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MS ENVIRONMENTAL BIOLOGY CAPSTONE PROJECT

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Hannah M. deKay

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

REGIS UNIVERSITY
May, 2022

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Hannah M. deKay

has been approved

May, 2022

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CHAPTER 1. LITERATURE REVIEW

Human-Wildlife Conflict: Comparing Lethal and Behavioral Mitigation in Agricultural Settings

Introduction

The conflict between humans and wildlife is primarily driven by competition for space and resources, and nowhere else is this seen more than within agricultural settings. Agricultural development accelerates the loss of habitat and forces wildlife into competition for adequate food, water, and prey availability (Treves, 2007). For example, elephants in both Asia and Africa have experienced extreme reductions in their traditional feeding grounds and migration corridors; foraging in the farmlands that have replaced their natural habitat (Shaffer et al., 2019). This pattern of conflict not only results in crop losses, but also both human fatalities and retaliatory killing of elephants. Between 2010 and 2017 over 200 human fatalities and over 120 elephant deaths were reported in Kenya as a result of these agricultural conflicts (Shaffer et al., 2019). As a keystone species, elephants engineer their surrounding habitat, supporting additional local biodiversity as a result (Fritz, 2018). This friction between conflict management and conservation efforts of the species has yet to be resolved (Fritz, 2018; Shaffer et al., 2019).

Because of conflicts between agriculture and wildlife, humans have historically resorted to forms of lethal control in an effort to lower wildlife populations and reduce damages, with varying degrees of success (Woodroffe et al., 2005). In their most extreme form, lethal methods have resulted in irreparable damage to ecosystems and caused the near extinction of many species (Baker et al., 2020; Nyhus, 2016; Woodroffe et al., 2005). In the face of targeted species conservation efforts, lethal methods may not always be an appropriate option.

Within the past 20 years, literature on conflict mitigation with wildlife and non-lethal alternatives has grown exponentially (Nyhus, 2016). One such alternative is behavioral modification: using a species' behavioral ecology to inform more effective and sustainable mitigation efforts (Blackwell et al., 2016; Greggor et al., 2016; Nyhus, 2016).

As we continue to develop and fragment wilderness spaces, solutions to manage our competition with the natural world and with wildlife must be addressed for both human and animal welfare. The purpose of this review is to evaluate the roles lethal control and behavioral modification play in terrestrial wildlife management as two distinct methodologies. Each method will be evaluated for efficiency, efficacy, and sustainability in relation to reducing conflicts to gain a better understanding of the appropriate uses and opportunities for improvements to management practices in agricultural settings.

Lethal Control

Historically, lethal control has been the only preventive method for reducing human-wildlife conflict (HWC) (McManus et al., 2015; Nyhus, 2016; Swaisgood, 2007; Treves & Karanth, 2003). Killing perceived pests or threatening wildlife affecting human welfare, safety, or economy is considered a method of population control to reduce the frequency of conflict (Woodroffe et al., 2005). Examples of consistently conflictive species include large terrestrial carnivores, such as wolves or predatory cats, who kill livestock (Treves & Karanth, 2003; Woodroffe et al., 2005). Wild herbivores, such as elephants, large wild ungulates, or smaller mammals and rodents, also may target crop fields for food (Treves, 2007; Woodroffe et al., 2005). Instances of successful long-term mitigation using lethal control methods only include those that eradicated endemic populations (Nyhus, 2016; Treves & Karanth, 2003; Woodroffe et al., 2005).

Lethal control is controversial because of the traditional context of eradication campaigns (Baker et al., 2020; Woodroffe et al., 2005), which are often referenced in opposition to lethal methods because they have resulted in severe population declines of many species (Nyhus, 2016; Peebles et al., 2013; Woodroffe et al., 2005). For instance, within a few decades, the American grey wolf was eliminated in over 90% of its historic range and this extensive extirpation was largely successful at reducing instances of livestock depredation (López-Bao et al., 2017).

Eradication events similar to the grey wolf are effective in reducing conflicts through the complete removal of target species, facilitating near extinction in many instances (Nyhus, 2016). Today, eradication campaigns are rare, and only within the past 50 years have public perceptions begun to shift towards restoration and preservation of these animals in contrast to their complete removal (López-Bao et al., 2017). Currently, other less severe programs for lethal control have been introduced depending on the severity of conflicts and management goals (Woodroffe et al., 2005).

The primary justifications for lethal control often center around agricultural losses, where depredation and crop-raiding cost farmers money and time (McManus et al., 2015; Woodroffe et al., 2005). Lethal control strategies are often misperceived to be effective because of the success of extreme historical exterminations. Damages vary year to year and are reported in the millions of dollars, giving the illusion that losses caused by wildlife are extremely high and catastrophic (McManus et al., 2015; Sunde et al., 1998). However, frequent, small-scale losses and other external factors are responsible for most of the cumulated economic costs (Treves, 2007). Over $1.6 billion dollars was spent by the US government between 1938 and 1998 on lethal predator control targeting wolves, bears, coyotes, and cougars to protect sheep. Yet, Berger (2006) found that waning societal demands for sheep products were primarily responsible for continuous stock population declines over that time period, not depredation. Additionally, the cost of lethal control significantly exceeds the use of other methods; McManus et al. (2014) found it costs three times as much per head of stock to implement lethal traps compared to a non-lethal alternative. Despite the economic inefficiencies, lethal control methods persist, partially due to the mistaken and biased belief that they are effective, based on the extreme historical exterminations (Berger, 2006; Woodroffe et al., 2005).

In attempts to mitigate reported economic losses, governments introduced public hunts and subsidized removals. However, these programs have been linked to higher incidences of conflicts globally (Berger, 2006; Peebles et al., 2013; Sunde et al., 1998). Unselective lethal control leads to altered population demographics in targeted species, where the removal of sensitized, older, and more stable individuals results in replacement by younger, bolder individuals; this is referred to as the perturbation effect (Shaffer et al., 2019; Sunde et al., 1998). In a study examining lightly hunted and heavily hunted populations of cougars in Washington state agricultural areas, Peebles et al. (2013) found complaints and depredation occurred significantly more often in heavily hunted areas. They also found the heavily hunted cougar population had a significantly altered age-sex demographic profile, with a higher occurrence of young males over stable, older males or any females. Young male cougars are responsible for the majority of conflicts and encounters with humans in any environment, as they tend to be more transient and bold compared to females or older males (Torres et al., 1996). Considering the perturbation effect in conjunction with cougar spatial and behavioral ecology, heavy lethal control in agricultural areas is not an effective method to reduce depredation conflicts (Peebles et al., 2013). Perturbation effects during lethal control have been documented across taxa including coyotes (Bonnell & Breck, 2017), bears (Elfström et al., 2014), elephants (Shaffer et al., 2019), lynx (Sunde et al., 1998), wolves (Bradley et al., 2015), and badgers (Woodroffe et al., 2005). General population control through subsidized or public hunting perpetuates conflicts rather than reducing them by exacerbating effects from problem individuals.

The perturbation effect occurs as a result of either selective or general lethal control programs, regardless of species. Only a few individuals are often responsible for the majority of HWC across taxa, despite the majority of the population having some access to human agricultural affairs (Honda & Iijima, 2016; Woodroffe et al., 2005)**.** The few problem individuals are often male, young, and transient, with offenses resulting from changes in resource availability in the surrounding area (Bradley et al., 2015; Elfström et al., 2014; Peebles et al., 2013; Torres et al., 1996)**.** Where problem individuals persist, selective removal can be used as an alternative to general population control (Honda et al., 2019; Honda & Iijima, 2016). In an effort to reduce the depredation of cattle in Wyoming, Idaho, and Montana, grey wolf packs were subject to selective removal programs of problem individuals (Bradley et al., 2015). However, the recurrence of depredation occurred within 64 days of selective removal, aligning with perturbation patterns of replacement, and additionally reduced viable breeding pairs for species recovery efforts (Bradley et al., 2015)**.**

Potential environmental consequences pose a serious problem and call into question the sustainability of lethal methods. Poisoning and trapping are often less laborious; however, they result in the killing of non-target species and do not reliably deal with bolder individuals responsible for the depredation or crop raiding (Nyhus, 2016). Lethal methods also alter age-sex demographics in a population, and the broader implications of this are not well understood

(Elfström et al., 2014; Peebles et al., 2013; Torres et al., 1996). More research is needed to identify how the changes in population ecology following lethal control methods affect the long-term viability of the species. Additionally, the removal of any species from its natural environment has cascading consequences in the ecosystem (Nyhus, 2016), making lethal control methods questionable in fostering sustainable coexistence between humans and wildlife.

Behavioral Modifiers

Behavioral modification is a non-lethal form of mitigation, whereby a species' behavioral ecology and tendencies are used as means to reduce HWC (Reed, 2004; Swaisgood, 2007). Using an animal's behavioral tendencies to inform management decisions is a relatively recent revelation, the merits of which are only beginning to be adapted into applied conservation practices (Blackwell et al., 2016). Specifically, recognition behavior through auditory deterrents, olfactory responses, and anthropogenic and predatory conditioning has been used to successfully reduce conflict. The application of these methods in addressing the problems caused by bolder individuals in conflict with agriculture is still in the process of scientific inquiry and refinement (Apfelbach et al., 2005; Baker et al., 2006; Bonnell & Breck, 2017; Honda et al., 2019; Kaplan et al., 2011; Kloppers et al., 2005; Reed, 2004).

Implementation requires an intimate knowledge of a species’ behavioral ecology, and is often why management efforts shy away from experimentally using these methods over lethal population control (Greggor et al., 2016). However, behavioral modifiers do not have to be expensive to mitigate and prevent future conflicts between humans and wildlife. For example, knowing that most predators kill their prey with lethal bites to the neck, McManus et al. (2014) found that using protective neck collars on cattle to prevent depredation was more cost-effective than trapping or poisoning potential predators and, as a result, pastoralists saved between 50% and 75% of their income that would have been lost due to depredation following the implementation of collars. Additionally, replacing time-consuming hazing methods with alternative food incentives to reduce crop raiding in habituated baboons proved effective: lowering mitigation costs and encounters between baboons and humans (Kaplan et al., 2011).

