THE APPLICABILITY OF PHYSIOLOGY FOR CONSERVATION AND MANAGEMENT PURPOSES: A CASE-STUDY USING THE BREEDING SEASON OF THE RED-WINGED BLACKBIRD (*AGELAIUS PHOENICEUS*)

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ABSTRACT

Physiology has the potential to play a unique and important role in conservation and management practices by helping identify the mechanistic responses of populations to environmental changes, and providing physiological tools and knowledge that can be applied to help solve conservation and environmental problems. It has previously been unclear, however, if the increase of physiology in conservation and management literature has translated into the application of physiological tools and knowledge into conservation and management plans. There were two purposes of this disquisition: 1) analyze how physiological tools have been integrated into applied conservation by reviewing USFWS endangered species recovery plans, and provide suggestions to help conservation scientists and physiologists work synergistically to solve conservation and management problems, and; 2) provide an example of how studying the physiology of a species can provide useful information for making management decisions, using the study of stress physiology in the red-winged blackbird (*Agelaius phoeniceus*) during their breeding season as our case study. Our results suggest that physiology is being underutilized in USFWS endangered species recovery plans. We hypothesize this absence of physiological tools and knowledge in conservation planning is primarily due to a deficit of physiological knowledge passing between physiologists and the cohort of federal agency recovery plan writers. We suggest the need for increased training of federal agency employees, the inclusion of authors with academic affiliations, increased integration of physiology and conservation research, and enhanced communication between all concerned parties. To illustrate how physiology can be useful, we exposed female red-winged blackbirds to predator and nest parasitism effigies, thus causing additional stress, during the breeding season. We were able to examine how females respond behaviorally and physiologically to stress, and how these responses alter their reproductive decisions. We found that females are responding both behaviorally and physiologically to the increased threat of predation and nest parasitism. There is potential to exploit these responses to manage the species, such as causing females to forgo breeding. We also determined that an individual’s stress physiology can be changed when held in captivity. We suggest using caution when trying to extrapolate captive data to wild populations.

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DEDICATION

I would like to dedicate this disquisition to my father, Jeff Mahoney. Without your love and support through the years, I would have never had the courage to pursue my dreams. You are my rock.

I would also like to dedicate this disquisition to the second most important man in my life, Jesse Poole. Our journey is only beginning, and I can’t wait to see how it unfolds.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropin Hormone.
AICc	Akaike Second Order Information Criterion.
ΔAICc	Delta Akaike Second Order Information Criterion.
ωAICc	Weighted Akaike Second Order Information Criterion.
CORT	Corticosterone.
DEX	Dexamethasone.
ESA	Endangered Species Act.
HPA	Hypothalamus-Pituitary-Adrenal.
NDSU	North Dakota State University.
NH	Natural History.
NMFS	National Marine Fisheries Service.
NRA	Non-Research Based Action.
PC1	Principal Component One of Nest Location.
PC2	Principal Component Two of Nest Location.
RBA	Research-Based Action.
USFWS	United States Fish and Wildlife Service.
USGS	United States Geological Survey.
UTMs	Universal Transverse Mercator Coordinate System.

LIST OF SYMBOLS

β	Beta (y-intercept).
Δ	Delta (change).
h	Height.
K	Number of Parameters.
ω	Omega (weight).
π	Pi.
r	Radius.
V	Volume.
Abs	Absorbance.
AC	Avian Control.
NP	Nest Parasite.
P	Predator.

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1. INTRODUCTION

Physiology has a unique role in conservation and management efforts. In the broadest terms, the study of physiology is the study of the functional and mechanistic responses of an organism. It can be studied at a wide range of scales, from organisms to cells, and a wide range of chemical and physical functions (Cooke *et al.*, 2013). Traditionally, conservation scientists and managers have focused on measuring either demographic characteristics of populations, or patterns of community dynamics to evaluate ecosystem function (Cooke *et al.*, 2013). Physiology can provide the mechanistic insight to help predict species' and individual responses to environmental change (Coristine *et al.*, 2014). In this manner, physiology represents a solution-based approach, where it can detect incremental effects on species or population viability, to help provide early warning for adaptive management and conservation plans (Coristine *et al.,* 2014).

In addition to helping identify the mechanistic responses to environmental changes, physiological tools and knowledge can also be applied to help solve conservation and environmental management problems directly. For example, researchers are using information about sensory biology in sharks to develop methods for reducing bycatch (Jordan *et al.*, 2013). Scientists are also examining ways to use their knowledge of avian sensory ecology to reduce aircraft bird strikes by exploiting avian vision with aircraft lighting (Blackwell *et al.*, 2010).

Although physiology has clear implications for conservation and management, and has been integrated into the scientific literature on conservation (Chidawanyida *et al.*, 2012; Cooke *et al.*, 2013; Lennox and Cooke, 2014), it is unclear if the increase in the literature has translated into the application of physiological tools and knowledge into conservation and management plans. There are two purposes of this disquisition: first, analyze how physiological tools have been integrated into applied conservation and provide suggestions to help conservation scientists, managers, and physiologists work synergistically to solve conservation and management problems, and; second, provide an example of how studying the physiology of a species can provide useful information for making management decisions, using the red-winged blackbird (*Agelaius phoeniceus*) as our study species.

The red-winged blackbird has been a species of interest in pest management for over forty years. This interest stems primarily from the $70 million worth of grain and crop damage caused by the blackbird nationally each year (United States Department of Agriculture *et al.*, 2015). Previously, the main areas of research for blackbird management include developing new scare techniques, improving evasion methods, and developing new methods to control populations (Linz *et al.*, 2011). Current management practices aimed at reducing damage to crops have had some success, but new approaches are needed to better manage crop damage (Linz *et al.*, 2011). With many consumers in today's market concerned with animal welfare, nonlethal management techniques have become more important. One such approach accepted by consumers is the study of nonlethal management techniques aimed at exploiting a prey species' fear of predation (Oh *et al.*, 2015). As part of developing the foundational knowledge on predator-prey interactions and their usefulness in population management, researchers are interested in understanding not only the behavioral interactions, but also the physiological responses of prey towards predators, and how such responses can be used to improve management techniques. The area of physiology that was the main concern of this disquisition was stress physiology in blackbirds, and how hormonal and behavioral responses to stress can affect reproduction.

An individual's physiological stress response is a "suite of physiological and behavioral mechanisms to cope with the stressor" (Wikelski and Cooke, 2006). The stress response is modulated by the hypothalamus-pituitary-adrenal (HPA) axis, such that during a stressful event an individual's adrenal gland secretes glucocorticoids, steroid hormones, via the HPA axis. In avian species, this process starts with the stimulation of the hypothalamus, which secretes corticotrophin-releasing factor to stimulate the pituitary (Rich and Romero, 2005). Once stimulated, the pituitary secretes adrenocorticotropin hormone (ACTH) which causes the adrenal gland to release corticosterone (CORT) (Rich and Romero, 2005). Once the stressful event has been mitigated, CORT acts on the pituitary in a negative feedback loop to quickly suppress further CORT release (Dickens *et al*., 2009a). Increased CORT levels help animals survive stressful situations, such as escaping a predator, by increasing energy in muscle tissue through facilitation of metabolic changes that activate glucose stores and inhibit additional glucose storage (Cyr *et al*., 2007). However, maintaining high levels of CORT during times of chronic stress has been linked with physiological consequences that endanger fitness, including hyperglycemia, neuronal cell death, and suppression of the immune and reproductive systems (Cyr *et al*., 2007).

An individual's physiological response to stressors can be measured using circulating plasma CORT levels, CORT levels found within the yolk of a female's egg, and even from CORT levels found in molted feathers and feces (Sheriff *et al*., 2011). Each method has its own pros and cons. For example, monitoring stress response from the egg yolk or molted feather is less invasive for the individual you are studying, but provides different information than plasma (Sheriff *et al*., 2011). Corticosterone from feathers provides a snapshot of what an individual's stress levels were during the previous molt (Legagneux *et al*., 2013), and yolk CORT levels can potentially provide information about a female's baseline CORT concentrations for the current season (Love *et al.*, 2008; Sheriff *et al.*, 2011), but does not provide you with the overall functionality of the HPA axis that measuring plasma levels can provide (Rich and Romero, 2005; Sheriff *et al.*, 2011).

In addition to being able to measure baseline CORT levels with plasma, we can also measure hormone profiles for individual birds (Table 1.1). When an individual is exposed to an acute stressor, CORT levels begin to increase shortly after exposure (usually within three minutes), peak approximately 30 minutes after exposure, and then being to decrease back to baseline levels (Rich and Romero, 2005). By measuring the natural profiles of individuals, we can evaluate if their HPA axis function deviates from this expected pattern. If they deviate from the norm, an individual may be exposed to higher levels of CORT for longer, which can be harmful for the individual (Dickens *et al*., 2009b). Deviations may also indicate normal variation of this response in different species, which can be help in understanding how different species respond behaviorally to environmental stressors. For example, some individuals may be able to modify their stress response based on the severity or duration of the stressor, thus potentially reducing the harmful effects of extended elevated CORT levels (Rich and Romero, 2005). Another option for examining the functionality of the HPA axis is to use an HPA challenge protocol, where you can individually measure different portions of the HPA axis by injecting different releasing factor hormones at different times (Table 1.1). By using an HPA challenge protocol you can identify exactly where the HPA axis has been modified to help individuals cope with chronic stress (Dickens *et al.*, 2009a; Sheriff *et al.*, 2011).

Table 1.1. Methods for Analyzing the CORT Stress Response of an Individual.

Protocol Type	Sample/Injection Protocol	Purpose	Example/Citation
Baseline blood sample collection	Blood sample within 3 minutes of the initiation of stress.	Basal CORT samples represent an individual's non-stressed CORT levels. Basal CORT levels may be altered during time of chronic stress.	Dickens *et al.*, 2009a Marra *et al.*, 1995 Rich and Romero, 2005 Sheriff *et al.*, 2011
Profile	Blood samples collected at 0, 15, 30, 45, and 60 minutes.	Shows the natural progression of the HPA axis after stimulation from an acute stressor. Can measure basal CORT, peak CORT, rate of CORT increase and decrease, and total CORT.	Cabezas *et al.*, 2013 Romero and Remage-Healey, 2000
DEX injection (Partial HPA Challenge)	Blood sample at 0-3 minutes DEX injected immediately Blood sample at 30 minutes Blood sample at 60 minutes.	DEX acts as a synthetic CORT. Shows if the negative feedback of the HPA axis is working correctly.	Sheriff *et al.*, 2011 Rich and Romero, 2005
ACTH injection (Partial HPA Challenge)	Blood sample at 0-3 minutes ACTH injected immediately Blood sample at 30 minutes Blood sample at 60 minute	ACTH acts on the adrenal to produce CORT. Shows if the adrenal is producing maximum CORT levels.	Rich and Romero, 2005 Romero and Rich, 2007
DEX – ACTH injections (Partial HPA Challenge – No Natural Stress Response)	Blood sample at 0-3 minutes DEX injection immediately Blood sample at 30 minutes ACTH injected immediately Blood sample at 60 minutes.	Shows if the negative feedback of the HPA axis is working correctly. Shows if the adrenal is producing maximum CORT levels. Show rate of CORT increase. Does not show the natural stress response to compare to maximum CORT levels.	Sheriff *et al.*, 2011 Rich and Romero, 2005
DEX – ACTH injections (Full HPA Challenge)	Blood sample at 0-3 minutes Blood sample at 15 minutes DEX injection immediately Blood sample at 45 minutes ACTH injection immediately Blood sample at 60 minutes	Shows if the negative feedback of the HPA axis is working correctly. Shows if the adrenal is producing maximum CORT levels. Shows rate of CORT increase. Can compare natural increase to maximum increase.	Dickens *et al.*, 2009a Dickens *et al.*, 2009b

For the purpose of this disquisition, we chose to use a combination of measuring yolk hormones, hormone profiles, and HPA challenges to examine how red-winged blackbirds respond to chronic stress during the breeding season.

