THE IMPACTS OF THE INVASIVE AMERICAN BULLFROG (*Lithobates catesbeianus*) ON THE WOODHOUSE TOAD *(Anaxyrus woodhousii)* POPULATION AT THE RIO MORA NATIONAL WILDLIFE REFUGE IN NORTHEASTERN NEW MEXICO

A THESIS

Presented to the Graduate Division

College of Arts & Sciences

New Mexico Highlands University

In Partial Fulfillment

of the Requirement for the Degree

Master of Science in Biology

By

Alfonso Trujillo

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Alfonso Trujillo

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ABSTRACT

American Bullfrogs (*Lithobates catesbeianus*) are one of the most aggressive invasive species causing ecological damage across many ecosystems. Bullfrogs can negatively impact native amphibians through either predation or competition when they become established in an ecosystem. This invasion leads to the loss of native biodiversity and can eventually drive some species into extinction. Invasive Bullfrogs were established in the Rio Mora National Wildlife Refuge (RMNWR) in Northern New Mexico during the last century. Bullfrogs were eradicated from an “Experimental” 2,600 meter section of the Mora River, creating an area virtually free of Bullfrogs. Another similar length of the river was left intact and referred as the "Control.” This study examines whether Bullfrogs have a negative impact on Woodhouse toads (*Anaxyrus woodhousii*) by using five methods to determine differences between the sites with Bullfrogs and without Bullfrogs: (1) A mark-recapture study to determine total abundance and population structure; (2) Random transects in the study area to estimate relative abundance; (3) Deterministic transects going perpendicular away from the river to evaluate habitat preference, either due to prey preference, vegetation choices, or avoidance of Bullfrogs; (4) Call surveys to define the relative abundance of active calling males; and (5) Tadpole abundance surveys to measure the larvae quantity. My results suggest that there are more Woodhouse toads in the area where Bullfrogs have been eradicated based on the calculated total abundance of Woodhouse toad adults in both sites. I also found more juveniles and tadpoles in our site with the absence of Bullfrogs. Also, I captured fewer female toads along road transects where Bullfrogs were removed, suggesting females may be utilizing the river more because they do not risk encounters with Bullfrogs. Females are larger in the area that has Bullfrogs (mean mass= 84.57g; mean SVL= 7.81 cm) than in the area without Bullfrogs (mean mass= 53.80g; mean SVL= 6.84 cm). This may be the result of increase recruitment on the early sizes where Bullfrogs have been eradicated. Overall, our study demonstrates that Bullfrogs have important impacts on the Woodhouse toad population in the RMNWR by affecting abundance on juveniles and tadpoles and causing the overall population to avoid areas that are heavily populated with Bullfrogs.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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DEDICATION

This thesis is dedicated to my grandparents and my Mima, for their wisdom, emotional support, and humor that they provided me throughout the years. Your wisdom of life lessons has helped me push forward on the days that I felt like giving up when life threw challenges at me. Your emotional support has always been there since I've scraped my knee as a child or encountered complications as an adult by providing me with comfort to continue on with my day. Your humor has taught me how to trigger positive feelings when everything seemed to collapse. Most importantly I would like to dedicate this to the memory of my beautiful grandmother Vidilia Trujillo for raising me to be the person I am today. One day I plan to follow each of your footsteps to carry on your wisdom and love that will be passed on to many generations

CHAPTER I: INTRODUCTION

The introduction of invasive species is one of the most significant factors leading to native species declines (Wilcove et al., 1998). These factors can have impacts on native species diversity by either direct (predation and competition) or indirect interactions (transmission of diseases, habitat changes, or assisting the success of another invader) that contribute to native population declines (White et al., 2006; McGeoch et al., 2010). Therefore, invasive species can have various effects on native species and communities by negatively impacting their behavior, adjusting population dynamics, altering ecosystem resilience functions, or disrupting the biotic community in which native species thrive (Olden et al., 2004; Lockwood et al., 2013).

When invasive species become well established in a new environment their population may increase beyond the carrying capacity, often due to lack of natural predators. Once the invaders have a large population size it gives them an advantage to replace and/or prey upon native species, especially if natives have no adaptations to deal with the intruder (Gurevitch & Padilla, 2004). In particular, the function of an invasive species may be different than that of a native organism's role in a community. When the native species is replaced by the invasive, it may result in substantial changes in ecosystem function within the food web (Gallardo et al., 2015). Most invasive species that become well established within an ecosystem end up competing with native species this may later lead to competitive exclusion (Huxel, 1999). Therefore, one of the competing species would have the advantage driving the other species into extinction or shifting the competitor to a different ecological niche. These ongoing challenges created by invasive species can reduce biodiversity and cause species extinction.

The decline of native biodiversity caused by invasive species reduces ecosystem resilience. This term ecosystem resilience is defined by the ability of an ecosystem to handle some amount of disruption without the system collapsing, or experiencing a type of conversion (Holling, 1973). If an ecosystem is resilient there is a chance that it will maintain structure and function when experiencing a disturbance like an invasion itself (Mitchell et al., 2000). Diverse ecosystems may be able to experience small disturbances and rebound quickly to their original state (Peterson et al., 1998). When an invasive species is introduced into a new environment the diversity may be lowered which in turn can decrease the ecosystem resilience. As invasive numbers increase, the ecosystem becomes more vulnerable to disturbances (O'Brien et al., 2015), native populations can be abolished (Zavaleta et al., 2001), and lead to regime shifts that cause reorganization of ecosystems into an alternate state (Brook et al., 2013). However, removing invasive species from a resilient ecosystem can sometimes help native communities thrive (SERI, 2004).

An increase in global trade and consumer goods has resulted in the accidental transport of invasive species into new habitats where they naturally do not exist (Everett, 2000). In addition to the ecological impact invasive species can produce, there may be economic consequences as well (Born & Rauschmayer, 2005). For instance, invasive Brown Treesnakes (*Boiga irregularis*) produce substantial economic losses in Guam by triggering power outages across the entire island (Fritts et al., 1987). Another example is the invasive pest in Florida, the Asian citrus psylidds (*Diaphorina citri*). This pest is responsible for transmitting citrus green disease to citrus fruits. The Florida citrus industry faces economic risks associated with the spread of this disease as it causes citrus fruits to discolor and taste bitter (Halbert & Manjunath, 2004). Yet another case is the invasion of Zebra mussels (*Dreissena polymorpha*) in the Great Lakes. The mussels have caused problems with water-dependent power generation systems and passages for drinking water treatment services by clogging up pipes and other equipment that affect the water flow pathways (MacIsaac, 1996). In the U.S. alone, invasive species cause around $137 billion per year in economic damages (Pimentel et al., 2000). The destruction caused by invasive species affects power production, agriculture, water passages, international trade, and many other economic circumstances.

The continental US has experienced numerous invasions from different taxa that negatively affect native species' habitats. Among these is the Asian Longhorned Beetle (*Anoplophora glabripennis*), which is native to China and Korea. It was detected in the United States in 1996 (Hu et al., 2009) and is responsible for damaging trees in the eastern part of the U.S. (Townsend & Scachetti-Pereira, 2004). Another example is the Musk Thistle (*Carduus nutans*), which is a noxious weed from Eurasia that was accidentally introduced in North America. This weed spreads throughout wildlife habitat causing a reduction in foraging habitat and destroying soil stability for other plant species (Han, 2012). Aquatic systems are also vulnerable to being colonized by invaders; the Common Carp (*Cyprinus carpio*) is an invasive aquatic species threatening native biodiversity by causing habitat degradation through moving sediments and creating mucky water (Vilizzi, 2012).

Although amphibians face worldwide declines either caused by habitat destruction, climate change, pollution, disease, and pet trade (Stuart et al., 2004), some amphibians can also be successful invaders (Peterson, 2013; Kats & Ferrer, 2003). One example of this is the Cane toad (*Chaunus marinus*), which was introduced in Australia as a biocontrol to feed on beetles affecting sugar cane crops (Mungomery, 1936). Besides just feeding on insects, Cane toads were discovered to cause population declines in native predators due to toxins being released from the toad during predation (Shine, 2010). Overall, this has benefited other species that would be consumed by these predators, but ultimately it changed the dynamics of the food web (Shine, 2010).

Besides Australia, there has also been documentation of invasive amphibians triggering harm in the United States. The American Bullfrog (*Lithobates catesbeianus*) is a native amphibian to eastern North America where their natural range extends from southern Canada to northern Florida, and from the East coast to Central (Northern Peninsula of Michigan, most of Wisconsin, southern Iowa, eastern areas of Nebraska and Kansas) and Southern (Eastern Oklahoma and Texas) United States (Bury & Whelan 1984; Gherardi, 2007). However, they are a known invader throughout the world, posing a major threat to indigenous amphibians (Kraus, 2009). In the early 1900s these frogs were introduced into California and other western states mainly for game, where they continue to flourish today (Boersma et al., 2006). In New Mexico, Bullfrogs were also documented at Carlsbad Caverns National Park in 1959 (Krupa, 2002). Bullfrogs are aggressive invaders because they occupy a large variety of habitats, such as lakes, marshes, rivers, and ponds allowing for a selection of a wide variety of prey (Babbitt et al., 2003). In addition, Bullfrogs can be resistant (or tolerant) to the most common amphibian diseases Chytrid fungus and ranavirus (Daszak et al., 2004), which are responsible for amphibian declines across the planet. Since Bullfrogs have been established on almost every continent they are known to be one of the top invasive species contributing to ecological damage (Lowe et al., 2000; Louette et al., 2014).

Invasive Bullfrogs have direct impacts on native species through competition and predation (Kats & Ferrer, 2003). Bullfrogs feed on a wide range of prey such as invertebrates, fish, reptiles, birds and small mammals (Bury & Whelan, 1984). In addition to these taxa, adult Bullfrogs have been documented to consume other frog species including Asiatic toads (*Bufo gargarizans*) in China and Spotted Frogs (*Rana pretiosa*) in the United States (Wu et al., 2005; Pearl et al., 2004). They are also known to prey on diverse age groups of frog species contributing to several declines in native frog populations (Doubledee et al., 2003; Kats & Ferrer, 2003; Boone et al., 2004; Pearl et al., 2004). Adult Bullfrogs are not the only problem; their tadpoles are also generalists that consume eggs or progeny of many species including other frogs (Snow & Witmer, 2010). In the Bullfrogs native range, the tadpoles utilize permanent waters over winter for at least one and up to three winters before they begin the process of metamorphosis into froglets (Cook et al., 2013). Bullfrog larvae are much larger than most other amphibian larvae so they easily outnumber others competing for resources (Lawler et al., 1999). Kupferberg (1997) discovered that competition of Bullfrog larvae reduces the survivorship and growth of both Pacific Treefrogs (*Pseudacris regilla*) and Yellow Legged Frogs (*Lithobates boylii*) larvae in northern California. When invasive Bullfrog populations increase, they can lower the survival of native species via competition. Some amphibians, such as Red Legged Frogs (*Lithobates draytonii*) cope with the presence of Bullfrogs by moving to different, suboptimal habitats where Bullfrogs are not commonly found. However, the Red Legged Frogs survived but have changed their habitat use, which likely lowers their survival (D'Amore et al., 2009). Ultimately, the impact of Bullfrogs on native species can range from competitive exclusion, predation, transmitting diseases, and forcing them into suboptimal habitats.

Bullfrogs are also known to spread diseases that contribute to population declines and extinctions (Daszak et al., 2003; Young et al., 2004; Schloegel et al., 2010). One of the pathogens that the Bullfrogs spread is the chytrid fungus *(Batrachochytrium dendrobatidis)*, which is an infectious disease responsible for large declines in amphibians across the world (Berger et al., 1998). There is concern about the Bullfrogs' resistance against this disease (Daszak et al., 2004), because they may act as vectors to other frog species (Bai et al., 2010). Garner et al. (2006) collected tissue samples from invasive Bullfrogs in four different continents and used polymerase chain reactions (PCR) and microscopic techniques. They determined that Bullfrogs were responsible for Chytrid infection in their respective localities. Since Bullfrogs are relatively resistant to Chytrid they are very efficient carriers of the disease. As Bullfrogs are introduced to new habitat this causes a problem by spreading the disease to other amphibian populations.