Recognition behavior employs the identification and discrimination of cues animals use to interpret their surroundings (Reed, 2004). The recognition of humans as threatening through various forms of hazing results in sensitization and ultimately reduces HWC, but it can be labor-intensive and requires long-term consistency (Breck et al., 2017; Kaplan et al., 2011). Other forms of recognition behavior include auditory cues. For example, African elephants are known to actively avoid beehives and signal to surrounding individuals to avoid the area (King et al., 2017). Using this recognition behavior in management involves placing real beehives, faux hives, and playing recordings along fence lines surrounding crop fields. This method proved successful in deterring elephants from raiding crops up to 80% of the time (King et al., 2017). These kinds of methods effectively deter all individuals, bold or otherwise, and do not have any direct effect on population demographics.

Another proposed recognition cue with mitigative potential is predator scent marking (Reed, 2004). Many predators communicate the boundaries of their territories and establish occupancy through scent marking (Gosling, 1982). Creating artificial boundaries with coyote urine was found to be an unsuccessful deterrent to reduce conflicts (Shivik et al., 2011), but coyotes may not have been the ideal territorial species for this method. Coyotes use urine to signal and attract neighbors and regularly intrude on surrounding territories as a consistent behavioral tendency (Gese & Ruff, 1997). As justification for their study, the authors also cited that human urine was an effective deterrent of wolves, which may not be as transferable to coyote ecology, nor has this method been substantiated in the literature. In an alternative study, the application of lynx urine around orchards was found to effectively reduce damages by woodchucks by over 95% (Swihart, 1991). The ultimate cause of this result is still unknown, however, lynx and woodchuck ranges overlap extensively, and genetic components to interspecific predator recognition in woodchucks may inform the observed results (Swihart, 1991).

Olfactory responses have been used to varying degrees of success as a deterrent to conflictive species in agriculture (Apfelbach et al., 2005). Some studies have observed wildly successful deterrents using a variety of different, species-relevant odors, where others have seen no effect at all or acclimatization to the effect (Apfelbach et al., 2005; Baker et al., 2006). Scents must directly pertain to the specific species and their specific recognition behaviors (Apfelbach et al., 2005; Reed, 2004). In territorial predators, replicating scent markings to mimic claimed areas in high conflict regions has the potential to be successful, but needs further investigation.

Conclusion

Agriculture is a direct source of conflict between humans and wildlife both competing for similar resources and space. Lethal control has been extensively used as it is difficult to reject the logical assumption that population control results in less conflict. However, lethal control has proved an expensive method that stimulates further conflict through perturbation effects in wildlife population demographics (Bradley et al., 2015; McManus et al., 2015; Peebles et al., 2013; Woodroffe et al., 2005). Additionally, lethal control carries conservation concerns as many targeted species are ecologically valuable to their surrounding habitat (Nyhus, 2016).

Alternatively, using the behavioral ecology of a target species to reduce depredation or crop-raiding frequencies can be cost-effective and efficient in preventing conflicts, provided it aligns correctly with relevant recognition cues (Apfelbach et al., 2005; Baker et al., 2006; King et al., 2017; Reed, 2004). Behavioral manipulation requires an intimate knowledge of a target species, and a series of trial and error to establish effective deterrents (Apfelbach et al., 2005; King et al., 2017; Reed, 2004). Farmers, pastoralists, and wildlife managers will need to balance the costs and benefits of each method, recognizing there is not any one-size-fits-all approach to HWC mitigation and prevention. The use of behavioral modification will rely on it delivering cost savings that ultimately outweigh the cost of experimentation before widespread use. However, as the world's population grows, recognition behavior based management in place of traditional, lethal methods will become necessary to maintain and promote sustainable coexistence between wildlife and agriculture.

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CHAPTER 2. GRANT PROPOSAL

Evaluating the Effectiveness and Impacts of Predator Odor Repellants for Elk Management in Colorado

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Section 1: Abstract

Increasing anthropogenic pressures and habitat infringement are forcing Rocky Mountain Elk to increasingly congregate and forage on croplands and urban vegetation, causing tens of thousands of dollars in crop losses and monetary compensation. Simultaneously, rapid declines in population abundance and distribution of elk in Colorado are creating a conflict of priorities, forcing managers to balance species conservation with damage mitigation. Olfactory repellants, such as predator urine, successfully discourage several species of cervids from problematic foraging in urban areas, but has yet to be quantified in elk specifically. However, some predators are attracted to intentional urine applications, and this also has not been extensively studied. This study will evaluate the effectiveness of predator urine as an elk deterrent to reduce crop damages and will also assess any effects on endemic predators in the area. Four different predator urine treatments will be evaluated with camera traps on plots in sunflower farms where elk have previously congregated and caused crop losses. The potential attractant qualities of predator urine olfactory cues may have unintended consequences on broader community ecology dynamics and localized human-predator conflicts. Quantifying patterns of elk visitation and related vegetation damages, as well as assessing the occurrence of endemic predators to treatment sites will provide necessary information for future management practices seeking to reduce crop losses without affecting the wider population of elk in an area, as well as reduce human-wildlife conflicts.

Section 2. Anticipated Value, Literature Review, Objectives and Hypotheses

Anticipated Value

The goal of this study is to quantify the effectiveness of predator odor repellant as a non-lethal method for reducing visitation and foraging by Rocky Mountain elk (*Cervus canadensis nelson*) and subsequent effects on non-target species. Wildlife management is currently challenged to find ways to balance damage mitigation with healthy population maintenance and monitoring. Currently, there are few quantitative studies evaluating the potential effects of predator urine on elk and still fewer on its effect on non-target species. Assessing olfactory repellants for both effectiveness and potential attractive qualities towards endemic predators must be a top priority for managers seeking to use non-lethal methods to balance conflict mitigation and conservation goals. In this study, I will evaluate both the positive and negative impacts of predator odor repellant to provide a comprehensive assessment of this practical management tool.

Literature Review

Rocky Mountain elk are highly valued in Colorado for their economic, aesthetic, and ecological value, and have been a target species for management efforts for over 100 years (B. K. Johnson, n.d.). In recent decades, elk populations experienced significant declines in Colorado and across North America (deCalesta & Witmer, 1994) as a result of human disturbances such as, habitat fragmentation and increased recreational use of wilderness areas (B. K. Johnson, n.d.). These anthropogenic disturbances forced many elk populations into urban and agricultural environments where they forage on crops and ornamental vegetation causing conflicts with humans (deCalesta & Witmer, 1994; B. K. Johnson, n.d.; H. E. Johnson et al., 2014). Additionally, disease transmission in social cervids like elk is exacerbated by congregations around anthropogenic food and water sources (Vander Wal et al., 2012; VerCauteren et al., 2007). There is significant tension between elk conservation efforts and conflict mitigation, forcing managers to explore non-lethal control and exclusionary methods to balance population maintenance with damage control.

Current exclusionary methods for elk control include permanent and temporary fencing, hazing, trapping, chemical taste repellants, or monetary compensation for crop losses (deCalesta & Witmer, 1994). These methods are often labor intesive, expensive, controversial, and/or not possible in an urban setting (deCalesta & Witmer, 1994; H. E. Johnson et al., 2014). Olfactory repellants successfully deter smaller species of cervid and are often cheaper and less labor intensive than other methods of deterrence (Apfelbach et al., 2005; Müller-Schwarze, 1972; Sullivan et al., 1985; Swihart et al., 1991). However, elk reactions to detectable olfactory signals from natural predators are hypothesized to be influenced by other spatial factors (Creel et al., 2005), and have not been quantitatively measured for fear response alone with olfactory cues.

The application of predator odors shows promise in other species of cervid (Apfelbach et al., 2005; Müller-Schwarze, 1972; Sullivan et al., 1985; Swihart et al., 1991) and may be a plausible alternative to deter elk from foraging on crops or urban vegetation without affecting wider population demographics and viability. The application of predator urine in particular reduces feeding rates and vegetation damages in white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemionus*), and red deer (*Cervus elaphus*) (Kuijper et al., 2014; Sullivan et al., 1985; Swihart et al., 1991; van Beeck Calkoen et al., 2021); this aversive behavior is apparent even in young, naïve individuals (Müller-Schwarze, 1972). In a comprehensive study of aversive behavior following predator scent application, Kuijper et al. (2014) found a 46% increase in vigilant behavior on plots treated with wolf feces as an olfactory cue, and as a result, vegetation damages were minimal as a function of the time trade-off between vigilance and foraging. Urine application from cougar (*Felis concolor*), coyote (*Canis latrans*), wolf (*Canis lupus*), lynx (*Lynx canadensis*), bobcat (*Lynx rufus*), and jaguar (*Panthera onca*) all reduced foraging damages to surrounding vegetation and aversive behavior in cervids (Müller-Schwarze, 1972; Sullivan et al., 1985; Swihart et al., 1991). However, cervids are not the only wildlife in any given treatment area, and other non-target species may also be affected by intentional urine application as a repellant.

Many North American predators communicate via scent-marking and urinating to delineate territorial boundaries, signal mating potentials, and other pertinent information (Gese & Ruff, 1997; Gosling, 1982; Shivik et al., 2011). Because of this behavior, predators revisit known scent sites. In a study evaluating the effects of intentional coyote urine application on subsequent individuals in the area, Shivik et al. (2011) found urine application increases coyote visitation. Conversely, top order predators suppress and deter mesopredators from areas via olfactory cues, suggesting complex interspecific predator interactions (Moseby et al., 2012). Evaluating the potential attractive or repellant qualities of predator odor is imperative to understand in the context of cervid visitation and vegetation damage as non-lethal control methods become increasingly necessary to balance conservation and human-wildlife conflict.

Objectives

This research seeks to evaluate the spatial and temporal response of elk to the application of large vertebrate predator urine in areas where congregation and vegetation damages are prevalent. Additionally, this study will evaluate potential effects of predator urine repellants on non-target species such as endemic predator occurrence. Analyzing the relative effectiveness of predator odor repellants to cervids and any attractive qualities influencing predator visitation will inform future management efforts that pursue this non-lethal dispersive method.

Specific Aims

- Analyze spatial and temporal patterns of elk visitation in response to repellant application
- Quantify the vegetation damages in elk congregation areas after predator odor application
- Analyze local predator visitation occurrence after repellant application

Hypotheses

1) Elk will avoid areas where predator urine is detectable due to inherent interspecific predator identification.
2) Elk avoidance will reduce vegetation damage as a result of less time spent foraging and more time spent vigilant where predator odor is detectable.
3) Predator odor application will increase endemic predator visitation to application locations because the odor is disjunct from natural and unknown territory boundaries and scent marking locations.

