric CO_2 at an exceptional rate due to their fine root system that absorbs carbon deposited in the soil (Pregitzer et al., 2000). Conifer species have a high surface-to-volume ratio and retain their foliage year-round, and therefore make them especially efficient sinks for pollutants as opposed to deciduous species (McPherson et al., 2007).

Although many tree species assist in the benefits of carbon sequestration and pollutant uptake, localized stressors may harm certain tree species. Trees' ability to handle certain stressors should influence which trees are planted in order to minimize the environmental cost of tree damage (Sæbø, Benedikz, & Randrup, 2003). Stressors include limited crown space and soil volume, air pollution, wind, droughts, and de-icing salt. North America uses about 14 million tons of road salts annually (Sanzo & Hecnar, 2006), and de-icing salt alters roadside soils (Blomqvist & Johansson, 1999). Deicing salts, namely, $NaCl$, $CaCl_2$, and $MgCl_2$ (Sanzo & Hecnar, 2006), are carried onto adjacent lawns by road runoff where they damage resident plants. Consequences of road salt to plants include the reduction in biomass, chlorosis (the reduction of the green coloration in the plants), and in extreme cases, mortality (Czerniawska-Kusza et al., 2004). By understanding the tolerance level of commonly planted trees to pollutants like deicers, managers can decide on the correct mix and location of trees to maximize the intended benefits and minimize the environmental costs.

One fast-growing city that uses trees to benefit its urban landscape is Denver, CO. The population in the Denver metropolitan area has grown 1.6 percent between 2010 and 2015 (Metro Denver EDC, 2018). Because of this recent population boom, Denver's air quality has declined because of increased motor vehicle emissions (Koken et al., 2003). Furthermore, temperature inversions, which result from Denver being geographically located at the bottom of the Rocky Mountains, exacerbate air pollution effects by trapping pollutants in cooler air near the ground (Koken et al., 2003). Because of the combination of these factors, measures to ameliorate the poor air quality in Denver demand attention; urban trees can mitigate these effects. However, managers must consider the environmental stressors, such as de-icing salts, that influence the success of certain tree species. In Denver, of the 260,924 publicly planted trees, the five most common species include green ash (*Fraxinus pennsylvanica*) at 9%, honey locust (*Gleditsia triacanthos*) at 8%, silver maple (*Acer saccharinum*) at 5%, white ash (*Fraxnius americana*) at 4%, and little leaf linden (*Tilia cordata*) at 4% (City and County of Denver, 2015). Although these trees beautify urban areas, they differ in their tolerance to soil salt concentrations. Green ash, honey locust, and white ash tolerate soil salt (Macfarlane & Meyer, 2005; Dirr, 1976; Townsend, 1980), while silver maple and little leaf linden are sensitive to high soil salt concentrations (Macfarlane & Meyer, 2005; Dirr, 1976).

These species may be unintentionally placed in areas of high soil-salt concentrations depending on the property manager's understanding of the species' traits. While public managers are informed about the effects that de-icing salt has on certain tree species (Pokorny et al., 2003), private homeowners may not be aware of this stressor. They may overlook this cost by instead valuing the shade or pleasant appearance that certain salt-intolerant species may bring to their streets (Schroeder & Canon, 1983). Due to the detrimental impacts that high salinity concentrations have on certain species, analyzing the tree distribution in relation to roads is necessary. This study can provide insight into whether private and public managers incorporate trees' salt tolerance into their decision-making process of where to plant particular species.

By analyzing a dataset of trees that are managed by the Department of Parks and Recreation in the City and County of Denver, I will assess the placement of these 5 species within different sites in Metro Denver. I hypothesize that a difference in species presence will exist between area types (i.e. park, golf course, street, and median) because managers will choose particular species based on their ability to thrive in areas of high soil salt concentrations. I predict that green ash, white ash, and honey locust are more likely to be distributed along streets and medians, due to their tolerance of salts and that little leaf linden and silver maple will be more common in the parks' interiors due to their salt intolerance. Furthermore, I predict that as distance from the nearest road increases, the probability of a tree being salt intolerant will also increase. Additionally, I predict that trees that are planted and cared for by public entities, as opposed to private maintenance, will be more likely to be intolerant to salt the farther their distance is to the nearest road. The results of this study can lead to more informed practices by private and public managers to consider the salt-tolerance of trees when choosing their location.

Methods

Data Collection

The Department of Parks and Recreation in the City and County of Denver manages an open tree inventory available online (City and County of Denver, 2017). The inventory managers recorded the species, location, site designation, diameter, number of stems, and the inventory date of the public trees that were planted in the city and county of Denver. Additionally, the city foresters compiled a separate dataset that lists specialized characteristics, including soil salt tolerance, for tree species that are used in the city (City and County of Denver, 2017). Trees are listed as tolerant, intermediate, or sensitive to soil salt.