In a prior study, stomach contents of Bullfrogs were analyzed, showing the presence of a juvenile Woodhouse toad *(Anaxyrus woodhousii*) suggesting Bullfrogs may exert top-down control on these toads. Woodhouse toads are endemic species to the United States and parts of Mexico (The United States Geological Survey, 2013), and can be found in prairies, grasslands, and woods that contain some source of permanent water (Ballinger et al., 2010). The native distribution of Woodhouse toads in the United States includes Central (Southern North Dakota; B: Majority of South Dakota, Nebraska, and Kansas), Southern (Majority of Oklahoma and central Texas), and Western (Majority of New Mexico and Utah; B: Eastern Arizona, Colorado, Wyoming, Nevada, and Montana; C: Isolated populations in Washington, Oregon, Idaho, and California) states (Lannoo, 2005). Woodhouse toads overlap in the native range of Bullfrogs in Texas, Oklahoma, Kansas, Nebraska, and Missouri. Since parts of the Bullfrogs and Woodhouse toad native ranges overlap in some states this has allowed for coadaptations. However, Woodhouse toad populations found outside of the Bullfrog native range may not have the adaptations to live amongst Bullfrogs. The breeding sites for Woodhouse toads include a range of habitats such as swamps, ponds, and riverbanks (Bragg, 1941) causing breeding site competition with Bullfrogs. Stebbins (1951) discovered the breeding of Woodhouse toads takes place around February to September but timing also reveals that it depends on the type of environment (e.g. populations that live in the desert breed as soon as it rains and others that live in the plains breed between June to September). In New Mexico, male Woodhouse toads call to attract mates around May through June. However, in the Rio Mora Wildlife Refuge, located in northeastern New Mexico, the author has heard Woodhouse toads calling as early as mid–March.

The purpose of this project was to examine whether Bullfrogs play a crucial role in Woodhouse toad abundance, demographic structure, and distribution. Woodhouse toad populations found with the presence of Bullfrogs were compared to a site with the absence of Bullfrogs. I posed the following question: How do invasive Bullfrogs impact Woodhouse toad populations? I hypothesize that the Bullfrog has a negative impact on Woodhouse toad population abundance and demographics. If this hypothesis is correct, I will see a higher density of toads in the sections of the river without Bullfrogs as opposed to the site with Bullfrogs. The two main goals of this project were to evaluate the Woodhouse toads: 1) population structure and abundance, and 2) habitat use and preference. In order to determine differences in Woodhouse toad abundance between sites with and without Bullfrogs I used five methods: 1) A mark-recapture study to determine population size; 2) Distance sampling using random transects to estimate relative abundance; 3) Deterministic transects to evaluate sex ratio habitat preference, either due to prey preference of vegetation choices, or avoidance of Bullfrogs more abundant in the river; 4) Call surveys to determine the relative abundance of active calling males; and 5) Tadpole abundance surveys to measure the larvae quantity.

CHAPTER II: METHODS

Table 1: List of methods conducted at different times of the year in 2014 and 2015.

Methods	Date
Call Surveys	May 2014 & May-June 2015
Random Transects	May-August 2014 & 2015
Tadpole Surveys	July-August 2015
Mark-recapture & Population Structure	May-September 2014 & 2015
Woodhouse toad Distribution &Bullfrog Avoidance	May-August 2015
Insect Abundance	September & October 2015
Vegetation Analysis	October 2015

Study Site

My study was conducted at the Rio Mora National Wildlife Refuge in Watrous, New Mexico (35°50'30.77"N, 105° 4'1.67"W). The major habitat within the refuge consists of short-grass prairies, pinon-juniper, and ponderosa pine forests. Moreover, the habitat along the river contains willows and cottonwood trees. In 2012 a long-term study began on the refuge to determine the impact of the Bullfrogs (an invasive species) on native species diversity. The Mora River runs through the refuge where it was divided into two 2.6 Km sections for the project. In one section of the river, the experimental site, Bullfrogs had been eradicated. The control zone was a comparable zone upstream where there was no manipulation in Bullfrog abundance. There was no barrier preventing Bullfrogs from re-colonizing the site without Bullfrogs, so they were continually being removed to maintain the area free of Bullfrogs. Between both sections, a segment of approximately 1.4 Km was left as a buffer zone between both treatments (Figure 1). This system allowed us to study the impact of Bullfrogs on the Woodhouse toad population by comparing its abundance and population demographic structure with Bullfrogs and without Bullfrogs. Both sections of the river were divided into 200-meter stretches for sampling and practical purposes.

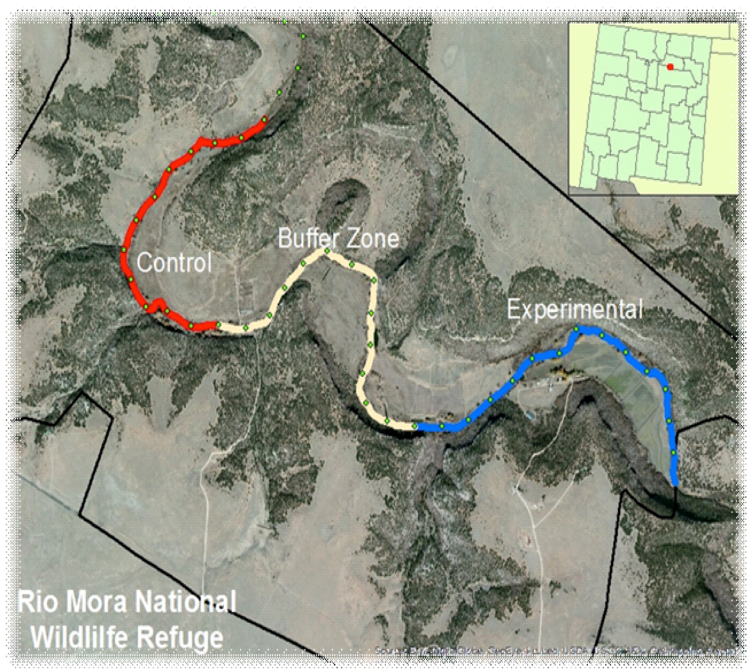

Figure 1: Aerial view of the Mora River at the Rio Mora National Wildlife Refuge in Northeastern New Mexico. The section of the river in red represents the presence of bullfrogs, blue is the area where bullfrog density is lowered, and yellow serves as a buffer zone between both sites.

Population Abundance and Structure

I studied the population of Woodhouse toads in both sites by conducting call surveys, relative abundance in random transect in the riparian area, trapping tadpoles within ponds, and mark & recapture. Before each data collection event I used a Kestrel (2500 Pocket Weather Tracker) to record wind speed (mph) and air temperature (^{o}C). I also used a thermometer to collect water temperature in areas where I conducted call surveys and tadpole surveys. When toads were captured for population abundance and structure I used a GPS (Garmin GPSmap 60CSx) to mark the location of all individuals.

Call Surveys (Male Relative Abundance)

Prior to my time of study, I previously heard Woodhouse toads calling in northern New Mexico around mid-March and during the monsoon season in July. Call surveys were conducted during May 2014 and May-June 2015 (Table 1). All of my technicians were trained to recognize Woodhouse toad calls prior to the surveys. I surveyed at nights approximately 20 minutes after sun down and no later than 1:00 am; unless the air temperature dropped below 2^{o} C (Fellers & Freel, 1995). The survey was canceled when temperature dropped because the activities of amphibians decline at lower temperatures (Olson et al., 1986). Upon arrival to the survey site I waited 5 minutes before counting calls at 10-minutes intervals (Zimmerman, 1991). I also documented the moon phase and weather condition during each survey. Furthermore, I analyzed the habitat type (e.g. pools and runs) at the sections of the river where I conducted the call surveys. For each section I observed 50 meters to the left and right of the river to categorize the habitat.

Relative Abundance Using Random Transects

I used the program ArcGIS to randomly select 10 parallel 100 meter transects within each site (Figure 2). Every transect was then divided into 10 meter stretches to better assess its width. Thus, the width of the subsection was determined by the visibility of the toad in every subsection of the transect. I walked along each transect at night side by side at a steady pace (Table 1). Two of us walked on each side of the transects through each 10 meter stretch until I reached 100 meters to record the total number of individual toads encountered (Jaeger, 1994; Yiming et al., 2011). When an individual was found within the transect, I measured the location in the relation to the distance of the starting point of the transect (Shirk et al., 2014).

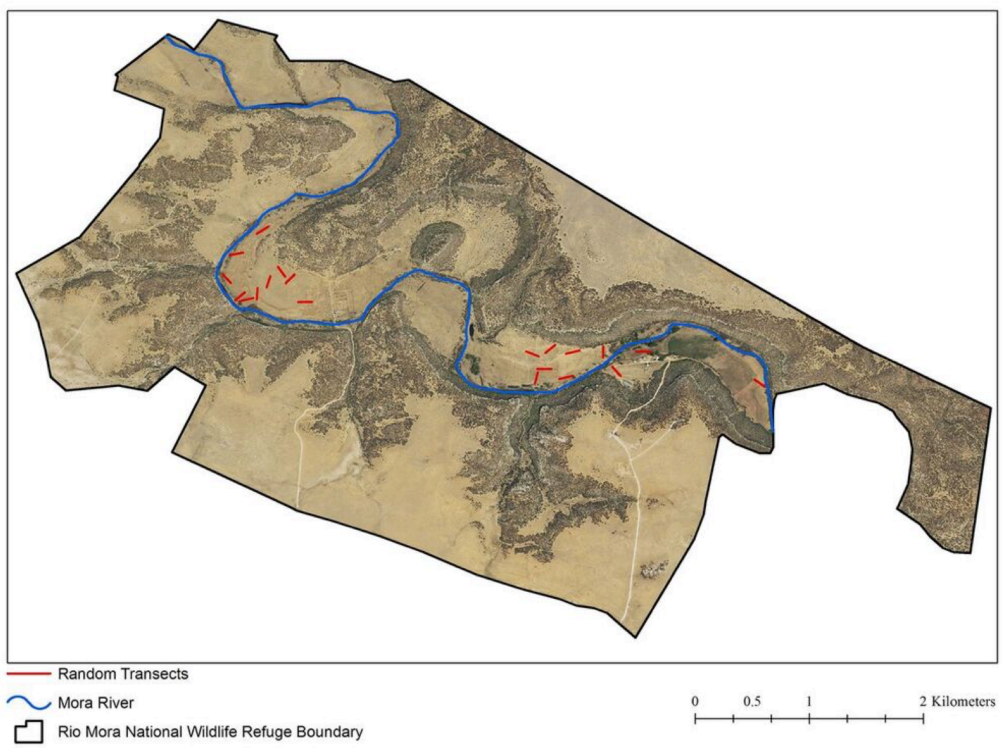

Figure 2: Rio Mora National Wildlife Refuge illustrating random transects in red to determine relative abundance of toads.

Tadpole Abundance Surveys

Based on Woodhouse toad populations in New Mexico, Gehlbach (1965) documented the amount of precipitation in the spring and summer allows these toads to have biannual breeding systems. Green (2005) discovered that American toad (*Anaxyrus americanus*) eggs hatch within 3–12 days. Therefore, Woodhouse toad eggs may have a similar time period for hatching. The large amounts of rainfall in July 2015 altered the breeding grounds of the Woodhouse toads, driving them to move out of the Mora River and into ponds. When the mating season began I waited a few days to allow eggs to hatch into tadpoles.

One-funnel traps were created with 2 litter plastic bottles. I assigned one pond in both sites with Bullfrogs and without Bullfrogs. Ten bottles were placed 1 meter from the water edge and 10 meters apart. The traps were set out for 4 hours to increase my tadpole capture rates. During each visit, I opened the traps and measured the tadpoles to classify them into four different development stages. I determined the different tadpole stages of each individual by measuring the total length (TL) and observing morphological features. After the information was collected the different stages were classified according to Gosner (1960) with the following Stages; Stage 1 the tadpoles are hatchlings, Stage 2 the tadpoles have no sign of limbs, Stage 3 tadpoles have the presence of hind limbs and absence of forelimbs, and Stage 4 tadpoles contain fully developed hind and fore limbs. I counted the different stages of tadpoles caught per trap twice a week until tadpoles were no longer present (Table 1).

Mark-Recapture and Population Structure

I did a mark-recapture study along dirt roads that run parallel with the river (Figure 3). Woodhouse toads were collected for 58 nights between the summers of 2014 and 2015. I gathered population dynamic information of toads by measuring snout urostyle length (SUL) and left hind leg (LHL) to the closest centimeter (cm). I also weighed toads in grams using spring scales (Pesola Light-Line Spring Scale). In order to assist adult male toads I sexed individuals by either encouraging vocalization or looking for the presence of dark throats (Scribner et al., 2001). I documented toads with a SUL less than 5.5 cm as juveniles and anything above were logged as adults (Olson et al., 1986; Carey et al., 2005). I gathered GPS coordinates of the exact locations toads were caught.

Furthermore, I placed ten cordless solar lights 50 meters apart in each site to collect a different dataset for mark-recapture within the prairie.

