ABSTRACT

ANTI-PREDATOR TRAINING AND SUBSEQUENT SURVIVAL OF CAPTIVE-HELD JUVENILE MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

By Brittney L. Wiggins

Identifying a cost efficient and effective anti-predator training program for captive-held or captive-bred animals is important for developing strong reintroduction programs. Since many reintroduction programs have high mortality due to predation, successful reintroduction programs may rely on reestablishing important anti-predator behaviors through training programs. Post-release survival is monitored, but perhaps is not monitored stringently enough, and many papers that have reported the effects of training have not done subsequent releases.

The meadow vole (*Microtus pennsylvanicus*) is a small, abundant rodent that can be used as a model organism for threatened or endangered species of similar size and ecological importance. For this research, voles underwent a training program where thirteen individuals were exposed to a gray wolf (*Canis lupus*) scented cloth and a rubber band shot. While trained and control voles had no difference in the number of explorations, trained voles learned from their training sessions as was shown by their responses to a very different predator cue: a red-tailed hawk (*Buteo jamaicensis*) call or silhouette. Control animals had similarly high responses to a call or silhouette prior to training sessions, whereas trained animals increased their responses from pre- to post-training tests.

To further determine the effects of training on survival, all voles were released into three one-acre enclosures in the Winnebago County Parks system. Voles had been ear tagged with unique identification numbers and survival was monitored via mark-recapture techniques. While results were not significant, trained voles and wild voles showed a trend to survive slightly better than control voles. A larger sample size may better elucidate the nuances of this trend. This research, along with other studies, suggests that training may have an impact on survival and should be implemented in captive breeding programs that have animals targeted for release.

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by

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A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of

Master of Science-Biology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

May 2012

COMMITTEE APPROVAL

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11 May 2012 Date Approved

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11 May 2012 Date Approved

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5-11-12 Date Approved

PROVOST
AND VICE CHANCELLOR

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5/21/12

Date Approved

FORMAT APPROVAL

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4/17/12

Date Approved

ACKNOWLEDGMENTS

I would like to acknowledge and thank Dr. M. Elsbeth McPhee for her instruction and direction in the development, conduction and writing of this thesis. I'd also like to thank Amaranta Kozuch for her tireless work in the field and the lab. Without funds and facilities from the University of Wisconsin Oshkosh, this research would not have been possible. Special thanks to the Vole Lab and to my committee members, Doctors Sheldon Cooper and Greg Adler. Further thanks to the Winnebgo County Parks Department, especially Rob Way and Bob Lohry, as well as Stacy Donnelly and the Menominee Park Zoo gray wolves, Rutger, Saleen and Caleb.

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CHAPTER I

INTRODUCTION

Conservation History

The term conservation is used so often these days that perhaps it does not have as strong of an effect as it should. Conservation is the act of preserving natural resources and communities, including plants, animals, fungi, etc., energy sources such as coal and natural gases and watersheds and groundwater. For my research and thus the purposes of this paper, I will focus on conservation for purposes of maintaining biological diversity of animal species. Various techniques have been developed or are in development as tools for conservation biologists, such as habitat restoration and protection, hunting restrictions, and captive populations and propagation. Controlled captive breeding and reintroductions are fairly new. Until the Endangered Species Act (ESA) was passed in 1973, there was no real controlled program to identify and manage threatened and endangered species (Scott et al. 2010; Harris et al. 2011). The ESA provides funds to create a list of endangered organisms and allocates these funds to the purchase and protection of habitat (Scott et al. 2010). Today, the U.S. Fish and Wildlife Service manages endangered species by determining which animals should be listed as endangered or threatened, when and if an animal should be delisted, designating habitat and recovery plans and even stopping development if threatened or endangered species are present on the planned areas of development (Harris et al. 2011).

The International Union for Conservation of Nature and Natural Resources(IUCN) was created in 1948 to maintain global biological diversity worldwide (Harris et al. 2011). The IUCN develops a Red List of all species and monitors animal populations in the wild and in captivity. This list is the most globally recognized, listing all known plants and animals as one of the following (from least to greatest concern): least concern, near threatened, vulnerable, endangered, critically endangered, extinct in the wild, and finally, extinct (Harris et al. 2011). This listing is an important process in developing strategies to maintain biodiversity. Both the IUCN and ESA lists have requirements to determine if and when an organism should be added to the list. The IUCN has five guidelines that determine if an animal is added to the list and its status once on the list: 1) decline in population size over time, 2) small or restricted geographic range, 3) small population in conjunction with decline of size and range, 4) very small population size, or 5) quantitative analysis (Harris et al. 2011). For the ESA, five threat categories are determined for the species in question: 1) overutilization of the animal, 2) loss of habitat, 3) increase predation due to new predators or disease, 4) inadequate regulatory mechanisms, or 5) any other reason (Scott et al. 2010). While both ESA and IUCN define five different categories required for listing, the categories differ, and both groups list animals differently. One review found that ESA, while a very useful tool, does not list the same species as endangered or threatened as does the IUCN—in fact, 40.3% of birds were absent from the ESA list when compared to the IUCN red list and >80% of other groups were under-represented on the ESA list (Harris et al. 2011).

Sadly, enormous portions of world species are listed as vulnerable, threatened or endangered. Of the 5,499 mammal species in the world, 1,138 are listed; 1,254 bird species are similarly classified of the total 10,052 species worldwide, and a large portion of reptiles and amphibians are also in danger of extinction (IUCN RedList 2011). Conservation biologists generally agree that development due to agriculture, urban sprawl, overexploitation, exotic species, and other human impacts are responsible for this dramatic loss of biological diversity (Carroll et al. 2009; Scott et al. 2010; Harris et al. 2011). Knowing that human interference is necessary to maintain current diversity and perhaps to reverse population losses, animals on the ESA list or the IUCN red list are often subjects for recovery plans (Harris et al. 2011). These recovery plans have species management goals that must be reached for a species to be delisted (Scott et al. 2010). Very few animals have been delisted from either the ESA or the IUCN red list; however, the ESA seems to have improved the conservation status of most listed species and may have prevented a large number of extinctions. Scott et al. (2010) reviewed conservation programs and the status of endangered or threatened species according to ESA. They found that by 31 December 2007, 15 of 1,136 listed species had been removed after reaching their designated goals. This is a low proportion of protected species, and Scott et al. (2010) believed that some animals had been delisted too soon. They suggested that although these goals could be met, these animals may require long-term conservation and therefore should not be delisted because they may eventually go extinct without this management. For instance, the Kirtland's warbler (*Dendroica kirtlandii*) in Michigan and the grizzly bear (*Ursus arctos horribills*) in Yellowstone National Park could not be maintained without further assistance (Scott et al. 2010).

Definitions

A complication with reintroduction and conservation is the terms used in publications. Many of these terms are used interchangeably although they may have different meanings. I will consequently define the terms that I will be using multiple times throughout this thesis: release, introduction, reintroduction, translocation, success, and failure.

Release is when any organism is intentionally or accidentally freed into a new environment. Release is usually used in association with captive breeding programs and is how I will use it in my thesis.

Introduction is often confused with translocation and reintroduction in the literature. Introduction best refers to the unintentional release of a species into an area other than its native habitat. These introductions often become invasive and problematic and sometimes are the cause of local extinctions or population declines.

Reintroduction generally calls to mind the release of individuals into a once historical range where individuals have been extirpated. The IUCN defines reintroduction as “projects that attempt to reestablish species within their historical ranges through the release of wild or captive-bred individuals following extirpation or extinction in the wild” (Seddon, Armstrong, and Maloney 2007). However, it is more useful to consider reintroductions as release of captive-bred individuals, rather than wild individuals (Griffin, Blumstein, and Evans 2000). This will help to eliminate the confusion that often arises from using reintroduction in exchange for translocation.

Translocations, then, are the movement of wild animals from their natal territory to a historical territory for either conservation or restocking game populations (Griffin, Blumstein, and Evans 2000). Oftentimes, translocated animals are held in captivity for a period of time before release.

There are many publications debating the definition of a successful release (Kleiman 1989; Griffith et al. 1989; Snyder et al. 1996; Seddon 1999; Seddon, Armstrong, and Maloney 2007). Some definitions include establishment of a self-sustaining population, breeding by first wild-born generations, persistence for a length of time (usually 3-5 years), breeding populations with recruitment exceeding mortality rates, and an unsupported population of 500 individuals (Seddon 1999). There is much debate about whether any of these definitions provide a good basis for determining if a released population will persist. Also, the definition of success can be very species-specific, which should be taken into account.

Failures are much easier to define. If a population fails to establish, if no breeding occurs or if all individuals die, then the release should be deemed a failure.

Types of Release

Soft release and hard release refer to whether or not animals are supported after release. During soft release, the animals are maintained in numerous ways. Often, supplemental food and water are provided for the released animals to reduce the risk of starvation and dehydration (Kleiman 1989; Seddon 1999). Sometimes, veterinary care or interference with predation events are used to promote survival. In extreme cases, animals may be released into a closed environment with restricted movement between the animals and their new environment (Kleiman 1989; Dobson and Lyles 2000; Biggins and Godbey 2003). This often means that animals are supplied with all previous aspects of soft release but are additionally protected from potential dangers in their new environment.