Section 3: Methods

Study site and species

This study will be conducted near Dove Creek, CO in three sunflower farms that have previously experienced monetary compensation for elk damages to crops (Colorado Parks and Wildlife, 2020). In prior years, farmers experienced 100% crop losses from cervid depredation (H. E. Johnson et al., 2014) and are interested in seasonal repellant methods to reduce crop damages and elk congregating on their land. All sites will be located on farms adjacent to wildland canyons and native vegetation in compliance with ideal elk habitat as defined by Vasquez et al., (2005)’s elk species evaluation. Daily and weekly assessments will occur from

July 2022 to September 2022. Eighteen random plots fitting the site criteria will be located using ArcGIS across the three farms and a 3x3m area will be set up using 6-ft t-posts. Plots will be at least 500m from each other to minimize scent contamination from other plots.

Vegetation Damage Assessment

To evaluate vegetation damage, I will transplant 20 sunflowers from the given farm, averaging 1m in height, into each plot. Only non-damaged sunflower will be planted to ensure all measured damages result from cervid grazing. Sunflowers will be assessed every two days for browsing damages to the terminal seeds, flower head, or trampling of the plants, quantifying the number of damaged sunflowers as ratio to the total plot (H. E. Johnson et al., 2014).

Elk Visitation Assessment

To evaluate the efficacy of predator urine as an elk repellant, all plots will be randomly assigned a urine treatment (three replicates for each treatment), with cow urine and water serving as controls and black bear (*Ursus americanus*), mountain lion (*Puma concolor*), coyote (*Canis latrans*), or grey wolf (*Canis lupus*) urine serving as repellants to match endemic predators in the area known to predate on elk (Vasquez et al., 2005). The grey wolf is historically a natural predator of elk but is endemically extinct in Colorado. All predator urines will be ordered online (www.predatorpee.com; Maine Outdoor Solutions, 2706 Union St., Hermon, Maine 05501 USA) and cow urine will be collected locally from volunteer pastoralists to be used as a non-threatening scent in the case that water is contaminated with human scent.

Treatments will be applied at each plot corner. A 6ft t-post with a dispenser mounted on top will be refilled with 12ml of urine every seven days throughout the treatment period in accordance with van Beeck Calkoen et al., (2021). A plastic covering will reduce possible scent diffusion by rain or field watering.

Elk visitation will be recorded with motion-activated camera traps placed 3m from the northern most corner of each plot with a clear view of the entire plot. Upon activation, the camera will record a 30 second video. Within each recorded interval, individuals who are visible the longest will be recorded for vigilance or foraging behaviors with continuous focal sampling. Vigilance is defined as an erect head without foraging and evidenced with olfactory, visual, or auditory scanning behavior (van Beeck Calkoen et al., 2021). Foraging is defined as an animal actively searching for food, feeding, or browsing (van Beeck Calkoen et al., 2021).

Predator Visitation Assessment

To evaluate endemic predator visitation to urine treatments, any non-target species presence and duration of visit will be recorded using the camera traps.

Statistical analysis

To determine differences in vegetation damages and non-target species visit duration averages between each treatment and the controls, I will conduct several one-way analyses of variance (ANOVAs) for each metric followed by any necessary post-hoc Tukey-tests for pairwise comparisons to measure individual significance. To analyze differences in proportions of foraging and vigilance behaviors between treatments and the controls, I will use a logistic regression. I will compare multiple linear regressions that look for an effect of predator urine treatments after accounting for the potential visitation of predators. All data analyses will be conducted using R 4.1.0.

Potential negative impacts

The potential negative ecological impacts of this study will be minimal as plots are temporary and any vegetation trampling that occurs will be contained to each plot. Some damage to crops may occur and any that occur outside of designated plots will be eligible for CPW-provided monetary compensation. Long term effects on the environment are not expected.

Research Schedule

Research Activity	July	August	September	October	November
Site selection and set up	X	X			
Field data collection	X	X	X		
Behavioral data coding		X	X	X	
Site break down			X	X	
Data analysis				X	X
Compile and submit report					X

Section 4: Budget

Item	Justification	Cost per Unit	Quantity	Total
Cuddeback C123 Triple Flash Trail Camera	To capture visitation and behavior data	$229.99	18	$5,399.82
Memory cards	For replacement in camera traps	$7.67	36	$276.12
AAA Batteries	For replacement in camera traps	$0.59	288	$169.92
6 ft t posts	For mounting urine treatments and camera traps	$4.99	90	$449.10
Urine	Treatment for plots	$125.00 for 64oz.	2 bottles for every urine type – 8 total	$1,000
Scent Dispenser	To allow urine scent to be detected	$7.00	18	$126
Purified water	Control treatment for plots	$2.99/gal	3	$8.97
Garmin eTrex 10 Handheld GPS Unit	To locate plot points in each farm	$99.99	1	$99.99
Air BNB	Place to stay during fieldwork	$38/night	34 nights	$1,292.00
Milage	For transportation to and from sites	$0.58/mile	---	$300.00

6-week Intern	To assist with research and procedures	$800	1	$800.00
Total resource expenditures				$9,921.92

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CHAPTER 3. JOURNAL MANUSCRIPT

Examining Post-release and Seasonal Movements in Wyoming Toads (*Anaxyrus baxteri*) for Current Recovery Efforts

Abstract

Movement ecology can be a useful tool to provide key data for reintroduced species and their conservation. The Wyoming toad is an extremely endangered species that has been the subject of reintroduction efforts for the past 30 years. Here, we described initial post-release movements of reintroduced individuals and characterize their dispersal patterns throughout the summer season. Using radio telemetry data from over 200 released individuals through 2019-2021, we fit mixed-effect models to describe toad movement rates. We found that both male and female toads initially disperse immediately following release but then movement rates decline, indicating establishment into the environment. This behavior is likely a combination of a flight-like response and necessary exploration for resources. Seasonally, male movement rates increased then decreased, while females moved at a more consistent rate throughout the summer. Females may display more consistent seasonal movements than males due to greater site fidelity. Our results suggest that it may be biologically useful to mitigate potential mortality during a released toad's initial dispersal period. Increased movement of males through the summer stresses the importance of site connectivity for increased population health and viability. Consequently, investigating additional opportunities for safe harbor agreements with private landowners near existing reintroduction sites may enhance the long-term recovery of the Wyoming toad.

Introduction

The reintroduction of endangered species into their native habitats has become an increasingly common tool conservation practitioners use for many taxa (Bubac et al., 2019; Germano & Bishop, 2009; Griffiths & Pavajeau, 2008). By translocating captive-bred or wild individuals from other populations, wildlife managers can re-establish healthy, self-sustaining populations in former habitats that have lost the ecosystem services provided by that species (Beck et al., 1994). Re-establishing amphibian populations is particularly important because of the ecosystem services they provide, including regulating insect population dynamics and altering nutrient cycling (Hocking & Babbitt, 2014). However, for amphibian species, rapid population declines in combination with data deficiencies and poor monitoring have forced a disproportionate reliance on captive breeding programs for species-specific conservation efforts (Bubac et al., 2019; Stuart et al., 2004). Unfortunately, the success of relocation efforts is low for herpetofauna, where only 22% of all reintroduction programs have resulted in stable populations that do not require active management (Griffiths & Pavajeau, 2008).

Unfortunately, many amphibian populations declined shortly after their discovery, thus halting the accrual of natural history data on these species (Dreitz, 2009; Scheele et al., 2021; Stuart et al., 2004; Tapley et al., 2015). Lacking basic natural history information, recovery efforts have relied on surrogate data from congeners or confamilials, which has contributed to very low reintroduction success rates in amphibians (Beck et al., 1994; Bubac et al., 2019; Cushman, 2006; Griffiths & Pavajeau, 2008). Moreover, the unwavering persistence of infectious diseases, such as chytridiomycosis, paired with systemic habitat disturbance and fragmentation significantly threaten species recovery and management efforts (Berger et al., 1998; Deguise, 2007; Gerber et al., 2018; Graeter et al., 2008). Factors influencing the success of reintroduction efforts can be investigated by observing the movement ecology of amphibians following their release into the wild.

Seasonal migration patterns and post-release movements of reintroduced individuals are often driven by their cryptic ecological needs and preferences that would otherwise be difficult to discern (Allen & Singh, 2016; Bailey & Muths, 2019; Pyke, 1983). Many amphibian species migrate annually to breed or hibernate in new habitats whose conditions meet their specific ecological requirements (Pittman et al., 2014). Anurans, for example, are constrained by narrow physiological requirements (Pittman et al., 2014; Sinsch, 1990), and consequently limit their migration to no more than 15 km on average (Sinsch, 1990). Over the course of the year, anurans in temperate climates use environmental conditions to cue movement between four distinct habitat types for hibernation, estivation, breeding, and feeding. For example, during its breeding season, the Western spadefoot toad (*Spea hammondii*) moved farther to reach and moved more frequently near ephemeral ponds, the toad's prime breeding habitat (Halstead et al., 2021). Therefore, studying spatiotemporal dispersal patterns can provide useful data that can inform managerial decision-making for data deficient species, such as identifying habitat features that contribute to survival of reintroduced animals at recovery sites (Allen & Singh, 2016; Bailey & Muths, 2019; Bowler & Benton, 2005).

The Wyoming toad (*Anaxyrus baxteri*) is an understudied species whose conservation and management may be improved by learning more about its movement ecology. Once found abundantly throughout the floodplains of the Laramie Basin, Wyoming, the species was listed as endangered under the Endangered Species Act (ESA) in 1984 (United States Fish and Wildlife Service [USFWS], 1984). The few remaining individuals were taken into captivity to establish a captive breeding program in 1994, and recovery efforts have persisted to this day (USFWS, 2015). Despite reintroduction and monitoring programs operating over the past 20 years, how these toads move after release is not well understood (Baxter et al., 1982; Geraud & Keinath, 2004). Typically, translocation of captive-bred amphibians into any novel environment may prompt a period of rapid movement upon release prior to relatively stable movement thereafter (Hammond et al., 2021; Rojahn et al., 2018; Zhang et al., 2019), but whether this is true for the Wyoming toad is not known. Moreover, sources describing seasonal movements of the Wyoming toad among key habitats differ on whether the toads migrate prior to hibernation (Geraud & Keinath, 2004). This short migration may help the toad find ideal burrowing areas in covered, saturated substrates or unoccupied rodent burrows for the wintering period (Geraud and Keinath, 2004). Understanding post-release movements as well as seasonal movements is imperative to identify where toads should be released in the future and to describe the spatial patterns exhibited in repatriated communities.