The breeding season is an energetically expensive time for female red-winged blackbirds. The risks associated with a breeding season include decreased survival through predation (Lima, 2009) or increased costs of self-maintenance (Drent and Daan, 1980), decreased survival of offspring through predation (Lima, 2009), and low food availability or brood parasitism (Payne, 1977). When a female is making reproductive decisions, she must not only consider the costs and risks associated with the current breeding attempt and her current season's reproductive output, but also future reproductive output (Searcy, 1979). In addition, the reproductive decisions a female makes during the breeding season may be in response to, or in avoidance of, alterations to her physiological stress response. For example, as increased CORT levels facilitate the use of energy reserves (Cyr *et al.*, 2007), females under chronic stress may need to focus their energy towards self-maintenance rather than towards their reproductive output during the current breeding season (Astheimer *et al.*, 1995). Other changes a female may make during the breeding season in response to stress may include moving her nesting location away from predation or parasitism risks (Lima, 2009), delay breeding (Perrins and McCleery, 1989), reduce her clutch size (Perrins and McCleery, 1989), reduce parental care via changes in nest structure (Lima, 2009), or reduce incubation time or feeding rate (Lima, 2009). In addition, a female's physiological response to stress during the breeding season may cause alterations to offspring phenotypes via the allocation of different hormones provided in the egg (Hayward and Wingfield, 2004). These alterations may reduce a female's reproductive success for the breeding season (Hayward and Wingfield, 2004). For example, in the Japanese quail (*Coturnix coturnix* *japonica*), females with high plasma CORT levels laid eggs with increased CORT levels, and their offspring grew slower than offspring from mothers with normal CORT levels (Hayward and Wingfield, 2004). All of these behavioral and physiological impacts of predation and nest parasitism stress during the breeding season could potentially impact reproductive success rates at the end of the season.

A change to the reproductive success rate of a breeding season also alters the number of birds recruited to the blackbird population in the fall. The majority of crop damage from blackbirds occurs in the fall as their diet shifts from high protein invertebrate prey during the breeding season to help fuel reproduction and offspring growth, to grains that help fuel migratory and overwintering physiology (Hintz and Dyer, 1970; Hintz, 2000). Thus, it is important to have a comprehensive study of how females balance multiples risks during the breeding season when making reproductive decisions, as those decisions will ultimately affect fall populations.

Another aspect to studying stress physiology in red-winged blackbirds is to understand how captivity affects blackbird behavioral and physiological responses to stress. This is especially relevant, as much of the initial research for the development of scare devices and methods for controlling populations involve initial behavioral and physiological studies in laboratory settings. A common practice for such research is to catch birds in the wild and study them in captivity, where individuals are easily accessible and environmental factors can be controlled to help focus research questions (Bateson and Feenders, 2010). Unfortunately, a significant limitation of captive studies is captive individuals are exposed to a completely different suite of stressors than natural populations, and likewise, may have a unique set of coping mechanisms (Archard and Braithwaite, 2010).

There are many forms of stress for wild individuals in captivity, such as confinement and reduced retreat space, abnormal social groups, and aversive sounds and odors (Morgan and Trombrog, 2007). These new stressors to wild individuals in captivity can potentially lead to chronic stress, and their physiological responses to stress may be modified to help them cope better (Koolhaas *et al.*, 1999). For example, the ability to suppress physiological sensitivity is beneficial to helping wild individuals cope with the stresses of captivity (Angelier *et al.*, 2016). In rock pigeons (*Columbia livia*), individuals with increased CORT stress responses lost more body weight, and had less success to adjusting to captivity than individuals with suppressed CORT responses (Angelier *et al.*, 2016).

By understanding how individuals respond to chronic stress during the breeding season and in captivity, we can help provide the foundational knowledge required to improve scare devices for managing the crop damage caused by red-winged blackbirds. Where previous studies have worked at the population level, our physiological data helps provide a clearer picture for the mechanisms governing population size and behaviors.

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2. THE USE OF PHYSIOLOGY IN USFWS AND NMFS ENDANGERED SPECIES RECOVERY PLANS

2.1. Abstract

Applying physiology to help solve conservation problems has become increasingly prominent in the field of conservation science. It is unclear, however, if the increased integration into the scientific community has translated into the actual application of physiological tools in conservation planning. We completed a review of the use of animal physiology in U.S. Fish and Wildlife Service's Endangered Species Act recovery plans developed between 2005 and 2016. Over those eleven years, 135 of the 146 recovery plans incorporated physiology, with 60% of them including it as background information on the natural history of the species and not as part of the recovery process. Of the 12 sub-disciplines of physiology previously described, immunology and epidemiology was used most often. Our review suggests a disconnect between available physiological tools and the potential role of physiology in developing conservation plans. We provide three suggestions to further guide conservation scientists, managers, and physiologists to work synergistically to solve conservation problems: (1) the breadth of knowledge within a recovery plan writing team can be increased by, for example, increased training of federal agency employees or the inclusion of authors with academic affiliations; (2) physiologists can make their research more available to conservation scientists and federal agencies by clearly linking their research to conservation; and, (3) communication can be enhanced between government conservation scientists and physiologists.

2.2. Introduction

Conservation scientists and managers are constantly faced with new challenges when preserving and protecting habitats and mitigating new threats to plant and animal populations.

These challenges are compounded as the number of undisturbed habitats diminishes exponentially, while at the same time, the number of anthropogenic impacts increases. Scientists predict that 15-37% of existing plants and animal species in threatened geographical areas will be extinct by the year 2050 (Thomas *et al.*, 2004). Although the field of conservation biology is integrative by including a wide range of social and scientific fields, conservation scientist and managers have traditionally focused on measuring either demographic characteristics of populations, or patterns of community dynamics to evaluate ecosystem function (Cooke *et al.*, 2013). Complex conservation problems will require managers and scientists to use all tools and information available to create innovative solutions. One potentially helpful field that has gained increasing interest in the conservation literature is physiology (Cooke *et al.*, 2013).

Conservation physiology is a sub-discipline of conservation biology, first defined in 2006 to help identify the important ways that physiological knowledge and tools can be used to help understand and solve conservation problems (Wikelski and Cooke, 2006). The most recent definition of the field describes conservation physiology as ‘an integrative scientific discipline applying physiological concepts, tools, and knowledge to characterizing biological diversity and its ecological implications; understanding and predicting how organisms, populations, and ecosystems respond to environmental change and stressors; and solving conservation problems across the broad range of taxa’ (Cooke *et al.*, 2013). Although the sub-discipline has only recently been defined, using physiology for management purposes is not a new concept. In the past, physiological data has helped conservation biologists and law makers develop legislation and regulations to protect both vulnerable habitats and species. For example, the U.S. Fish and Wildlife Service (USFWS) have been studying the physiological effects of lead poisoning from lead shot and lead sinkers on wildlife since the 1930s (National Wildlife Health Center, 2016).

Their research helped provide the scientific support for banning lead shot for waterfowl hunting in 1991 (National Wildlife Health Center, 2016). Environmental toxicology studies on lead have also provided the scientific support for individual states such as Washington, Maine, and New York to regulate or ban the use of lead fishing sinkers (National Wildlife Health Center, 2016).

The field of conservation physiology includes areas of research such as bioenergetics and nutrition as well as toxicology, stress and reproductive physiology. Previous reviews of the field identify the potential sub-disciplines of physiology that can be used in conservation efforts (Wikelski and Cooke, 2006; Cooke *et al.*, 2013; Madliger and Love, 2015). One area of conservation physiology that has received considerable attention and has potential to affect management plans is the use of stress physiology as an early warning system for negative population responses (i.e. decline) to environmental changes. Managers respond to declining populations by changing management approaches without always clearly understanding the mechanisms of the population's decline. However, population declines might be detected earliest by understanding the physiological responses of individuals. For example, in zebra finches (*Taeniopygia guttata*), increases in stress hormones early in life are correlated with decreased adult lifespans (Monaghan *et al.*, 2012). By monitoring individuals at the physiological level, researchers can provide mechanistic insight to help managers not only better detect, but also identify and predict species' responses to changing environments (Coristine *et al.*, 2014).

Researchers have identified two main ways that physiological research is most applicable for conservation: case-directed and tool refinement (Madliger and Love, 2015). When physiology is used in a case-directed manner, there is a specific conservation question to which managers incorporate existing physiological principles and information. In case-directed scenarios, conservation biologists and managers are responsible for identifying, understanding, and applying the physiological information and tools to their problem (Madliger and Love, 2015). However, when physiology is integrated into conservation using tool refinement, physiologists identify and generate potential tools that can be useful for conservation biologists and managers, thus making it easier for conservation biologists to incorporate physiological knowledge and tools into their management plans (Madliger and Love, 2015).

Case-directed flow of information has been the traditional way that physiology is used in conservation projects; but, tool refinement has gained increasing use. For example, stress physiology has been extensively studied in the field of physiology. Researchers have identified how tools to quantify physiological stress can be used for conservation as outlined in the review by Dantzer *et al.* (2014). In a recent bibliometric analysis on the integration of physiology and conservation research, Lennox and Cooke (2014) suggest physiologists interested in contributing to conservation efforts submit research articles to conservation-themed journals. Physiologists should also focus on providing easily accessible information for those charged with developing conservation plans (Lennox and Cooke, 2014). With the creation of the topic focused journal "Conservation Physiology," the applicability of physiological tools to conservation are accessible to conservation biologists and other authors of management plans more than ever before.

Since the sub-discipline of conservation physiology was defined, applying physiology to help solve conservation problems has become increasingly more prominent in the field of conservation science (Cooke, 2014; Lennox and Cooke, 2014). The increasing interest in the field has prompted reviews that have used the scientific literature to categorize the different ways that physiology can be useful for conservation (Wikelski and Cooke, 2006; Cooke *et al*., 2013; Madliger and Love, 2015). Additionally, a conceptual framework has been developed to guide conservation physiology and promote research cultivating conservation-motivated policy (Coristine *et al.*, 2014). It is unclear, however, if the increased integration into scientific literature has translated into the application of physiological tools in conservation planning and management. In light of this missing link, the purpose of this review was to analyze how physiological tools have been integrated into applied conservation by examining the U.S. Endangered Species Act (ESA) recovery plans developed between 2005 and 2016.

2.3. Methods

2.3.1. Reasoning

The USFW's database of ESA recovery plans was analyzed for the use of physiology in final and draft plans created between 2005 and 2016. Although previous scientists have linked physiological regulations to an animal's ability to adapt to changing environments for decades (Carey, 2005), we selected the start year for our analysis based on the fact that the first time physiology was clearly articulated in the literature as an important conservation tool was in 2005, when Carey (2005) suggested that 'physiological principles, concepts and methods that are rooted in traditional basic research in physiology, physiological ecology, and evolutionary physiology are fundamentally important in understanding the causes of population declines and in conservation planning.' Although we focused solely on animals, conservation physiology can also be an important field for research and management plans focused on endangered plant species (Wikelski and Cooke, 2006). In cases where multiple species were included in a single report, each species was counted as an individual report.

We focused on the recovery plans of federally listed species for a few reasons. First, recovery plans are a required document for all threatened and endangered species. Second, plans are developed by experts in the field of conservation and wildlife biology. These plans are routinely submitted to federal and state agencies that have direct knowledge of the conservation problem, so that feedback can be provided to develop a thorough and accurate report and plan. Thus, these plans should provide accurate insight into the tools and information that conservation biologists and managers across the nation find most important and appropriate to use in conservation efforts. Third, the National Marine Fisheries Service (NMFS) and the USFWS developed a joint document outlining the guidelines for developing and implementing recovery plans that all participating agencies must follow (National Marine Fisheries Service, 2010a). However, planners are given considerable discretion, and the guidelines clearly state that planners should view this discretion as an ‘opportunity to use their creativity and ingenuity to craft the most effective and practical recovery program for each species in their care’ (National Marine Fisheries Service, 2010a). Thus, all recovery plans follow similar outlines, but still show important variation, making them easy to compare. Finally, all of the recovery plans are housed in a national database, making them easily accessible for analysis purposes.