GIS Analysis

Prior to running statistical analyses, some manipulation of the data was needed in ArcGIS to calculate trees' distances from the nearest road (ESRI, 2011). I used Denver's open catalog (City and County of Denver, 2017) to acquire the location and length of roads in the city. I projected both the road polylines and tree points to the UTM Zone 13 projected coordinate system, then used the 'Near' tool to calculate the distance each tree was from the nearest road and stored it as a column in the tree dataset. I exported the attribute table for further statistical analysis. Additionally, nineteen trees were greater than 7 km away from a road and were therefore removed from the analysis. They were removed because after examining their location in ArcGIS, they were not planted in Denver, however they were in Denver's tree database. For visualization purposes, I added in a base map and color-coded trees according to their tolerance values (Figure 1).

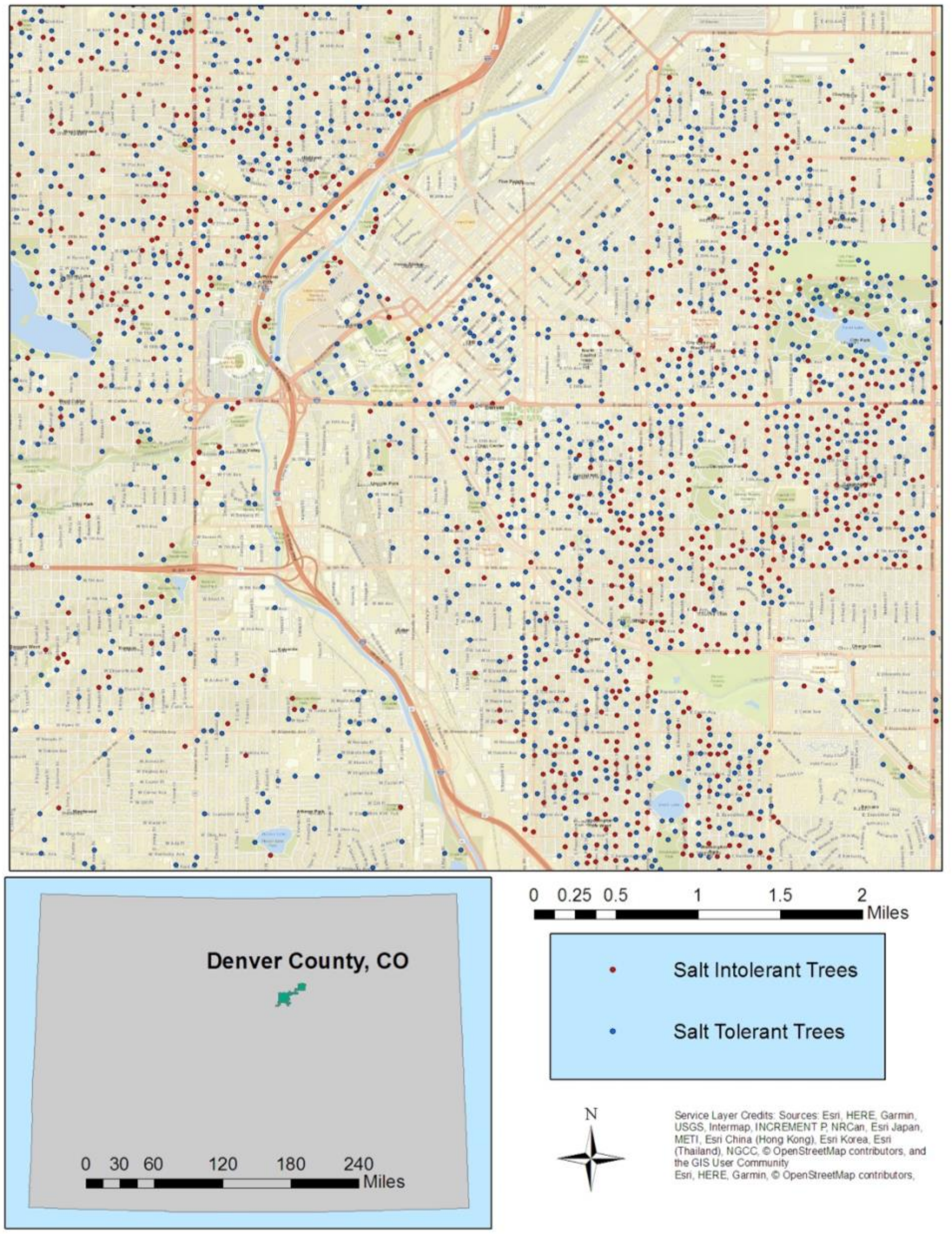

Figure 1. The distribution of salt-intolerant and salt-tolerant trees in the City and County of Denver (ArcGIS). Only the 5 most commonly planted trees were considered in this analysis.

Statistical Analysis

I conducted all statistical analyses in R version 3.4.3 (R Core Team, 2017). I categorized the 5 dominant tree species (*A. saccharinum, F. americana, F. pennsylvanica, G. triacanthos,* and *T. cordata*) based on their tolerance levels provided by Denver's tree characteristic database using the dplyr package (Wickham et al., 2017). I categorized medians, sidewalks, and any street as 'streets' and parks, natural areas, and golf courses as 'parks' as two different location types in my analysis. I grouped street trees by their maintenance designation recorded in the original open catalog. Trees that were along "Privately Maintained Streets" were labeled as "private" and the rest were designated as "public".