We used radio telemetry data to characterize the movement patterns of Wyoming toads immediately following their release and throughout the breeding, post-breeding, and pre-hibernation seasons. After release in both male and female toads, we expected an initial dispersal period, characterized by large, exploratory movements followed by a notable reduction in movement indicative of an individual's relative establishment into the selected habitat. Although we expected movement rates would be reduced during the breeding season because toads are restricted to ideal breeding habitats, we did predict that females would move more quickly than males because they seek out stationary calling males (Geraud and Keinath, 2004). We also expected movement rates to increase approaching the pre-hibernation period as toads searched for appropriate sites for hibernacula. Finally, we expected significant site differences in toad movements because land use and habitat vary across current locations where toads are released in Albany County (USFWS, 2015). We expected toads to move at a faster rate in sites dominated by lentic water bodies and ephemeral ponds than in sites with perennial lotic water bodies because of time constraints imposed by ephemeral pond drying.

Methods

Study sites

Field data were collected by Rachel Arrick, who also designed the study, and numerous technicians associated with both the University of Wyoming and the U.S. Fish & Wildlife Service. The four sites currently used for reintroductions were selected by the Wyoming Ecological Services Field Office of the U.S. Fish & Wildlife Service (WYES) in accordance with the revised Wyoming toad recovery plan from 2015 (USFWS, 2015). Mortenson Lake National Wildlife Refuge (MLNWR) is the location where the last remaining individuals were discovered in 1987 (USFWS, 2015). The three additional release sites are located on private property whose owners have signed a Landowner Cooperative Management Agreement (LCMA) as part of the Wyoming toad Safe Harbor Agreement (SHA) aptly named The Buford Foundation SHA (site 2), Lindzey SHA (site 3), and Outrider SHA (site 4) (USFWS, 2015). All four sites are located within Albany County, Wyoming and differ in the relative amount of appropriate habitat conditions for the toads (Geraud & Keinath, 2004; USFWS, 2015). The Buford Foundation SHA and MLNWR have large, perennial lotic water bodies with open canopy, whereas Lindzey SHA and Outrider SHA have lentic water bodies, ephemeral ponds, and denser canopy (Figure 1).

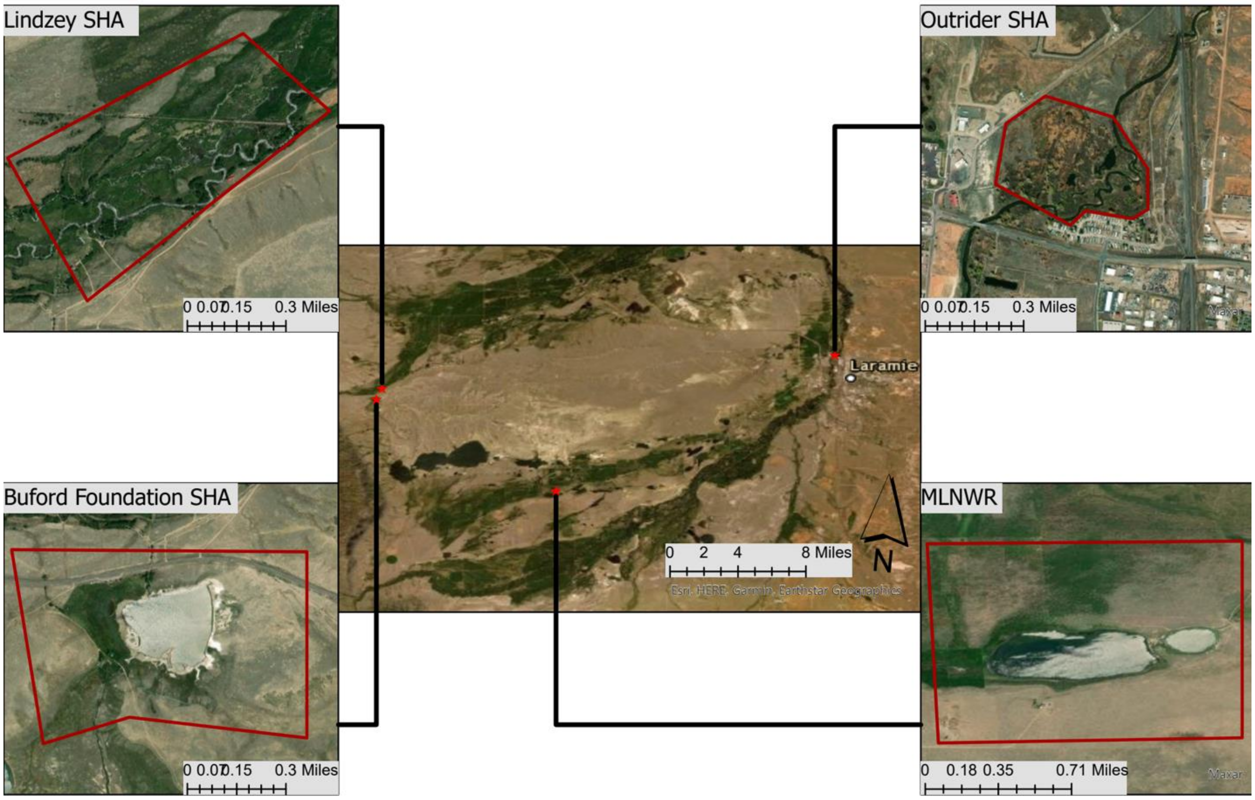

Figure 1. Map of all four release sites. Red stars indicate site location and red outlines represent the general boundaries of the site and are not indicative of official property lines. Map was created using ArcGIS Pro (Version 2.5) (2020).

Study Animals and Radio Transmitters

Captive-bred individuals are reintroduced annually throughout the summer, and from 2019 through 2021 included toads fitted with telemetry transmitters. Focal individuals for each year were randomly selected from different cohorts at the Saratoga Fish Hatchery, Leadville Fish Hatchery, and Cheyenne Mountain Zoo. Each toad was weighed, measured for snout-vent length (SVL), and swabbed for baseline pathogen load, bacterial community composition, and genetic material approximately one week prior to release. The unique PIT-tag identification numbers were recorded for each individual and a shorter ‘toad ID’ was assigned for streamlined data collection. Radio transmitters were attached via belts and the total weight of the units were less than 10% of the toad’s total body weight at the time of release (Bartelt & Peterson, 2000). Belts included a Holohil BD-2 transmitter and polyethylene surgical tubing connected with fishing eyelets and superglue and was designed for quick-release if necessary (Bartelt & Peterson, 2000). Toads were monitored for belt-fit prior to release and throughout the season and refitted when necessary. Within two hours of collection, toads were transported to the release sites in containers whose temperature was maintained between 70°F and 80°F. The IACUC study number is: 20190524MM00371-01 with WGF Chapter 33 Permit 1348 and USFWS ESA Permit TE37953D.

Reintroduction and Post-Release Monitoring

Toad releases occurred annually throughout the summer and were staggered to take place approximately mid-June, late-June, mid-July and mid-August. Released individuals were spread roughly evenly throughout the four release sites (Table 1).

Table 1. Number of radio-tagged toads released at each site during the 2019, 2020 and 2021 field seasons.

YEAR	DATE	SEX	MORTENSON	BUFORD	LINDZEY	OUTRIDER
2019	**May 31**	Males	0	4	3	0
		Females	0	4	3	0
	June 10	Males	0	4	4	4
		Females	0	5	4	5
	August 14	Males	1	1	2	2
		Females	1	1	1	1
TOTAL 2019			**2**	**19**	**17**	**12**
2020	**June 12**	Males	3	2	2	2
		Females	4	4	3	3
	June 26	Males	2	3	2	2
		Females	3	4	4	3
	July 8	Males	2	2	3	2
		Females	3	3	4	4
	August 11	Males	1	2	0	2
		Females	2	1	0	2
TOTAL 2020			**20**	**21**	**18**	**18**
2021	**June 11**	Males	3	3	2	3
		Females	3	3	4	3
	June 25	Males	1	3	2	3
		Females	4	2	3	3
	July 9	Males	2	2	2	2
		Females	4	4	4	4

	August 3	Males	3	0	0	0
		Females	4	0	0	0
TOTAL 2021			**24**	**17**	**17**	**18**

Toads were relocated 1-2 times weekly at various times of the day with a Communications Specialist R-1000 receiver and a Yagi 3-element antenna. After finding a toad, the GPS coordinates of the toad's location were recorded as precisely as possible. If the toad could not be found, its location was estimated as accurately as possible. Toads that could not be visually sighted were prioritized for accurate, visual relocation in subsequent weeks.

Upon every other discovery of an individual, a visual health assessment was conducted and *Batrachochytrium dendrobatidis* (*Bd*) and microbiome swabs were collected. The toad's mass, SVL, body temperature, microsite temperature, activity status and habitat assessments of the microsite and surrounding area within 15 and 30 meters of the located toad were recorded. Temperatures were measured with an IR thermometer. Before release, transmitter belt status was evaluated, and refitting occurred if necessary using vitamin E as a treatment to prevent belt chafing. Transmitter battery life only lasted between 9 and 12 weeks, and belts were replaced before the batteries died to ensure that the toad could be relocated during the fall and its transition into hibernacula.

Toads were actively tracked until dropped transmitters were located, predation and mortality occurred, or transmitters needed to be removed due to visible skin irritation. At each relocation event the toad status was recorded as: actively tracking, confirmed mortality/predation, removed transmitter, lost signal, unconfirmed/inaccessible transmitter, recovered transmitter, or tracking to hibernation. For mortalities, when a body was recovered, information on the nature of its death was recorded including notes on the surrounding area and body and transmitter condition. Dead toads were either frozen or fixed in formalin and sent to

Dr. Allan Pessier at the Washington Animal Disease Diagnostic Laboratory for necropsy to ascertain the cause of death. If a toad was tracked into hibernation in the fall, and the location was confirmed over several weeks, a hibernation enclosure crafted from mesh wiring (approximately 2-3 feet wide x 2-3 feet tall) was placed around the location and was partially buried to ensure stability throughout the winter. Additionally, game cameras and HOBO loggers were placed and buried near the toad to monitor body temperature over the hibernation period.

Breeding, post-breeding, and pre-hibernation seasonal timings are currently unconfirmed and remain hypothesized periods of time for the Wyoming toad. Therefore, we used toad survey records from the Fish & Wildlife Service to determine the date criteria for each season. The latest egg masses observed were recorded on June 20th, 2014, so any period prior to June 30th was categorized as the breeding season (Per. Comm. Doug Keinath). Dates between July 1st and August 20th were categorized as post-breeding season, and any movement after August 20th was categorized as occurring in the pre-hibernation season. Few toads (34 toads, 85 relocations) remained actively tracked into pre-hibernation, however, toads are hypothesized to enter hibernacula from mid-September through late October depending on environmental conditions (Geraud & Keinath, 2004; USFWS, 2015).