2.3.2. Procedure

Our first objective was to identify the fraction of recovery plans that incorporated some aspect of physiology. To accomplish this, each recovery plan published between 2005 and 2016 was read and any portion that incorporated one of the physiological sub-disciplines outlined in past reviews was highlighted (Wikelski and Cooke, 2006; Cooke *et al.*, 2013). The sub-disciplines included were: bioenergetics and nutritional physiology, cardio-respiratory physiology, chemical physiology, comparative physiology and biochemistry, environmental and ecological physiology, environmental toxicology, evolutionary physiology, immunology and epidemiology, locomotor performance physiology, neurophysiology and sensory biology, physiological genomics, and reproductive physiology (Wikelksi and Cooke, 2006; Cooke *et al.*, 2013). Common words we looked for included (but were not limited to): physiology, temperature, oxygen, toxins, pollutants, disease, parasite, reproduction, hormone, nutrition, and stress. If one of these terms was found, further review was completed to confirm that the term was used while discussing physiology specifically.

Our second objective was to classify how physiology was applied to conservation efforts within each report. We had three main classifications. Our first we termed “Natural History.” In this classification, physiology was used when describing the life history, natural history, or background of the species. For example, in the final recovery plan for the Mexican spotted owl (*Strix occidentalis lucida*), authors discuss the potential physiological impacts climate change may have on the species. They suggest if climate change results in increased periods of time where habitat temperatures exceed the lower or upper limit of the species, it will impact ‘key physiological processes like thermoregulation and water balance’ (U.S. Fish and Wildlife Service, 2012). Our last two classifications were termed research-based action or non-research action. A research-based action is described as when a recovery plan clearly stated that continued physiological research was required for the species of concern. For example, in the recovery plan for the ocelot (*Leopardus pardalis*), authors discuss the need for baseline physiological data. As part of the recovery strategy, they recommend establishing a protocol for physiological assessment and identification, where every ocelot handled will have physical data collected from them to monitor their health and reproductive status (U.S. Fish and Wildlife Service, 2016b). If a recovery plan included a non-research action, it included a plan to use a physiological tool to help in the recovery or monitoring of the species. Using the recovery plan for the ocelot again for an example, the authors recommended conducting serology and pathology surveys on all handled ocelots to determine their ‘overall condition, and the presence and effect of diseases and parasites' (U.S. Fish and Wildlife Service, 2016b). These surveys would be done by collecting blood, hair, urine, and fecal samples. In the ocelot example, the research-based action differs from the non-research action in that the former is collecting baseline information from individuals that may potentially be used latter to help monitor a population's health, whereas, the latter is already using tools to monitor individuals via their physiology and aggregating the data to evaluate the population's health. Another example of a research-based action comes from the draft recovery plan for the Laurel dace (*Chrosomus saylori*). The authors suggest the need for research on how changes in habitat quality (e.g., water temperature, dissolved oxygen, water pH) will affect the physiology of the species (U.S. Fish and Wildlife Service, 2016a). After all reports were categorized, the frequency of use of the three classifications was compared using a chi-square analysis.

To evaluate the relative contribution of traditional population level management techniques compared to physiological approaches, all reports were analyzed for how the term "stress" was used. Environmental stress, or stressors, can be defined as aversive stimulus (Romero, 2004; Dantzer *et al.*, 2014), whereas, the physiological stress response of an individual is a 'suite of physiological and behavioral mechanisms to cope with the stressor' (Wikelski and Cooke, 2006). If recovery plans recognize different environmental stressors as threats to different species, and also try to establish the link between cause and effect of the stressors at the physiological level, effects can be measured and monitored to act like an early warning system against future environmental and anthropogenic changes that may cause population declines. We classified reports on if the link between stressors and the physiological response was or was not made apparent. If the link was made, then that supports the idea that there is a disconnection between the authors of the reports and available physiological techniques and approaches.

Finally, our last objective was to examine what sub-disciplines of physiology were being used the most in the recovery plans. This information could be important for helping future physiologists decide where they should focus their research questions to help conservation efforts. For this, we focused on examining all physiology uses classified as action-based. We then separated them using the sub-disciplines described in Cooke *et al.* (2013) and Wikelski and Cooke (2006) (see Table A1. in the Appendix). The frequency of use of the sub-disciplines was then compared using a chi-square.

2.4. Results

Out of the total 146 recovery reports that were published between 2005 and 2016, 135 (or 92.5%) included the use of physiology. Of those that did include physiology, 55.6% used physiology in the form of describing the natural history of the species, but did not also use an action-based form (Figure 2.1). Overall, of the 135 reports including physiology, the number of reports including physiology in the form of natural history was significantly greater than the number of reports including an action-based form of physiology ($\chi^2 1 = 91.934$, $p < 0.0001$). Of the 135 reports that included physiology, nearly all, 95.6%, used a natural history form of physiology, 44.4% used at least one action-based form of physiology, 31.9% used at least one research-based action, and 17.0% used at least one non-research action. In some instances, recovery reports used a combination of all three forms of the use of physiology (Figure 2.2). When comparing just the reports that used an action-based form of physiology (60 reports, or 44.4% of reports including physiology), research-based actions were used significantly more than non-research actions ($\chi^2 1 = 12.428$, $p = 0.0004$; Figure 2), such that research-based actions were used in 71.7% of reports and non-research actions were used in 38.3% of reports.

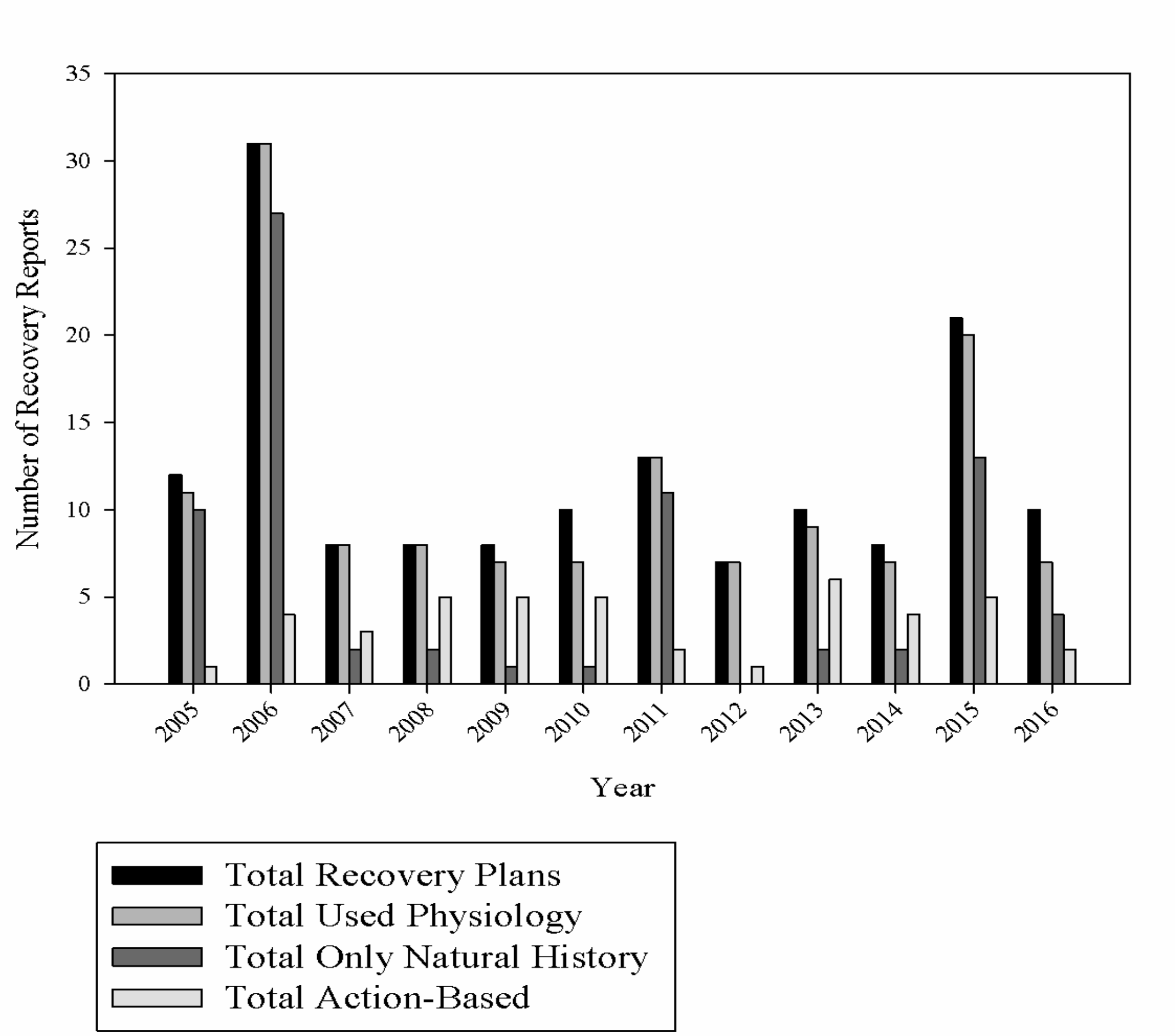

Figure 2.1. The Use of Physiology across Years
Note: The use of physiology in ESA recovery plans across year. Bars represent the total number of recovery plans published in a particular year including those that lacked information on physiology (black), those that included physiology in general (light grey), those that used physiology in the form of natural history, but not an action-based form (dark grey), and those that used at least one of the action-based forms of physiology (white).

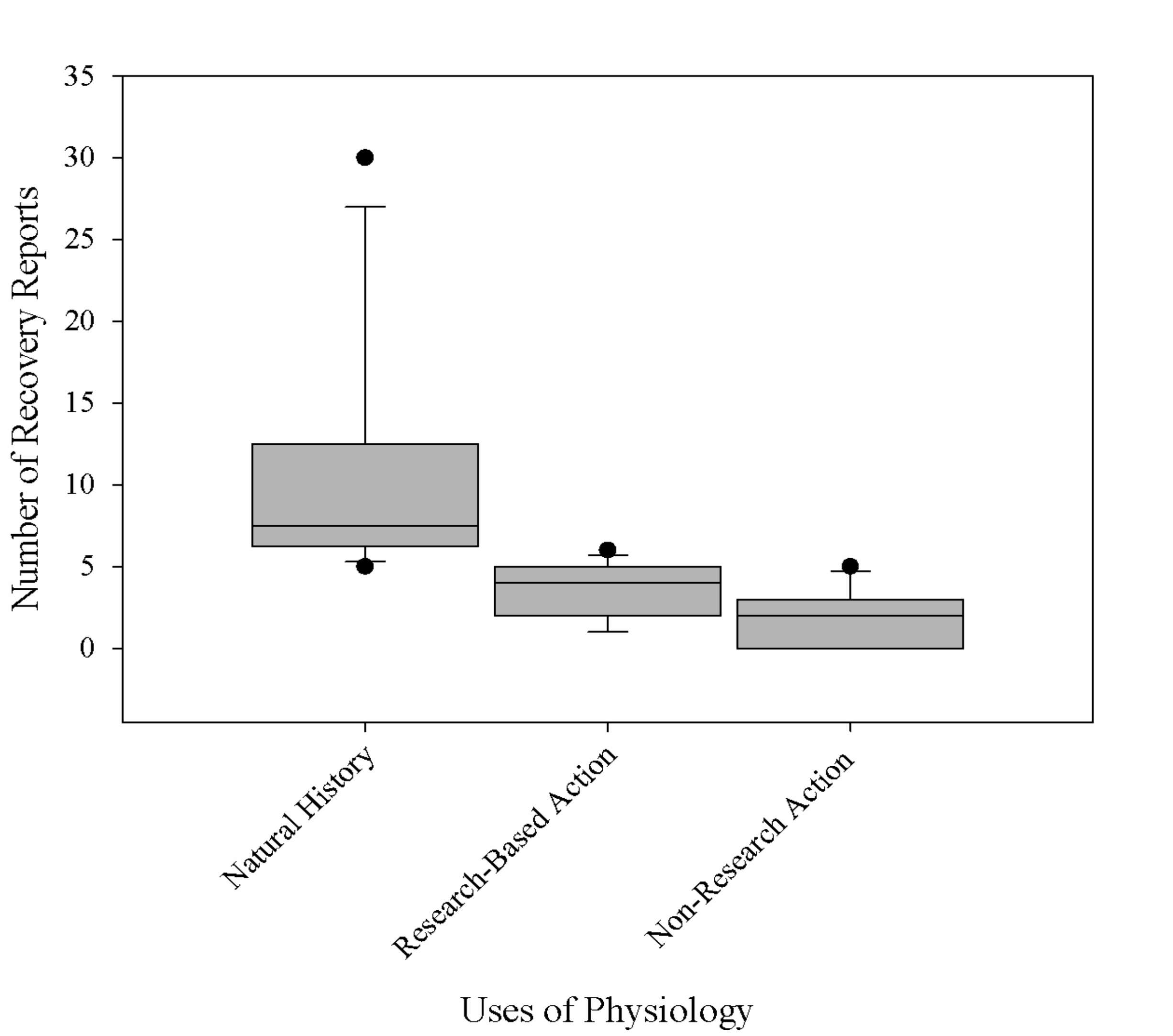

Figure 2.2. A Comparison of Physiology Uses
Note: Number of ESA recovery plans across years that used natural history (NH), research-based action (RBA), or non-research action (NRA). In some instances, reports used more than one form of action-based physiology.