To determine if the distribution of trees was associated with location (i.e. street or park), I conducted a chi-squared test. To assess whether street and park locations differed in the proportion of salt-tolerant tree species, I fit a binomial generalized linear model assuming tolerance class was a function of site type. I initially fit this relationship as a binomial mixed model with the trees' addresses as a random variable, however the model failed to converge due to the large number of individual addresses. To quantify the relationship between tolerance status and distance (m) from the nearest road, I fit a binomial generalized linear model, where tolerance status was assumed to be a function of $\log_2$(distance + 1).

To determine whether privately and publicly maintained streets differed in the proportion of salt-intolerant tree species, I fit a binomial mixed model assuming tolerance class was a function of maintenance type. I also fit a binomial mixed model assuming the trees' address as a random variable, however this model failed to converge due to the large number of individual addresses. To quantify the relationship between tolerance status and distance (m) from the nearest road, I fit a binomial generalized linear model, where tolerance status was assumed to be a function of $\log_2$(distance + 1). I also included an interaction between distance and street type, because private and public entities may differ in the relationship between tolerance class and distance to the nearest street.

Results

Of the 260,924 trees recorded in the Denver database, I analyzed 81,935 (31%) individual trees that comprised the 5 most abundant tree species (Table 1). 69% of the tree species that I analyzed were salt tolerant, and 89% were located in street designated areas (Table 1). Of the trees that were planted in street designated areas, 89% were planted along privately maintained streets (Table 1). The average distance to the nearest road, regardless of the trees' tolerance level, was 12.6 m (95% CI: 12.4 – 12.8). For tolerant trees only, the average was 13.2 m (95% CI: 12.3 – 14.1), and for intolerant trees the average distance was 11.3 m (95% CI: 11.0 – 11.5).

Table 1. Summary of tree locations and salt tolerance of the 5 most abundant tree species in the city and county of Denver.

TOLERANT		**INTOLERANT**	
All Trees			
Green ash	23,475	Little leaf linden	12,254
Honey locust	21,529	Silver maple	13,020
White ash	11,657	**TOTAL**	**25,274**
TOTAL	**56,661**		
Park Trees			
Green ash	2,642	Little leaf linden	1,973
Honey locust	3,008	Silver maple	1,017
White ash	751	**TOTAL**	**2,990**
TOTAL	**6,400**		
Street Trees			
Privately Maintained			
Green ash	18,737	Little leaf linden	8,499
Honey locust	15,611	Silver maple	11,797
White ash	9,913	**TOTAL**	**20,296**
TOTAL	**44,261**		
Publicly Maintained			
Green ash	2,096	Little leaf linden	1,782
Honey locust	**2,910**	Silver maple	206
White ash	**993**	**TOTAL**	**1,988**
TOTAL	**5,999**		

I found that there was a significant association between the tolerance classification of trees and their location sites (i.e. street or park) (p = 0.028, Pearson's Chi-squared test; Figure 2). The probability of a street tree being intolerant is 0.307 (95% CI: 0.304 – 0.311, p < 2e-16, binomial generalized linear model), while a park tree has a 0.318 (95% CI: 0.309 – 0.327) probability of being intolerant.

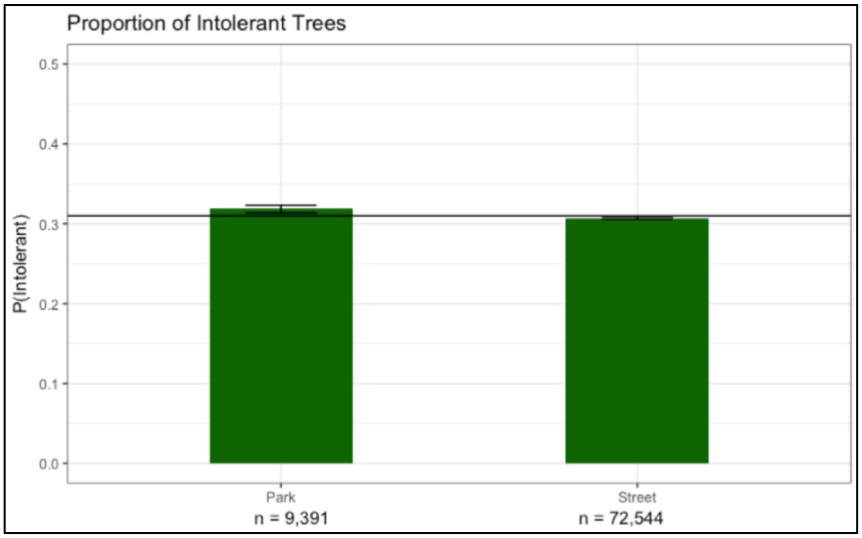

Figure 2. The proportion of trees that are salt intolerant at sites categorized as parks and streets. Only the 5 most commonly planted tree species were considered for this analysis. At both sites, approximately 30% are salt intolerant, but there is a significant difference between the two sites (p = 0.028). The horizontal line indicates the overall proportion (31%) of salt-intolerant trees across both sites.