Statistical Analysis

The total data set consisted of 1,020 relocations (excluding release locations). A total of 221 relocations were removed from the analysis due to GPS location inaccuracies and instances where the transmitter signal was lost, or the toad was inaccessible. The removed relocations included all observations for nine toads due to missing release location information. This resulted in a final dataset of 981 locations events for 219 toads.

Distance moved and elapsed time between each successive toad relocation were calculated for each toad using the *move* package in R (Kranstauber et al., 2021). Movement rates were then calculated by dividing distance moved by time elapsed and reported between relocation events in meters per day (Kranstauber et al., 2021).

To understand how quickly toads moved pre- and post-establishment, each toad's first relocation was categorized as its pre-establishment period into the new environment (approximating a one-week time-period), and all subsequent relocations were categorized as post-establishment (i.e., seasonal) movement. Linear mixed effects models (LMM) were fit to describe movement rate as a function of pre- and post-establishment periods for each year separately and for all three years combined. Movement rates were log10-transformed (+1 added to 0 responses) to normalize variance. Fixed effects included sex, period (pre- and post-establishment), and their interaction. In the analysis combining all three years, year and its interaction with period were also included as fixed effects to account for annual differences in movement rates. Toad ID was fit as a random effect to describe variation in movements between individual toads. We also explored site and release date as fixed effects by conducting maximum likelihood ratio tests for their addition to the model, then describing the final model based on restricted maximum likelihood (Zuur et al., 2009)

To understand the movements of toads during the post-establishment period, relocations were modeled as seasonal movements in a separate LMM for each year and for all three years combined, with all pre-establishment relocations removed for the analysis. First, we assumed that seasonal movements would follow a quadratic function of cumulative time since 'emergence'. This emergence period began May 1st when temperatures regularly exceed 70°F, a threshold for emergence (Geraud & Keinath, 2004). In addition to cumulative time since emergence, the toad's sex and an interaction between sex and cumulative time since emergence for both the linear and quadratic terms were fit as fixed effects to account for any potential differences between the sexes and their rate of movement. Year was included as a fixed effect in the model that combined all three years. Again, we included individual toad IDs as a random effect, however in the model specific to a single year, rank deficiencies prevented us from fitting these effects. We also explored site as a fixed effect by conducting likelihood ratio tests for their addition to the model. For all additional parameters, we used maximum likelihood ratio tests for their addition to the model, then described the final model based on restricted maximum likelihood (Zuur et al., 2009). The 95% confidence intervals for vertex peaks were calculated through permutation as $\frac{-\beta_1}{2\beta_2}$, by drawing 1000 random coefficients assuming the coefficient estimates followed a multivariate normal distribution.

All statistical analyses were conducted using R version 4.1.0 (R Core Team, 2021) in RStudio version 1.4.1717 (RStudio Team, 2021), confidence intervals of estimates were derived from generalized linear hypothesis tests, and effects were considered significant based on a Satterthwaite's derived alpha of 0.05. AICs were calculated and compared to the best model and a significance threshold of a Δ AIC value of 4 was used.

Results

On average, both male and female toads were relocated four times (SD = 3.3). From release to final relocation males moved an average of 413.6 meters total (95% CI: 272.16 - 555.10 meters). From release to final relocation females moved an average of 246.5 meters total (95% CI: 186.63 – 306.36 meters), traveling on average 167.1 meters less than males (95% CI: 13.96 - 320.29 meters, p-value = 0.032).

Pre-establishment Movement

Using AIC-based model selection, we found that the best fitting model was one that did not include any additional parameters beyond the categorization of pre- and post-establishment (Table 2, AIC = 540.9, weight = 0.502). The best model indicated that toads regardless of sex moved significantly faster ($p = 0.005$) in the pre-establishment period (9.6 m/day, 95% CI: 7.4 – 12.6 m/day) than in the post-establishment period (6.5 m/day, 95% CI: 3.2 – 11.4 m/day). However, the second-best fitting model was one that included the addition of toad sex and its interaction (Table 2, AIC = 542.9, weight = 0.182), within a ΔAIC of 4. Therefore we chose to further describe the effects from this model that included sex, because this is a variable of key interest to our hypotheses. The addition of year and site as parameters to predict toad movement rates did not statistically improve the fit of the model and did not significantly affect the movement rates of toads between pre- and post-establishment (Table 2).

Table 2. AIC model selection table for relevant models containing log-transformed toad movement rates as a function of pre- and post-establishment movement classifications (ReleaseMov). Each model included individual toad movement as a random effect. Colons in column names imply interactions between variables.

Model Rank	ReleaseMov	Sex	ReleaseMov: Sex	Year	ReleaseMov: Year	Sex: Year	Site	ReleaseMov: Site	AIC	ΔAIC	Weight
1	*								540.9	0.00	0.502
2	*	*	*						542.8	1.99	0.186
3	*	*	*	*					545.7	4.84	0.121
4	*	*	*				*		545.7	4.87	0.120
5(null)									546.7	5.88	0.026
6	*	*	*	*			*	*	547.0	6.13	0.023
7	*	*	*	*	*				547.6	6.72	0.017
8	*	*	*	*	*	*			550.9	10.02	0.003
9	*	*	*	*	*	*	*	*	555.4	14.55	0.000

After accounting for individual toad differences, females but not males moved more slowly in the post-establishment period than in the pre-establishment period. Female toads moved significantly faster (Figure 1, t = -2.89, p = 0.0041) during the pre-establishment period (median rate: 10.0 m/day, 95% CI: 6.7– 14.9 m/day) than they did during the post-establishment period (median rate: 5. 9 m/day, 95% CI: 4.9 – 7.7 m/day), representing a reduction in median movement rate of 58% (95% CI: 41 – 85 %) between the pre- and post-establishment periods. Female toads traveled an average of 43.5 meters (95% CI: 26.7-70.9 meters) within the pre-establishment period. Male toads moved slightly faster (Figure 1, t = -1.105, p = 0.270) during the pre-establishment period (median rate: 9.2 m/day, 95% CI: 5.6– 14.9 m/day) than they did during the post-establishment period (median rate: 7.2 m/day, 95% CI: 5.4 – 9.5 m/day), representing a reduction in median movement rate of 21% (95% CI: 48.2 % decrease – 20.5 % increase) between the pre-and post-establishment periods. Male toads traveled an average of 45.6 meters within the pre-establishment period (95% CI: 25.2 - 82.7 meters). Additionally, after accounting for individual toad differences, male and female movement did not significantly differ during either the pre-establishment period (Figure 1, t = -0.333, p = 0.74), nor the post-establishment period (t = 1.032, p = 0.30).

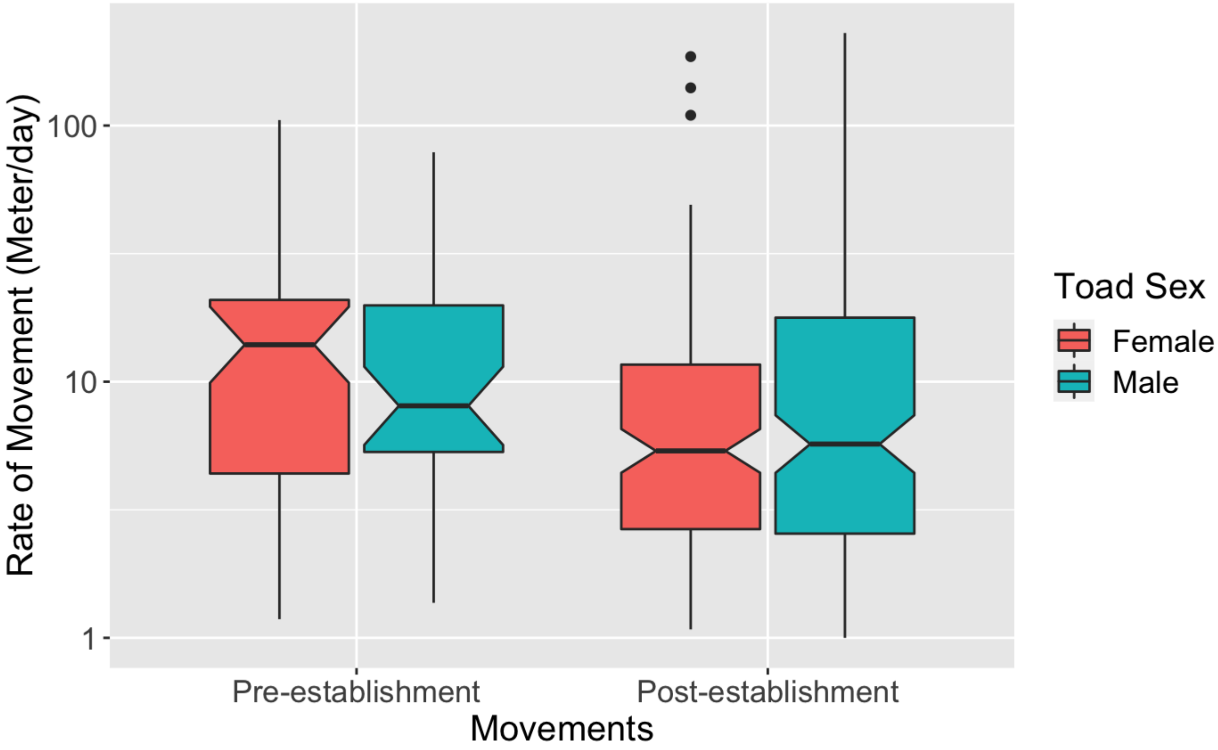

Figure 2. The established movement rate (meters/day) of females was significantly lower after an initial post-release period and males followed a similar through statistically insignificant trend. The black bars indicate the median, and the upper and lower quartiles. Whiskers extend to the minima and maxima and outliers are depicted as points.

Seasonal Movement

Using AIC-based model selection, we found that the best fitting model with seasonal movement rates as the response was a quadratic model of cumulative time since emergence that included toad sex and its interaction with cumulative time since emergence, and site (Table 3, AIC = 793.6, weight = 0.662).

Table 3**.** AIC model selection table for relevant models containing log-transformed toad movement rates as a function of cumulative time since emergence (CTE). Each model included individual toad movement as a random effect.