The term "stress" was used with clear physiological meaning in 63.4% of reports that included the term (Figure 2.3). An example of using stress with clear physiological meaning comes from the Kemp's Ridley sea turtle (*Lepidochelys kempii*) recovery plan, where authors discuss how being entangled in fishing gear, and forcibly submerged in water causes 'respiratory and metabolic stress that can lead to severe disturbance of their biochemistry' (National Marine Fisheries Service *et al.*, 2011). An example of using stress with no clear physiological meaning comes from the recovery plan for the St. Andrew's beach mouse (*Peromyscus polionotus peninsularis*), where authors identified each potential threat to the species as a stressor without ever identifying the physiological effects of those stressors on an individual (U.S. Fish and Wildlife Service, 2010).

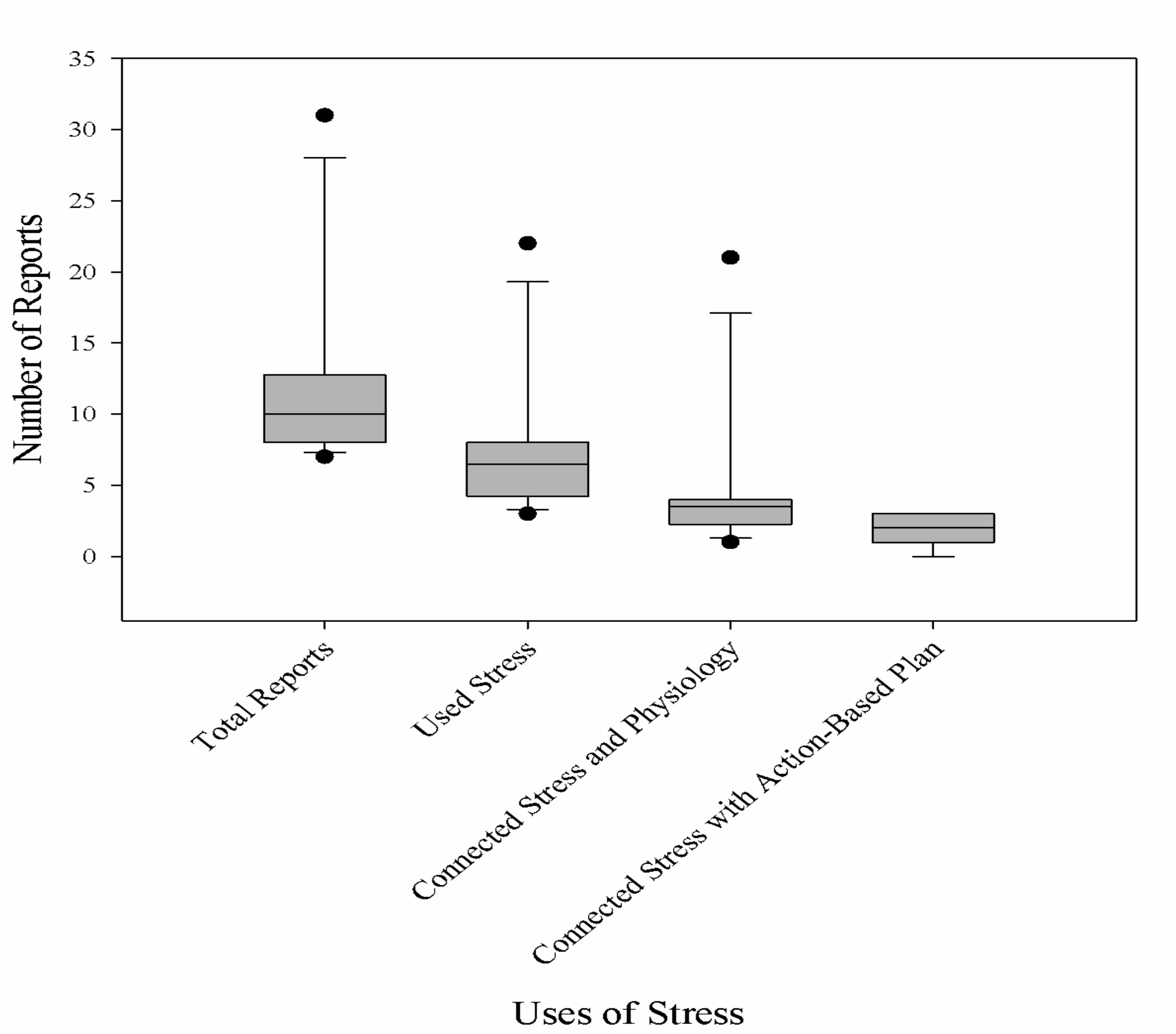

Figure 2.3. The Use of the Term Stress in Recovery Plans
Note: A breakdown of how the term stress was used within the ESA recovery plans for the 11 year period (2005 – 2016).

To further examine the connection between the use of the word stress and its connection to physiology, we also examined how often the word stress was used in conjunction with an action-based use of physiology. In which case, only 22.6% of all recovery plans describing a threat with the terms stress or stressor then proceeded to describe a physiological action-based plan to manage the said threat (Figure 2.3).

Of the eleven sub-disciplines of physiology defined as important to conservation, only eight were in an action-based form for all of the recovery plans. There was a significant difference in the frequency of use of the sub-disciplines ($\chi^2 11 = 162.650$, $p < 0.0001$). The sub-discipline immunology and epidemiology was used the most (63% of reports), followed by reproductive physiology (31% of reports; Figure 2.4).

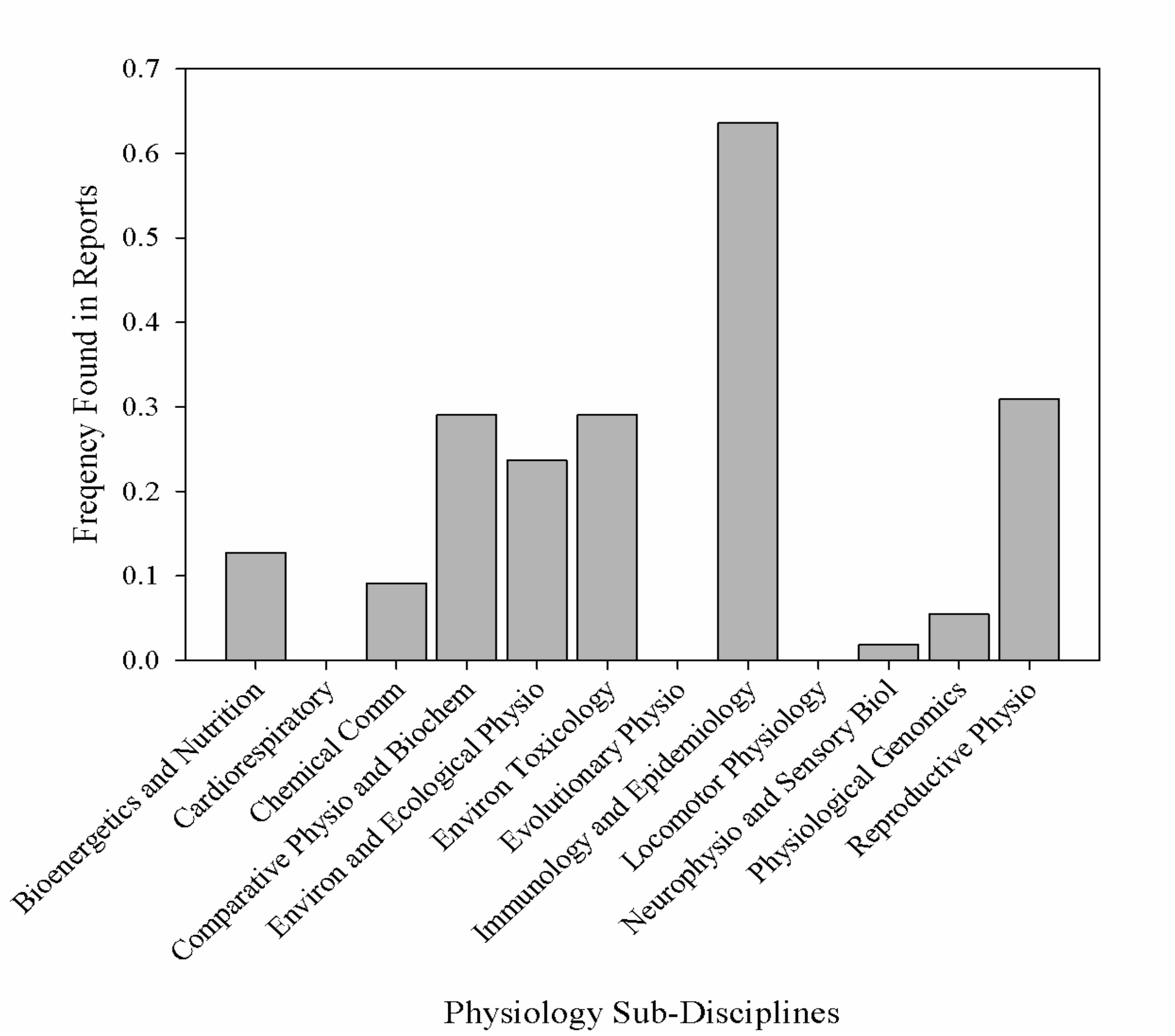

Figure 2.4. Frequency of Sub-disciplines Found in Recovery Plans
Note: The frequency of physiology sub-disciplines used in all ESA recovery plans from 2005 – 2016 that used an action-based form of physiology.

2.5. Discussion and Conclusions

In the past decade, applying physiology to help solve conservation problems has become increasingly prominent in the field of conservation biology. Even so, it is unclear if the increased integration into the scientific community has translated into the actual application of physiological tools in conservation planning. By reviewing USFWS's database of recovery plans, we have provided insight into how managers and conservation scientists are actually using physiology. During the review process, we discovered that there is a major deficit of knowledge passing between physiologists and the federal agency authors assigned to write recovery plans. We identified three main recommendations to further guide conservation scientists, managers, and physiologists to work synergistically to solve conservation problems: (1) the breadth of knowledge within a recovery plan writing team can be increased by, for example, increased training of federal agency employees or the inclusion of authors with academic affiliations; (2) physiologists can make their research more available to conservation scientists and federal agencies by clearly linking their research to conservation; and, (3) communication can be enhanced between government conservation scientists and physiologists.

The importance of physiology for understanding species – environment interactions needs to be communicated to recovery plan authors prior to (preferably), or during, the peer-review process. Written input on how current environmental stressors affect an individual at the physiological level, and thus potentially lead to a cascade of other affects at the population level would be beneficial. The lack of expert input is supported by the fact that although the use of physiology was seen in most recovery plans, it was mainly found in the portion of the plan describing the natural history of the species. Further, the portions of the reports describing the actions required for the recovery of the species rarely utilized physiological tools or methodologies. Lack of awareness could be resolved by forming interdisciplinary teams to ensure that both recovery plans undergoing updates and future plans connect the terms ‘stress’ or ‘stressors’ to an action-based use of physiology. These teams might include university based scientists with specialized expertise pertaining to the physiology of the subject threatened species.