During a hard release, by contrast, animals are simply released into their new environment without any supplemental support or interference from researchers. Hard releases are often difficult for captive-bred animals, due to the high mortality rate due to behavioral deficiencies (Kleiman 1989; Britt, Welch, and Katz 2003). In the cases of translocations, hard releases are the normal method of release; these animals are often wild and are best served if they are released as quickly as possible into their new homes (Fischer and Lindenmayer 2000; Hayward et al. 2006). These animals already have the necessary skills to survive. Hard releases may be too difficult for captive-born individuals to survive and do not usually result in a successful reintroduction.

Rearing Techniques

The way captive animals are raised can have a negative impact on behaviors and subsequently survival. Hand-rearing can quickly lead to taming, domestication, or improper bonding in certain species (Snyder et al. 1996). Different rearing techniques have been developed and tested to determine what their negative effects may be and how best to avoid or mitigate them. For many bird species, these techniques are exceptionally important. Wallace (1994) examined three widely-used rearing techniques—1) parent-rearing, 2) cross-fostering, and 3) isolation-rearing—and reviewed studies of different bird species reared under these techniques. I will briefly discuss the techniques and examples Wallace used in his review.

Parent-rearing is where either biological or conspecific foster parents raise the young. This is the most natural form of rearing and often ensures appropriate behavioral development such as proper social behaviors. This form of rearing coupled with a soft release method has been fairly successful for species such as Bali mynah (*Leucopsar rothschildi*), thick-billed parrots (*Rhynchopsitta pachyrhyncha*), Peregrine falcons (*Falco peregrinus*), and Guam rail (*Gallirallus owstoni*). While parent-rearing is a preferred method for raising young, more often than not it is not a viable option and other techniques must be implemented.

Cross-fostering is another successful tool, as it often results in proper behavioral responses, or at the very least, provides the young with an opportunity to develop naturally. Cranes (*Grus* spp.) have often been cross-fostered but with limited success and mixed results. Wild sandhill cranes (*G. canadensis*) were used as cross-foster parents for their endangered relative, the whooping crane (*G. americana*). While sandhill crane parents were able to raise the young whooping cranes with limited survival, the young whooping cranes did not develop proper social behaviors and were unable to form pair bonds with conspecifics, resulting in lack of reproduction. A successful cross-fostering program for masked bobwhites (*Colinus virginianus ridgwayi*) focused more on predator training rather than raising the young to adulthood. A male Texas bobwhite (*Colinus virginianus*) was placed with a group of juvenile masked bobwhite to teach proper predator responses; this training was successful at promoting survival of the juveniles after release.

Isolation-rearing is a very costly, laborious, time consuming and difficult method but is sometimes the only tool available to conservation biologists. This type of rearing is famously known as the 'puppet method.' Caretakers of certain species, such as California condors (*Gymnogyps californianus*), Hawaiian crows (*Corvus tropicus*), and—perhaps most famously—whooping cranes, use either hand puppets or full-body costumes to prevent habituation to humans or other maladaptive behaviors from developing. These puppets or costumes allow keepers to feed and raise young as realistically as possible. Many programs have had success using this method, especially birds of prey such as California and Andean (*Vultur gryphus*) condors, Peregrine falcons, and bald eagles (*Haliaeetus leucocephalus*). Other species, such as trumpeter swans (*Cygnus buccinator*) and whooping cranes have been taught proper feeding and migrating behaviors by model parents, such as decoys and gliders painted like adult conspecifics. While this method of rearing has been fairly successful, it should only be used when completely necessary to the survival of a species and after other methods have failed, as it is very time consuming and costly.

One interesting point to note is that while there have been many of studies focusing on the effects of rearing techniques on birds, there have been very few studies on raising mammals. It is generally agreed that more handling and interaction with humans quickly leads to habituation as compared to animals that are rarely handled (Snyder et al. 1996).

Difficulties in Captive Breeding Programs

Captive breeding and translocations are essential tools of conservation biologists. These tools have shown promising results for increasing total population numbers in an assortment of species. Small mammals and birds often do well in captivity. For instance, black-footed ferret (*Mustela nigripes*) populations rebounded from 18 captive founders in 1986 to over 3000 captive individuals in 1999 (Dobson and Lyles 2000). Golden lion tamarins (*Leontopithecus rosalia*), after an initial period of no breeding in a population of 83 captive individuals in 1975, the population boomed, reaching 500 individuals in the late 1980's and is now stable and managed (Ballou et al. 2002). Similar population increases in captivity (albeit with limited long-term success) are noted for bald eagles, whooping cranes, California condors, Arabian oryx (*Oryx leucoryx*), European otters (*Lutra lutra*), and American bison (*Bison bison*) and other species (Kleiman 1989; Snyder et al. 1996; Meretsky et al. 2000; Freese et al. 2007).

Captive breeding is often only a first step in a recovery program for threatened and endangered species. The next step logically flows to reintroduction of individuals from a captive population either into an established wild population or into areas where the species was found historically but has since been extirpated. In 1998, IUCN's red list had over 200 species listed as current reintroduction projects (Seddon 1999). These reintroductions again seem to be a promising tool in the conservation biologists' handbag of biodiversity restoration, but many times, reintroductions are fatal to the animals released into the wild and success of these programs is highly restricted. Limiting factors for survival of released individuals often fall to behavioral deficiencies of the individuals (Kleiman 1989; Britt, Welch, and Katz 2003. 2003). Another problem cited in the literature involves the quality of habitat into which individuals are being released; releasing animals into unsuitable habitat due to introduced predators and disease, unreliable food sources, destruction or fragmentation of habitat, or even lack of education of native peoples often lead to high mortality rates of reintroduced individuals (Kleiman 1989; Grifftih et al. 1989; Seddon 1999; Seddon, Armstrong, and Maloney 2007). Available data for releases conducted in the 1970s and 1980s show consistent failure to establish populations (Seddon, Armstrong, and Maloney 2007). Further, reintroductions of untrained, captive-reared animals are often reported as failures, regardless of species (Britt, Welch, and Katz 2003. 2003). One major problem that arises with releasing animals is the lack of long-term monitoring of populations. Without long-term monitoring, determining long-term success of release programs is difficult (Seddon 1999; Seddon, Armstrong, and Maloney 2007). For instance, Arabian oryx released in the 1970s into Oman were determined to be a successful flagship reintroduction effort; however, 20 years later, the oryx population has decreased due to poaching to the point where it is no longer viable without intervention (Seddon 1999).

Commensurate with problems with post-release survival, animal behavior can change rapidly under captive conditions. Captivity does not exert the same selective pressures on individuals as living in the wild does. McPhee (2003) stated that behavior, physiology, and morphology evolve in complex environments, and individuals from a captive environment have behavioral deficiencies that may decrease survival upon release. This decrease in post-release survival can have dire consequences for conservation efforts. The focus of this thesis is to show that training can improve post-release survival of captive-held animals. It has been suggested, with great argument for and against, that training can have a positive impact on animal survival post-release for a number of species (McLean, Lundie-Jenkins, and Jarman 1996). Birds and mammals have both been trained to avoid predation, increase foraging, and other survival skills. Anti-predator skills are incredibly important for animal survival, as an animal must react appropriately the first time a predator is encountered (McLean, Lundie-Jenkins, and Jarman 1996). Further discussion of this particular conservation tool will occur later in my thesis.

Examples

There are several success stories of animals being saved from extinction due in part to captive breeding, such as the bald eagle and the gray wolf (Carroll et al. 2009), the California condor and whooping crane (Meretsky et al. 2000; Wallace 1994), and the black-footed ferret (Dobson and Lyles 2000; Biggins and Godbey 2003). These captive-bred individuals are often reintroduced to wild populations to increase the species' ability to persist over time (Britt, Welch, and Katz 2003. 2003).

One fairly well-known and supported captive breeding program is that for the California condor. In 1980, captive breeding began as conservation biologists realized that the wild population was declining catastrophically (Meretsky et al. 2000). In 1987, all free-flying birds (n=27) were captured and brought into captivity, and captive breeding began in earnest with artificial incubation, allowing the population to grow exponentially (Meretsky et al. 2000). By 1999, the population exceeded 150 animals, and reintroductions began with 88 birds released between 1992 and 1999 (Meretsky et al. 2000). While condor breeding has been successful at saving the population from complete extinction, this program is expensive and not nearly as effective as many others. Condors raised in captivity must be raised by puppets to avoid malimprinting (Wallace 19994; Meretsky et al. 2000). This form of captive rearing is expensive, time consuming, and preferably only used as a last resort to save a species.

Another species that has been saved from extinction in a similar manner as the California condor is the whooping crane. A once abundant species, whooping crane populations dwindled to 21 individuals in 1944, leading to the creation of the original Endangered Species Act by congress (Scott et al. 2010). Captive breeding increased the population to 145 captive birds and 354 wild birds in 2006 (Department of Natural Resources 2006). Wallace (1994) reported that without rigorous methods and the use of the full-body puppet costumes, releases resulted in failure. Whooping cranes have been successfully raised, trained, and released using the puppet and isolation-rearing methods; however, survival of these released birds is low, and reproduction and recruitment remain low (Wisconsin Department of Natural Resources 2006).

Very few animals have been removed from the ESA list of threatened and endangered animals. Two such delistings that have been met with jubilation and trepidation are the bald eagle and the gray wolf (Carroll et al. 2009). Bald eagles, the national bird of the United States, were once highly endangered due to environmental contamination, human persecution, and habitat loss (Simons et al. 1988). Breeding raptors in captivity is difficult, and bald eagles were not exceptions (Maestralli and Wiemeyer 1975). After years of intense breeding and releases to suitable, albeit fragmented habitat (Simons et al. 1988), eagles were delisted in 2007 and are currently found throughout the contiguous United States and Alaska (Carroll et al. 2009).