Model Rank	CTE	CTE^2	Sex	CET:Sex	CTE^2:Sex	Site	CTE:Site	CTE^2:Site	Year	CTE:Year	CTE2:Year	AIC	ΔAIC	Weight
1	*	*	*	*	*	*						793.6	0.00	0.662
2	*	*										796.4	2.78	0.165
3	*	*	*	*	*							797.5	3.93	0.093
4	*	*	*	*	*				*			799.0	5.41	0.044
5	*	*	*	*	*	*	*	*				801.0	7.46	0.016
6	*	*	*	*	*				*	*	*	802.1	8.56	0.009
7(null)												802.8	9.18	0.007
8(linear)	*											803.8	10.24	0.004
9	*	*	*	*	*	*	*	*	*	*	*	807.6	13.99	0.001

After accounting for individual toad differences, toad movement in males but not females displayed a significant hump-shaped response, initially increasing and then decreasing later in the summer. We found that seasonal movement rates of male toads were significantly parabolic (Figure 2, $t = - 2.003$, $p = 0.0457$). Male toads also exhibited a more defined shift in movement over the summer than females, and the greatest movement rates for male toads occurred on August 8th, 99 days (95% CI: 91 – 111 days) after their hypothetical emergence on May 1st. Female toads did not display a hump-shape through the summer ($t = -0.301$, $p = 0.7631$). Females did have a mathematically identifiable peak, occurring August 4th, 95 days (95% CI: -10.7 – 291.0 days) following their emergence on May 1st, but the estimate was quite uncertain (Figure 2). Additionally, after accounting for individual toad differences and the quadratic effect of time since emergence, we found toads at Outrider SHA displayed significantly lower movement rates than those at any other field site (MLNWR: $t = -2.102$, $p = 0.037$; Buford Foundation SHA: $t = -2.973$ $p = 0.015$; Lindzey SHA: $t = -2.667$, $p = 0.037$) even though the other three sites showed similar movement rates ($p > 0.05$)

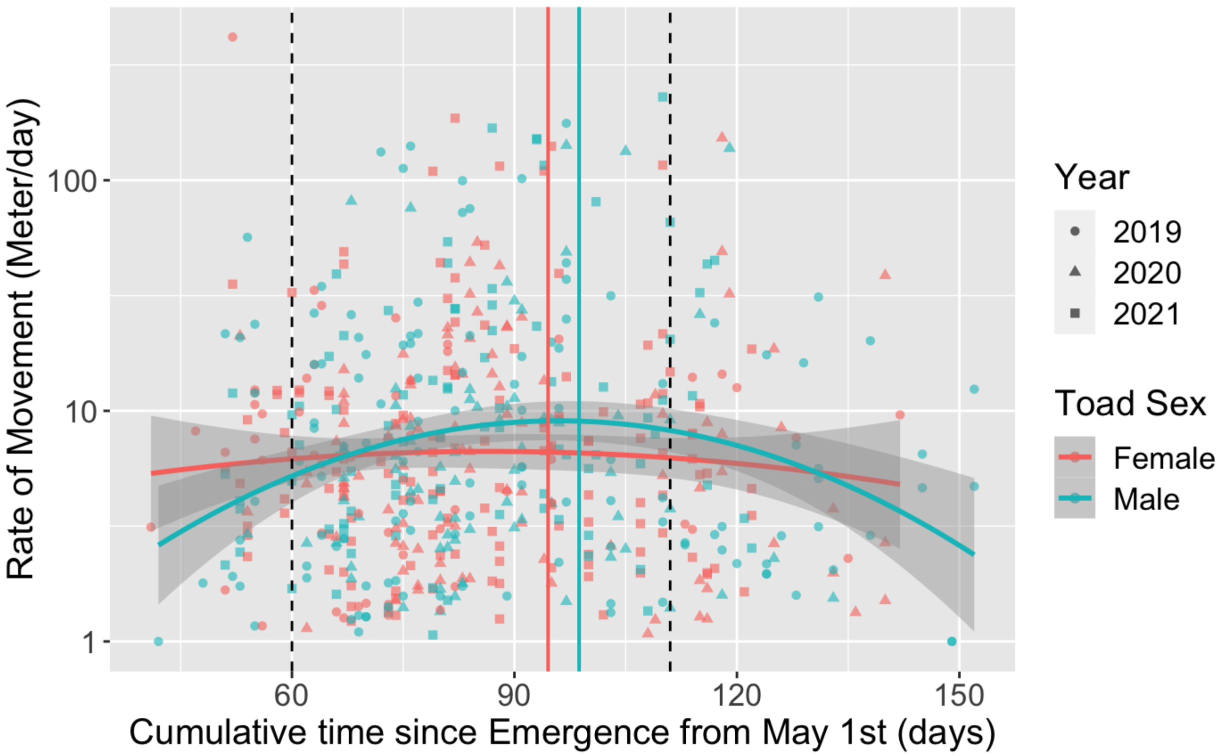

Figure 3. Scatter plot of movement rates as a function of cumulative time from emergence. Male and female movement rates peaked in early August. The grey-shaded areas represent the 95% confidence intervals from a quadratic model. The solid-colored lines represent the peaks in for the matching parabola. The black dashed lines signify the boundaries of the hypothesized breeding, post-breeding, and pre-hibernation seasons respectively.

Discussion

The recovery of the critically endangered Wyoming toad is ongoing, and the goal of this study was to evaluate the movement rates of Wyoming toads relative to their release date and characterize their patterns of movement. This information will help inform conservation and management decisions, such as the selection of future safe harbor sites to facilitate population connectivity. While movements of Wyoming toads have been briefly examined in past studies, an analysis over multiple years provides a more robust investigation of toad movements and dispersal rates in these reintroduced populations.

In accordance with our hypotheses, we found greater rates of movement during the first week following reintroduction compared to subsequent movements as well as significant variation in male movement rates through the summer season.

Across both captive-bred and wild taxa, exploratory movements following release are common in reintroduced populations as individuals establish themselves into an unfamiliar environment (Berger-Tal & Saltz, 2014). As anuran species are bound by strict habitat requirements to fulfill physiological needs for moisture (Pittman et al., 2014; Sinsch, 1990), the initial dispersal may aid in efficiently locating resources like cover and water, and explains the similarity of initial dispersal patterns between the sexes. Wyoming toads are released within or nearby suitable habitat (USFWS, 2015), so the relatively small initial dispersal movements we observed are unsurprising compared to other amphibian species. Following release, captive-reared Sierra Nevada yellow-legged frogs (*Rana sierrae*) did not disperse more than 50 m beyond their original release site and displayed greater release site fidelity compared to their wild counterparts (Keung et al., 2012). Similarly, captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) released in suitable core habitat, settled within 50 m of their initial release site following an initial dispersal and exploration period of 21 days (Bodinof et al., 2012). The initial dispersal period we observed in Wyoming toads was similar to Bodinof et al. (2012) and is likely a combination of flight-like response to search for habitat similar to captive conditions or to explore for resources (Berger-Tal & Saltz, 2014). Moreover, the observed movement rate pattern was consistent regardless of the release site or year of release, suggesting this initial dispersal pattern is regular and predictable for reintroduced Wyoming toads, regardless of interannual weather and habitat variability.

Although we predicted female movement would be greater than males throughout breeding and post-breeding periods, instead we found that female toads moved at a generally consistent rate throughout the summer while movement rates of males increased until mid-summer and then decreased later in the season. Peak movement rate for both sexes occurred in early August, earlier than we expected and greatly preceding the suspected pre-hibernation period (late August-September). Our results on peak movement characterized the late season with reduced movement rates and may suggest that hibernation sites are selected as a short-term decision. Differences in dispersal between sexes is occasionally observed in other amphibians (Bartelt et al., 2004; Helfer et al., 2012; O'Brien et al., 2021) but the direction and strength of the differences depend on the species in question (Dervo et al., 2016). In a recent analysis of red-backed toadlet (*Pseudophryne coriacea*) movement, male movement corresponded strongly with precipitation events, whereas female movement corresponded more closely to breeding calls (O'Brien et al., 2021). Additionally, male western toads (*Bufo boreas*) display greater site fidelity than females because larger females have greater water storage capacity, allowing them to disperse greater distances when foraging in the terrestrial ecosystem (Bartelt et al., 2004). Thus, males may prioritize suitable habitats due to their more stringent physiological needs and to gain pre-emptive reproductive advantages. However, despite similar sexual dimorphism in Wyoming toads, dispersal differences between sexes in our study yielded the opposite pattern, suggesting that other factors may have a stronger influence on sex-specific movements for this species.

Habitat selection could play a major role in the dispersal differences of male and female Wyoming toads. For example, female anurans consistently demonstrate greater microhabitat selectivity to meet their immediate thermoregulatory or water needs and to seek greater cover

(Bartelt et al., 2004; Helfer et al., 2012; O’Brien et al., 2021). In a laboratory setting, male common toads (*Bufo bufo*) moved more through resource-limited environments to search for resources, whereas females movements were unaffected by resource availability (Ogurtsov et al., 2018). If the release area proves suitable within the first week, perhaps females remain relatively close to their selected site throughout the rest of the season compared to males, whose movements may correspond with foraging-driven behaviors

Though we initially predicted toad movements would differ between the four sites as a function of differences in habitat and land use of the reintroduction site, we found that toads reintroduced to Outrider SHA moved more slowly than toads at other sites whose movement rates were similar. The differences detected at Outrider SHA are likely a result of far fewer relocations per toad than other sites. Toads released at Outrider SHA were only relocated an average of three times across all three years compared to four relocations at other sites. This may also additionally be due to an apparent abundance of snake-related mortalities associated with this site (Keinath, pers. comm., 2022). Beyond this difference, toads behave similarly across all reintroduction sites regardless of the general habitat profile (e.g., cattle grazing, lentic or lotic water bodies present, willow cover, etc.). Year was also found to have no significant effect on movement rates of toads, solidifying that any climatic or precipitation differences between these years does not play a significant role in the observed dispersal rates of male and female Wyoming toads. This also suggests early August as a consistent period of greater movement rates for the Wyoming toad.