Previous analysis of the process and success of recovery plans suggests that increasing training for writing ESA and recovery plans in federal agencies would increase quality and efficacy of these plans (Clark *et al.*, 2002). Recommendations by the Society for Conservation Biology's recovery plan project pointed out that recovery plans underused modern conservation biology tools (Clark *et al.*, 2002). They also suggested that underutilization was partially due to the tools being relatively new, and the tools had been developed by biologists outside the USFWS and the NMFS. Because the tools were developed outside of the federal agencies, federal employees often lacked the current training to know how to effectively use the tools (Clark *et al.*, 2002). An example of a physiological tool that may require specialized training is the collection and interpretation of glucocorticoid levels, or stress hormone levels, in endangered species (Dantzer *et al*., 2014). There are many mediums that can be used to collect physiological stress data, and when and how to use each technique may require specific training (Dantzer *et al.*, 2014)

Diversifying the authorship of the recovery plans by engaging individuals with academic affiliations can also increase the breadth of physiological tools and knowledge available to the writing teams. According to the "Interim Endangered and Threatened Species Recovery Planning Guidance," federal agencies have the option of developing recovery teams to write recovery plans; and, there have been multiple reviews and analysis of recovery plans that suggest diverse teams make the best and most productive recovery plans (Boersma *et al.*, 2001; Gerber and Schultz, 2001; Clark *et al.*, 2002). By including at least one author with an academic affiliation and physiological background, the number of recovery plans examine the cause and effect relationships between physiology and environmental stressors may increase. In fact, one review that examined how authorship influenced the biology used in recovery plans found that recovery plans with at least one author with an academic affiliation were more likely to clearly link the biology of the species with recovery criteria and monitoring strategies, as opposed to having no clear biological link to the recovery criteria and monitoring strategies (Gerber and Schultz, 2001). The relationship between physiologists and the field of conservation biology should be reciprocal in nature. The time requirements for someone with academic affiliations actively participating as a member of a recovery plan writing team may be extensive, causing potential conflicts between their different responsibilities. However, there are also many advantages to being a part of a writing team such as having access to knowledge of new potential research topics and new avenues for funding (Lennox and Cooke, 2014).

Another possible argument for the lack of physiology in recovery plans is that there just has not been enough relevant physiological research on endangered and threatened species to be of current use for recovery plans. Lennox and Cooke (2014) estimated that between 2006 and 2012, there was only 2% integration between current physiological research and conservation research in 16 prominent conservation and biodiversity, animal physiology, plant physiology, and ecology journals. The lack of integration is supported by our analysis, which indicates that more research-based actions were suggested than non-research based actions in the recovery plans. In many instances, more information about a particular method or how to use a tool for a particular species was needed before the authors could suggest that a method or tool be applied in a recovery plan.

As part of the lack of integration between physiological research and conservation, recovery plan authors may find identifying useful resources difficult. For example, in some instances, the use of physiology in conservation efforts may be frowned upon due to the invasive nature of collecting physiological data (Lennox and Cooke, 2014). Many recovery plans even stipulate the need to better regulate the use of the species for research purposes, such as in the recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*). The authors specify that they will only permit research that may ‘result in infrequent injury or mortality’ (U.S. Fish and Wildlife Service, 2011). They go on to discuss how invasive procedures associated with obtaining physiological data can cause significant stress and possible death for individuals (Berry *et al.*, 2002; U.S. Fish and Wildlife Service, 2011). However, if the resources describing the many different noninvasive methods for collecting physiological data were made more easily accessible, more action-based research may be included in recovery plans. Examples of such tools include: fur and feathers (molted or new) can be used to analyze stable isotope and stress response (i.e., corticosterone or cortisol) in birds and mammals (Bortolotti *et al.*, 2008; Richards *et al.*, 2008; Carlitz *et al.*, 2016); and, fecal samples can be used for analyzing stress and reproduction (Millspaugh and Washburn, 2004; Schwarzenberger, 2007).

Our second recommendation is that physiology researchers can link their research to conservation more clearly to help recovery plan writers identify available physiological data, tools, and methods. By examining previous reviews on the uses of physiology in conservation, physiologists can get a better idea of what type of research is useful for recovery plans (Wikelski and Cooke, 2006; Cooke *et al.*, 2013). To go one step further, our results specifically show what sub-disciplines of physiology recovery plan authors use the most – immunology and epidemiology, comparative physiology, environmental toxicology, environmental and ecological physiology, and reproductive physiology.

Physiological data can be helpful for conservation recovery and management plans, but there is a need to continue to use tool refinement to improve and refine physiological sampling methods and tools for increased applicability (Madliger and Love, 2015). Increased conservation physiology research may not be followed by increased use in recovery plans if the writers are not aware of the new methods and tools available to them. Thus, physiology researchers can also increase accessibility of knowledge by developing thorough reviews of methods and tools. Such reviews can be thought as “one-stop shopping” for writers where they can find a plethora of information in one location.

For the solutions discussed above to be successful, they must all share a common denominator – communication. Thus, our final recommendation is that in order for recovery plans to be successful, physiologists, conservation biologists, and federal agencies need to communicate with each other. For physiologists to produce useful research for conservation they need to have a clear understanding of what is needed from them; and in order for agencies to do their due diligence for the ESA and recovery plans, they need to continue to incorporate new and useful tools and methods in their plans. By increasing publications, attending one-on-one meetings, participating in the recovery plan review process, and attending professional meetings and conferences we can all work together to make recovery plans effective tools for solving conservation problems.

Conservation physiology is a relatively new field, and has great potential for helping solve and monitor conservation issues. However, thus far, conservation plans have sorely underutilized the tools and techniques that the field of physiology has to offer. We believe that this disconnect is mainly due to a deficit of knowledge about physiology from the authors of the recovery plans. By increasing the training of federal agency employees, diversifying writing teams to include authors with academic affiliation, using tool refinement to make physiological research more available to conservation scientists and federal agencies, and by improving the modes of communication between conservation scientists, federal agencies, and physiologists, conservation physiology can be used to its full potential in recovery and other management plans in the future.

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3. FEMALE BLACKBIRDS' RESPONSE TO STRESS DURING BREEDING: POSSIBLE IMPLICATIONS FOR FUTURE MANAGEMENT

3.1. Abstract

Blackbirds are reported to cause between one to two percent crop damage per year, but the distribution of damage is not uniform, with some sunflower fields, this number can be as high as twenty percent. With many consumers in today's market concerned with animal welfare, nonlethal management techniques have become more important. Many of these techniques exploit natural predator-prey systems. One area of research that has not been thoroughly addressed is the physiological response of birds to visual and auditory scare devices designed to imitate predators. This study is part of a series of studies aimed at developing knowledge of both physiological and behavioral trade-offs of female red-winged blackbirds when exposed to predation risk as a chronic stressor. Breeding colonies were exposed to an avian predator, avian nest parasite, or a non-threatening avian effigy and corresponding bird call at the beginning of the breeding season. Behavioral and physiological responses (plasma corticosterone concentration) were monitored across the season, including general response to the predators and reproductive trade-offs. We predicted female response to perceived predation risk would be greater than response to a parasite or control treatment, and females would make a reproductive trade-off in favor of the current breeding season when presented with the perceived risk and stress of predation. Results suggest red-winged blackbirds do have stronger behavioral responses to the perceived risk of predation than to the parasites or control treatments. Females also respond by reducing their secretion of corticosterone when exposed to the threat of nest parasitism and predation. Females did not alter their reproductive behavioral trade-offs based on treatments. Future work should focus on examining potential reproductive trade-offs made

during the breeding season under stress, specifically how a female's physiology is connected to her reproductive decisions. Results will help provide a basis for applied research aimed at improving bird damage management.

3.2. Introduction

The primary diet of the red-winged blackbird (*Agelaius phoeniceus*) during the breeding season is high protein invertebrate prey that fuel reproduction and offspring growth. Diets shift across the season to grains and seeds, such as sunflower seeds, that fuel migratory and overwintering physiology (Hintz and Dyer, 1970; Hintz, 2000; Linz *et al*., 2017). Crop grain maturation often occurs simultaneously with shifts in the foraging and diets of the blackbirds (Dolbeer, 1990). The usual amount of regional sunflower crop damage averages 1-2% but can be over 20% in some fields (Peer *et al*., 2003; Klosterman *et al*., 2011).

Current management practices aimed at reducing damage to crops have had some success, but new approaches are needed to better manage crop damage (Linz *et al*., 2011). Wild bird populations are exposed to a variety of predators, and predation can come in the form of adult and fledgling predation or nest predation (Lima, 2009). Nonlethal management techniques aimed at exploiting a prey species' (e.g. red-winged blackbird) fear of predators (e.g. raptors) is an area of research accepted by consumers concerned about animal welfare (Oh *et al*., 2015). Thus, scientists and managers are evaluating predator-prey interactions to develop improved nonlethal approaches to protect crops. As part of this foundational knowledge, researchers are interested in understanding not only the behavioral interactions, but also the physiological responses. By having a thorough understanding of the physiology, or internal mechanisms, that drive an individual's response to the threat of predation, we can develop more efficient population management techniques by directly exploiting how those mechanisms function.

The majority of crop damage caused by the red-winged blackbird occurs in the fall when the species forms large pre-migratory flocks. Population recruitment for the fall flocks begins in the spring with the breeding season, and fall population size can be altered by reducing the reproductive success of breeding females. The fitness costs of reproduction include trade-offs a female must consider between her current season's reproductive output and her future reproductive output (Searcy, 1979). Within a current mating season, risks include decreased survival through predation (Lima, 2009) or self-maintenance (Drent and Daan, 1980), and decreased survival of offspring through predation (Lima, 2009) or brood parasitism (Payne, 1977). Although a few studies have evaluated these risks separately, showing that females can respond and react differently to the risk of parasitism and predation (Neudorf and Sealy, 1992), there has not been a comprehensive study of how females balance multiple risks when making reproductive decisions, which has important implications for the numbers of birds recruited to the fall blackbird population that is involved in depredation of grain crops.

When assessing the risk of predation during the breeding season, the female red-winged blackbird has to assess both the survival risk for herself, and the survival risk for her offspring, which can also be thought of in terms of her reproductive success. Because nest predation is one of the leading causes of reproductive failure in avian species, most studies focus on how females assess and react specifically to nest predators (Lima, 2009). Numerous studies have shown breeding birds have the ability to respond proactively to the presence of nest predators by selecting nest sites that are of lower quality, but may possibly reduce the ability of predators to access the nest (Milks and Picman, 1994; Forstmeier and Weiss, 2004; Eggers *et al*., 2006). However, a few studies have focused on adult predation risk, and have shown that females can select territories that are located further from a predator's nest (e. g., Suhonen *et al*., 1994;

Norrdahl and Korpimäki, 1998), or respond with changes in normal behavior such as call rate or intensity (Beletsky, 1991).

Brood parasitism is also of concern to many species of songbirds such as the red-winged blackbird. When a parasitic bird species lays its eggs in the host's nest, the host often raises the parasite as its own, depleting its own energy and decreasing reproductive success (Payne, 1977). The most common brood parasite of the central United States, the brown-headed cowbird (*Molothrus ater*) (Mayfield, 1965; Friedmann *et al*., 1971; Clotfelter and Yasukawa, 1999), lays its egg in a host's nest, often removing one of the host bird's own eggs, which further lowers the host's reproductive success (Clotfelter and Yasukawa, 1999).

Breeding seasons are usually limited to a short period of time during the year, time constraints can become an issue a female must consider (Verhulst and Nilsson, 2008). The female must be able to weigh the costs and benefits to determine the optimal time to start breeding (Drent and Daan, 1980). If she does not wait until she has enough stored energy before mating, she decreases both her and her offspring's chances of survival. However, if she waits too long before mating, both her offspring's fitness and her own decrease because of the lack of time available to prepare for winter. The presence of an extra risk like a predator or nest parasite may cause a female to delay egg-laying (Morosinotto *et al*., 2010), creating a shorter period of time for her to raise her offspring and prepare for winter migration.