Every Woodhouse toad has its own unique pattern on their back allowing us to distinguish individuals. Each toad was placed in the center of a white background and photographed multiple times with a digital camera to obtain the pattern. I released all animals in the place of capture after processing. I selected photos with the clearest patterns from each individual toad and the image was then modified to standardize the placement of snout, anus and width of the toads for photo identification purposes. These photos were then uploaded into the program Wild-ID (Version 1.0), to automatically identify recaptures (Bolger et al., 2011). All new photos were imported into the program and given a percentage representing how similar the new photo is to a pervious individual that I photographed. This is determined by achieving a value that compares patterns; the higher the percentage this means the pattern seems similar. Each photo was compared and a capture history for each individual was saved into a databank by confirming photo recognitions. I used the capture history of each toad to create a matrix of captures and the Schnabel method was applied to determine the population size.

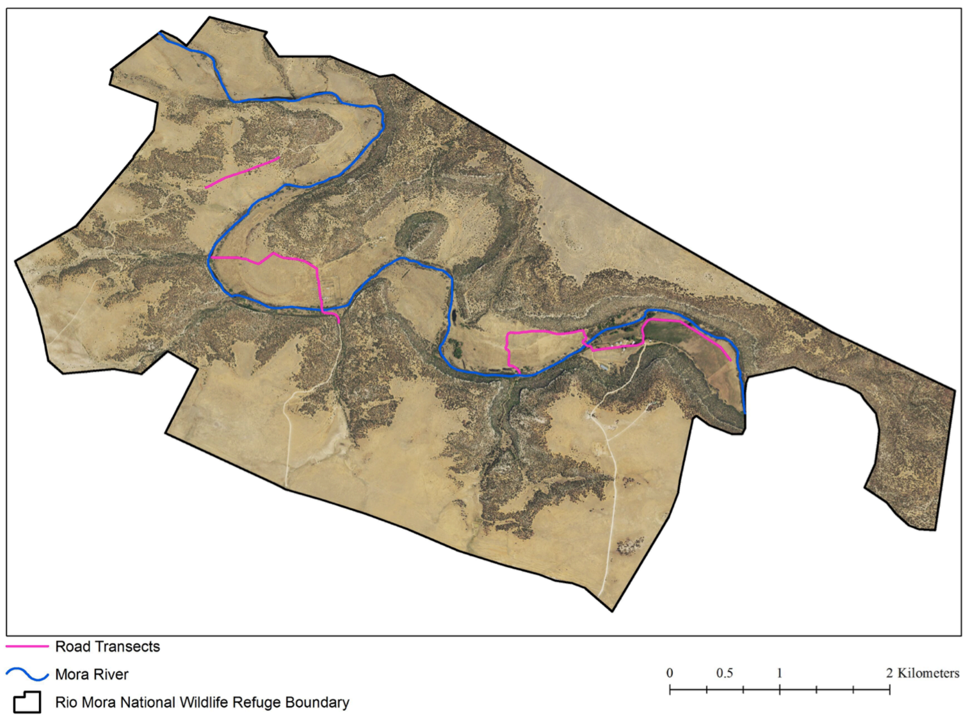

Figure 3: Rio Mora National Wildlife Refuge depicting the road transects in pink used for mark-recapture and population structure of Woodhouse toads.

Habitat Use and Preferences

Abundance of Bullfrogs and Woodhouse Toads within Deterministic Transects Perpendicular to the River

I randomly assigned 100 meter transects that run perpendicular to the river and were separated by 50 meters. The starting point of the transect begin at the rivers flood plain and extended into the prairie. Thus, transects started near the river, passed by the riparian area and ended up in the river's flood plain (Figure 4). I scored these transects the same way I recorded the random ones above. I counted and sexed the Woodhouse toads found within the transects to determine the distribution of Woodhouse toads away from the river. Additionally, I also counted Bullfrogs and Woodhouse toads found at the first 10 meters of each transect to evaluate Bullfrog avoidance.

Toad Prey Availability (Invertebrate Abundance)

I sampled invertebrates in September and October 2015 in the same transects that Woodhouse toad sex ratios were examined (Figure 4 & Table 1). I collected insects by sweep netting between 10 meter section of each transect to observe prey availability (Spungis, 2002). I used this information to identify if there may be a preference in prey abundance away from the river to justify toad distribution. All new species of insects were euthanized and identified at the family level (Eaton & Kaufman, 2007). I counted the number of each insect family found in each subsection of the deterministic transects.

Insect Vegetation Choice Analysis

Once again, I used the same transects to identify percent vegetation cover in one fall season of October 2015 (Figure 4 & Table 1). I used the line point intercept method (Bonham, 1989) to quantify if there was a change in percent cover of vegetation from 10m to 100m. At each 10m section, I dropped a pinpoint every 1m and recorded if it landed on either bare ground or vegetation. I documented the total frequency the pinpoint landed on vegetation to estimate the percent vegetation cover.

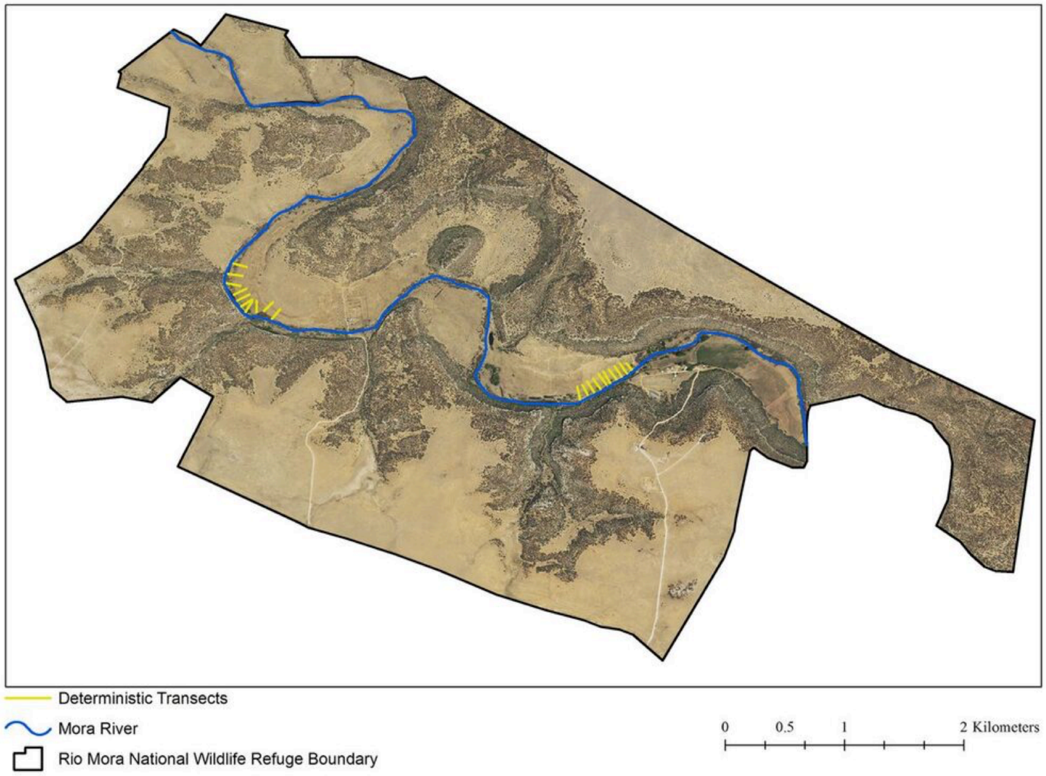

Figure 4: Rio Mora National Wildlife Refuge illustrating deterministic transects in yellow to determine Woodhouse toad sex distribution, Woodhouse toad avoidance of Bullfrogs, prey availability, and insect vegetation choice in percent cover.

Statistical Analysis

All statistical analyses were conducted in a spreadsheet (Microsoft Excel). I calculated the differences in Woodhouse toad abundance and population structure between both sites with an unequal variance T-test. I also used an unequal variance T-test to determine if there was a difference in the average number of calling male toads in pools and runs with the presence and absence of Bullfrogs. The average number of individuals encountered in the subsections of the deterministic transects were compared with using a chi-square to examine the sex ratio and the distance they were located. I also used a chi square test to determine if there was a significant association between the habitat utilization and the total number of males, females, and juveniles within the deterministic transects. I used a single linear regression to examine the relationship between distance from the river to the prairie with insect abundance and percent vegetation cover. Additionally, I conducted another linear regression to identify if there was a relationship with insect abundance and percent vegetation cover.

CHAPTER III: RESULTS

Call Surveys (Male Relative Abundance):

In the mating season, I detected male toads calling during the months of May and June. I found no significant difference between the average number of Woodhouse toad calls per sampling period of 10 minutes without Bullfrogs (9.29) and with Bullfrogs (11.43) (T-test: t-stat =0.47, df=12, p-value=0.65, Figure 5). In addition, I found no significant difference in the average number of male toads calling in pools (T-test: t-stat= 0.95, df=7, p-value=0.38) and runs (T-test: t-stat= 0.27, df=7, p-value=0.79) with the presence and absence of Bullfrogs (Figure 6).

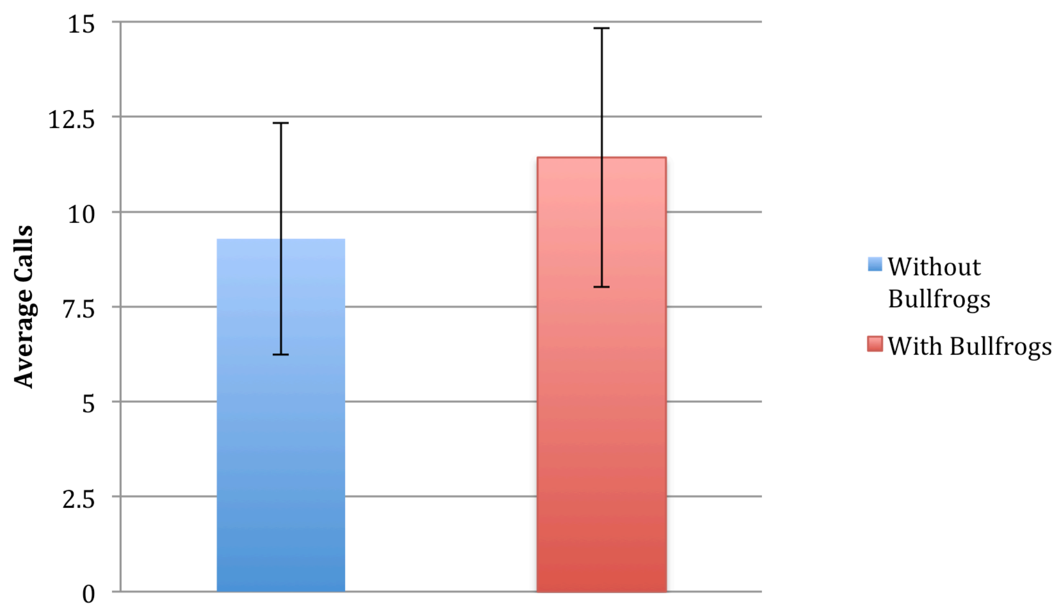

Figure 5: The average number of calls from male Woodhouse toads within river transects without Bullfrogs (blue) and with Bullfrogs (red) sites. T-test: t-stat =0.47, df=12, p-value=0.65.

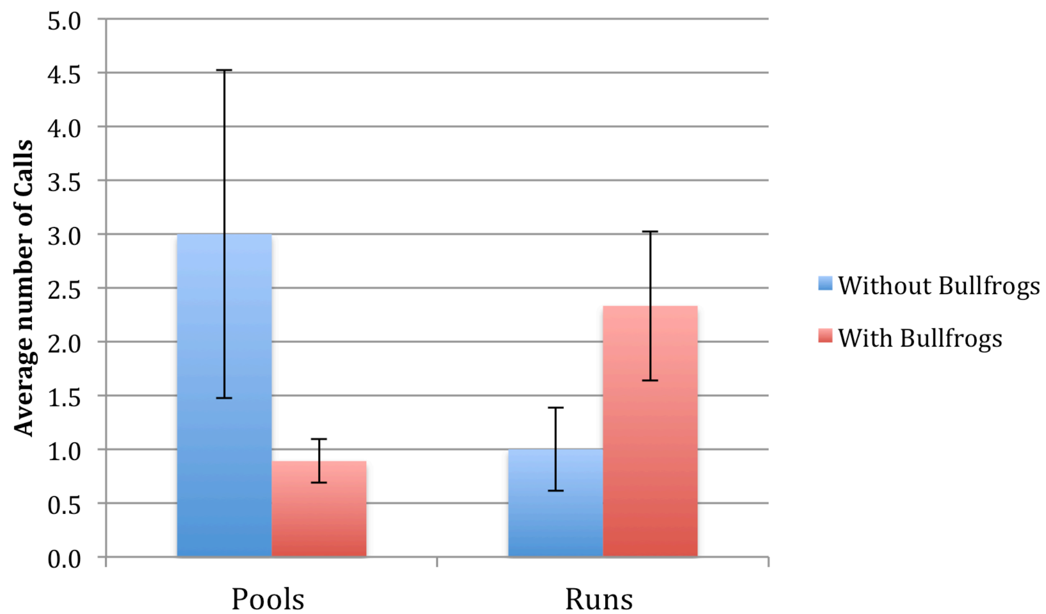

Figure 6: The average number of male Woodhouse toad calls within different breeding habitats with Bullfrogs (Red) and without Bullfrogs (Blue) (Pools T-test: t-stat= 0.95, df=7, p-value=0.38) and (Runs T-test: t-stat= 0.27, df=7, p-value=0.79).