Unfortunately, several caveats exist within our analyses. Because the Wyoming toad is extremely endangered, and the nature of the study prevented daily relocations, a quick-release function of the transmitter belt was imperative to prevent unnecessary mortalities. As a result, many toads slipped or broke their belts preventing additional relocations throughout the entire season (especially at Outrider SHA). Additionally, high mortality rates from predators and amphibian chytrid fungus, *Bd*, caused many toads to be removed from the analysis, lowering the number of relocations per toad and negatively influencing the strength of our interpretations. Moreover, the long-term captive breeding of the Wyoming toad may produce non-adaptive traits and behaviors (Geraud & Keinath, 2004), such as irregular dispersal, that contribute to mortalities following release. Therefore, our inferences are likely only applicable to captively-bred individuals as opposed to natural Wyoming toad populations.

More research is needed to fully understand movement patterns of the Wyoming toad; however, it may be biologically useful to investigate means to mitigate initial dispersal of released toads. Whether or not the dispersal detected in our study is a flight response or exploratory movement, it may result in lower survival rates due to increased predation or dispersal in an unfamiliar environment. The use of soft release enclosures in suitable habitat for tadpole releases of Wyoming toads results in larger individuals upon metamorphosis, thereby improving the likelihood of survival post-release (Polasik et al., 2015). Additionally, captive-reared Ozark hellbenders that exhibited greater release-site fidelity had 1.5 times greater annual survivorship (Bodinof et al., 2012). Typically, reintroduced species dispersing greater distances from their release sites may end up in less-than-suitable habitat, further decreasing the likelihood of survival and fitness following release (Berger-Tal & Saltz, 2014). Soft-release enclosures for adult toads may reduce initial dispersal responses and increase general survival through a season, but more focused research is needed to confirm this.

The detected dispersal through the summer stresses the importance of site connectivity for increased population connectivity. While it is unclear why males exhibited greater rates of dispersal, site connectivity may aid in population viability. Subject to over 30 years of reintroduction efforts, the Wyoming toad has yet to display the necessary population viability in any of the reintroduction sites to relieve the WYES of their toad recovery efforts. There are certainly many factors contributing to the continued need for captive-bred propagation into the wild, including but not limited to *Bd* presence across all reintroduction sites (Keinath, pers. comm., 2022). However, if sites were perhaps within closer proximity to one another, dispersal of male toads may result in migratory behavior necessary for increased population viability and metapopulation health. Site connectivity observed in other anuran species such as the common toad, the canyon treefrog (*Hyla arenicolor*), red-spotted toad (*Anaxyrus punctatus*), and Mexican spadefoot (*Spea multiplicata*) translated to increased genetic population connectivity (Bounas et al., 2020; Halley et al., 1996; Mims, 2015). Therefore, it may be worth investigating additional opportunities to draft safe harbor agreements with private landowners near existing reintroduction sites to increase site connectivity as a part of the long-term recovery of the Wyoming toad.

The reintroduction of endangered amphibians faces numerous threats on both environmental and anthropogenic fronts, with habitat fragmentation, *Bd* presence, and data deficiencies playing major roles in unsuccessful current species recovery efforts (Griffiths & Pavajeau, 2008). These hinderances are difficult to combat from a managerial viewpoint, but understanding how reintroduced individuals move post-release can help managers assess whether the assumptions under which they operate reintroduction programs are justified. The Wyoming toad is no exception and has been subject to reintroduction and monitoring efforts for over 30 years with limited success (Baxter et al., 1982; Geraud & Keinath, 2004). This study expands the knowledge of the movement characteristics of this repatriated species with actionable implications for future management practices and their ultimate success.

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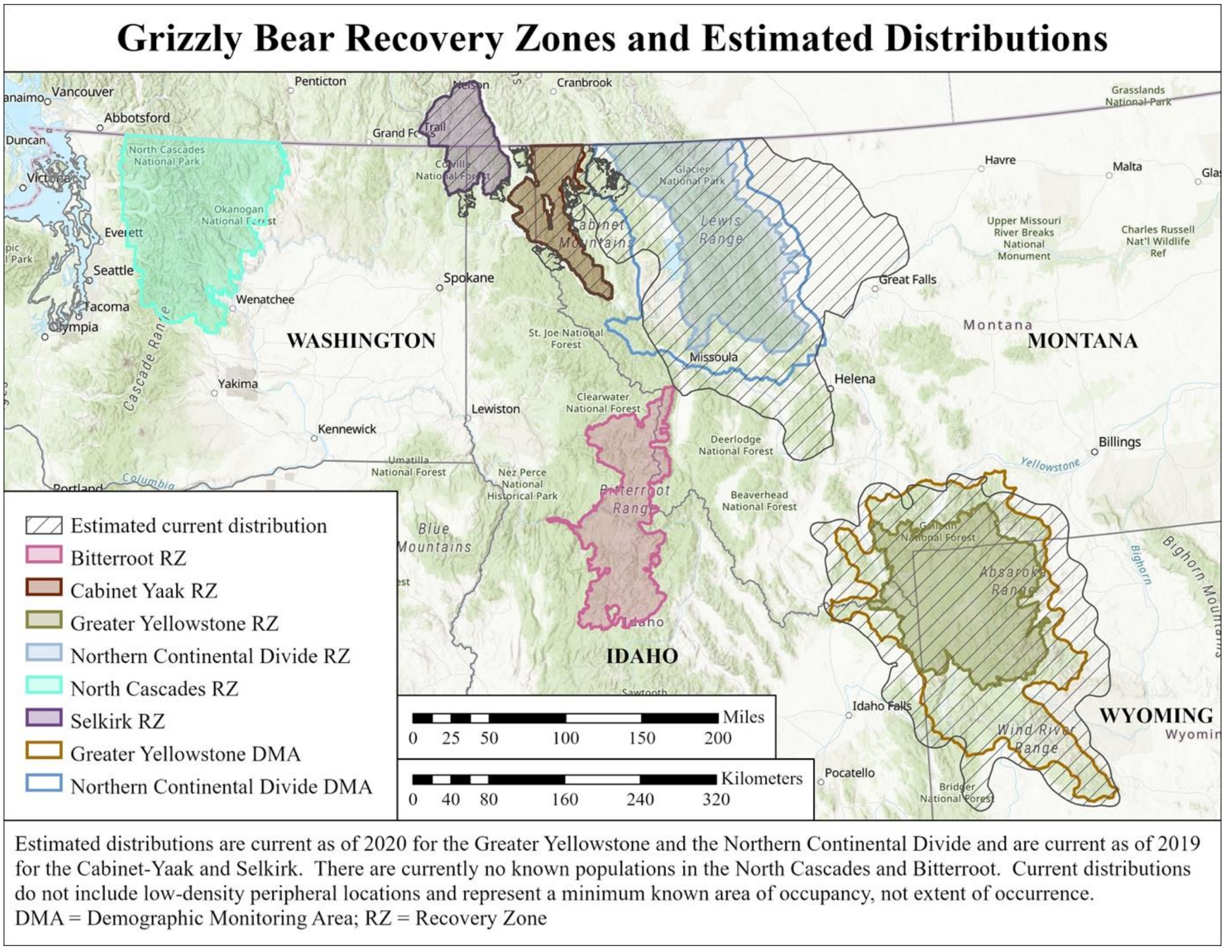
CHAPTER 4

Resolving the Success of Grizzly Bear Conservation in the Greater Yellowstone and Northern Continental Divide Ecosystems: Learning to Live with Large Terrestrial Predators

Introduction

The grizzly bear (*Ursus arctos*) was once abundant throughout western and central North America; its population was estimated to total over 50,000 in the lower 48 states prior to extensive European settlement (U.S. Fish & Wildlife Service, 2021; Wilkinson, 1998). With the arrival of European settlers in the West during the mid-19th century, the bear's range shrank and its population rapidly declined. After the bear's population was nearly extirpated by the 1970s, the species was listed as "threatened" under the Endangered Species Act (ESA) (Mattson & Merrill, 2002; U.S. Fish & Wildlife Service, 2021; Wilkinson, 1998). The U.S. Fish & Wildlife Service (USFWS) implemented on-the-ground monitoring and management efforts in 1983 for remnant populations and in a 1993 species recovery plan identified six ecosystems of contiguous habitat spanning Washington, Idaho, Montana, and Wyoming (USFWS) (Figure 1) (U.S. Fish & Wildlife Service, 2021). Through USFWS's ongoing monitoring and reintroduction efforts, populations in the Northern Continental Divide and Greater Yellowstone Ecosystems (NCDE and GYE, respectively) have increased and have approached a sustainable threshold of over 1,000 bears (U.S. Fish & Wildlife Service, 2021).



Estimated distributions are current as of 2020 for the Greater Yellowstone and the Northern Continental Divide and are current as of 2019 for the Cabinet-Yaak and Selkirk. There are currently no known populations in the North Cascades and Bitterroot. Current distributions do not include low-density peripheral locations and represent a minimum known area of occupancy, not extent of occurrence.
DMA = Demographic Monitoring Area; RZ = Recovery Zone

Figure 4. Current grizzly bear ecosystems and distributions in the conterminous United States. Retrieved from https://www.fws.gov/mountain-prairie/es/grizzlybear.php.

Unfortunately, the increase in grizzly bear populations has resulted in a corresponding increase in encounters and conflicts with humans (Ciarniello et al., 2007; Mattson & Merrill, 2002). The presence of reintroduced bears not only increased predation on livestock and human deaths, but contact with humans also increased bear mortality (Mattson & Merrill, 2002), sparking controversy about future grizzly bear management and policies. ESA delisting has been proposed by both the USFWS and stockgrower associations to mitigate human-bear conflict; however, grizzly bear populations have only recovered within the past 10 years. Current estimates approximate 1,800 bears which occupy only 2% of their historic range in the contiguous US (U.S. Fish & Wildlife Service, 2021). Years of conservation work may be at risk if the bears’ protected status is reduced, especially in the ecosystems where they have yet to exhibit signs of growth and establishment.

However, grizzly bears were absent from the NDCE and GYE areas just 30 years ago but have since rebounded as a result of successful reintroduction efforts managed by USFWS. Because grizzly bears are one of the largest carnivores in North America, there is no denying the growing risk they pose to livestock, residents, and recreationalists as their populations resurge in the ecosystems bears inhabit. This multifaceted human-wildlife conflict is ongoing and will likely persist for many years to come, emphasizing the necessity for solutions and compromise now. As grizzly bears approach sustainable population thresholds in the GYE and NCDE, states must prepare for the communication and management that will be necessary when the bears are delisted in those areas and bear management returns to state control. Moreover, public education and outreach should exploit the potential economic advantages of ecotourism and promote healthy coexistence with bears in the ecosystems they occupy.