A negative relationship existed between the trees' intolerance to salt and its distance from the nearest road. The odds of intolerance decreased significantly as distance from the nearest road increased ($p = 1.44 \times 10^{-6}$, binomial generalized linear model). The odds of a tree being intolerant decreased by 8.3% (95% CI: 6.7% – 9.9%; Figure 3) as distance to the nearest road doubles. When at the edge of a road, the probability of a tree being intolerant is 0.37 (95% CI: 0. 0.36 – 0.38) ($p < 2e-16$, binomial generalized linear regression). Additionally, the probability of a tree being intolerant is 0.30 (95% CI: 0.19 – 0.43) when at 12.6 m from the street, the average distance away from the nearest street.

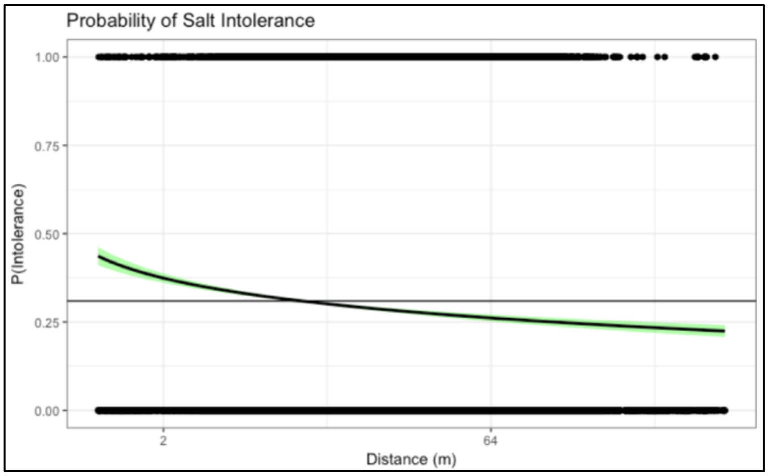

Figure 3. The negative relationship between distance from the nearest road (m) and the probability of a tree being intolerant. The odds of a tree being intolerant decreased by 8.3% (95% CI: 6.7% – 9.9%) as distance to the nearest road doubles. The green shading represents the 95% confidence bounds of the relationship. The horizontal line (31%) indicates the overall proportion of salt-intolerant trees.

However, publicly maintained streets are less likely to have intolerant trees than privately maintained streets. The probability of a tree being intolerant at a publicly maintained street site is 0.249 (95% CI: 0.239 – 0.259), whereas the probability of a tree being intolerant is 0.314 (95% CI: 0.311 – 0.318) at a privately maintained street site (Figure 4). Additionally, the relationship between a street trees' intolerance and distance to the nearest road yielded different results for privately and publicly maintained areas. Although the odds of a street tree being intolerant decreased by 30% (95% CI: 27% – 33%) as distance from the nearest road doubled, this relationship was not as strong for publicly maintained areas as it was for privately maintained areas. The odds of a tree being intolerant decreased by 34% (95% CI: 31% - 37%; Figure 5) as distance from the nearest privately maintained street doubles. For publicly maintained streets, the odds of a tree being intolerant decrease by 12% (95% CI: 5.8% - 19%; Figure 5).

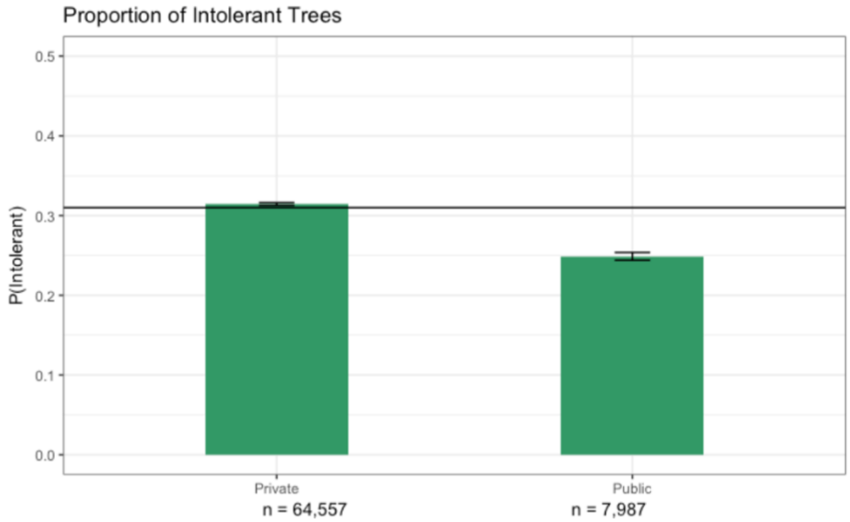

Figure 4. The proportion of trees that are salt intolerant along streets that are privately and publicly maintained. Only the 5 most commonly planted tree species were considered for this analysis. Along privately maintained streets, approximately 30% are salt intolerant. Along publicly maintained streets, 25% of the trees are intolerant. A significant difference in these proportions exist (p<2e-16). The horizontal line (31%) indicates the overall proportion of salt-intolerant trees.