Relative Abundance Using Random Transects:

Woodhouse toads were detected in 8 out of 10 random transects in each one of the sites. I found a significant difference in the average number of toads within the transects without Bullfrogs (1.5 toads) and with Bullfrogs (3.2 toads, T-test: t-stat= -3.29, df=9, p-value=0.01; Figure 7). I further identified the relative abundance between 2014 (n= 3 with the absence of Bullfrogs & n=17 with the presence of Bullfrogs) and 2015 (n= 11 with the absence of Bullfrogs & n=15 with the presence of Bullfrogs; Figure 8). The relative abundance in 2014 at both sites showed a significant difference due to toads utilizing Bison wallows (T-test: t-stat= -2.81, df=9, p-value=0.02), but in 2015 the Bison wallows were overgrown with vegetation from high rains and I saw no significant difference in the abundance of toads between both sites (T-test: t-stat= -0.88, df=9, p-value=0.4).

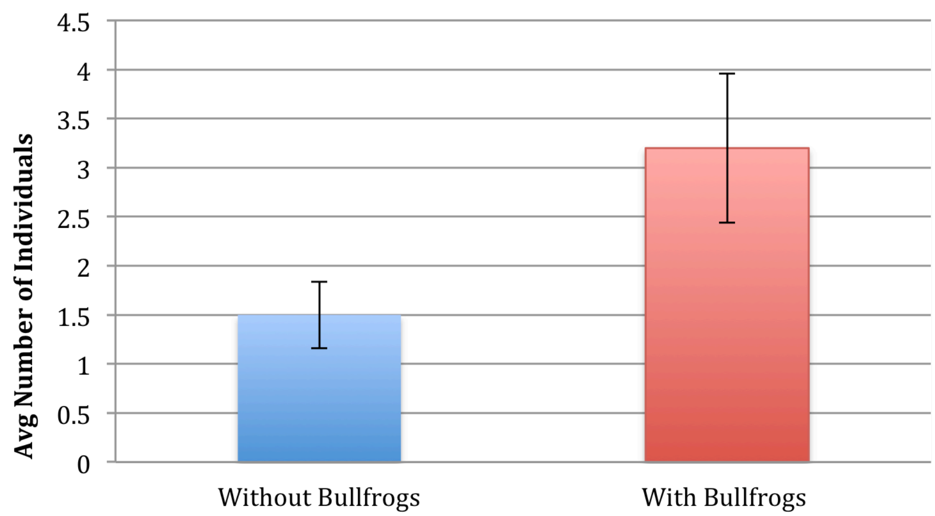

Figure 7: The total number of Woodhouse toads found within random transects without Bullfrogs (Blue) and with Bullfrogs (Red). T-test: t-stat= -3.29, df=9, p-value=0.01.

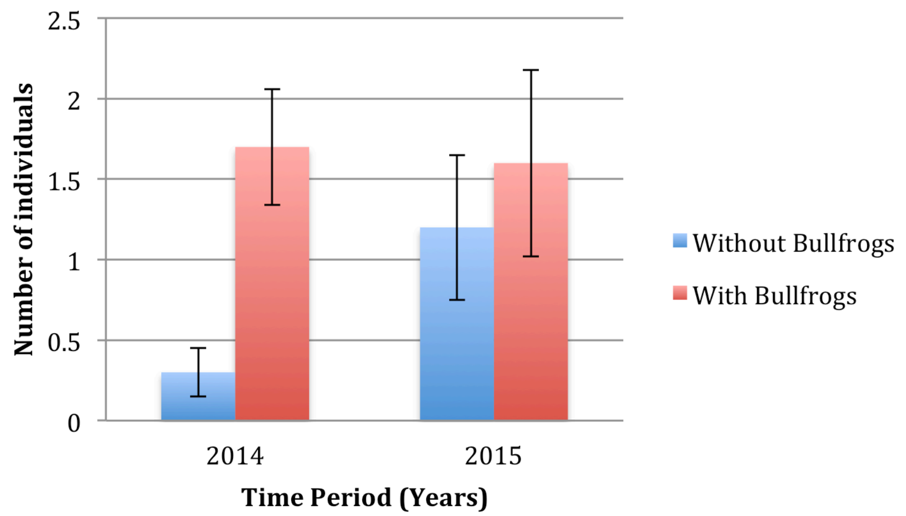

Figure 8: The number of individual Woodhouse toads found within the random transects in 2014-2015 without Bullfrogs (Blue) and with Bullfrogs (Red). 2014 T-test: t-stat= -2.81, df=9, p-value=0.02 and 2015 T-test: t-stat= -0.88, df=9, p-value=0.4.

Tadpole Abundance Surveys:

Between the two breeding ponds, Woodhouse toad tadpoles were significantly more abundant with the absence of Bullfrogs (77.1) than with the presence of Bullfrogs (20.9; T-test: t-stat =3.13, df=18, p-value=0.006; Figure 9). Where Bullfrogs are being removed, I observed the lower stages to be more abundant than the higher stages; whereas with the presence of Bullfrogs the highest abundance of tadpoles was observed in the stage two phase (Figure 10). The life cycles of tadpoles also differed significantly between both sites (Chi-square: x^2= 45.16, df=3, and p-value=0). Tadpoles seem to have survivorship curve type II when Bullfrogs are absent but a survivorship curve type III when they are present.

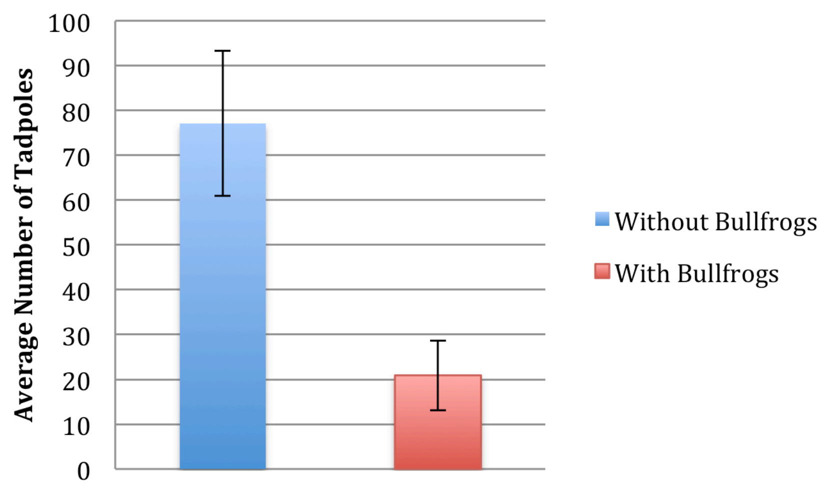

Figure 9: The total number of Woodhouse toad larvae caught in one-funnel traps without Bullfrogs (Blue) and with Bullfrogs (Red). T-test =3.13, df=18, p-value=0.006.

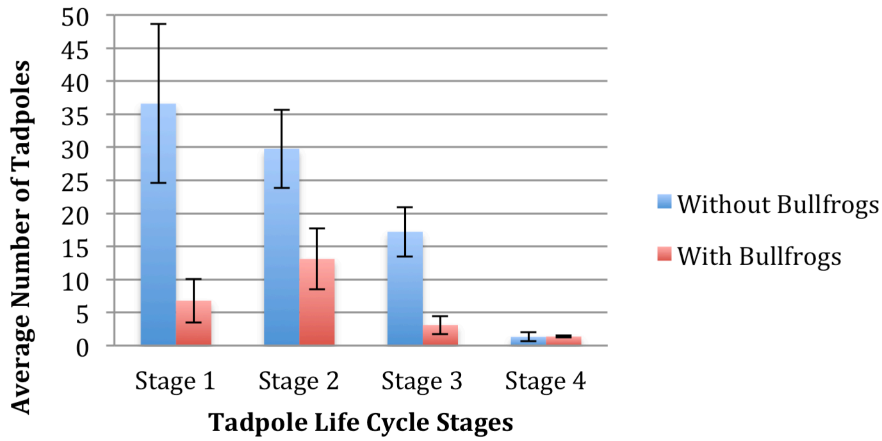

Figure 10: The average number of different life stages of Woodhouse toad tadpoles captured without Bullfrogs (Blue) and with Bullfrogs (Red). Chi-square: x^2= 45.16, df=3, and p-value=0.

Mark-Recapture and Population Structure:

I photographed 369 individual Woodhouse toads in 2014 and 2015 (213 females, 57 males, and 99 juveniles) on my road surveys. I captured 181 toads (78 females, 32 males, and 71 juveniles) in the area without Bullfrogs and 188 with Bullfrogs (135 females, 25 males, and 28 juveniles) (Figure 11 & 12). The relative abundance of Woodhouse toads with the absence of Bullfrogs (4.64 toads per day) does not differ from the mean abundance of toads with the presence of Bullfrogs (4.82 toads per day) (T-test =0.16, df=76, p-value=0.44). Furthermore, there was a significant difference in the amount of females and juveniles caught per day between both sites (T-test: t-stat=1.94, df=39, p-value=0.03 for females; t-stat=3.45, df=40, p-value=0.001 for juveniles). However, it was revealed that there was no significant difference in the average males caught per day with and without Bullfrogs (T-test: t-stat=0.44, df=39, p-value=0.33).

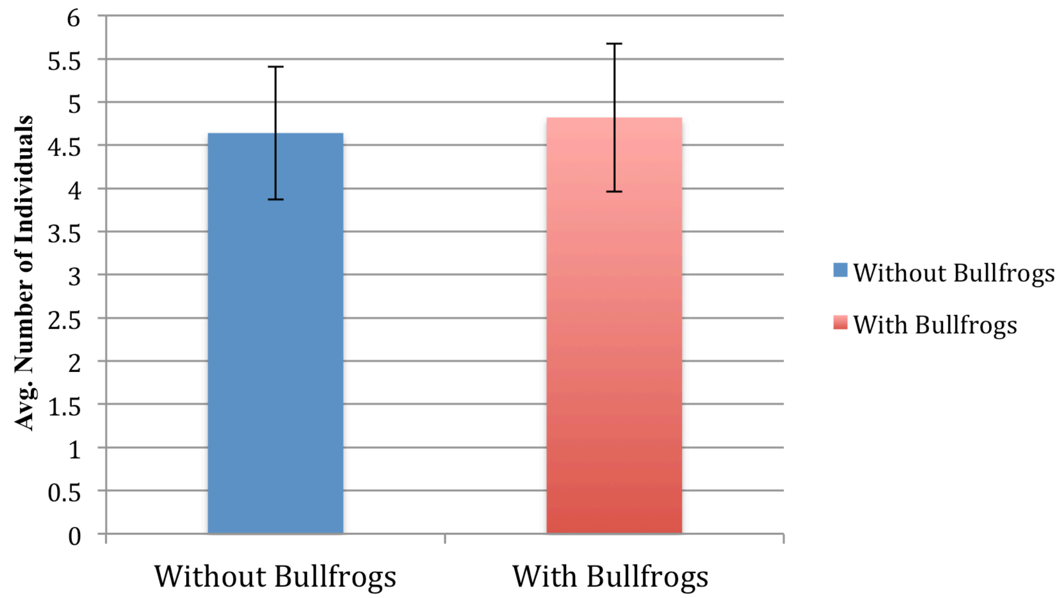

Figure 11: The overall number of individual Woodhouse toads caught within the sites without Bullfrogs (Blue) and with Bullfrogs (Red). T-test =0.16, df=76, p-value=0.44.