An important aspect of understanding red-winged blackbird response to these risks is to understand how an individual responds to risk of predation physiologically as well as behaviorally. All of the risks discussed above can cause stress during the breeding season. When an individual is stressed, the hypothalamus – pituitary – adrenal (HPA) axis regulates their physiological stress response by secreting glucocorticoids (Rich and Romero, 2005). In avian species, after exposure to an acute stressor, the hypothalamus secretes corticotrophin-releasing factor to stimulate the pituitary (Rich and Romero, 2005). The pituitary then secretes adrenocorticotropin hormone (ACTH), which finally causes the adrenal gland to release corticosterone (CORT) (Rich and Romero, 2005). After the stressful situation is mitigated, CORT acts in a negative feedback loop to quickly suppress further secretion of CORT (Dickens *et al*., 2009). For limited amounts of time, elevated levels of CORT can be beneficial for an individual. During exposure to an acute stressor, increased CORT concentrations helps an individual survive by increasing energy in muscle tissue and facilitating metabolic changes. These changes promote behavioral changes such as those required for escaping (Sapolsky *et al*., 2000; Cyr *et al*., 2007). However, maintaining elevated levels of CORT during times of chronic stress can lead to decreased fitness. Extended periods of elevated CORT levels have been linked with hyperglycemia, neuronal cell death, and suppression of the immune and reproductive systems (Cyr *et al*., 2007; Dickens *et al*., 2010).

This is the first of a series of studies with the purpose of integrating and improving the effects of visual and auditory scare devices for protecting fruit and grain crops. This study focuses on examining how red-winged blackbirds respond behaviorally and physiologically to the perceived risk of predation and parasitism during the breeding season using avian effigies and calls as scaring devices. Additionally, this study evaluates potential reproductive decisions – such as egg-laying date, clutch size, and nest success – made under the perceived risk of predation and nest parasitism. By understanding how female red-winged blackbirds respond at the behavioral and physiological level to perceived threats we can potentially develop management tools that take advantage of the blackbirds' responses during the breeding season to reduce population levels. We predict females will show a greater behavioral and physiological response to the predator effigy than the other effigies. In addition, we predict females will make a greater reproductive trade-off in favor of the current breeding season when presented with the perceived risk and stress of predation.

3.3. Methods

3.3.1. Study Organism

The red-winged blackbird is a polygynous species that nests in large breeding colonies in wetlands found in most of North America and Central America. Clutches range in size between two and six eggs (Beletsky, 1996), and the incubation and nesting period is between 22 and 27 days. Females are known to raise one or two clutches through the breeding season, and will often attempt a second clutch if her first clutch fails (Beletsky, 1996); however, we monitored nests closely to ensure our results were from first nesting attempts only.

At our field site, males arrive on the breeding grounds well in advance of the females at the beginning of May; and after arriving, females wait several weeks before selecting a mate (Beletsky and Orians, 1996). The breeding season lasts through July.

3.3.2. Field Site

Our field site is located in a coulee system, or drainage ditch, which is part of the Sheyenne River watershed in Mapleton, ND, located in Cass County. The surrounding area is mainly used for agricultural purposes, with corn, soybean, and wheat as prominent crops. The coulee itself is ephemeral, and due to the overgrowth of cattail (*Typha spp.*), is stagnant. Other avian species found in the area include brown-headed cowbirds, marsh wrens (*Cistothorus palustris*), purple martins (*Progne subis*), and yellow-headed blackbirds (*Xanthocephalus xanthocephalus*). Waterfowl, large birds of prey such as the great-horned owl (*Bubo virginianus*), and other wetland species common to the area are also be found at our field site.

3.3.3. Behavioral and Hormone Study Protocol

3.3.3.1. 2013 Field Season

At the start of the blackbird breeding seasons of 2013 we identified breeding colonies in a coulee system in Cass County, ND (46.818324, -97.000946) by locating adult male red-winged blackbirds defending territories. The breeding colonies were separated into four stress treatment groups: control (no effigy), avian control (purple martin), nest parasite (brown-headed cowbird), and predator (great-horned owl). Each treatment group had three sites. All sites were at least 30 m long and separated by at least 60 m, further than previous studies (Olendorf *et al*., 2004). In addition, we confirmed sites as active if a male was spotted on the site. Sites were assumed to be physically similar; however, to control for any possible dissimilarities between sites, sites were randomly assigned to a treatment.

Once females were observed on territories, the sites were presented with their corresponding effigy and avian call for one hour a day, for four days over no more than a two week period. To control for the fact great-horned owls are a crepuscular species, we randomly presented to each treatment group twice at sunrise and twice two hours before sunset.

An observation protocol was designed to monitor specific behaviors common to red-winged blackbirds (i.e. attacking the effigy, alarm calling, non-interactive behavior, and approaching the effigy). The protocol was used to observe individual male territories during the effigy and avian call presentation. During each observation, a random male was selected within a site. To avoid observing the same male twice, the male's territory was mapped out on the observation sheet so that territory could be avoided during future observations. Observations lasted for 60 minutes, and were divided into 5 minute observation periods. The behaviors we focused on were attacking the effigy and the use of alarm calling. Behaviors were recorded as either present or absent during each 5 minute observation period, and the fraction of observation periods with the behavior present was calculated. A similar protocol has been used successfully with other bird species (Coslovsky and Richner, 2011).

3.3.3.2. 2014 Field Season

At the start of the 2014 breeding season, sites were selected using the same protocol as in 2013. However, we removed the control (no effigy) stress treatment. The remaining three stress treatments had three sites each. The protocol for exposing sites to their corresponding effigy and call was also the same as 2013, but we did not conduct behavioral observations in 2014.

After exposing the sites to their effigy and call, the remainder of the breeding season was spent monitoring for active nests. We located nests with recorded UTMs (Universal Transverse Mercator coordinate system; Garmin GPSMAP62sc) and marked nests with neon orange flagging tape within 30 cm of the cattail and reed [mainly canary grass (*Phalaris canariensis*)] cluster supporting the nest. Active nests were monitored for reproductive data (i.e. lay date, clutch size, and nest fate).

After sites were exposed to the treatment effigy and call, we placed mist nets and nest traps at each site to trap females for hormone analysis. All nets and traps were observed from approximately 30 m away. Once a female was caught, she was removed and an initial blood sample was taken within three minutes of capture to collect a plasma sample to measure the basal CORT concentration. Females were banded with a colored band approved by the United States Geological Survey (USGS) bird banding laboratory and a United States Fish and Wildlife Service (USFWS) silver band for future identification. Their physical condition was analyzed by collecting mass and tarsus length data. We approximated the age of female red-winged blackbirds using the Munsell® Soil Color Chart (Miskimen, 1980a; Miskimen, 1980b). Females were separated by age into three different categories based on their epaulet color: yellow (first molt), yellow-red (second to third molt), and red (fourth molt or older).

3.3.3.3. 2015 Field Season

At the start of the 2015 breeding season, sites were selected using the same protocol as in 2013. However, we removed the control (no effigy) stress treatment. The remaining three stress treatments had six sites each. The protocol for exposing sites to their corresponding effigy and call was also the same as 2013 and 2014, but we did not conduct behavioral observations in 2015.

In the 2015 field season, we also trapped females for hormone analysis. The same measurements were taken in 2014 and 2015, but in 2015 females also underwent an HPA axis challenge to examine how the HPA axis was functioning after exposure to the threat treatments. If the HPA axis is not functioning normally, females may be exposed to extended periods of increased CORT levels (Dickens *et al*., 2009). After the initial basal blood draw, females were injected with 5 ug/kg body mass of dexamethasone (DEX), a synthetic form of CORT, to initiate the negative feedback and suppress the pituitary from signaling the adrenal to secrete additional CORT (Rich and Romero, 2005). Females were then placed in an opaque bag for 30 minutes, and then bled a second time to examine if the negative feedback loop was working. Afterwards, females were injected with 100 IU/ kg body mass of ACTH to stimulate the adrenal gland to secrete CORT and measure peak CORT secretion. Females were placed back into the opaque bag and bled 30 minutes after the ACTH injections. After the final blood draw females were released. All injections were done intramuscularly. All weight-dependent doses were standardized for a 45 g bird and diluted in 10 ul of phosphate buffered saline. In addition, the DEX was first dissolved in EtOH (Rich and Romero, 2005).

All blood samples were taken from the brachial vein, and were rotated between the left and right wing for every sample. Samples were collected using Microvette® CB 300 LH, containing lithium heparin, and approximately 50 ul of blood were taken for each sample for a total of 250 ul of blood per bird. Female blackbirds weigh on average between 40 and 55 g (Beletsky 1996) and the total blood sample accounts for between 9% and 7 % of their total blood volume. Blood samples were kept on ice until they could be spun down at 4000 g for 5 minutes to separate the plasma from blood cells. The plasma was extracted, and frozen for future analysis.

Corticosterone was extracted from plasma from each sample, and concentrations were analyzed using an enzyme-linked immunosorbent assay, an ELISA kit, (ENZO Life Science, ADI-900-097). Corticosterone was extracted from plasma samples by diluting 12 ul (or as much as was available if less than 12 ul) of plasma in 200 ul of double distilled water, and extracted with 1.5 ml diethyl ether. After quickly freezing the water phase, the ether phase was decanted, and the process was repeated three times. The ether phases were then dried down on a heating block set at 20^{o}C, and a multi-probe drying rack with nitrogen gas. Once samples were dry, they were suspended in 338 ul of assay buffer from the ELISA kit and kit directions were followed. Females were randomly assigned to one of three ELISA plates, and samples were plated in triplicate.

3.3.4. Data Analysis

3.3.4.1. Behavioral Protocol

All analyses were performed using JMP® version 11 (SAS Inc.). For the behavioral study in 2013, the fraction of observational units (5 minute blocks) including an alarm call or an effigy attack was calculated. The data was not normally distributed for either behavior, thus, a contingency analysis was used to analyze the behavioral data. Alarm call and effigy attacking behaviors were compared between morning and evening observations, and among the stress treatments.

In 2014 the average lay date was evenly distributed across sites, and compared among treatments using an ANOVA. Clutch size was compared among treatments using a Chi-square test. Nest success was compared among treatments using Program MARK. Encounter occasions, or number of days nest success data was collected in 2014 was 48 days. Nest success data was divided into three attribute groups (the stress treatment groups), and general linear models were developed to compare nest success among treatments (Table 3.1). Models were ranked using the delta Akaike second order information criterion (ΔAICc), weighted Akaike second order information criterion (ωAICc), and the number of estimated parameters (K) calculated for each model, such that a $\Delta\text{AICc} < 2$ was considered a significant model based on the parameters of our data, and ωAICc represents the relative likelihood of the model based on the parameters of our data.

Table 3.1. Treatment Models Compared in Program MARK.

Model
Null
All Treatments Differ
Avian Control and Nest Parasite Differ from Predator Treatment
Avian Control and Predator Differ from Nest Parasite Treatment
Nest Parasite and Predator Differ from Avian Control Treatment

3.3.4.2. Hormone and Female Quality Analysis

Female physical quality was calculated using the residuals of mass by tarsus regression, and data was normally distributed ($F_{1,25} = 80.085$, $p = 0.0001$, $R^2 = 0.762$, $N = 27$). We evaluated if female condition differed between years, among age groups, across the season, and among stress treatments using a mixed model. To control for variation among sites, site was nested within treatment in both mixed models. However, if site was not a significant parameter, site was removed, and the model was rerun.

Plasma CORT concentrations after the ACTH injections were not normally distributed, and were transformed using the natural log for all analysis. Plasma basal CORT concentrations and plasma CORT concentrations after DEX injections were normally distributed.