Gray wolves were delisted from six western U.S. states in 2009 (Carroll et al. 2009). This delisting has been a subject of heated debate, because wolves may be an example of an animal that met the goals to be removed from the list but may need long-term conservation management. Gray wolves are a better example of translocations rather than of captive breeding efforts. Wolves were once found throughout the contiguous United States, but due to conflicts with ranchers and settlers, they were eradicated in most of their natural range by the 1930’s (Chadwick 2010). Lone wolves began moving unaided into the western United States from Canada in the mid-1980’s and were later aided by translocations from Canada into Yellowstone National Park in 1995 and 1996 (Chadwick 2010).

Black-footed ferrets are an excellent example of successful conservation efforts through captive breeding and training programs. This once-widespread species was historically found throughout the Western United States from Canada to Mexico. Ferrets rely heavily on prairie dog colonies for food. As a specialist hunter, ferrets suffered catastrophic population declines correlated with the presumed villainy of prairie dogs by ranchers. Prairie dogs have been poisoned, trapped, and extirpated since farmers began settling into the prairies of the Midwest. Introduction of plague (*Yersinia pestis*) also destroyed entire colonies of prairie dogs, thereby removing the vital food sources of the ferrets. Black-footed ferrets are also susceptible to plague and canine distemper virus (*Canine distemper*). These factors led to the believed extinction of wild ferret populations in the 1970’s. However, a small population was rediscovered in 1981 by a rancher on his land in Wyoming (Dobson and Lyles 2000). At this point, all remaining wild individuals (n=18) were brought into captivity, and the breeding program was initiated. Captive breeding has been very successful after a few initial setbacks. The initial breeding population of six ferrets died from distemper before breeding occurred (Dobson and Lyles 2000). A second breeding population of 18 ferrets began breeding in 1987, and by 1999 the population had reached over 3000 ferrets in captivity (Dobson and Lyles 2000).

After initial reintroduction attempts failed due to inability of captive ferrets to forage properly and avoid predators, training programs were implemented (Miller et al. 1990; Vargas and Anderson 1999; Dobson and Lyles 2000; Biggins and Godbey 2003). Hunting success was incredibly low when ferrets were first introduced into the wild; this was due to the animals never having had experienced hunting for live prey. Feeding captive ferrets live hamsters and using soft-release methods onto an active prairie dog colony greatly improved post-release survival (Vargas and Anderson 1999; Dobson and Lyles 2000). Black-footed ferrets are also susceptible to predation by owls, badgers, and coyotes; of 130 Siberian polecats (the ferrets' closest living relative) and black-footed ferrets released, 93% of fatalities were attributed to predation (Biggins and Godbey 2003).

My Research

Conservation biologists often suggest that anti-predator training can increase survival in captive-raised or captive-born animals upon release into the wild. For some species, training to visual predator cues has improved survival (Miller et al. 1990; van Heezik, Seddon and Maloney 1999; Biggins and Godbey 2003; Shier and Owings 2006 and 2007). Some attempts have been made to train animals to recognize predators using odors (McLean, Lundie-Jenkins, and Jarman 1996; Griffin, Blumstein, and Evans 2000). In nature, however, animals adjust their behaviors in response to many types of predator signs. Predators are not always seen, even when they are in the area. This suggests a need to train animals to a variety of cues to fully ensure that training will promote survival by teaching released individuals proper behavioral adjustments in the presence of predators.

To my knowledge, whether or not training to recognize one kind of predator cue influences response to other predator cues in mammals is unknown; accordingly, I asked two questions: 1) can anti-predator behaviors be learned through laboratory training, and 2) does exposure to predator odor increase response to predator calls or visual predator cues? Chapter 1 examines learning through training and whether or not there is a link between responses to odor and other cues. Juvenile meadow voles (*Microtus pennsylvanicus*) were exposed to a pre-training stimulus of either a silhouette or a call of a red-tailed hawk (*Buteo jamaicensis*) to determine a base-line alert response. Voles were then subjected to a training regimen using odor and were again exposed to the silhouette or call in a post-training test.

Few studies have monitored post-release survival (van Heezik, Seddon and Maloney 1999; Shier an Owings 2006, 2007). Chapter 2 focuses on post-release survival of trained, control, and wild voles. After testing and training, all voles were released back to the field site where they had originally been captured. Voles were recaptured to monitor survival and all trapping histories were analyzed using Program MARK (http://warnercnr.colostate.edu).

This unique attempt at testing responses to visual and aural cues after training with olfactory cues could create a promising method to train a variety of species to avoid predators. If trained voles have stronger alert response post-training to other predator cues than untrained voles, it may suggest that predator recognition is linked across the senses. Furthermore, if trained voles survive better than their control counterparts, it would be safe to suggest that training is an important tool that should be employed in the preparation of endangered or threatened captive-bred animals for release.

CHAPTER II

ANTI-PREDATOR TRAINING IN CAPTIVE-HELD JUVENILE MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

ABSTRACT. Captivity alters natural behaviors, especially predator avoidance behaviors, and training captive animals has been suggested as a way to reverse this loss of behavior. Juvenile meadow voles (*Microtus pennsylvanicus*) were brought into the lab and trained to recognize and avoid predator odors. Pre-training tests were conducted to determine a base-line alert response to either a red-tailed hawk (*Buteo jamaicensis*) call or silhouette. Voles were then exposed to either a wolf-scented cloth and a negative stimulus in the form of a rubber band shot for trained animals or a clean cloth and no stimulus for control animals. Post-training tests showed a significant increase in alert behaviors when compared to pre-training tests for trained voles. Control animals had no change in alert behaviors. This suggests that voles can learn to recognize predator odors and training to these odors increases responses to very different predator stimuli.

Introduction

Humans have changed the face of the planet and therefore have greatly impacted wildlife, whether intentionally or inadvertently. Losses of habitat, pollution, exploitation of resources, and introduction of invasive species and disease have all had a hand in the decimation of natural populations of numerous species. The survival of these threatened and endangered species rests heavily on human intervention through captive breeding, habitat restoration, education, and other programs. Currently, human intervention tends toward captive breeding programs in zoos, aquaria, and conservation programs worldwide. For instance, captive breeding is part of the recovery plans for 64% of U.S. threatened and endangered wildlife (Snyder et al. 1996).

There have been tangible benefits to captive breeding programs. A majority of zoo animals are currently bred in captivity and therefore are not being removed from natural populations (Beck 1995). In countless cases, captive breeding is used as an attempt to bolster global populations, both in captivity and in the wild. The Association of Zoos and Aquariums (AZA) oversees zoos and aquaria and is especially important in conservation programs because it provides many reintroduction programs (Beck 1995). The Species Survival Plan (SSP) of the AZA focuses on coordinating breeding programs throughout accredited facilities has bolstered captive populations with potentially limited inbreeding (Conway 1995).

Natural behaviors evolve in complex environments, but captive environments are notably simpler than natural environments (McPhee 2003). In captivity, the goal is to keep all animals in the populations alive and healthy; hence, predators and disease are eliminated or controlled, food and water are abundant and easy to find, and substrates are static and unnatural so keepers can maintain a clean environment. This often results in a population that is at an inherent disadvantage when released back into natural settings (McPhee 2003). This loss of behavior has been documented in a number of species and has manifested itself in various ways. In golden lion tamarins (*Leontopithecus rosalia*), loss of locomotion and orientation while climbing and decrease in foraging ability, improper social skills, and predator avoidance have been noted in captive populations (Beck et al. 2002; Stoinski and Beck 2004). An increase in boldness or a lack of fear has been noted in the swift fox (*Vulpes velox*) (Bremner-Harrison, Prodohl, and Elwood 2004), the Siberian polecat (*Mustela eversmanii*) (Miller et al. 1990), and the black-footed ferret (*Mustela nigripes*) (Biggins and Godbey 2003). This lack of fear represents an inability to recognize and avoid predators; such a lack of predator recognition has resulted in massive mortality of released in golden lion tamarins (Beck et al. 2002), black-footed ferrets (Miller et al. 1990), Siberian polecats (Biggins and Godbey 2003), houbara bustards (*Chlamydotis undulata)* (van Heezik, Seddon and Maloney 1999), New Zealand robins (*Petroica australis*) (McLean, Holzer, and Studholme

1999), and black-tailed prairie dogs (*Cynomys ludovicianus*) (Shier and Owings 2006 & 2007).

Anti-predator training is a frequently debated method of improving success after release (Dobson and Lyles 2000; Seddon, Armstrong, and Maloney 2007). Behavioral biologists generally agree that the losses of anti-predator behaviors are reversible to some extent (Blumstein et al. 2002). However, for training to be effective, the individuals must recognize predators by sight, sound, or smell and must learn to appropriately deal with a predator once recognition has occurred (McLean, Lundie-Jenkins, and Jarman 1996). Because such small numbers of endangered animals are reintroduced, a single predator can easily exterminate a newly reintroduced population (McLean, Lundie-Jenkins, and Jarman 1996). Few studies have attempted to determine the effects of training on captive-bred or captive-held animals.