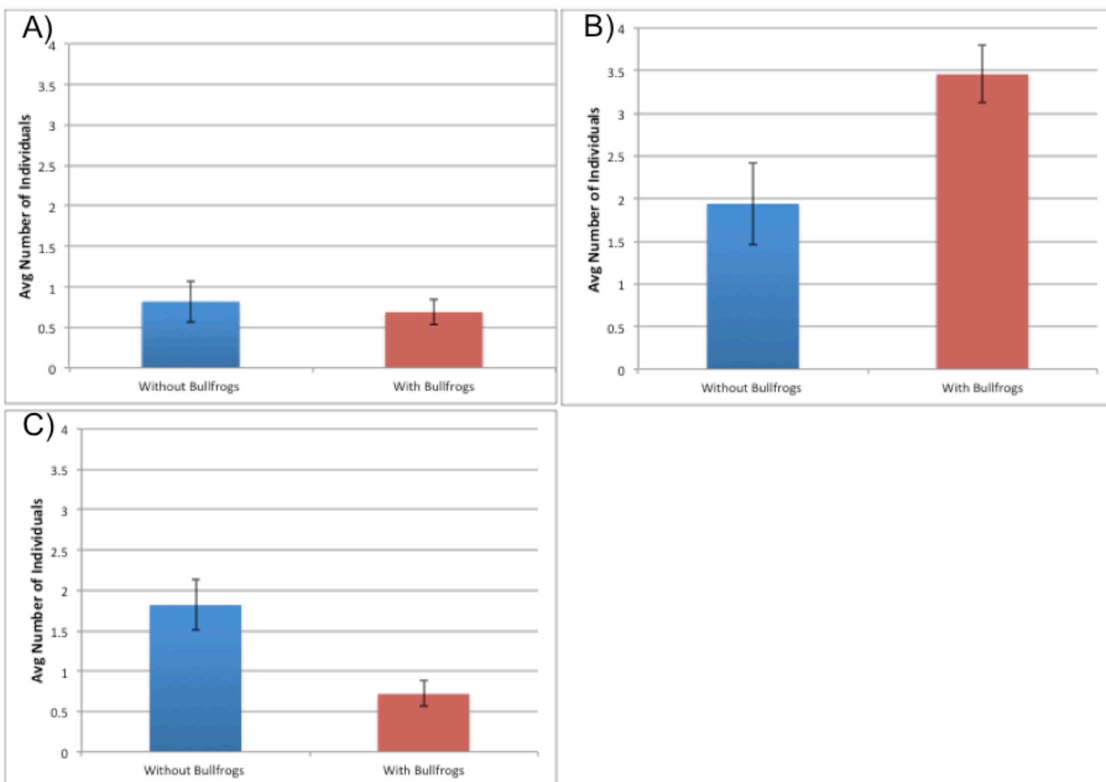

Figure 12: The total number of A) male, B) female, and C) juvenile Woodhouse toads caught in sites without Bullfrogs (Blue) and with Bullfrogs (Red). T-test: t-stat=0.44, df=39, p-value=0.33 for males; t-stat=1.94, df=39, p-value=0.03 for females; t-stat=3.45, df=40, p-value=0.001 for juveniles.

I was able to identify 29 unique individuals (18 females, 8 males, and 3 juveniles) using Wild ID. Woodhouse toad recapture rates between both sites were 9.94% without Bullfrogs and 5.85% with Bullfrogs. The recapture rate of toads caught multiple times with the absence of Bullfrogs include 3.86% males, 4.42% females, 1.66% juveniles, whereas with the presence of Bullfrogs the recapture rates were 0.53% males, 5.32% females, and 0% juveniles. One individual female (AW177) was caught a total of 12 nights in the site with Bullfrogs. AW177 was found with another female (AW288) for a total of five nights. There was no apparent reason for this association between these individuals, except that they were always found in the same physical location.

The Schnabel method estimates of Woodhouse toad population size displayed a dramatic difference between both study sites. The area where Bullfrogs are being removed had a Woodhouse toad population estimate of 820 and 700.4 in the area with Bullfrogs. As for my light points, I did not have a successful mark-recapture rate due to only one toad being captured. There were Woodhouse toad feces located at two light points in the control, which suggests that the lights had some activity.

I did not find any difference in male toad size with absence of Bullfrogs (7.59 cm; range= 5.6-8.9 cm; n=32; t-test =0.15, df=53, p-value=0.44) and presence of Bullfrogs (7.58 cm; range= 6.2-9.4 cm; n= 25; t-test =0.15, df=53, p-value=0.44). However, without Bullfrogs I found female toads to be significantly smaller (6.84 cm SVL; range = 5.6-10.8; n=78) than females that coexist with Bullfrogs (7.81 cm SVL; range =4.2-11.6; n=135; t-stat=4.03, df=206, p-value=0.00).

Overall, I found more Woodhouse toads with a smaller population structure with the absence of Bullfrogs compared with fewer individuals but larger toads in the site with the presence of Bullfrogs (Figures 13,14, 15, 16 ; Table 2 & 3). The site where Bullfrogs are removed I captured 31 males with an average of 65.78g (range 25.5-105g) and I caught 25 males with an average of 68.56g (range 38.5-100.5g) with the presence of Bullfrogs. The females without Bullfrogs averaged 53.80g (range 16.5-190g, n=78) and the average mass with Bullfrogs was 84.57g (range 17.5-261g, n=135). The mass of males between the sites without Bullfrogs and with Bullfrogs was not significantly different (T-test: t-stat=0.57, df=55, p-value=0.28 for males). The t-test for females revealed that toads with the presence of Bullfrogs (68.56g at average) were larger than toads with absence of Bullfrogs (53.80g at average) (T-test: t-stat=3.63, df=206, p-value=0.00 for females).

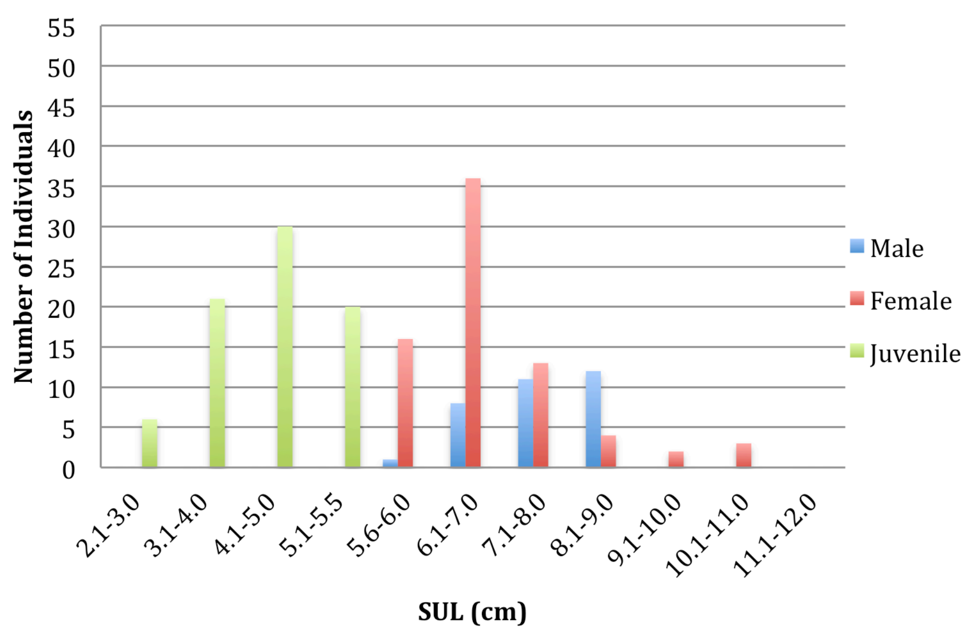

Figure 13: The overall body size (SUL) in centimeters of males (Blue), females (Red), and juveniles (Green) collected from the area without Bullfrogs. Males: t-test =0.15, df=53, p-value=0.44 and Females: t-test: t-stat=4.03, df=206, p-value=0.00.

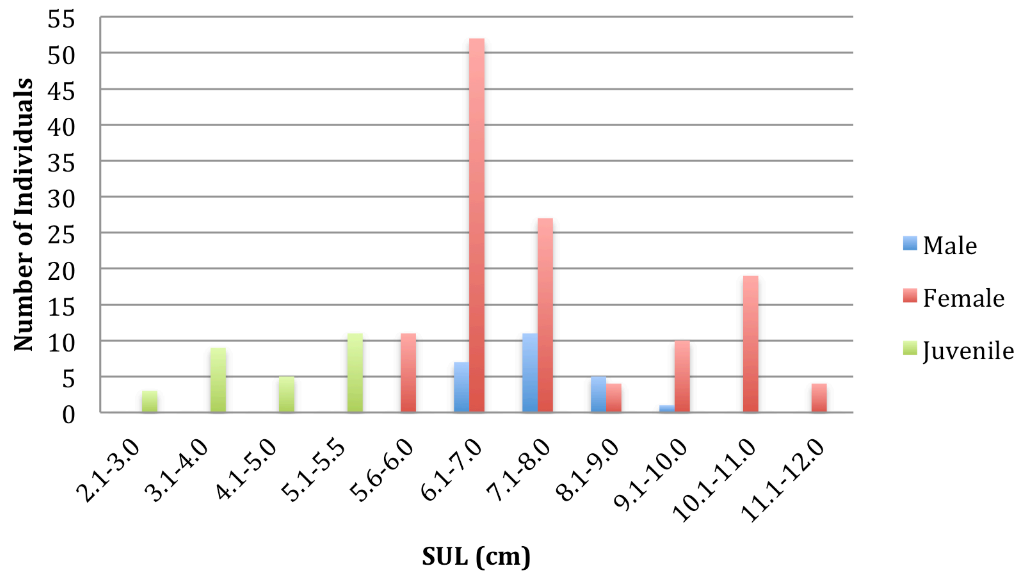

Figure 14: The overall body size (SUL) in centimeters of males (Blue), females (Red), and juveniles (Green) collected from the area with Bullfrogs. Males: t-test =0.15, df=53, p-value=0.44 and Females: t-test: t-stat=4.03, df=206, p-value=0.00.

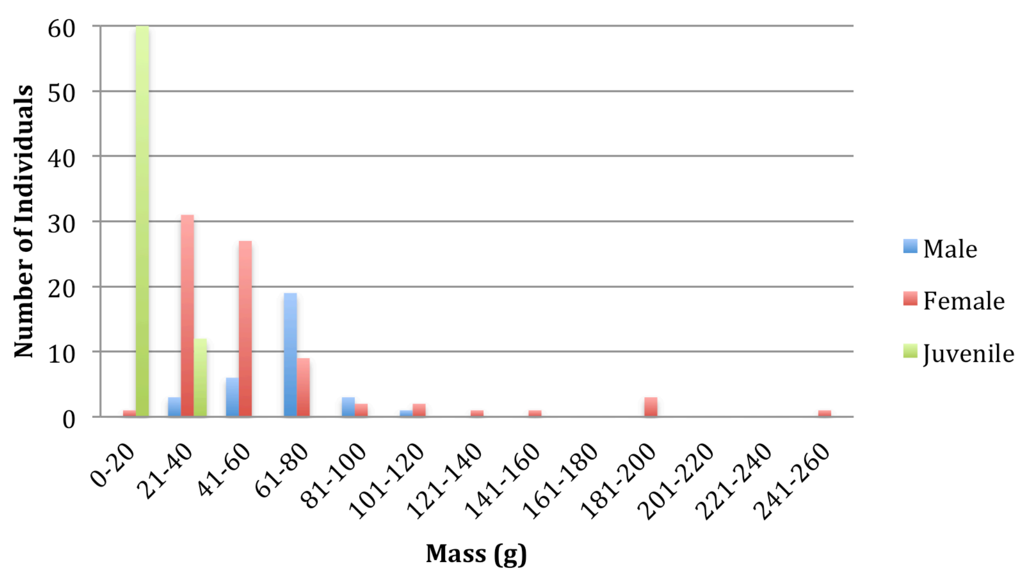

Figure 15: The overall mass of male (Blue), female (Red), and juvenile (Green) Woodhouse toads collected in site without Bullfrogs. T-test: t-stat=0.57, df=55, p-value=0.28 for males. T-test: t-stat=3.63, df=206, p-value=0.00 for females.