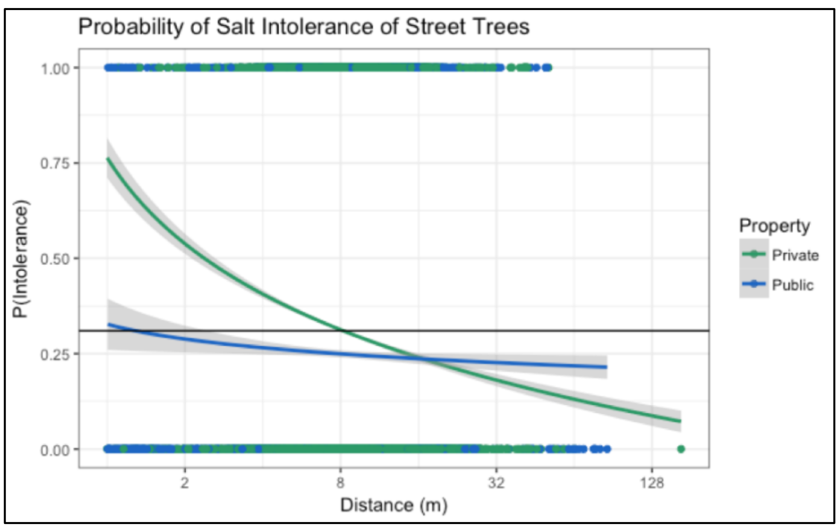

Figure 5. The negative relationship between salt intolerance and distance from the nearest road for privately and publicly maintained streets. The odds of a tree being intolerant decreases by 34% (95% CI: 31% - 37%)as distance from the nearest privately maintained street doubles. For publicly maintained streets, the odds of a tree being intolerant decrease by 12% (95% CI: 5.8% - 19%). The gray shading represents the 95% confidence bounds of the relationships. The horizontal line (31%) indicates the overall proportion of salt-intolerant trees.

When comparing the odds of trees being intolerant at the street's edge and at the average distance from the nearest road, the results differed between streets' maintenance type. When at the edge of a privately maintained street, the probability of a tree being intolerant is 0.62 (95% CI: 0.58 – 0.65), whereas the probability of a tree being intolerant along a publicly maintained street is 0.34 (95% CI: 0.28 – 0.39). Also, the probability of a tree being intolerant on a privately maintained street is 0.324 (95% CI: 0.322 - 0.325) when at the overall average distance (7.4 m) from the nearest street. When at the same distance, the probability of a tree being intolerant on a publicly maintained street is 0.254 (95% CI: 0.253 – 0.254).

Discussion

Contrary to my predictions, the salt-intolerant tree species (little leaf linden and silver maple) were planted closer to streets than salt-tolerant trees (green ash, honey locust, and white ash) in the City and County of Denver. Furthermore, private managers tended to plant intolerant trees closer to the street edge than public managers, which implies they may consider and value different traits when placing tree species. Private and public managers alike may not consider the soil-salt tolerance of the five most common species when deciding which trees to plant along streets. In turn, the soil-salt concentration may prevent these sensitive trees from fulfilling their intended purposes in urban environments.

The finding of dissimilar distribution patterns of salt-intolerant trees between privately and publicly maintained street trees could imply differing management practices or preference for tree species between the two groups. I found that private land managers tend to plant salt-intolerant trees closer to roads, whereas public land managers are simply just planting the trees randomly, regardless of their soil-salt sensitivity. The two salt-intolerant trees that I analyzed (*A.*

saccharinum and *T. cordata*) are both commonly used as ornamentals in urban areas because of their ease of establishment (Nesom, 2000). Their aesthetic value to private landowners, who may be uninformed about their salt sensitivity, could explain the difference in the planting patterns between privately and publicly maintained streets. Tree beauty can contribute to increased property value (Morales, 1980), and the visual effect of tree-lined streets creates a pleasant atmosphere in residential areas (Schroeder & Canon, 1983). This may explain why many private managers had a higher concentration of these salt-intolerant species close to streets than the public managers.

Yet even publicly managed areas tended not to have tolerant trees closer to roads. One reason for this could be the irrigation system in place. In an effort to conserve water, Denver Parks and Recreation utilizes recycled water in their urban parks as a low-cost, sustainable irrigation source (Denver Parks and Recreation, 2018). The average soil salinity in areas irrigated with recycled water is higher than those treated with potable water by approximately 19% (Chen et al., 2013). Managers could be strategically not planting salt-intolerant trees in parks where this technique is implemented.