Plasma CORT concentrations begin to rise shortly after an individual is stressed (Romero and Romero, 2002); however, at capture, we were not able to bleed all females within the standard three minute period. Thus, only samples taken within four minutes of capture were kept ($N = 17$, 10 females with a bleed time ≤ 3 minutes, $\bar{x} = 2.97$ min.). Within the remaining samples, there was not a significant relationship between time from capture and CORT concentration ($F_{1,27} = 1.490$, $p = 0.233$, $R^2 = 0.052$, $N = 17$). Due to small sample size, samples were pooled within a treatment, rather than using site averages.

We examined if there was a year, seasonal, condition, age, or treatment effect on plasma CORT concentration samples taken at capture (baseline) using a mixed model. We also analyzed if there were condition, age, seasonal, or treatment effects on plasma CORT concentrations 30 minutes after the DEX injection, and 30 minutes after the ACTH injection using mixed models analysis. To control for variation among sites, site was nested within treatment in both mixed model. However, if site was not a significant parameter, it was removed, and the model was rerun.

3.4. Results

3.4.1. Behavior

The behavioral response of red-winged blackbirds does not differ between morning and evening observations for effigy attacks ($\chi^2_1 = 0.152$, $p = 0.697$, $N = 63$), such that 9.52% of morning observations include an effigy attack, and 7.94% of evening observations include an effigy attack. In the presence of an effigy, blackbirds are more likely to attack the predator effigy than any of the other stress treatment effigies ($\chi^2_3 = 16.294$, $p = 0.001$, $N = 63$; Figure 3.1), such that 45% of observations of the predatory effigy include an observation of a blackbird attacking the effigy, 7.69% of observations of the nest parasite effigy include an observation of a blackbird attacking the effigy, 6.25% of observations of the avian control include an observation of a blackbird attacking the effigy, and none of control observations include an observation of a blackbird attacking the effigy.

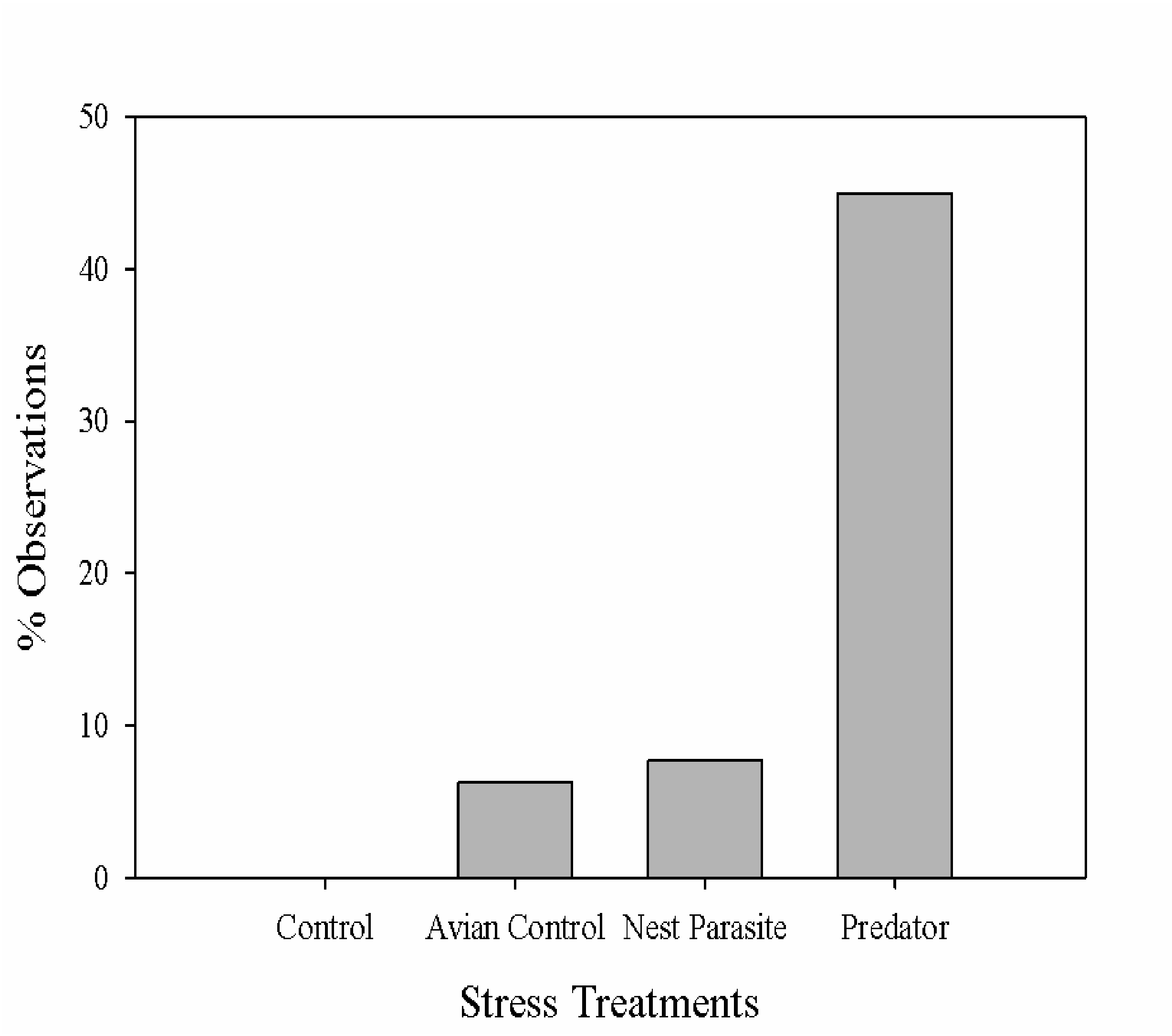

Figure 3.1. Comparison of Effigy Attacks between Stress Treatments.
Note: The percent of 60 minute observations to include an effigy attack in each of the four stress treatments: control, avian control, nest parasite, and predator.

Blackbirds also do not differ in their alarm call response between morning and evening observations ($\chi^2_1 = 0.391$, $p = 0.532$, $N = 63$), such that 23.81% of observations in the morning include an alarm call, and 28.57% of observations in the evening include an alarm call. Blackbirds are more likely to alarm call in the presence of a predator effigy than any other stress treatment effigy ($\chi^2_3 = 14.058$, $p = 0.003$, $N = 63$; Figure 3.2), such that observations of the predator effigy include an alarm call observation 85% of the time, 30.77% of the nest parasite observations include an alarm call, 43.75% of the avian control observations include an alarm call, and 35.71% of the control observations include an alarm call.

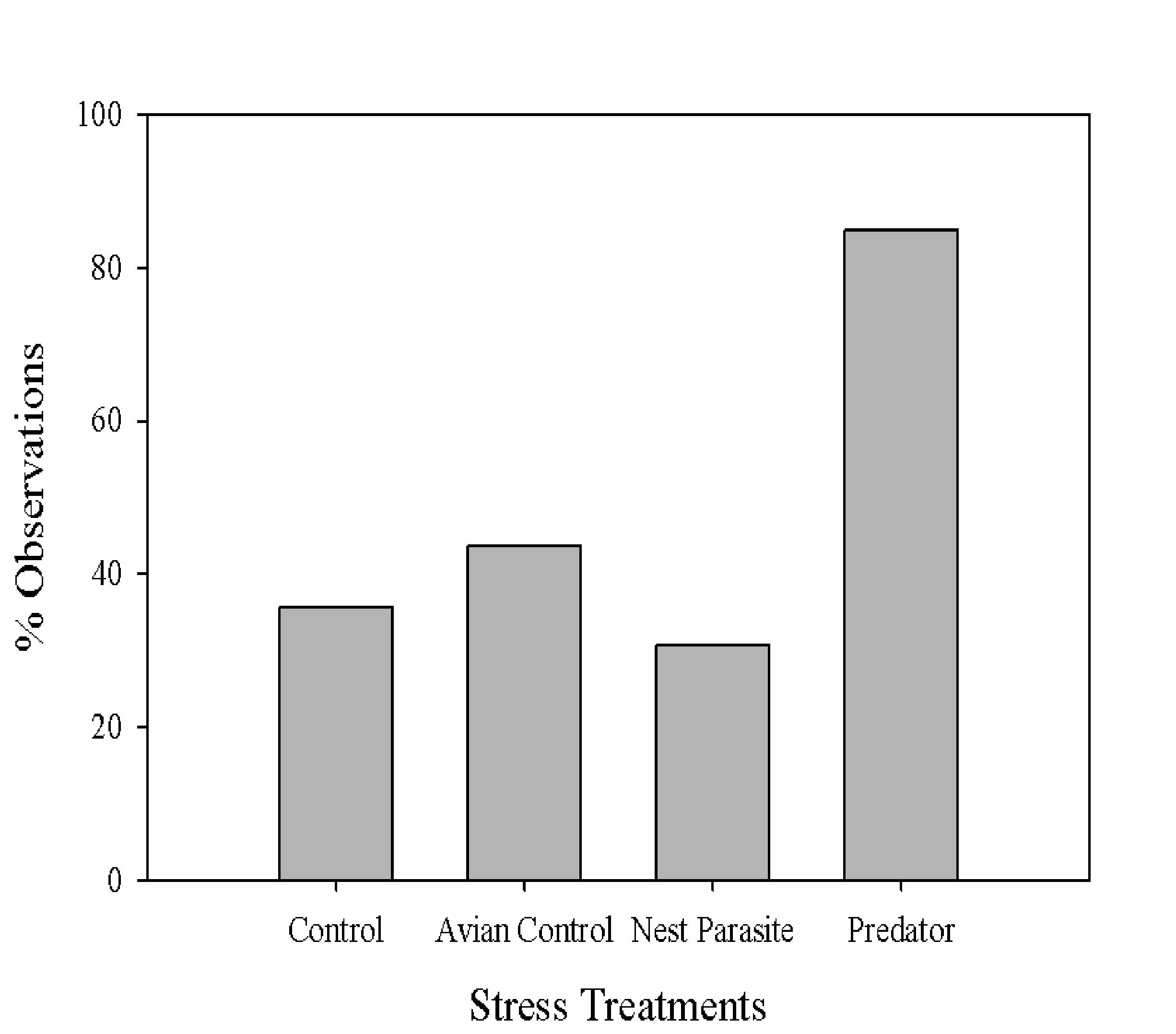

Figure 3.2. Comparison of Alarm Calls between Stress Treatments.
Note: The percent of 60 minute observations to include an alarm call in each of the four stress treatments: control, avian control, nest parasite, and predator.

None of the reproductive effort measurements monitored show significant differences among treatments. Lay date does not differ among treatments (predator: $\bar{x}$ = 148.25 days, SE = 4.639; nest parasite: $\bar{x}$ = 144.40 days, SE = 4.639; avian control: $\bar{x}$ = 148.50 days, SE = 3.788; F2,4 = 0.266, p = 0.779). Clutch size does not differ significantly among treatments (χ^2_2 = 3.993, p = 0.136, N = 8), such that 100% of avian control sites have an average clutch size of 3 eggs, 33.33% of nest parasite sites have an average clutch size of 3 eggs, and 50% of predator sites have an average clutch size of 3 eggs.

Nest success does not differ between treatments (Table 3.2). The null model (model 1) is ranked as the best model (ΔAICc = 0. 00). Both models 3 and 4 have a ΔAICc < 2; however, the ωAICc, or model likelihood for both models 3 and 4 are lower than the null model.

Table 3.2. Treatment Model Comparison Output in Program MARK.