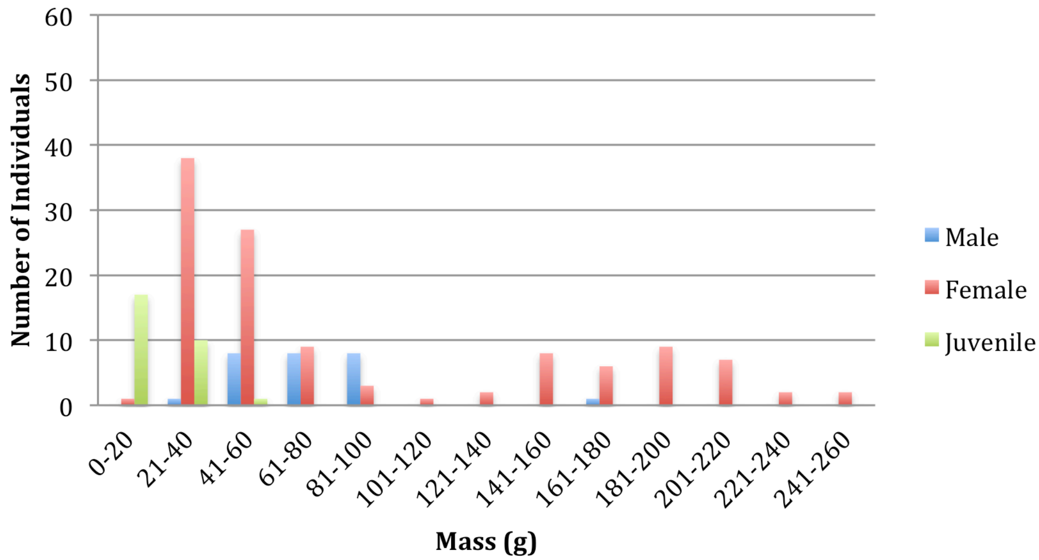

Figure 16: The overall mass of male (Blue), female (Red), and juvenile (Green) Woodhouse toads collected in the site with Bullfrogs. T-test: t-stat=0.57, df=55, p-value=0.28 for males. T-test: t-stat=3.63, df=206, p-value=0.00 for females.

Table 2: Population structure of the number of captured males, females, and juveniles in both sites for SUL range and average SUL.

Sex	Without Bullfrogs (Captures)	With Bullfrogs (Captures)	Without Bullfrogs (SVL Range)	With Bullfrogs (SVL Range)	Without Bullfrogs (Average SVL)	With Bullfrogs (Average SVL)
Males	31	25	5.6-8.9 cm	6.2-9.4 cm	7.59 cm	7.58 cm
Females	78	135	5.6-10.8 cm	4.2-11.6 cm	6.84 cm	7.81 cm
Juveniles	71	28	2.5-5.5 cm	2.2-5.6 cm	4.29 cm	4.43 cm

Table 3: Population structure of the number of captured males, females, and juveniles in both sites for mass range and average mass.

Sex	Without Bullfrogs (Captures)	With Bullfrogs (Captures)	Without Bullfrogs (Mass Range)	With Bullfrogs (Mass Range)	Without Bullfrogs (Average Mass)	With Bullfrogs (Average Mass)
Males	31	25	25.5-105 g	38.5-100.5 g	65.78 g	68.56 g
Females	78	135	16.5-190 g	17.5-261 g	53.80 g	84.57 g
Juveniles	71	28	2-40 g	2.6-42 g	14.37 g	15.80 g

Abundance of Bullfrogs and Woodhouse Toads within Gradients Perpendicular to the River:

I found a total of 59 Woodhouse toads within the deterministic transects. All juveniles encountered in the deterministic transects were yearlings or older. The total encounter rates of toads were higher with Bullfrogs (17 females, 9 males, and 5 juveniles) than without Bullfrogs (13 females, 6 males, and 9 juveniles) (Figure 17). Between the study sites, I uncovered more males and females with the presence of Bullfrogs and more juveniles with the absence of Bullfrogs. The average number of males (9 without Bullfrogs; 6 with Bullfrogs) and females (17 without Bullfrogs; 13 with Bullfrogs) found within the deterministic transects in both sites was non-significant (T-test: t-stat=0.52, df=9, p-value=0.31 for males; t-stat=0.15, df=13, p-value=0.44 for females) and significant for juveniles (T-test: t-stat=2.14, df=8, p-value=0.03 for juveniles).

As for the site without Bullfrogs, the number of toads found between 10 and 100 meters of the deterministic transect range from 0-5 individuals (Figure 18). I found most of the males in the first 10 meters while females and juveniles were found in 7 out of 10 subsections. As for juveniles, the highest abundance was located in both 10 and 100 meters. Furthermore, the number of toads counted in the site with the presence of Bullfrogs ranged between 0-4 individuals (Figure 19 & Figure 20). A chi-square test shows that there is a significant difference between the distributions of Woodhouse toads found with Bullfrogs in the first 10 meters of the deterministic transects in both study sites (Chi-square: x^2= 6.33, df=2, and p-value=0.04).

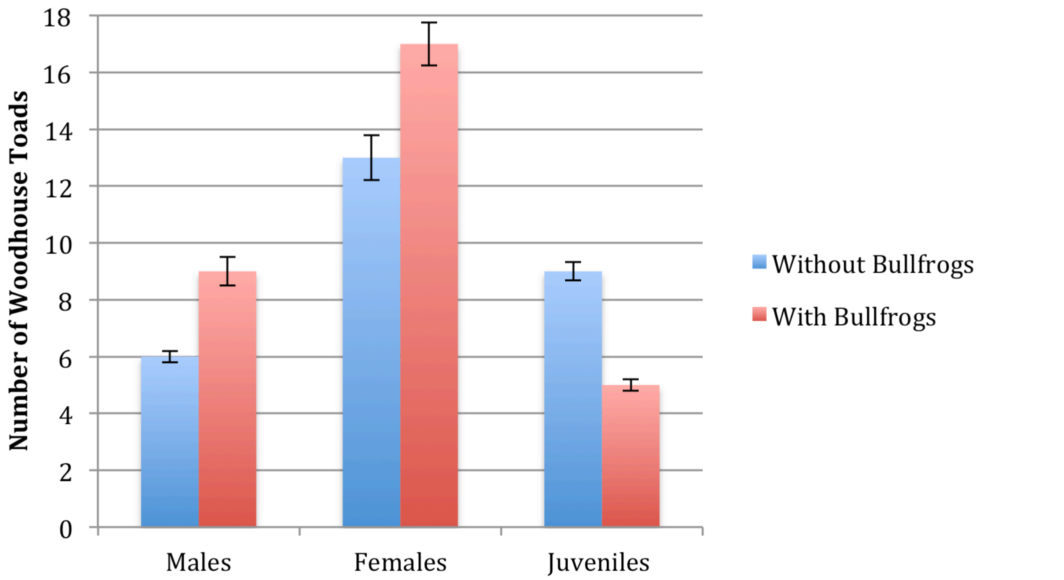

Figure 17: The total number of males, females, and juvenile Woodhouse toads encountered in the deterministic transects without Bullfrogs (Blue) and with Bullfrogs (Red). T-test: t-stat=0.52, df=9, p-value=0.31 for males; t-stat=0.15, df=13, p-value=0.44 for females and t-test: t-stat=2.14, df=8, p-value=0.03 for juveniles.

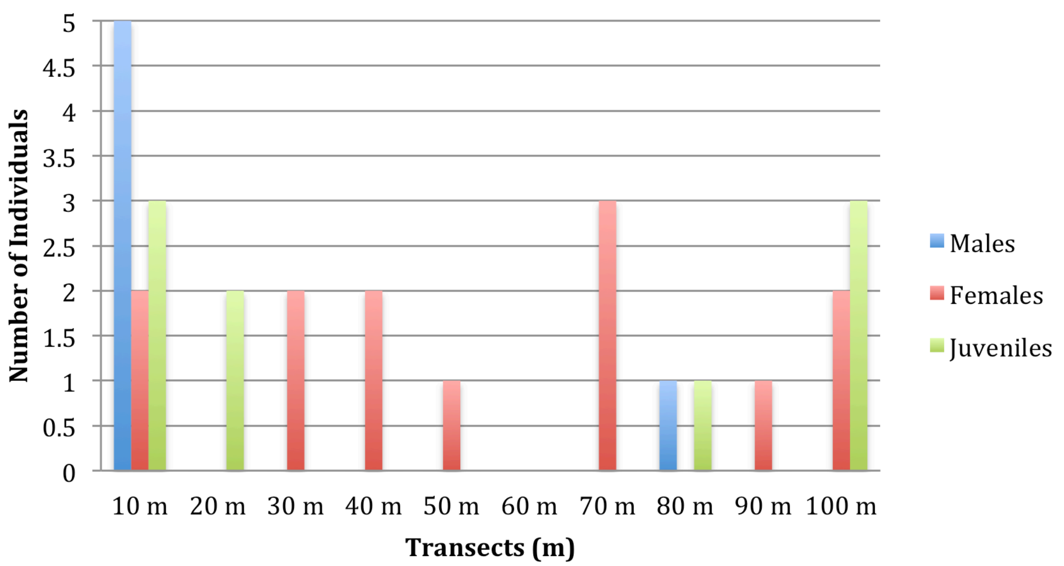

Figure 18: The total number of male (Blue), female (Red), and juvenile (Green) Woodhouse toads detected at different distances of the deterministic transects in the site without Bullfrogs.

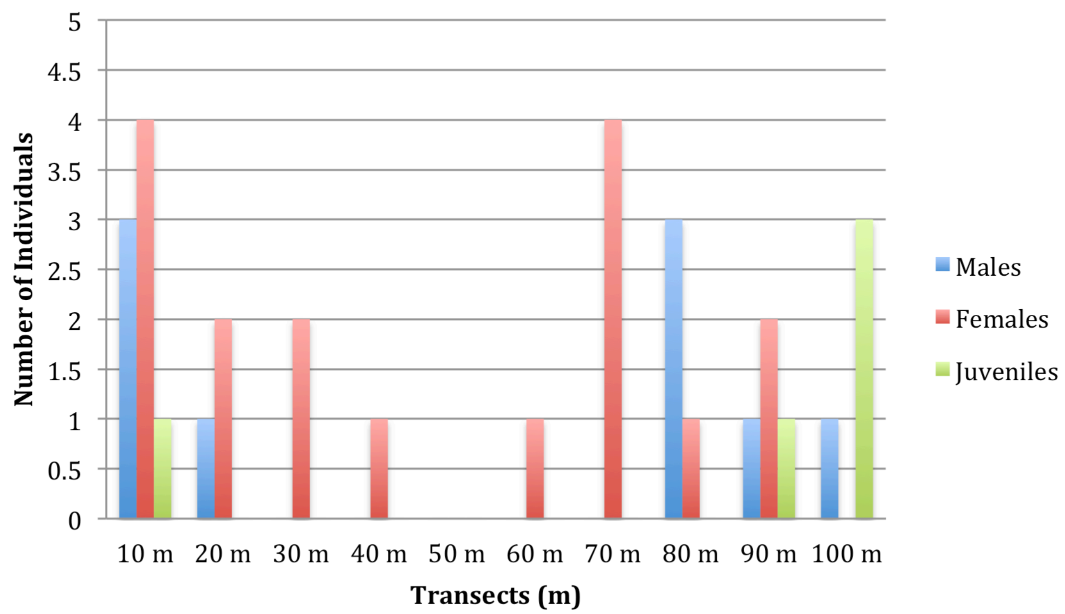

Figure 19: The total number of male (Blue), female (Red), and juvenile (Green) Woodhouse toads detected at different distances of the deterministic transects in the site with Bullfrogs.

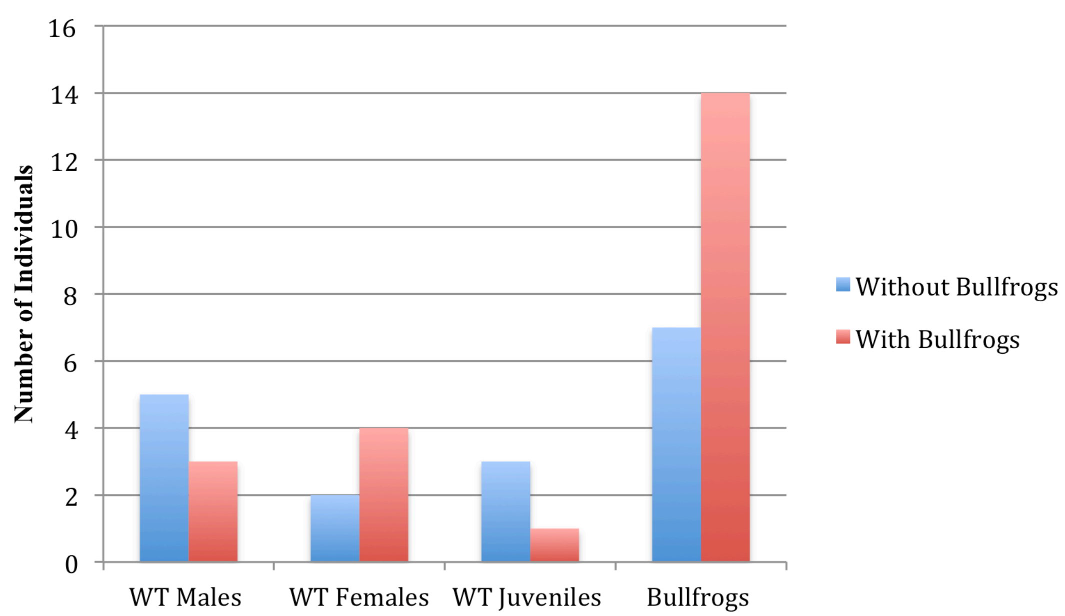

Figure 20: The presence of Woodhouse toads (males, females, and juveniles) and Bullfrogs found together at the 10-meter section of the deterministic transects in both sites without Bullfrogs (Blue) and with Bullfrogs (Red). Chi-square: x^2= 6.33, df=2, and p-value=0.04.