Additionally, managers may be prioritizing other benefits and costs when placing trees in Denver. Benefits of salt-intolerant trees, such as adding aesthetic value (Nesom, 2000), aiding in cooling (Gilman & Watson, 1993), and carbon sequestering (Moser et al., 2015), could outweigh the cost of their intolerance, and therefore, this could be the underlying explanation of why they are distributed close to streets. For example, little leaf linden, which is salt intolerant, provides ecosystem services, such as carbon storage, shading, and cooling (Moser et al., 2015), which could be useful close to roads where these services can mitigate the effects from vehicle emissions. Silver maple, which is also salt intolerant, grows rapidly and is most commonly used for shading and cooling (Gilman & Watson, 1993). Because these two species are proficient in shading and cooling, their location near streets could have possibly been influenced by their ability to cool street surfaces that tend to have high solar radiation. Regardless of the benefit that the tree provides, both public and private managers should be informed of the damaging impacts that soil salt has on certain species.

The Tree Team from Colorado State University provides classes to private and public managers on tree issues and teaches them how to diagnose plant diseases and identify pests (Littlefield, 2016). The Tree Team also advises homeowners on when to call an arborist if they have major issues with the health of their trees. Furthermore, the Denver Botanic Gardens provides courses so that individuals can obtain a certificate in Rocky Mountain Gardening (Denver Botanic Gardens, 2018). In this educational program, individuals learn how to identify trees and to select the best species in certain landscapes and areas. With programs like these, both groups of managers can be educated on the best practices for urban trees.

My results indicate that tree managers in Denver may not consider the salt tolerance of trees when planting alongside streets. Failing to consider this characteristic of certain tree species could be potentially harmful due to deicing salt's permeability in the soil of lawns adjacent to streets. Although I did not measure the amount of de-icing salt that penetrates the soil adjacent to streets nor whether the trees are actually damaged nor did I know which streets receive this treatment, we do know that the city utilizes a de-icing mixture, 90% of which comprises chloride salts, on approximately 77% of all streets (City and County of Denver, 2018). Given that we know the detrimental effects of de-icing salt on certain tree species (Czerniawska-Kusza et al., 2004), more informed practices of tree planting in Denver could be in place. Although *A. saccharinum* and *T. cordata* bring many benefits to urban areas, their proximity to high salinity concentration in soils restricts their ability to grow to their fullest potential (Kansas Forest Service, 2016; Czerniawska-Kusza et al., 2004). The higher concentration of Na^+ and Cl^- ions in the soil, the higher their accumulation in *A. saccharinum* and *T. cordata*. Consequently, this leads to reduced biomass and chlorosis in individual trees (Sullivan, 1994; Czerniawska-Kusza et al., 2004). Therefore, the trees may not meet their full potential to sequester carbon, provide shade, or add beauty if their growth is inhibited (Nowak et al., 2013). Further studies of Denver trees and de-icing salt use are necessary to fully comprehend the effects of road salts to salt-intolerant species.

In summary, I found that on average salt-intolerant trees were located closer to roads than at farther distances in the city and county of Denver. This implies that the salt sensitivities of five tree species examined in this study are not actively considered when being planted along roads. The trees' benefits, such as aesthetic value, carbon sequestering, and pavement shading, may be the deciding factor when managers or homeowners plant them near streets. However, their proximity to streets may impede their ability to fulfill these tasks if they are being affected by road salt. Therefore, an in-depth assessment of commonly planted urban trees' benefits and costs is necessary to make informed decisions on their placement in cities, such as Denver, that use urban forestry to mitigate the effects of rapid urbanization.

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CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: ADVOCATING FOR FROG CONSERVATION AND GOVERNMENTAL REGULATIONS IN GUATEMALA'S MAYA BIOSPHERE RESERVE

Introduction

In recent decades, a multitude of amphibian populations have rapidly declined and several have gone extinct due to major threats such as habitat loss, overexploitation, and diseases. To mitigate the ongoing decline of frog populations, nature reserves are imperative for numerous frog species to survive. Guatemala's Maya Biosphere Reserve (MBR), a reserve established by the Guatemalan government and United Nations Educational, Scientific and Cultural Organization UNESCO in 1990, holds the largest remaining natural forest left in Mesoamerica (Rainforest Alliance 2018). This 5 million-acre reserve provides the ideal habitat for dwindling frog populations. However, conflicting interests exist among the people and entities involved in the management and control of MBR. After assessing these differing interests, I recommend that proper conservation practices and stricter governmental regulations on illegal activities should be established to prevent the degradation of MBR potentially allow it to host amphibian research and become a habitat for declining frog populations.

Concerns about frog conservation have arisen in the past few decades, after many species have become locally or globally extinct. Diseases, international pet trade, habitat loss, and pesticides cause the decline of frog species. Specifically, species endemic to Central America have been recorded to have mass mortality events due to the disease chytridiomycosis, caused by the highly-transmittable fungus *Batrachochytrium dendrobatidis* (Bd) (Boyle et al. 2004).

Although many etiologies for this disease have been proposed, none have been validated (Berger et al. 1998). To make matters worse, the international pet trade has substantially contributed to the spread of Bd globally (Garner, 2009). Furthermore, habitat fragmentation decreases the viability of certain species (Cushman, 2006), and pesticide use suppresses frogs' immune system and can lead to mortality (Relyea & Diecks, 2008). Such factors have been known to have interactive effects (Beebee & Griffiths, 2005), and therefore the rate of population declines have been intensified.