Miller et al. (1990) attempted to train Siberian polecats to recognize and fear model predators in the form of stuffed badgers and owl silhouettes with limited success in a controlled laboratory setting. Tamar wallabies (*Macropus eugenii*) were tested to determine if predator-naïve translocated animals could recognize predator odors without any training, but results were inconclusive in a laboratory setting (Blumstein et al. 2002). Houbara bustards have been successfully trained to avoid live foxes under controlled conditions (van Heezik 1999). Black-tailed prairie dogs and New Zealand robins also learn to recognize predators in laboratory settings (Shier and Owings 2006 & 2007; McLean, Holzer, and Studholme 1999).

Because pre-release anti-predator training does seem to be a promising way to reverse loss of appropriate predator avoidance behaviors in captivity, it is imperative that an effective and cost-efficient plan that could potentially be applied to a number of species be developed and tested. Using captive-held juvenile meadow voles (*Microtus pennsylvanicus*), I asked if: 1) anti-predator behaviors can be learned and retained, and 2) exposure to one type of predator stimulus can improve responses to other types of stimuli. To determine whether or not there is a link between responses to odor and other cues, meadow voles were exposed to a pre-training stimulus of either a silhouette or call of a red-tailed hawk (*Buteo jamaicensis*) to determine a base-line alert response. Voles were then subjected to a training regimen using odor from gray wolves (*Canius lupus*) and a negative stimulus in the form of a rubber band shot and were again exposed to the silhouette or call in a post-training test. This training will possibly elucidate whether or not juvenile meadow voles can learn anti-predator behaviors in a laboratory and whether training influences responses to a variety of predator cues. This could be potentially useful for young individuals of threatened and endangered species.

METHODS

Subjects.

Study subjects were juvenile meadow voles trapped between 31 May and 14 June 2011 on a 2.8-hectare plot of grassland in Oshkosh, WI. Three 0.4-hectare enclosures were constructed of aluminum flashing buried approximately 30.48 centimeters in the ground with another 30.48cm above ground. Stakes were placed within the enclosures at 10-meter intervals. Each stake designated a trap location in a transect line. Sherman traps (7.62 x 8.89 x 22.86cm) were placed at each stake. Stakes were also established around the outside perimeter of each enclosure to monitor possible escapes from the enclosures. Traps were set in the evening between 1600 and 2000. They were baited with peanut butter and rolled oat balls and provided with a cotton ball for warmth. Traps were checked between 0600 and 0800 the following morning. If temperature was below 10 degrees Celsius or if thunderstorms were in the area, traps were not set for the safety of the voles and the researchers.

Ages were delineated by weight; any vole under 20 grams was deemed to be a juvenile (Krebs et al. 1969; Myers and Krebs 1971). Juvenile voles are typically aged two to three weeks and were chosen because these animals would have had the least predator experience in their short time in the wild. When initially brought into the laboratory, all voles were kept in quarantine for two weeks after receiving Ivermectin and Adam's™ Flea and Tick treatment to remove internal and external parasites, respectively. After a two-week quarantine, all voles were moved into the colony until testing and training in July.

Twenty-eight juvenile voles were assigned to either trained or control groups, for a total of 14 animals in each group. Of these, 13 trained animals and 12 control animals survived through all testing and training sessions. Voles in both groups were housed in standard mouse cages (27.94 x 17.78 x 12.7cm) topped with wire hoppers. Cages were lined with 500 mL of Sani-Chip ® bedding and voles were given a nesting square for enrichment. Food and water were given *ad libitum*. Cages were fully changed at least once a week, with additional changes performed as necessary.

Training.

Thirteen experimental and twelve control voles were subjected to training and testing. Training for anti-predator behaviors was conducted on three separate days— 6, 13, and 20 July 2011. I used a one-week break between training sessions to minimize stress while maximizing retention of the training; this interval was determined from the literature (Miller et al. 1990), the short life spans of voles, and pilot studies.

Cloth was used to introduce the predator odor to the voles. Blankets were purchased from St. Vincent de Paul, Oshkosh, WI and with permission, placed into the gray wolf enclosure at the Menominee Park Zoo, Oshkosh, WI for one week in December 2010. Wolves began rolling and urinating on the blankets immediately. As this was winter, blankets were frozen, thus keeping the scents fresh. Upon retrieval from the wolf enclosure, they were cut into small squares and were placed into Ziplock® bags and kept frozen until needed for testing. Control blankets were also cut into small squares, stored in Ziplock® bags, and frozen.

Because many predator avoidance behaviors are learned, I exposed voles to a negative stimulus in conjunction with a predator odor. Rubber bands were shot near the voles when they came within 1.3cm of a cloth with the odor. I used rubber bands (Miller et al. 1990) so the voles would be startled in association with a predator odor but would not be injured.

Voles were placed in an uncovered Tupperware® container with deep sides (57.15 x 40.64 x 30.48cm) for training; this allowed me to shoot a rubber band freely but did not allow the voles to escape. The arena was separated from the camera and researcher by an approximately 91.44cm blind to reduce possible interactions between voles being tested and the researcher. Odors from the blankets hung noticeably in the air after testing, and, to avoid confounding variables, all control group animals were tested before experimental group animals. All voles were placed into the Tupperware® container in the far right corner away from the video camera and blind. Prior to training, all voles were randomly assigned a blanket placement (Fig. 1-1).

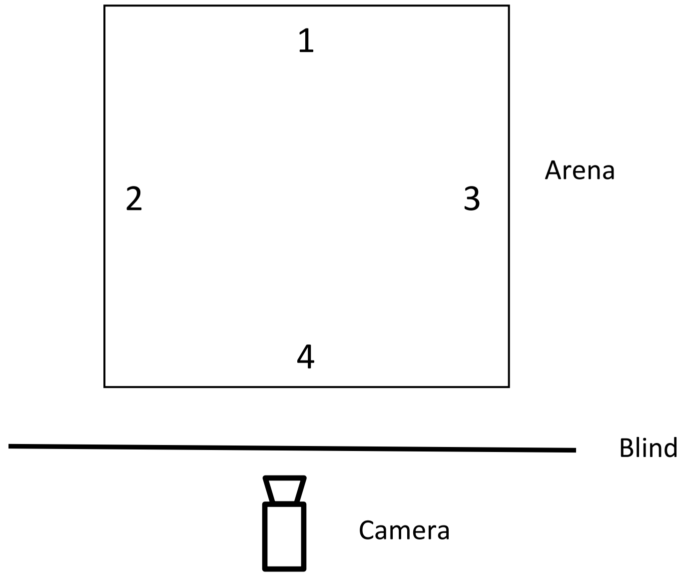

Figure 1-1. Locations of both control and predator scented cloth squares in relation to the camera's position.

The cloth was placed along an edge of the arena because voles would spend most of the time along the edges of the enclosure (Wiggins unpublished data). After a one-minute acclimation, voles were contained in a large, opaque, plastic beaker, and the cloth was introduced. For control animals, voles were watched for approaches to the clean cloth. If an animal approached or explored the cloth three times, sat on the cloth for one minute, or the timer reached six minutes, the training session was ended.

For trained animals, a predator-scented cloth was introduced in the same manner as the control cloth. Extra precautions to prevent odor from entering the colony were taken, including spraying a paper towel with Atmosklear® and covering the vent, keeping Ziplock® bags closed at all times, and spraying garbage bags with Atmosklear®. Rubber bands were used to inflict a negative stimulus upon the vole when it approached the cloth. The rubber band was shot from approximately one meter above the arena. Approaches or explorations resulted in a rubber band being shot near the vole to startle it. The training sessions ended after three shots or six minutes, whichever came first.

At the end of each training session, voles were removed and returned to the colony. Predator-odor blankets were removed and placed into a Ziplock® bag designated as trash and sprayed with Atmosklear®. Control cloths were simply discarded. All Sani-Chip® was disposed of in the laboratory garbage. Predator-odor blankets were discarded outside of the laboratory to remove odors. The room was sprayed with Atmosklear®; all Tupperware® was sanitized with Nolvasan® between voles and Sani-Chip® was replaced.

All vole training sessions were coded using *JWatcher* (www.jwatcher.ucla.edu). Total numbers of rubber band shots and total time spent in the arena were recorded during the training sessions. Behaviors were coded as one of the following: *freezing, sniffing, grooming, exploring, hiding, climbing, running,* and *other. Freezing* was the number of observations in which the vole was not moving at all. *Sniffing* was when the vole smelled the air by moving its head back and forth. *Grooming* was when the animal was cleaning itself using its tongue and forepaws. *Exploring* was used in training sessions as when the animal approached, sat on or near, or in any way interacted with the cloth. *Hiding* was used in testing sessions to describe when a vole was out of view in a PVC pipe. *Climbing* was when the vole was jumping at the sides of the enclosure or when the vole was on top of the PVC pipe during testing sessions. *Running* was any forward movement, whether slow or fast. *Other* described any other behavior.

Testing.

Thirteen trained and 12 control voles were tested for anti-predator response to either visual or audio predatory stimuli. Each vole was tested twice—once before exposure to predator odors or a clean cloth depending on group assignment and once after exposure. Visual stimulus was presented in the form of a red-tailed hawk (*Buteo jamaicensis*) silhouette constructed of a piece of cardboard attached to a 60.96cm long dowel. Aural stimulus was a red-tailed hawk call played from the Cornell Lab of Ornithology website (http://www.allaboutbirds.org/guide/red-tailed_hawk/id). A 2009 MacBook Pro was used to access the website and the call was played on a medium volume, which pilot studies deemed sufficiently loud to startle voles but not too loud to permeate the colony (Wiggins unpublished data). Trained and control voles were randomly assigned to receive either a call or silhouette and were split as evenly as possible in both groups while maintaining random assignment (Table 1-1). These assignments were then used for both testing sessions.