Toad Prey Availability (Invertebrate Abundance):

Within the two months of sampling in the fall of 2015 I collected 21 different insect families throughout the deterministic transects (Tables 4 & 5). Along with insects I also captured multiple arachnids. This suggests arthropods are uniformly distributed along the transects, so food supply is not likely an explanation of the distribution of Woodhouse toads. The most abundant insect families found in Rio Mora were Miridae and Acrididae. Insect abundance showed a decline as I moved away from the river in both sites (R^2=0.80, p-value=0.00 without Bullfrogs; R^2=0.58, p-value=0.01 with Bullfrogs; Figure 21).

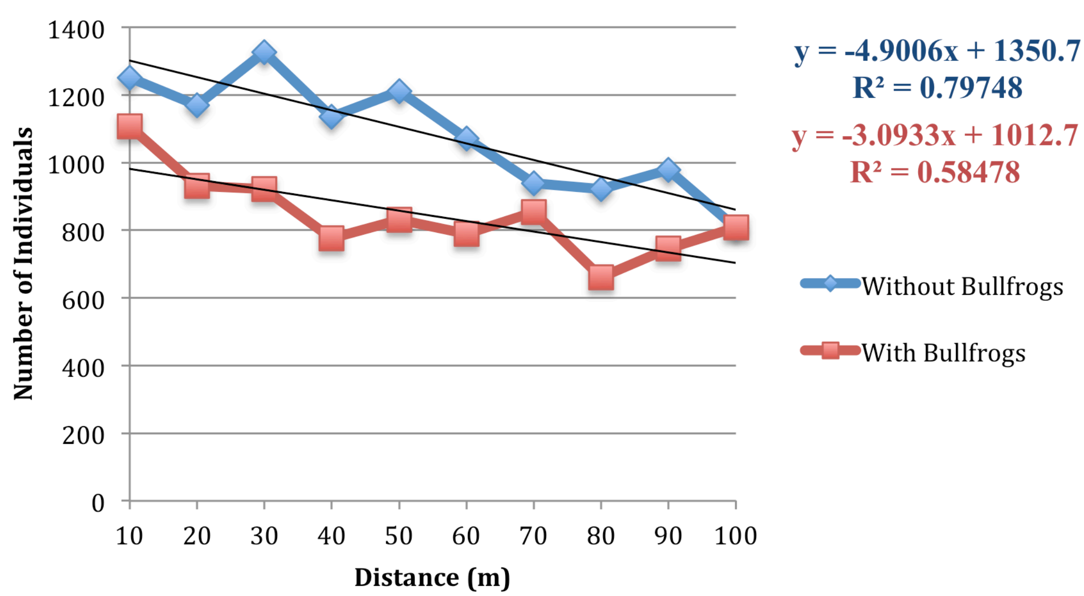

Figure 21: Insect abundance of the least and most abundant insects combined with the relation of distance in both sites. Regression: r^2=0.80, p-value=0.00 without Bullfrogs; r^2=0.58, p-value=0.01 with Bullfrogs.

Table 4: The total number of insects of 21 families captured during insect sweeps in the deterministic transects at different distances from the river in the site without Bullfrogs. The table also includes the abundance of arachnids within the transects.

Insect Families	10m	20m	30m	40m	50m	60m	70m	80m	90m	100m
Miridae	1003	906	1038	805	899	7852	661	627	700	535
Acrididae	199	240	251	268	266	232	229	265	237	234
Baetidae	0	0	0	1	0	0	0	0	0	0
Chrysomelidae	1	3	5	3	2	3	0	4	3	3
Heteronemiidae	0	1	0	2	0	1	0	0	0	0
Mantidae	1	0	1	0	0	0	0	0	0	0
Lestidae	0	0	0	1	0	1	0	0	0	0
Cicadellidae	4	0	2	0	0	0	0	0	0	0
Membracidae	0	0	0	1	0	0	1	0	0	0
Reduviidae	0	0	1	3	2	0	0	3	0	0
Largidae	0	0	0	0	0	0	2	0	0	0
Petatomidae	5	7	11	17	22	29	25	12	10	10
Carabidae	0	3	1	8	3	2	0	2	2	3
Coccinellidae	0	0	1	1	2	1	6	1	0	1
Cantharidae	0	0	2	0	1	1	2	0	2	0
Geometridae	0	1	1	0	1	0	0	1	2	0
Calliphoridae	2	8	8	9	3	6	9	5	12	4
Megachilidae	0	0	0	0	0	0	0	0	0	0
Formicidae	35	0	4	10	0	0	0	0	0	14
Sphecidae	0	0	0	1	1	2	0	0	1	1
Oecophoridae	0	0	0	1	5	3	1	2	4	0
Arachnid	1	0	1	4	5	6	3	0	5	2

Table 5: The total number of insects of 21 families captured during insect sweeps in the deterministic transects at different distances from the river in the site with Bullfrogs. The table also includes the abundance of arachnids within the transects.

Insect Families	10m	20m	30m	40m	50m	60m	70m	80m	90m	100m
Miridae	863	715	678	567	597	593	599	435	532	587
Acrididae	205	200	218	179	206	166	226	199	200	212
Baetidae	0	0	1	1	0	0	0	0	0	0
Chrysomelidae	0	2	3	3	1	5	1	0	1	2
Heteronemiidae	0	0	1	1	0	0	0	0	0	0
Mantidae	0	0	0	0	0	0	0	0	0	0
Lestidae	0	0	0	0	1	0	0	0	0	0
Cicadellide	0	1	1	1	3	0	1	0	0	0
Membracidae	1	0	0	0	2	0	0	0	0	0
Reduviidae	0	0	0	0	0	0	1	2	1	3
Largidae	0	0	0	0	0	0	2	1	0	0
Petatomidae	6	5	13	5	8	9	12	13	4	0
Carabidae	2	2	1	2	0	5	1	3	0	0
Coccinellidae	0	0	0	1	0	0	0	0	0	0
Cantharidae	0	0	1	0	0	0	2	1	0	1
Geometridae	0	0	0	0	0	2	0	0	0	0
Calliphoridae	10	0	3	12	6	2	6	3	8	1
Megachilidae	0	0	0	0	0	0	0	0	0	0
Formicidae	18	6	0	0	0	0	0	0	0	0
Sphecidae	0	0	0	0	0	1	0	0	0	0
Oecophoridae	0	1	1	2	1	1	3	2	0	0
Arachnid	2	0	0	3	6	5	0	2	0	2

Insect Vegetation Choice Analysis:

The average percent vegetation cover within the subsections of the deterministic transects ranged from 45-68% in the site without Bullfrogs and 41-66% with Bullfrogs (Figure 22). Surprisingly, the average percent vegetation cover between the both sites is homogenous except for the drastic drop at 70 meters in the control due to slopes without vegetation. My percent vegetation cover between both sites did not have any significant difference (T-test: t-stat=0.0, df=198, p-value=0.5). As for the linear correlation between distance versus percent vegetation cover there was no significance (Single Regression:

r^2=0.11, p-value=0.35 without Bullfrogs; r^2=0.19, p-value=0.2 with Bullfrogs). Moreover, I found a significant difference between the abundance of insects with percent vegetation (R^2=0.11, p-value=0.35 without Bullfrogs; R^2=0.19, p-value=0.2 with Bullfrogs) (Figure 23 & 24).

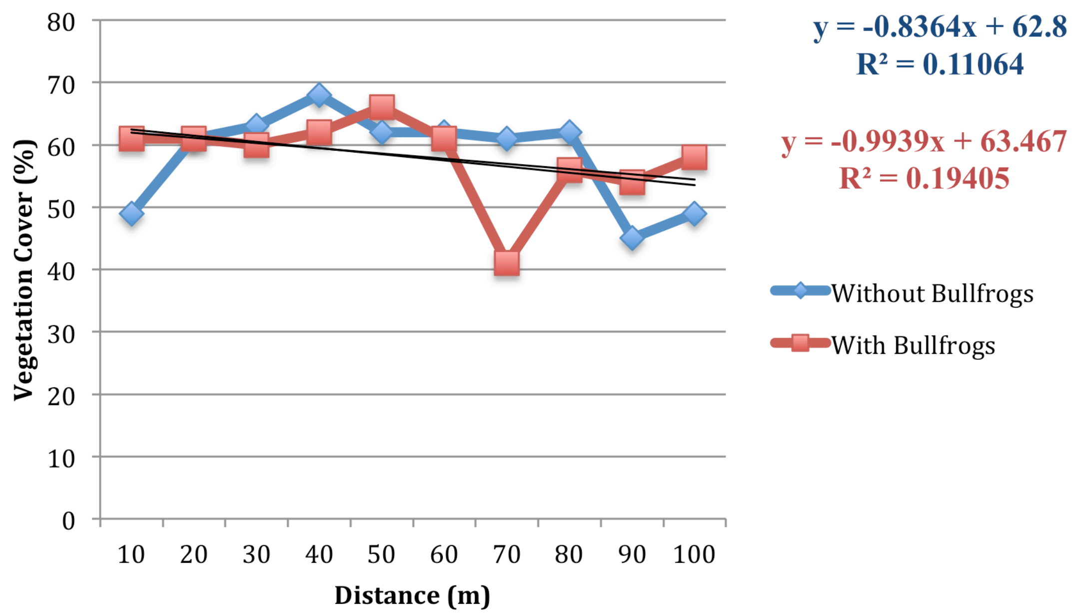

Figure 22: The changes of percent vegetation cover at different distances from the river to the prairie in both the experimental (Blue) and control (Red) sites. Regression: R^2=0.11, p-value=0.35 without Bullfrogs; R^2=0.19, p-value=0.2 with Bullfrogs.

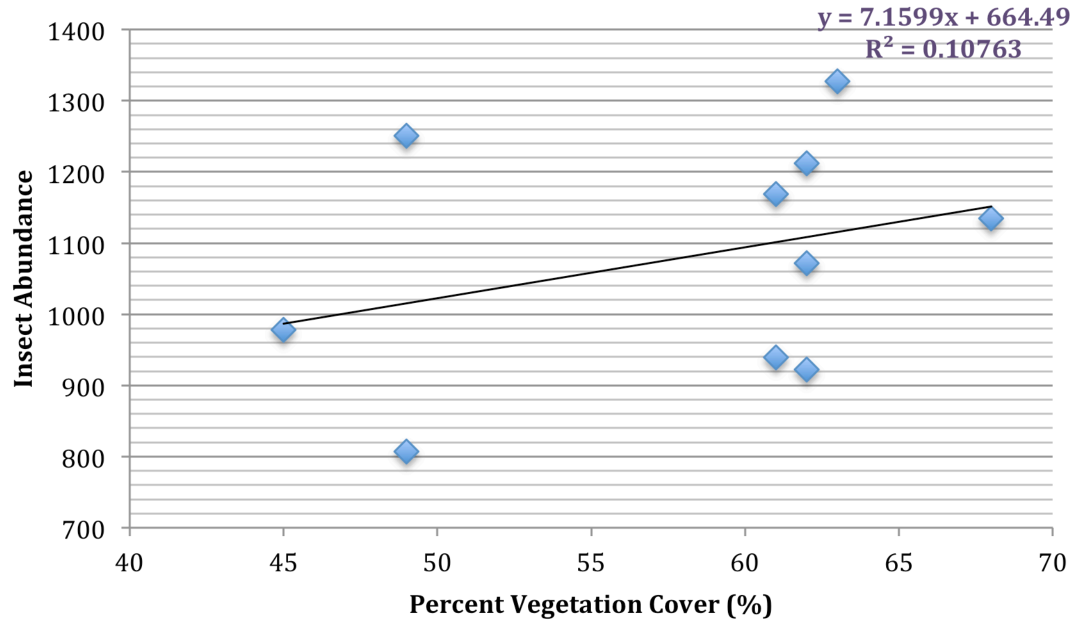

Figure 23: The relationship between insect abundance and percent vegetation cover in the site without Bullfrogs. Single Regression: r^2=0.11, p-value=0.35.