The focus of frog species conservation is of particular importance because they are known as biological indicators, meaning that frog species diversity mirrors the overall health of an ecosystem. Their high sensitivity to environmental changes indicate habitat alteration, fragmentation, or presence of disease (Pineda et al., 2005). By conserving these species in protected habitats, we can potentially save one of the most meaningful and informative biotas on our planet.

Currently, MBR provides a safe haven for sixty-two amphibian species, along with protected birds, reptiles, mammals, and plants (Wildlife Conservation Society, 2018). Its conservation success is due to collaboration among local communities, the Guatemalan government, and non-profit conservation groups such as the Wildlife Conservation Society (Wildlife Conservation Society, 2015). Although the main focus of the reserve is the conservation of natural land, jaguar (*Panthera onca*) populations, and overall biodiversity (Cuffe, 2016), currently no detailed management strategies exist to ensure the longevity of frog species in MBR. In order to protect the frog species present and to potentially release endangered frog species in the area, conservation management practices and species monitoring must expand to amphibians as well. However, the MBR appeases many different stakeholders, and opposing interests and threats arise during their conservation efforts.

MBR currently does not run as a typical protected area; although it is overall managed by Guatemala's National Council of Protected Areas (in Spanish: Consejo Nacional de Areas Protegidas, CONAP), the land is a network of many subdivisions that are controlled by twenty-four different units (Rainforest Alliance, 2018). Three typical zones exist: The Core Zone (CZ) which is restricted to conservation, research and low-impact ecotourism, the Multiple-use Zone (MUZ) which is used for exploitation of natural resources, and the Buffer Zone (BZ) which buffers MBR from outside agriculture, forestry, and livestock productions in the nearby area (Selva Maya, 2018). These zones, along with the people and organizations involved in each one, are important to consider when proposing more conservation efforts inside the reserve.

Stakeholders

CONAP, which was created in 1989, oversees MBR (Alarcon, 2018). Led by an Executive Council, CONAP is responsible for forest management at the national level, however it cannot raise its own revenues and it is severely under-resourced (Cuffe, 2016). Because of this, it partners with municipalities, universities, and NGOs (Alaron, 2018) for managing the reserve and the activities that occur in each zone type. The values of CONAP aligns with most conservationists involved in MBR; CONAP limits destructive activity in the forest and aims to protect the wildlife inhabiting the reserve.

Approximately 75% of the citizens of MBR live in the BZ, however this is not reflective of their zone of work or influence (State of the Maya Biosphere Reserve, 2018). Most of the citizens rely on timber production, agricultural products, traditional craft production, and remittances from outside MBR as sources of income (State of the Maya Biosphere Reserve, 2018). For the indigenous population, MBR has provided socio-economic incentives for the local community. However, population is increasing by approximately 7% per year (Campbell, 1999). This boom in population has led to deforestation by immigrants to the area who are unknowledgeable about the importance of conserving MBR and see no harm in consumptively exploiting the forest for economic gain (Campbell, 1999). However, in more recent years, deforestation for land development and cattle has be limited to only the BZ (Cuffe, 2016).

Fourteen of the twenty-four management units in MBR are forestry concessions in the MUZ. The forestry concessions sustainably extract timber, fruit, and nut products from the forest. Although these businesses abide by governmentally-enforced standards set forth by the Forest Stewardship Council (Rainforest Alliance, 2018), consultation with these managers is necessary to monitor the frog species and their habitat in MBR. Most of the involved forestry units are run by locals, who primarily make their living through this timber production, but who also have a strong incentive to protect it (Rainforest Alliance, 2018). The practices implemented by the locals, such as only extracting one tree per acre every few years (Rainforest Alliance, 2018), leads to a deforestation rate of only 0.4 percent annually (Cuffe, 2016). This ensures the stability of their jobs by not fully deforesting MBR.

While the indigenous community recognizes the significance of conserving the massive forest, major battles over the control and management of the land have been ongoing for many years. Some of the most dangerous threats that are currently looming in MBR include the illegal activities done by oil-palm plantation owners, drug traffickers, cattle ranchers, and jaguar poachers (Primack, 1998). These groups are the hardest obstacle to overcome in efforts to conserve the land and to protect frog species.

Some conservationist and local community leaders have expressed their concerns about the expansion of neighboring oil-palm plantations in the MBR (Cuffe, 2016). A few plantations already occur in the BZ, but fears exist about their potential illegal spread into MUZ or CZ. Not only does this effect wildlife and conservation efforts, but the plantations displace indigenous people who rely on the MBR for sustainable jobs (Alonso-Fradejas, 2012). In 2015, the Guatemalan government ordered a suspension of the operations of a particular oil palm company called Reforestadora de Palmas AC (REPSA), however, major oil spills and illegal activity continued in the MBR region (Cuffe, 2016; Conant 2018). This battle between plantation owners and conservationists is an ongoing issue not only in MBR but throughout Guatemala.