Table 1-1. Numbers of voles assigned to each type of test for each group.

Group	Silhouette	Call
Trained	6	7
Control	7	5

All control and trained animals were tested in the same manner on 5 and 21 July 2011. The testing arena was a large, shallow black arena with a Plexiglas cover (88.9 x 60.96 x 22.86cm). Lights were set up on the sides of the arena to fully illuminate the arena without causing glare off of the cover. The bottom of the arena was covered with ~3500 mL of Sani-Chip® bedding, and a piece of PVC pipe was placed in the upper left corner.

Each vole was introduced to the upper right corner of the arena. After introduction, voles were allowed a five-minute acclimation period to explore their new surroundings. At the five-minute mark, the call or silhouette was presented to the vole. The silhouette was “flown” so the shadow moved and crossed the vole for one minute. The call was played for one minute. After exposure, the vole was left in the arena for five minutes post-exposure to see how quickly the vole returned to non-alert behaviors, such as grooming. Videos were coded with *JWatcher* (www.jwatcher.ucla.edu) using the behaviors described previously.

Results

Variation was similar between pre- and post-tests in control and trained animals (F=1.3912, p=0.59), but data were not normally distributed; therefore paired Wilcoxon tests were run to compare changes in all alert behaviors. Alert behaviors were defined as *freezing*, *sniffing*, and *running*.

Control animals had no significant change in alert behaviors from pre-training to post-training tests (Wilcoxon, p=0.24). Trained animals showed an increase in alert behaviors from pre-training tests to post-training tests (Wilcoxon, p=0.01) (Fig. 1-2). Variances were not significantly different from pre-test to post-test in trained (F-test, p=0.59) or untrained animals (F-test, p=0.21).

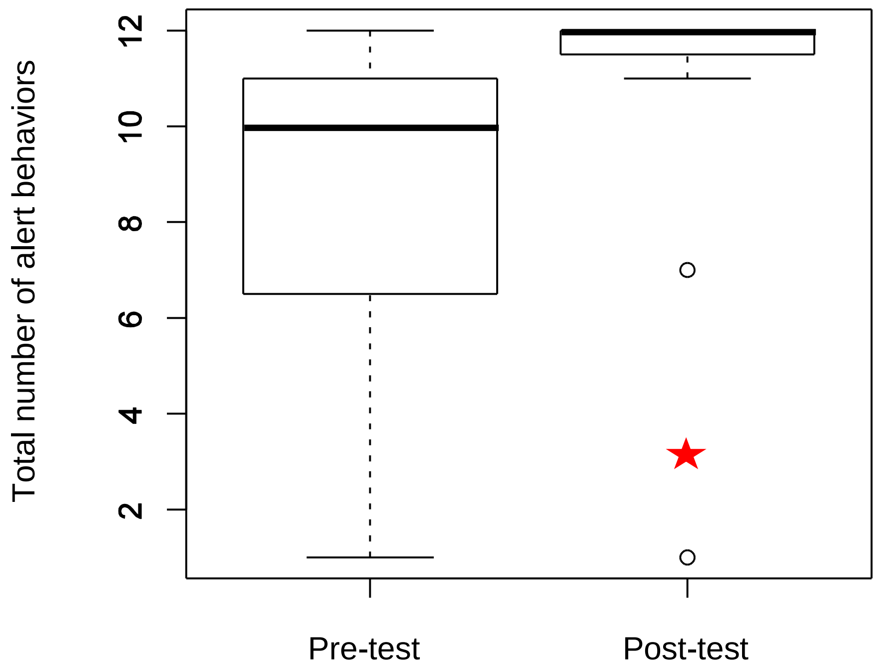

Figure 1-2. Boxplot comparing changes in alert behaviors from pre-training to post-training in trained voles. The red star indicates significance.

Discussion

Learning and retention.

The first question I tested, whether or not anti-predator behaviors were learned and retained, was analyzed by the number of times all voles approached and explored the cloth. Control and trained animals had inconsistent and seemingly random explorations of the cloth. I had predicted that trained animals, because of the negative influence from the rubber band, would show a marked decrease in total number of explorations in each subsequent training session. I also predicted that eventually trained individuals would refuse to approach the cloth at all, especially by the third and final training session. Finally, control voles were predicted to consistently explore the cloth because it was clean and no harmful stimulus was introduced. After analysis of the videos, it was clear that voles in both groups had inconsistent explorations; therefore, all of my predictions for this portion of my research were unsupported.

There are several explanations possible for this unexpected result. Many individuals in both groups failed to explore the cloth during any of the training sessions. For instance, four predator and two control voles did not explore the cloth and often sat in the opposite corner from the cloth during all three training sessions. Some voles in both groups failed to explore during one of the three training sessions but did not consistently avoid the cloth. Many that failed to explore exhibited alert behaviors, especially freezing and sniffing. This may have been a result of problems with the length of acclimation period. Voles usually exhibited non-alert behaviors, such as grooming, after a one-minute acclimation period. Therefore, I allowed the voles one minute to adjust to the arena before introducing the cloth; a longer period may have increased willingness to explore in animals with zero explorations.

Another potential problem could have been the use of an opaque beaker to restrain the vole while the cloth was introduced. Pilot tests determined that being exposed to a researcher made the voles panic and exhibit extreme behaviors that could have potentially led to self-harm. To avoid potential self-injury, I decided to momentarily detain voles within a 500 mL opaque beaker. This often caused the voles to freeze, thereby allowing me to introduce the cloth and then release the vole into their now altered arena. This interaction may have startled some voles too much for them to overcome their fear and start exploring the novel objects.

Some animals were possibly bolder than others. Bremner-Harrison et al. (2004) showed that captive red foxes (*Vulpes velox*) that were more likely to approach and explore novel objects in a shorter time span were determined to be bold as compared to foxes that did not approach or explore novel objects. If a trained vole was exceptionally bold, rubber band shots may not have had sufficient impact to deter exploration of the scented cloth.

Researcher presence may have also influenced the number of approaches exhibited by the voles. Voles have a keen sense of hearing, and although I attempted to minimize interaction by using a blind and reducing movement and sound, some animals may have been aware of my presence, regardless of any precautions taken. This could have resulted in the voles from both groups refusing to approach the cloth during the training sessions.

Finally, for trained animals, the scent may have been overwhelming. Voles have an acute sense of smell, and even without exploring the cloth, it is very likely that the vole smelled the wolf odor and decided to avoid the perceived threat, thereby bypassing any physical training with a rubber band.

Pre- to post-training.

While training sessions did not differ in explorations between control and trained voles, results indicate that training to recognize a predator increased the total number of alert behaviors to visual and audio predator cues. To my knowledge, there is no current research investigating the link between recognizing various predator cues. Of importance to note is that control animals had a much higher total number of alert behaviors during their base-line test than did the trained voles, but there was no change between pre- to post-testing. This may have been due to age or behavioral differences between the different groups. In any case, trained voles significantly increased alert behaviors to a new stimulus after being trained with rubber bands and wolf odor.

This finding was surprising. Recognition of a mammalian predator scent between different predators may not change or be difficult for an animal to learn due to the high content of sulfurous metabolites in the predator urine and feces due to their meat-heavy diet (Blumstein et al. 2002). However, the fact that training with these odors seemed to increase response to aural and visual cues from an avian predator, which does not often leave olfactory cues, is fascinating. If this is indeed the case, anti-predator training could be a promising tool for conserving and training populations of endangered animals to recognize predators. Many zoos and conservation societies have access to natural predator odors for prey species targeted for release. If training to an odor increases alert behaviors for other predator cues, it is possible to easily and cheaply train animals to recognize and avoid predators, especially without having to expose the prey physically to the predator, which has often been suggested as the best way to train some species (van Heezik et al. 1999).

One point of interest with these data is that in the trained animals, the total number of alert behaviors from pre-testing to post-testing increased; however, two individuals greatly increased their alert behaviors, while six others slightly increased behaviors, and three stayed at the same number. The two that greatly increased their alert behaviors went from 1) a total of one to a total of 11 and 2) from a total of six to a total of 12. These two animals could have greatly influenced the increase of alert behaviors for the trained group. However, because a majority of trained animals increased their behaviors, it is still possible that training was responsible for this increase, especially because both animals with large increases had received two to three rubber band shots during each training session.

Conclusions.

My research supports the supposition that training may be necessary to improve survival of captive-held animals. While I was unable to test captive-bred individuals due to initial difficulties breeding meadow voles in captivity, I was able to essentially test translocated individuals. Translocated individuals can be considered as good models for first generations of captive-bred individuals since translocated individuals are often held in captivity during a crucial period of behavioral development. Using translocated individuals to show that training can be effective is a promising step towards developing a training regimen for captive generations of species with reintroduction as part of their Species Survival Plans.

CHAPTER III

POST-RELEASE SURVIVAL OF ANTI-PREDATOR TRAINED JUVENILE MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

ABSTRACT. Post-release survival is vital for the success of reintroduction programs and anti-predator training may improve post release survival. To test this, juvenile meadow voles (*Microtus pennsylvanicus*) were trained to recognize predator odors and tested for alert responses to a red-tailed hawk call or silhouette. After training, three groups of voles were released: trained, control and wild. Survival monitoring was conducted in Fall 2011 by mark-recapture techniques. Survival estimates were generated using Program MARK. While results were not significant, likely due to small sample sizes, wild and trained groups had similar survival rates, both of which were higher than the survival estimate of the control group. This suggests that post-release survival can be improved by training in controlled laboratory settings.