The Grizzly Bear

Grizzly bears are a keystone species in the numerous ecosystems they occupy because they are apex predators that also scavenge and excavate when they forage for food (Doak & Loso, 2003; Rosenbaum et al., 2004; U.S. Fish & Wildlife Service, 2021). These activities aid in seed dispersal, maintain soil composition, and aid in the maintenance of healthy fish and terrestrial prey populations. Because of the key roles they play in ecosystems and the variety of habitats they require, grizzly bears are also an umbrella species whose protected status effectively protects other organisms that co-occur with them (Cicon, 2019; Noss et al., 1996; Roberge & Angelstam, 2004). When large reserve areas are protected to support wide-ranging grizzly bear populations, anthropogenic stressors are minimized, and habitat for many other species is likewise protected (Cicon, 2019; Noss et al., 1996).

Loss of large carnivores results in trophic cascades that destabilize ecosystems when top-down control is removed (Palazón, 2017). Loss of apex predators propagates to lower trophic levels when mesopredator populations increase to unsustainable levels and impact smaller prey populations; plant communities respond in turn and geochemical cycling can be disrupted within the ecosystem, (Palazón, 2017). For this reason, restoring and protecting grizzly bear populations enhances the biodiversity of many species at lower trophic levels. However, the tension between stakeholders resulting from unreliable management regimes ultimately threatens the benefits of bears and their persistence in the contiguous US.

Grizzly bear recovery is challenged by the push-and-pull among local, state, and federal jurisdictions. For example, in 2001 the federal Bitterroot Ecosystem reintroduction plan was scrapped due to major opposition from the state of Idaho and local residents and organizations who argued bears are dangerous to the lives of humans and livestock (Burnham & Mott, 2021). In 2007 grizzly bears within the GYE were delisted only to be relisted in 2009 following a ruling by a federal judge in Missoula, Montana, that argued delisted bear management was inadequate because the USFWS didn’t account for the decline of the bear’s key dietary and habitat necessities (such as whitebark pine) in their management plans (Burnham & Mott, 2021). In 2017 grizzlies were delisted again, only to be relisted for reasons similar to those in 2009 (Burnham & Mott, 2021). Groups such as the Alliance for the Wild Rockies (AWR) conservation group are specifically concerned that delisting would result in a loss of over 30 years of reintroduction and recovery efforts. AWR files lawsuits that challenge each delisting proposal and has acted as a major voice in maintaining the protected status of the species.

Stakeholders

The U.S. Fish & Wildlife Service

Although the management of grizzly bears transcends state borders and remains an interagency effort, primary jurisdiction falls upon the USFWS, bound by federal law and the ESA. The USFWS functions as the largest proponent of on-the-ground reintroduction efforts and is responsible for monitoring grizzly bear recovery and recommending future policies and management actions. The USFWS as a government entity must strike a delicate balance among conflicting goals: 1) the preservation of bear populations, 2) the conservation of livestock as the center of ranchers' livelihoods and as an important economic resource, and 3) the protection of human safety and recreation.

The USFWS has previously supported delisting efforts in Montana and Wyoming, which would ultimately return grizzly bear population management to each state. However, in 2020, the USFWS initiated a five-year review of the grizzly bear's status throughout the six recovery zones identified in 1993 (Burnham & Mott, 2021). In 2021, the USFWS released a formal species assessment that recommended the grizzly bear continue to be listed as "threatened" (U.S. Fish & Wildlife Service, 2021). The USFWS justified this decision by citing future conservation uncertainty in states and ecosystems where the bears have not recovered. They argued that limited habitat and range connectivity, and an increasing number of primarily human-caused mortalities may hinder future recovery efforts and the ultimate sustainability of delisted populations (U.S. Fish & Wildlife Service, 2021).

Alliance for the Wild Rockies and WildEarth Guardians

Conservation groups such as AWR and WildEarth Guardians (WEG) are primarily invested in the protection of the grizzly bear and the ecosystem services they provide. While not proponents of on-the-ground monitoring efforts, these two conservation organizations act as voices for the bears in court by appealing delistment proposals and advocating for continued protection of grizzly bear populations (Nokes & McMillan, 2020). These two groups also aim to increase the bear's range and ecosystem connectivity, which would enhance genetic exchange and metapopulation dynamics necessary for a more robust recovery of the bears (Nokes & McMillan, 2020). Additionally, these groups maintain the grizzly bear as an iconic species in the US. They argue the protection of the bear supersedes the values of governing administrations and could represent an important legacy of ESA success.

Ranchers

While grizzly bear conservation is important, a major consequence of increased population size in Idaho, Wyoming, and Montana is livestock depredation. In areas where the bear's estimated range includes public land grazing allotments, increased depredation occurrences correlate with increasing bear populations (Wells et al., 2018; Wells, 2017). Grizzly bears opportunistically prey upon smaller mammals (U.S. Fish & Wildlife Service, 2021), and when they co-occur in high density with livestock, depredations occurred more frequently (Wells, 2017). Additionally, depredation events were more frequent in grazing allotments near open forests, ecosystems known as high-quality grizzly bear habitat (U.S. Fish & Wildlife Service, 2021; Wells et al., 2018). Annual livestock losses due to grizzly bear depredation average between 2-3% each year, and between 2012 and 2016, the state of Wyoming paid over $1.5 million USD in grizzly bear damage and stock loss claims ($358,492 each year) (Wells et al., 2018). These costs will likely increase alongside recovering bear populations.

Ranchers and stockgrower associations such as the Montana Stockgrowers Association (MSGA) and the Blackfeet Stockgrowers Association (BSGA) lobby state and national legislators to mitigate depredation, primarily through delisting of the bear from the ESA (Bolton, 2020). The federal protection level overrides state legislation, and retaliation against bears for depredation is illegal (Bolton, 2020; U.S. Fish & Wildlife Service, 2021). Monetary compensation from the USFWS is available for both the loss of the individual animal and for depredation prevention equipment (electric fencing, steel shipping containers, guard dogs, etc.) (Bolton, 2020). However, the value of the lost livestock to its owners beyond the monetary value is often overlooked (Bolton, 2020). Losing animals to bears is difficult in the sense that it is an animal under someone’s care and protection and compensatory funding only treats the symptoms of depredation and not the ultimate cause: an increasing grizzly bear population that was simply non-existent 30 years ago.

Residents and Recreationalists

With grizzly bear populations increasing within the GYE and the NDCE, residents and tourists imagine greater risks to humans. However, despite a clear relationship between depredation events and bear density, bear attacks against humans are rare. The National Parks Service reports the likelihood of a grizzly bear attack in developed areas of Yellowstone National Park as one in every 59 million visits (Park & U.S. National Park Service [USNPS], n.d.), while in the undeveloped backcountry one attack occurs in 1.7 million overnight visits, (an average of less than one attack per year) (USNPS, n.d.). Public education and outreach on bear behavior and safety has reduced risk of injury and mortality to a minimum (USNPS, n.d.). However, poor public opinion on grizzly bears can be perpetuated by these rare attacks.

Bears also serve as a key attraction in Yellowstone National Park. Because grizzly bears provide ecosystem services as keystone species, ecosystem engineers, and apex predators, their presence enhances the environment which many visitors delight in. In fact, the highlight of many

Yellowstone visitors is to see a bear, generating revenues from those millions of visitors fascinated by bears every year. In 2016, the Wyoming Game and Fish Department reported over $235 million from bear and other wildlife viewing alone (*Grizzly Times* | *Bear Finance*, n.d.). Therefore, in contrast to safety concerns, bears attract visitors and generate revenues for surrounding areas, demonstrating the value of the bears to surrounding communities and recreationalists.

Solution

There is a disconnect between federal legislation, including the ESA, and decisions made by states with different grizzly bear populations and conditions. While the ESA provided the framework for grizzly bear conservation and restoration, a framework for post-restoration will be largely up to the states to manage. Maintaining the ecosystem-level benefits grizzly bears provide and minimizing human conflict with grizzly bears necessitates increased inter-state communication, consistent education on bear safety and their ecological importance, well-defined encounter regulations, and additional depredation mitigation funding.

It is important to recognize the decades in which grizzly bears were absent and the impact a swift 30-year recovery made on residential, recreational, and ranching communities. Delisting the grizzly bear in the contiguous US may become a very real possibility as the population grows, but is not feasible at this time because their recovery across all ecosystems has yet to materialize. However, when delisting is feasible, management will ultimately be returned to state governance. Because grizzly bears play key roles in their respective ecosystems and are wide-ranging, future interstate cooperation and mitigation policies should be agreed upon before delisting occurs. Current cooperation efforts are brokered through a consortium of agencies in an Interagency Grizzly Bear Committee (IGBC) that includes both state and federal representatives, but this committee could be expanded to more broadly include states where grizzly bears have yet to recover (e.g. Northern Cascades ecosystem in Washington). Through enhanced interstate communication, the six zones identified for grizzly bear recovery efforts can be more uniformly prepared for state-level management, an accomplishment which will improve conditions for bears and the people who live alongside them.

Conservation practices in two recovery zones established by the USFWS (GYE and NCDE) have successfully increased grizzly bear populations to self-sustaining levels for at least the next 100 years (U.S. Fish & Wildlife Service, 2021). Regardless of the grizzly bear's protection status, ranchers, residents, and recreationalists need to embrace the presence of bears and use available mitigation funding and tools to minimize the effects of conflicts which will continue to exist under increasing bear populations. Additional state and federal funding for expensive depredation prevention methods, such as electric fencing and steel storage containers, should be made available to those who have lost or may lose stock each year to grizzly bears. In states such as Alaska, where grizzly bears are not listed under the ESA and have existed alongside humans for centuries in stable populations, bear encounter policies include regulated hunting and clear self-defense and defense of property laws to kill bears. These regulations also include well-funded educational programming within parks and protected areas that promote bear safety and explains the important role bears play in ecosystems.

Additionally, much of the funding for educational programming and campaigns detailing bear safety is fronted by conservation associations in Alaska with state capital granting (Alaska Wildlife Conservation Center, 2012). Public education significantly lowers the risk of human-bear encounters and injuries (Herrero, 1970). Therefore, to balance the state-level governmental solutions in the lower 48 states, conservation associations that have previously invested in litigation against delisting, should instead invest in the educational programming necessary to mitigate the potential damages from bear encounters. More widespread public educational campaigns such as these will help reduce bear mortalities and will help maintain the ecosystem services bears provide. Common ground exists across all stakeholder groups and leveraging potential partnerships will help the transition toward a mindset of living alongside bears in the GYE and NCDE.

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