Another menacing group in MBR is the illegal cattle ranchers and the drug industry (Elbein, 2016) that they support. The influx of drugs and weapons in MBR and the deforestation between 2006 to 2010 are no coincidence. As a front for the high profits made from producing and distributing drugs, they clear-cut the forest for cattle ranches or palm plantations within MBR. While clear-cutting is technically illegal in MBR, these "nacroranchers" either slip through the cracks of Guatemala's security or bribe government officials to register their business as a legal entity (Allen, 2012; EFE, 2017). Furthermore, these illegal organizations maintain their power in MBR through violent threats and confrontations with park guards (Elbein, 2016). Since 2000, illegal ranchers deforested about 8% of MBR by cutting and burning the native acacia and mahogany trees (Elbein, 2016). This will not only make the goal of conserving frog populations impossible, but it further threatens the existence of the old growth forest as a whole.

Additionally, just south of the reserve, Chinese criminals conduct logging and jaguar poaching for illegal trade in Asia (Allen, 2012). CONAP believes that in the near future these activities will infiltrate MBR, and much like the "nacrorancher" situation, the poachers will take extreme measures to gain control of areas of land (Allen, 2012). This will subsequently cause the endangerment, or extinction, of the jaguar population, and in turn make conservation efforts tremendously difficult to continue or increase.

On the other hand, many people from outside of MBR appreciate the value that it offers. MBR is not only home to a plethora of wildlife species, but also contains Tikal, the largest excavated site in Central America, which contains numerous archaeological remains of the ancient Maya civilization (Tikal Park, 2015). Tikal National Park, established in 1955, attracts archeologist and tourists from all over the world. Archeologists recognize that this area is not only relevant historically, but necessary for wildlife conservation. With new technologies, archeologists are able to discover novel findings about the ruins without excessive excavation. In particular, the Foundation for Maya Cultural and Natural Heritage (in Spanish: Fundacion Patrimonio Cultural y Natural Maya, PACUNAM) has made substantial efforts in archeology while avoiding habitat degradation. PACUNAM is a Guatemalan non-profit organization dedicated to historical preservation and environmental conservation. Recently they utilized laser scanning and mapped over 800 square miles of MBR. This allowed them to discover an extensive network of previously-unknown structures (Liptak, 2018) while having little to no impact on the environment.

MBR has only been opened for ecotourism within the past decade (Hearne & Santos, 2005). Ecotourism not only helps people to become aware of the importance of conservation efforts, but it also brings in needed money to keep the reserve running. While ecotourism supplies financial needs to support the reserve, tourists may inadvertently pollute or harm the reserve. With more than usual traffic through the reserve, the likelihood of damaging effects from anthropogenic impacts increase. Therefore, ecotourism can be both beneficial and harmful for conservation efforts. However, it is essential for the monetary demands of conserving MBR and frog species populations.

Recommendations

With all of the conflicting stakeholders involved in MBR, proposing and implementing a more extensive conservation plan to include frogs would be a challenging task. However, MBR may be the last natural area that could support declining frog populations. To support more intensive conservation practices, the entrance fee for ecotourists could be raised and fines could be given for those who litter or deliberately harm wildlife. With the sufficient funding from ecotourism, management practices focusing on frogs can be implemented. More specifically, conservationists can initially monitor the current frog species that inhabit MBR. By using a software system that was developed to recognize animal vocalizations to the species level (Grigg et al., 1996; Aide et al., 2013), researchers can determine which frog species are present and where in the forest they exist. After a clear understanding of which species inhabit MBR, scientifically sound decisions can be made by CONAP, WCS, and other conservation entities to potentially reintroduce endangered frog species.

However, newly-implemented frog research alone will not guarantee their viability in MBR. The dangers of deforestation by illegal entities still threaten the forest habitat as a whole. In order to eliminate corrupt plantation owners, drug traffickers, cattle ranchers, and jaguar poachers, the Guatemalan government must intervene by enacting stronger laws and enforcement. This includes removing politicians who accept bribes from criminals. Additionally, since approximately 75% of the drugs from Guatemala are transported to the U.S. (Guatemala

Human Rights Commision, n.d.), the U.S. Drug Enforcement Agency (DEA) could intensify their involvement in this issue. The DEA already assists in drug control in Guatemala by giving the International Narcotics Control and Law Enforcement over $40 million since 2004 (Guatemala Human Rights Commision, n.d.). They also currently aide the Guatemalan police officers in raids and arrests of drug traffickers (Latin American Herald Tribune, 2018). By increasing the DEA’s involvement and ridding of corrupt politicians, steps to ensure that MBR stays intact could be easier to achieve. With MBR protected, frog conservation efforts could therefore be obtainable.

In summary, the long list of stakeholders involved in the control and management of MBR make conservation practices complicated to achieve. Funding to support research of frogs and MBR can be acquired from ecotourism. However, significant political changes are required to rid of entities who are overexploiting MBR’s resources. Despite the conflicting views towards the use of MBR, it makes for an ideal safe haven for frog populations to exist without the threats of disease, pet trade, and pesticide use. Financial support, minimal deforestation, and a community desire to protect the reserve can in turn support scientific research and conservation of frog species in MBR.

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