Introduction

Humans have changed the face of the planet and therefore have impacted wildlife, whether intentionally or inadvertently. Losses of habitat, pollution, exploitation of resources, and introduction of invasive species and disease have all had a hand in the decimation of natural populations of numerous species. The survival of many of these threatened and endangered species rests heavily on human intervention, which currently strongly trends toward captive breeding programs in worldwide zoos, aquaria, and conservation programs. For instance, captive breeding is part of the recovery plans for 64% of U.S. threatened and endangered wildlife (Snyder et al. 1996).

There have been definite benefits to captive breeding programs. In many cases, captive breeding is used as an attempt to bolster world populations, both in captivity and in the wild. These captive-bred individuals are often reintroduced to wild populations to supplement existing populations or to reestablish historical populations (Snyder et al. 1996; Seddon 1999; Britt, Welch, and Katz 2003. 2003).

Translocations are another important conservation tool in the recovery and management of threatened and endangered species. Translocations take wild animals from their home territory, and often after a period in captivity, introduce them into a historical habitat (Fischer and Lindenmayer 2000; Seddon, Armstrong, and Maloney 2007; Griffith et al. 1989). Generally, people think of translocations as a tool to remove problem animals from areas of human development and reintroduce them to a better habitat (Fischer and Lindenmayer 2000). However, translocations have been used as a conservation tool for the gray wolf (*Canis lupus*) and the grizzly bear (*Ursus arctos horriblis*) (Fischer and Lindenmayer 2000). Importantly, the short period of time these individuals are held in captivity could potentially alter their behaviors. For instance, wild bears that are captured for relocation and held in captivity for a short period of time may develop stereotypies, or repetitive behaviors typical of captive animals, which may decrease their chance for survival once they are released (Vickery and Mason 2003). Factors that could also alter behaviors are if the individuals are fundamentally shy, calm, or if these individuals are young and are therefore captive during a necessary part of their natural behavioral development.

Loss of behavior has been documented in a number of species and has manifested itself in various ways. In golden lion tamarins (*Leontopithecus rosalia*), loss of locomotion and orientation while climbing, decrease in foraging ability, and improper social skills and predator avoidance have been noted in captive populations (Beck et al. 2002; Stoinski and Beck 2004). An increase in boldness or a lack of fear has been noted in the swift fox (*Vulpes velox*) (Bremner-Harrison, Prodohl, and Elwood 2004), the Siberian polecat (*Mustela eversmanii*) (Miller et al. 1990) and the black-footed ferret (*Mustela nigripes*) (Biggins and Godbey 2003). This lack of fear of represents an inability to recognize and avoid predators. A lack of predator recognition has resulted in massive mortality of released individuals in golden lion tamarins (Beck et al. 2002), black-footed ferrets (Miller et al. 1990), Siberian polecats (Biggins and Godbey 2003), Houbara bustards (*Chlamydotis undulata***)** (van Heezik, Seddon and Maloney 1999), New Zealand robins (*Petroica australis*) (McLean, Holzer, and Studholme 1999), black-tailed prairie dogs (*Cynomys ludovicianus*) (Shier and Owings 2006 & 2007), and many others.

Anti-predator training is a debated method of improving success after release. Behavioral biologists generally agree that losses of anti-predator behaviors are reversible to some extent (Griffin, Blumstein, and Evans 2000). However, for training to be effective, the individuals must recognize predators by sight, sound, or smell and must learn to appropriately deal with a predator once it has been recognized (McLean, Lundie-Jenkins, and Jarman 1996). Because such small numbers of endangered animals are reintroduced, a single predator can easily eradicate a newly-established population (McLean, Lundie-Jenkins, and Jarman 1996). Few studies have attempted to determine the impacts of training on survival of captive-bred or captive-held animals. New Zealand robins trained to predator models responded fearfully but failed to survive after reintroduction (McLean, Holzer, and Studholme 1999). Masked bobwhite (*Colinus virginianus ridgewayi*) (Wallace 1994), black-tailed prairie dogs (Shier and Owings 2006 & 2007), and houbara bustards (van Heezik, Seddon and Maloney 1999) exposed to live predators showed higher post-release survival.

Post-release survival is often difficult to measure because individuals can move long distances and may not be re-sighted, recovered, or recaptured (Cooch and White 2012). If we are to determine whether training has truly altered behavior and promoted survival, post-release monitoring is necessary. To determine whether anti-predator training was effective in captive-bred meadow voles (*Microtus pennsylvanicus*), I monitored and compared survival for three released groups: trained, control and wild. Animals were brought into captivity and trained prior to release (Wiggins Chapter 2 this volume). Wild and control animals were not trained, and wild animals were in the lab only briefly. I predicted that wild and anti-predator trained voles would survive better than control voles due to retention and restoration of anti-predator behaviors, respectively.

METHODS

Subjects.

Study subjects were juvenile meadow voles trapped between 31 May and 14 June 2011 on a ~2.8-hectare plot of grassland in Oshkosh, WI. Three 0.4-ha enclosures were built out of aluminum flashing buried approximately one foot in the ground with another foot above ground. Stakes were placed within the enclosures at 10 meter intervals. Each stake designated a trap location in a transect line. Sherman traps (7.62 x 8.89 x 22.86 cm) were placed at each stake. Stakes were also set up around the perimeter of each enclosure to capture any voles that might have escaped the enclosures. Traps were baited in the evening between 1600 and 2000 with peanut butter and rolled oat balls along with a cotton ball to provide warmth. Traps were checked between 0600 and 0800 the following morning. If the temperature was below 10 degrees Celsius or if thunderstorms were in the area, traps were not set for the safety of the animals and lab members.

Twenty-eight juvenile voles were ear-tagged with a distinct identification number and then randomly and evenly put into a control or trained group. Of the 14 in each group, 11 trained animals and 10 control animals survived through all testing and training sessions. Voles in both groups were housed in standard mouse cages (27.94 x 17.78 x 12.7 cm) topped with wire hoppers. Cages were lined with 500 mL of Sani-Chip ® bedding and voles were given a nesting square for enrichment. Food and water were given *ad libitum*. Cages were fully changed at least once a week, with additional changes as necessary.

Fourteen wild voles were brought into the lab the week prior to the release. These animals were held under the same housing conditions as predator and control groups, but were not treated with anti-parasitics or handled. These animals were used as a wild control against both groups.

Release.

After testing and training (Wiggins Chapter 2 this volume), voles were released into the three 0.4-ha outdoor enclosures on 17 August 2011. Voles were assigned to an enclosure prior to release date. Voles from the control group, the trained group, and a wild group were assigned to a transect point with males and females alternating. One third of all groups were randomly assigned to one of the three enclosures; however, each animal was assigned to a specific stake without complete randomization to keep all animals from the same group being placed in the same area or the same enclosure.

On the day of release, voles were placed into clean Sherman traps containing a piece of apple and a nesting square. Each trap was labeled with the previously-assigned transect location and enclosure. Voles were in the trap prior to release for less than one hour. Once transported to the field site, voles were released at their designated stakes between 1600 and 1630. Voles were released using a soft release method—all traps were locked and left open until the first survival monitoring period. This allowed the vole access to shelter from the elements for the first week that they were in the wild.

Trapping to monitor survival began on 25 August 2011 using the same trapping methods described above. Any voles without tags were removed from the enclosure and released in a prairie approximately 1.6 kilometers away. Voles with torn ears were assumed to be released voles with lost ear tags, but they could not be identified; therefore, they were noted and released back into the enclosure but not included in the survival data.

Voles recaptured during survival monitoring were examined for ear tags with personal identification numbers, and females were checked for reproductive activity. All voles were placed in a cloth bag and weighed using a 100-gram

Pescola scale. After weighing, voles were immediately released back into the enclosure at point of capture. A total of 12 trapping days was recorded from 25 August to 29 September 2011.

Mark-recapture analysis.

Survival and recapture rates were analyzed using Program MARK (http://warnercnr.colostate.edu). In Program MARK, ϕ is used to denote the probability of survival, and ‘p’ is used to denote the probability of recapture. Models of interest were defined *a priori* and run on the data. Variables of interest were defined as group (g), time in wild (t), and null (.) (Table 1).

Table 2-1. Definition of models tested using Program MARK.

Model	**Definition**
ϕ(.)p(.)	Survival(no effect), Recapture(no effect)
ϕ(g)p(.)	Survival(group effect), Recapture(no effect)
ϕ(g)p(g)	Survival(group effect), Recapture(group effect)
ϕ(t)p(.)	Survival(time effect), Recapture(no effect)
ϕ(.)p(t)	Survival(no effect), Recapture(time effect)
ϕ(t)p(t)	Survival(time effect), Recapture(time effect)
ϕ(.g*t)p(.)	Survival(group*time effect), Recapture(no effect)
ϕ(g*t)p(g*t)	Survival(group*time effect), Recapture(group*time effect)

Results

From Program MARK, I determined that my data set was under-dispersed. However, the model $\phi(.)p(.)$ appeared to fit the data set best with an AIC of 311.6345 with two parameters. Another model, $\phi(g)p(.)$, also fit the data (AIC value 313.0802). A likeness ratio test determined that these two models were sufficiently similar to be considered the same, and because $\phi(g)p(.)$ had two more parameters, I used this model to explore survival estimates. Figure 2-1 shows that trained and wild groups had similar point survival estimates as well as similar variations in 90% confidence intervals (CI), whereas the control group had a slightly lower point survival probability as well as a larger 90% CI. Predator-trained and wild animals both had a point estimate of 0.97 (90% CI: trained 0.94-0.99; wild 0.94-0.99), whereas control animals had a point estimate of 0.94 (90% CI: control 0.88-0.97).