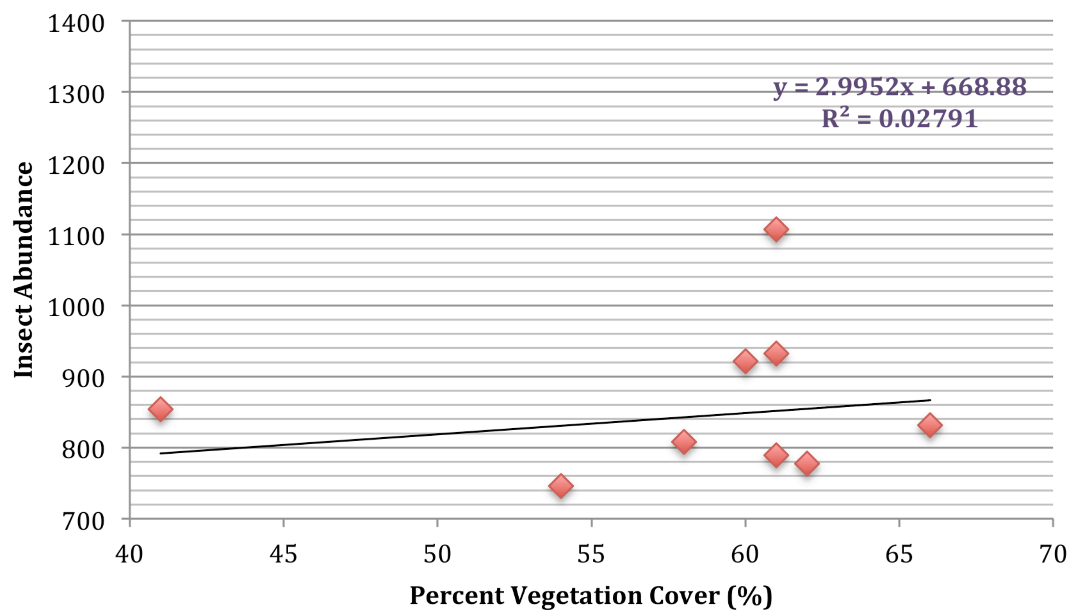

Figure 24: The relationship between insect abundance and percent vegetation cover in the site with Bullfrogs. Single Regression: r^2=0.19, p-value=0.2.

CHAPTER IV: DISCUSSION

My results indicate that invasive Bullfrogs within my study site impact the population of Woodhouse toads in northeastern New Mexico. There are significant differences in Woodhouse toad and tadpole abundance between the sites with and without Bullfrogs. I also observed the impacts of Bullfrogs affecting the Woodhouse toad population structure and riparian habitats utilized by adult and juvenile Woodhouse toads. Moreover, Woodhouse toads altered their behavior to avoid areas with the presence of Bullfrogs by retreating further into the prairie. Overall, it appears that Woodhouse toad populations are different between the study sites and shows a correlation with the presence of Bullfrogs. Several studies also confirm that invasive Bullfrogs have direct and indirect impacts on native amphibian populations (Kiesecker & Blaustein, 1998; Lawler et al., 1999; Kats & Ferrer, 2003; Boone et al., 2004; Schloegel et al., 2010).

Relative Abundance:

During my call surveys, Woodhouse toad calls were only detected on two nights of May 2014, potentially due to snowfall in late May and early June. I suspect that the snowfall could have affected calls by causing toads to go back into coverage for a short period of time until the snow was gone. However, later into the season of 2014 there were no calling males. In 2015, males were calling between early May and late June. Heavy flooding caused the toads to retreat away from rivers and colonize ponds for breeding instead. I documented more calls with Bullfrogs (Average: 11.43 calls) compared to the site without Bullfrogs (Average: 9.29 calls) with no significant difference. There is an apparent contradiction between the fact that Woodhouse toads are more abundant in the area without Bullfrogs but the number of calls is comparable between both sites. A possible explanation for this is that Woodhouse toads do not reach sexual maturity until they are 3-4 years of age and do not call for females (Kellner & Green, 1995). Since the eradication started in 2012 it is likely that the effects are not yet present in the adult male toad population.

While evaluating the Bullfrog's influence on Woodhouse toad abundance, I came across some surprising results. In 2014, Woodhouse toads were not found in half of the random transects in the site without Bullfrogs, which were also areas Bison (*Bison bison*) were excluded. However, in the site with Bullfrogs, Bison roam free and within one transect I came across 4 individual Woodhouse toads in a wallow. These wallows are created by Bison often pawing and rolling on the ground resulting in the creation of new habitat (Knapp et al., 1999). Therefore, the presence of wallows causes a buildup of water collected from snow melts and rainfall, producing temporary pools that provide a habitat. These temporary pools offer breeding habitats for amphibians such as Spadefoot toads (*Spea bombifrons*) and Great Plains toads (*Anaxyrus cognatus*) (Corn & Peterson, 1996; Bragg 1940); I surmise that Woodhouse toads were using this depression because of the higher moisture. This was not the case in 2015; Bison are currently roaming the areas that they were once excluded from and wallows are not found within the transects due to rainfall improving vegetation growth.

Woodhouse toad and Tadpole Abundance:

My observations on tadpole surveys demonstrated more tadpoles captured per day in the pond without Bullfrogs (Average: 77.1 tadpoles) than the pond with Bullfrogs (Average: 20.9 tadpoles), suggesting that Bullfrogs are predators to egg masses, tadpoles, or breeding pairs (Figure 9). I also found tadpoles in the site without Bullfrogs to have a type II survivorship curve showing a constant chance of dying at different tadpole life stages. However, they showed a type III survivorship curve when Bullfrogs are present (Figure 10). Ehrlich (1979) has documented invasive Bullfrogs consuming newly hatched native Plains leopard frog (*Lithobates blairi*) tadpoles (Ehrlich, 1979), which I also see Bullfrogs preying on newly hatched (first stage) Woodhouse toad tadpoles. Other than predation, there is evidence that Bullfrog larvae have an impact on breeding ponds with American toad (*Anaxyrus americanus*) and Southern Leopard frog (*Lithobates sphenocephalus*) tadpoles due to competition for either resources or space (Boone et al., 2008); this might also be the case for Woodhouse toad tadpoles.

There are two other ways Bullfrogs may be affecting abundance of Woodhouse toad tadpoles. First, Bullfrogs may prevent nesting pairs of toads from laying eggs. If Woodhouse toads do not come to the water to breed, there will be fewer clutches. Second, Bullfrogs may be preying on toad eggs. In the Bullfrog stomach contents I frequently found a white mucinous substance that could have been denatured protein from toad egg masses that Bullfrogs had consumed.

Other than tadpoles, the sex ratios of toads in Bullfrog-absent areas indicate more male and juvenile Woodhouse toads, whereas larger females are more abundant with the presence of Bullfrogs (Figure 17). Smaller animals are more susceptible to Bullfrog predation, so size may be affecting toad abundance by displacing them to other habitats, ultimately producing other indirect effects. Since males need to stay near water to reproduce, they may suffer stronger Bullfrog predation than females. On the other hand, juveniles are highly abundant in the site without Bullfrogs suggesting that the management of Bullfrogs is effective in lowering tadpoles and juvenile predation. As for females, I caught more in the site with Bullfrogs away from the river compared to the site without Bullfrogs suggesting that females may avoid areas with Bullfrogs and spread out more into the prairie. Consistent with the higher number of tadpoles and juveniles, the Schnabel method displayed an overall higher abundance of Woodhouse toads without Bullfrogs (820) than with Bullfrogs (700.4).

Prey Availability:

Insect abundance was extremely high throughout both sites in the deterministic transects. There was no significant difference in insect abundance with distance from the river to the prairie. There was also no significant difference between percent vegetation cover and insect abundance indicating there is no relationship linking the two. Perner et al. (2005) also found that insect abundance and plant species richness did not have a correlation. Insects exposed a wide distribution throughout the landscape and did not prefer areas with higher vegetation. There was a small drop in their density away from the river, but Woodhouse toads were found even in these areas with fewer insects. Therefore, this suggests Woodhouse toad distribution is not correlated with insect abundance.

Bullfrog Avoidance:

I believe that Woodhouse toads within the deterministic transect are avoiding Bullfrogs rather than following their prey. I found Woodhouse toads away from the river in areas where Bullfrogs were present but not in areas without Bullfrogs (Figure 17). Male toads found with Bullfrogs may also be a signal to attract females; if they can survive the presence of Bullfrogs, it advertises good genes to potential female mates. The idea of this principle explains how males contain exquisite traits demonstrating the ability of their survival leading to sexual selection by females (Zahavi, 1975). This has also been seen in Bibron's toadlets (*Pseudophryne bibronii*) in Australia; these little toads enhance their calls to be louder when females are around to attract more mates (Byrne, 2009), however, this increases their exposure to predators.

Other amphibians respond to Bullfrogs presence by occupying different habitats. Northern Leopard frogs (*Lithobates pipiens*) and Northern Green frogs (*Lithobates clamitans*) sought more areas near the shoreline amongst thick vegetation with the presence of Bullfrogs (McAlpine & Dilworth, 1989). Also, Northern Red Legged Frogs (*Rana aurora*) have a Bullfrog avoidance response by decreasing their activity levels and changing their habitat use (Kiesecker & Blaustein, 1998). Modifying the habitat use in order to avoid introduced predators has been documented in other amphibians (Lima & Dill, 1990). This predatory avoidance could be the result of natural selection or behavioral plasticity (Chivers et al., 2001; Freidenfelds et al., 2012). In the case of Southern toads (*Anaxyrus terrestris*), their behavior changed by increasing their movement patterns to avoid injury from invasive Red-imported fire ants (*Solenopsis invicta*), but this can also lead to negative impacts on toad fitness by reducing reproductive and foraging success (Long et al., 2015).

Thus Bullfrogs can also exert indirect effects by changing their preys' behavior. When Bullfrogs are absent, male toads seem to occupy the riparian areas more frequently. However, male toads often occupy areas distant from the river when Bullfrogs are present. This phenomenon of prey species changing behavior and habitat use in response to predation has been documented with reintroduced Gray Wolves (*Canis lupus*) in Yellowstone National Park. While the wolves were absent, the elk (*Cervus elaphus*) population increased and browsed more on aspen (*Populus tremuloides*) which affected the tree height. However, when the wolves were reintroduced elk reduced their browsing to avoid areas more visible to wolves and the height of the aspen increased (Ripple & Beschta, 2007).

I was surprised that female toads in areas where Bullfrogs have been eliminated were smaller than females that coexist with Bullfrogs. A likely explanation is that the absence of Bullfrogs helps increase recruitment of young females that are more abundant, dragging down the average size of the female populations. This is because Bullfrogs' mouths are only big enough to prey on juveniles and smaller animals. Therefore, female toads found in the area with Bullfrogs are too large to consume.

Challenges and Future Investigations

One challenge that remains in this study is to investigate why there are smaller female toads in areas without Bullfrogs. The deterministic transects have shown us the distribution of females but the transect starting point was at the rivers flood plain which did not allow us to detect if larger females are utilizing the river more after the eradication of Bullfrogs. In order to investigate this I would need to conduct river transects to count and measure these females to determine if there was a response to the removal of Bullfrogs. Given that Bullfrogs prey on tadpoles, I question whether there has been predation on Woodhouse toad egg masses. In the past, dissected Bullfrogs contained an unidentified mucinous substance in their gut, I summarize that may originate from the gel layer of Woodhouse toad egg masses. In order to further investigate this I would need to conduct river transects and count the total number of Woodhouse toad egg masses and compare the sites.

General Conclusion:

Throughout the world many amphibians are threatened and it is essential to understand the factors contributing to their declines. The introductions of invasive species such as the Bullfrog are playing a crucial role in native amphibian population declines through predation, competition, and habitat displacement. My study provides information on the direct impacts that Bullfrogs have on Woodhouse toad demographics. Multiple lines of evidence point to the notion that Bullfrogs negatively affect the ecology of Woodhouse toads by lowering their density at different stages of development, likely via predation, as well as affecting their behavior displacing them to suboptimal habitats. Therefore, my hypothesis is supported. As climate change continues in New Mexico this may cause Bullfrogs to increase and have a stronger impact on Woodhouse toads. These toads may be in trouble, as long periods of droughts generate severe problems as the reduction of habitat and resources leads to increased competition and predation pressure by Bullfrogs. Avoidance of aquatic predators may not be easy in a world where all water bodies are reduced. Therefore the combination of Bullfrogs and climate change may possibly have a greater effect on the Woodhouse toad population by diminishing ecosystem resilience. RMNWR is positioned within the rain shadow on the eastern part of the Sangre de Cristo Mountains where stronger effects of droughts have been shown. The study area provides an insight of future weather conditions that can roughly happen throughout New Mexico as climate change advances it can cause the weather to become drier. Therefore, Bullfrogs and climate change can have synergistic impacts on Woodhouse toad populations. Overall, these invaders appear to influence Woodhouse toads in a negative way that require proper management plans to eradicate Bullfrogs to help save the Woodhouse toad before there are major population declines.

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