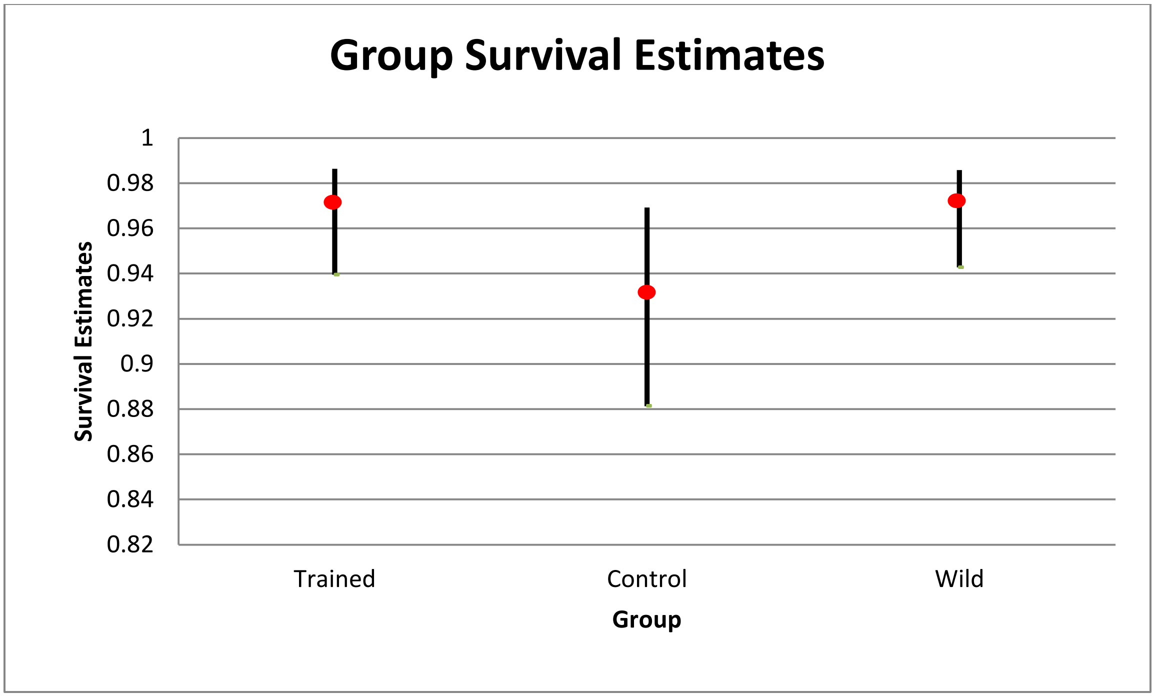

Figure 2-1. Comparison of survival probabilities with 90% confidence intervals between trained, control, and wild voles. Red circles indicate point estimates.

Discussion

One of the greatest threats to small mammals and birds during reintroductions is predation by unrecognized predators (Snyder et al. 1996; Biggins and Godbey 2003; Seddon, Armstrong, and Maloney 2007). My prediction that trained animals would have a higher post-release survival than control voles was not supported through my mark-recapture analysis. The results of mark-recapture analysis were not significant, although there was a slight trend towards wild and trained voles to survive similarly.

There are several possible reasons for this potential trend, including training, time in captivity, human interactions, age of the vole, and wild factors, such as disease. First, training may have impacted survival by promoting proper anti-predator defenses in the captive-held juvenile voles. However, I was unable to distinguish this factor from other possible variables, such as time in captivity. Wild voles were only held in the lab for two weeks, whereas trained and control voles were held in the lab for a little over two months. This lengthened period of time may have resulted in a greater loss of behavior in control voles that received no training to promote predator avoidance. Human interactions were also much higher in trained and control animals. Every Friday, cages were cleaned, during which time the vole was caught, weighed and moved. If cages were dirty on Mondays, cages were changed again. During training sessions, voles were caught by hand and introduced into the arenas. Wild animals, by contrast, received minimal cleaning and handling for the two weeks they were in the lab facilities. This may have had an impact on vole behavior. Trained and control animals often attempted to escape or bite handlers. A recent change to our lab protocol requires researchers to move voles with a beaker to reduce the amount of stress during cleaning.

The age of the animals during their time in captivity could have had an impact on post-release survival. Juvenile voles were used for both control and trained groups. This means the animals developed into mature individuals while in captivity, thereby bypassing any natural behaviors that they would have potentially learned in the wild. Wild animals were all adults, and many of the females had pups while in the lab. Wild animals were adults and reproduced while in the wild and therefore may have learned and retained better survival skills than the control and trained animals.

Non-predation wild factors, such as the inability to forage or the presence of parasites and disease may have influenced survival and recapture the most. I only trained animals to learn to recognize predators; however, lack of foraging and social skills can also develop during time held in captivity. For instance, golden lion tamarins had a remarkable inability to forage and act properly when released into the wild (Beck et al. 2002; Stoinski and Beck, 2004). Research by Kozuch (unpublished thesis 2012) has shown that time in captivity can affect foraging ability in adult meadow voles. My vole groups were possibly unable to forage efficiently and therefore suffered reduced survival. Another possibility was that parasites killed the voles. Several voles were trapped during the summer that had bot flies. Other parasites may have had an impact on the released voles' survival as well but were not observed. There were several voles found dying or dead in traps with unknown causes. These voles may have contracted some sort of disease which decreased their survival. Attempting to determine the cause of death of a meadow vole is incredibly difficult. Therefore some of my released voles may have died for reasons other than predation. Anti-predator trained individuals still trended towards greater survival than control individuals and therefore my training may have still impacted survival.

Another potential problem arises with recapture probabilities. Animals may learn to be trap prone or trap shy, thereby influencing the ability to accurately assess survival. MARK analysis determined that there was no effect and therefore no difference between groups on recaptures probabilities; therefore, it is unlikely that this happened in my release.

Finally, perhaps the biggest influence on survival probabilities was the loss of the individual ear tags in several voles and the small sample size. The loss of ear tags resulted in the inability to identify an animal, which meant that several animals that were caught and had torn ears may have been a part of one of my study groups. This reduced my already small sample size, which may have also influenced the results. A larger sample size may have produced visible, statistically significant differences in the trained, wild and untrained groups.

While there may be several possible explanations for the slight trend in survival differences between released groups, the most likely explanation seems to hinge on anti-predator training. MARK analysis supports this, and trained voles had increased anti-predator behavioral responses after training (Wiggins Chapter 2 this volume). This supports the idea that training might have an impact on survival of released animals and should be incorporated into the Species Survival Plans (SSP) of captive-bred species targeted for release back into native areas.

Currently, released animals typically have low survival rates, and very few studies have reported post-training survival rates. Exposing juvenile black-tailed prairie dogs to model predators in conjunction with conspecific alarm call recordings seemed to improve post-release survival (Shier and Owings 2006 and 2007). Rufous hare-wallabies increased caution to cat and fox after fright training; these animals were not released, so whether these behavioral changes would have promoted survival is unknown (McLean, Lundie-Jenkins, and Jarman 1996). Captive-bred New Zealand robins improved responses to predators after training, but since all birds (trained and untrained) died within six months of release, it is unclear whether training really did improve survival (McLean, Holzer, and Studholme 1999). Most training programs use model predators, as the use of live predators has severe ethical implications. However, using live fox significantly increased post-release survival in captive-bred houbara bustards as compared to bustards trained with model fox (van Heezik, Seddon and Maloney 1999).

My study is one of only a few to examine post-release survival after in-lab training. I developed a training regimen that is cost and time efficient and effective. My trained animals survived almost as well as their wild counterparts. This suggests that training to a model with a negative stimulus can improve survival and therefore help to reestablish behaviors lost in captivity. Survival is a very important goal of conservation, and training may be a powerful tool in improving the survival of captive-held and captive-bred animals.

CHAPTER IV

CONCLUSIONS

Laboratory studies conducted on a variety of species have shown that training can improve behavioral responses in laboratory settings. In Siberian polecats (*Mustela eversmanii*), training improved fright responses to owl silhouettes and stuffed badgers (Miller et al. 1990). Houbara bustards (*Chlamydotis undulate*), black-tailed prairie dogs (*Cynomys ludovicianus*), and New Zealand robin (*Petroica australis*) also recognize and respond appropriately to predators in laboratory settings (van Heezik, Seddon and Maloney 1999; Shier and Owings 2006, 2007; McLean, Holzer, and Studholme 1999).

In Chapter 1, trained juvenile meadow voles (*Microtus pennsylvanicus*) increased alert behaviors in the presence of a red-tailed hawk (*Buteo jamaicensis*) call or silhouette. While the number of approaches in training did not differ between trained and control groups, and in fact, were seemingly random, post-training responses suggest that training did promote learning in the juvenile meadow voles. Further studies should be conducted to test larger sample sizes, as well as other species of animals. However, I suggest that predator training for captive-bred threatened and endangered animals should incorporate several types of predator cues from different predators to better promote learning under laboratory conditions.

While laboratory learning has been documented, post-training survival of released animals is rarely reported. Shier and Owings (2006 and 2007) reported an increase in post-release survival of juvenile black-tailed prairie dogs that had been trained to recognize and avoid predators under controlled laboratory settings. Houbara bustards exposed to live predators survived better post-release than bustards trained with a model or birds that did not receive training (van Heezik, Seddon and Maloney 1999).

Although not significant, my research showed a trend for trained animals to survive better than control animals. Larger sample sizes may result in significance and further research and monitoring is necessary to determine if this result is repeatable and therefore supported. Further research is needed to elucidate the full effects of training on post-release survival.

REFERENCES

Ballou, J.D., Kleiman, D.G., Mallinson, J.J.C., Rylands, A.B., Valladares-Padua, C.B., and Lues, K. 2002. History, management and conservation role of the captive lion tamarin populations. In: *Lion tamarins: biology and conservation*: 95—114. Kleiman, D.G. and Rylands, A.B. (Eds). Washington, DC: Smithsonian Institution Press.

Beck, B.B. 1995. Reintroduction, zoos, conservation, and animal welfare. In: *Ethics on the Ark*: 155—163. Norton, B.G., Hutchins, M., Stevens, E.F., and Maple, T.L. (Eds). Washington, DC: Smithsonian Institution Press.

Beck, B.B., Castro, M.I., Stoinski, T.S., and Ballou, J.D. 2002. The effects of prerelease environments on postrelease management on survivorship in reintroduced golden lion tamarins. In: Kleiman, D.G. Rylands, A.B. editors. In:*Lion tamarins: biology and conservation*: 283—300. Washington, D.C.: Smithsonian Institution Press.

Biggins, D. E., and Godbey, J. L. 2003. Challenges to reestablishment of free-ranging populations of black-footed ferrets. *Comptes Rendus Biologies.* 326:104—111.

Blumstein, D.T., Daniel, J.C., & Evans, C.S. 2000. JWatcher. Accessed October 1, 2011:http://wwww.jwatcher.ucla.edu.

Blumstein, D.T., Mari, M., Daniel, J.C., Ardron, J.G., Griffin, A.S., and Evans, C.S. 2002. Olfactory predator recognition: wallabies may have to learn to be wary. *Animal Conservation*. 5:87—93.

Bremner-Harrison, S., Prodohl, P.A. and Elwood, R.W. 2004. Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive bred swift fox (*Vulpes velox*). *Animal Conservation*. 7:313—320.

Britt, A., Welch, C., and Katz, A. 2003. Can small, isolated primate populations be effectively reinforced through the release of individuals from a captive population? *Biological Conservation.* 115:319—327.

Carroll, C., Vucetich, J.A., Nelson, M.P., Rohlf, D.J., and Phillips, M.K. 2009. Geography and recovery under the U.S. Endangered Species Act. *Conservation Biology.* 1—9.

Chadwick, D.H. 2010. Wolf Wars. In: *National Geographic*: 34—55.

Conway, W. 1995. In: *Ethics on the Ark*: 1—9. Norton, B.G., Hutchins, M., Stevens, E.F., and Maple, T.L. (Eds). Washington, DC: Smithsonian Institution Press.

Cornell University. 2011. Red-tailed hawk. Accessed July, 2011: http://www.allaboutbirds.org/guide/Red-tailed_Hawk/id.

Cooch, E. and White, G. (Eds). 2012. Program MARK: “A gentle introduction”. 11th edition. Online book. Accessed May 20, 2011: http://www.phidot.org.

Cooch, E. and White, G. (Eds). 2012. Program MARK. Accessed June 3, 2011: http://www.phidot.org.

Dobson, A. and Lyles, A. 2000. Black-footed ferret recovery. *Science*. 288(5468):985—988.

Fischer, J. and Lindenmayer, D.B. 2000. An assessment of the published results of animal relocations. *Biological Conservation.* 96:1—11.

Freese, C.H., Aune, K.E., Boyd, D.P., Derr, J.N., Forrest, S.C., Gates, C.C., Gogan, P.J.P., Grassel, S.M., Halbert, N.D., Kunkel, K., and Redford, K.H. 2007. Second chance for the plains bison. *Biological Conservation.* 1—10.

Griffin, A. S., Blumstein, D. T., & Evans, C. S. 2000. Training captive-bred or translocated animals to avoid predators. *Conservation Biology.* 14(5):1317—1326.

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. 1989. Translocation as a species conservation tool: status and strategy. *Science.* 245(4917):477—480.

Harris, J.B.C., Reid, J.L., Scheffers, B.R., Wanger, T.C., Sodhi, N.S., Fordham, D.A., and Brook, B.W. 2011. Conserving imperiled species: a comparison of the IUCN Red List and the U.S. Endangered Species Act. *Conservation Letters.* 5:64—72.

Hayward, M.W., Adendorff, J., Moolman, L., Hayward, G.J., and Kerley, G.I.H. 2006. The successful reintroduction of leopard *Panthera pardus* to the Addo Elephant National Park. *African Journal of Ecology.* 45:103—104.

International Union for Conservation of Nature and Natural Resources. 2011. *The IUCN Red List of Threatened Species. Version 2011.2*. <http://www.iucnredlist.org>. Downloaded on 15 March 2011.

Kleiman, D. G. (1989). Reintroduction of captive mammals for conservation. *BioScience*. 39(3):152—161.

Kozuch, A. 2012. Unpublished thesis.

Krebs, C.J., Keller, B.L., and R.H. Tamarin. 1969. Microtus population biology: demographic changes in fluctuating populations of M. ochrogaster and M. pennsylvanicus in Southern Indiana. *Ecology.* 50(4):587—607.

Maestrelli, J.R., and Wiemeyer, S.N. 1975. Breeding bald eagles in captivity. *The Wilson Bulletin.* 87(1):45—53.

Meretsky, V.J., Snyder, N.F.R., Beissinger, S.R., Clendenen, D.A., and Wiley, J.W. 2000. Demography of the California condor: implications for reestablishment. *Conservation Biology.* 14(4):957—967.

McLean, I. G., Lundie-Jenkins, G., & Jarman, P. J. 1996. Teaching an endangered mammal to recognise predators. *Biological Conservation.* 75:51—62.

McLean, I. G., Holzer, C., & Studholme, B. J. 1999. Teaching predator-recognition to a naive bird: implications for management. *Biological Conservation.* 87:123—130.

McPhee, M. E. 2003. Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation.* 115:71—77.

Miller, B., Biggins, D., Wemmer, C., Powell, R., Calvo, L., Hanebury, L. 1990. Development of survival skills in captive-raised Siberian polecats (*Mustela eversmanni*) II: Predator Avoidance. *Journal of Ethology.* 8:95—104.

Myers, J.H. and Krebs, C.J. 1971. Genetic, behavioral, and reproductive attributes of dispersing field voles Microtus pennsylvanicus and Microtus ochrogaster. *Ecological Monographs.* 41(1):53—78.

Scott, M.J., Goble, D.D., Haines, A.M., Wiens, J.A. and Neel, M.C. 2010. Conservation-reliant species and the future of conservation. *Conservation Letters.* 3:91—97.

Seddon, P. J. 1999. Persistence without intervention: assessing success in wildlife reintroductions. *TREE.* 14(12):503.

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. 2007. Developing the science of reintroduction biology. *Conservation Biology.* 21(2):303—312.

Shier, D. M., & Owings, D. H. 2006. Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). *Biological Conservation.* 132:126—135.

Shier, D. M., & Owings, D. H. 2007. Effects of social learning on predator training and postrelease survival in juvenile black-tailed prairie dogs, *Cynomys ludovicianus*. *Animal Behaviour.* 73:567—577.

Simons, T., Sherrod, S.K., Collopy, M.W., and Jenkins, M.A. 1988. "Restoring the bald eagle." *American Scientist.* 76:253—260.

Snyder, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., et al. 1996. Limitations of captive breeding in endangered species recovery. *Conservation Biology.* 10(2):338—348.

Stoinski, T. S., & Beck, B. B. 2004. Changes in locomotor and foraging skills in captive-born, reintroduced golden lion tamarins (*Leontopithecus rosalia rosalia*). *American Journal of Primatology.* 62:1—13.

van Heezik, Y., Seddon, P. J., & Maloney, R. F. 1999. Helping reintroduced houbara bustards avoid predation: effective anti-predator training and the predictive value of pre-release behaviour. *Animal Conservation.* 2:155—163.

Vargas, A., & Anderson, S. H. 1999. Effects of experience and cage enrichment on predatory skills of black-footed ferrets (*Mustela nigripes*). *Journal of Mammalogy.* 80(1):263—269.

Vickery, S. S., & Mason, G. J. 2003. Behavioral persistence in captive bears: implications for reintroduction. *Ursus.* 14(1):35—43.

Wallace, M. 1994. Control of the behavioral development in the context of reintroduction programs for birds. *Zoo Biology.* 13(5):491—499.

Wiggins, B. 2012. Unpublished data.

Wisconsin Department of Natural Resources. 2006. Wisconsin Whooping Crane Management Plan, Section 3: History and Current Distribution. Accessed March 15, 2012: <www.dnr.wi.gov.>