Model	**Deviance**	**K**	**AICc**	**ΔAICc**	**ωAICc**
Null	89.3493	1	91.3587	0.0000	0.39771
All Treatments Differ	88.5422	3	94.5989	3.2402	0.07870
Avian Control and Nest Parasite Differ from Predator Treatment	88.6927	2	92.7210	1.3623	0.20126
Avian Control and Predator Differ from Nest Parasite Treatment	88.9451	2	92.9734	1.6147	0.17739
Nest Parasite and Predator Differ from Avian Control Treatment	89.3492	2	93.3775	2.0188	0.14494

3.4.2. Hormone and Female Quality

The parameter, site nested within treatment, is not a significant predictor of female condition (whole model: $F_{17,9} = 1.524$, p = 0.264, $R^2 = 0.742$, N = 27; site nested in treatment: $F_{11,11} = 0.741$, p = 0.685). When site nested in treatment is removed from the model, the model examining the effects of year, season, age, and treatment on female body condition is significant ($F_{6,20} = 3.450$, p = 0.017, $R^2 = 0.509$, N = 27). Year is the only significant main effect in the model (F1,1 = 5.164, p = 0.034) , such that female body condition is overall higher in 2015 ($\bar{x}$ = 4.414, SE = 3.566) than in 2014 ($\bar{x}$ = -7.503, SE = 3.893). Treatment, female age, and the date female condition was measured are not significant predictors (treatment: $F_{2,2} = 1.852$, p = 0.183; female age: $F_{2,2} = 1.182$, p = 0.327; date measured: $F_{1,1} = 0.175$, p = 0.680).

The mixed model examining the effects of year, season, condition, age, and treatment on plasma CORT concentration samples taken at capture (baseline CORT) is significant ($F_{7,6}$ = 5.926, p = 0.023, R^2 = 0.874, N = 14; Figure 3.3). Treatment is the only significant main effect in the model ($F_{2,2}$ = 9.377, p = 0.014), such that female baseline CORT concentrations are significantly lower in the nest parasite treatment ($\bar{x}$ = 426. 067 pg/mL SE = 67.779) than in the avian control treatment ($\bar{x}$ = 991.061 pg/mL, SE = 96.261), but CORT concentrations in the predator treatment are not significantly different from concentrations in the nest parasite or avian control treatments ($\bar{x}$ = 461.460 pg/mL, SE = 149.718). Neither female age nor year are significant predictors within in the model (age: $F_{2,2}$ = 3.616, p = 0.093; year: $F_{1,1}$ = 4.455, p = 0.079). Female condition and the date samples were collected are not significant predictors within the model (condition: $F_{1,1}$ = 0.602, p = 0.467; seasonal effect: $F_{1,1}$ = 1.252, p = 0.306).

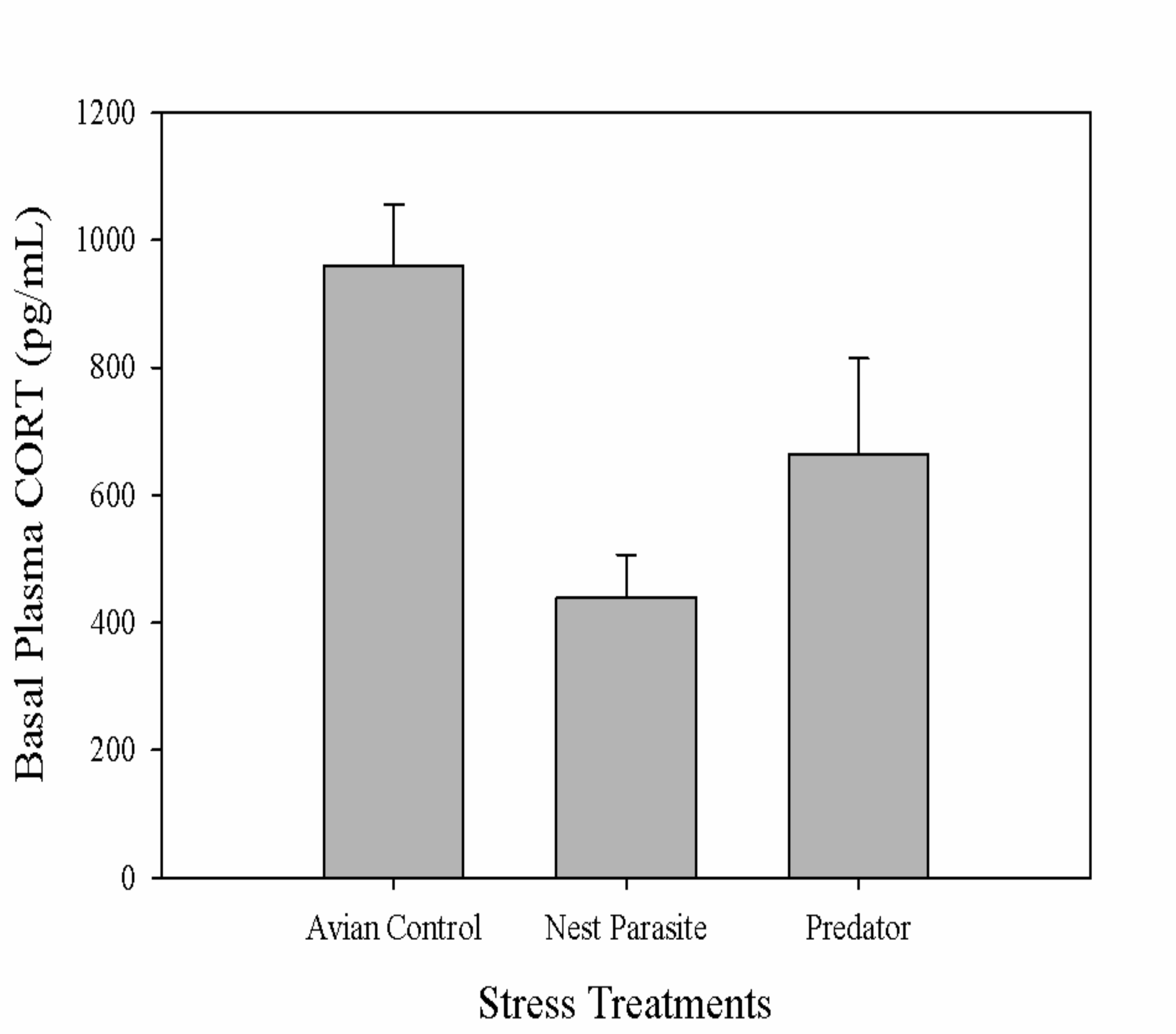

Figure 3.3. Baseline Plasma CORT Concentrations among Stress Treatments.
Note: Difference in baseline plasma CORT concentration among females exposed to an avian control, nest parasite, and predator stress treatments. Data shown are least-square means ± 1 S.E.M. from the full model, which included year, date samples were taken, condition, age, and stress treatment.

The parameter, site nested in treatment, is not a significant predictor within the mixed model examining the effects on plasma CORT concentrations samples 30 minutes after a female was injected with DEX (whole model: $F_{11,4} = 0.594$, $p = 0.777$, $R^2 = 0.620$; site nested in treatment effect: $F_{5,5} = 0.299$, $p = 0.891$). When the effect of site nested in treatment is removed, the mixed model examining the effects of season, condition, age, and treatment on plasma CORT concentration samples 30 minutes after a female was injected with DEX is still not significant ($F_{6,9} = 1.376$, $p = 0.320$, $R^2 = 0.478$).

The parameter, site nested in treatment, is not a significant predictor within the mixed model examining the effects on plasma CORT concentrations samples 30 minutes after a female was injected with ACTH (whole model: $F_{10,1} = 3.600$, $p = 0.390$, $R^2 = 0.973$; site nested in treatment effect: $F_{4,4} = 1.059$, $p = 0.613$). When the effect, site nested in treatment is removed, the mixed model examining the effects of season, condition, age, and treatment on plasma CORT concentration samples 30 minutes after a female was injected with ACTH is significant ($F_{6,5} = 5.055$, $p = 0.048$, $R^2 = 0.858$). Both treatment and date measured are significant predictors within the model (treatment: $F_{2,2} = 8.848$, $p = 0.023$; Figure 3.4; date measured: $F_{1,1} = 8.170$, $p = 0.036$; Figure 3.5). Females in the avian control treatment have significantly higher plasma CORT concentrations across the season ($\bar{x} = 4651.756$ pg/mL, SE = 1.8507) than females in the nest parasite ($\bar{x} = 1586.047$ pg/mL, SE = 1.215) and predator treatment ($\bar{x} = 1310.154$ pg/mL, SE = 1.202) groups, and plasma CORT concentrations decrease across the season (slope = -0.097, SE = 0.034).

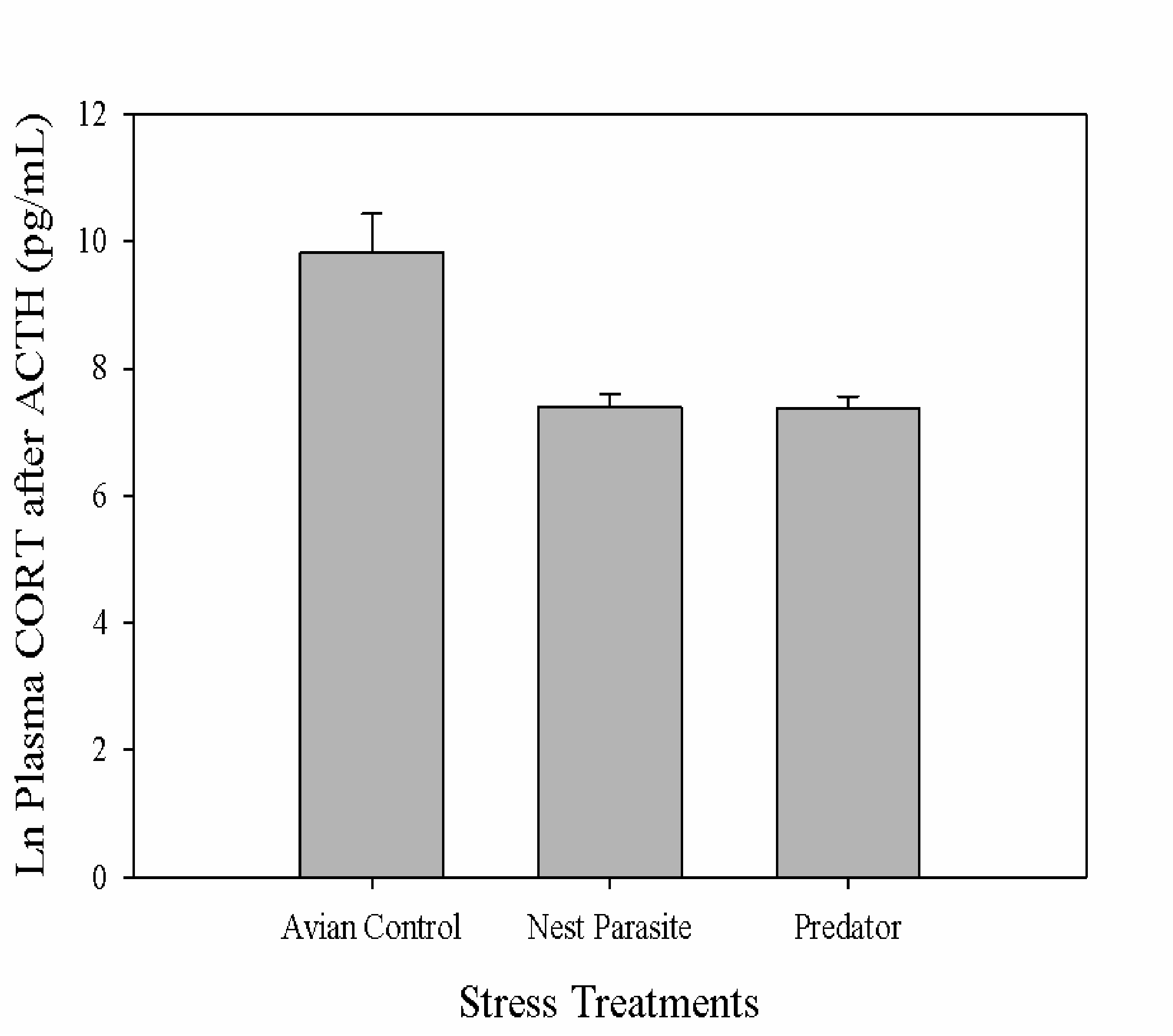

Figure 3.4. Plasma CORT Concentrations after ACTH Injections among Stress Treatments. Note: Difference in plasma CORT concentration 30 minutes after ACTH injections among females exposed to an avian control, nest parasite, and predator stress treatments. Data shown are least-square means ± 1 S.E.M. from the full model, which included year, date samples were taken, condition, age, and stress treatment.

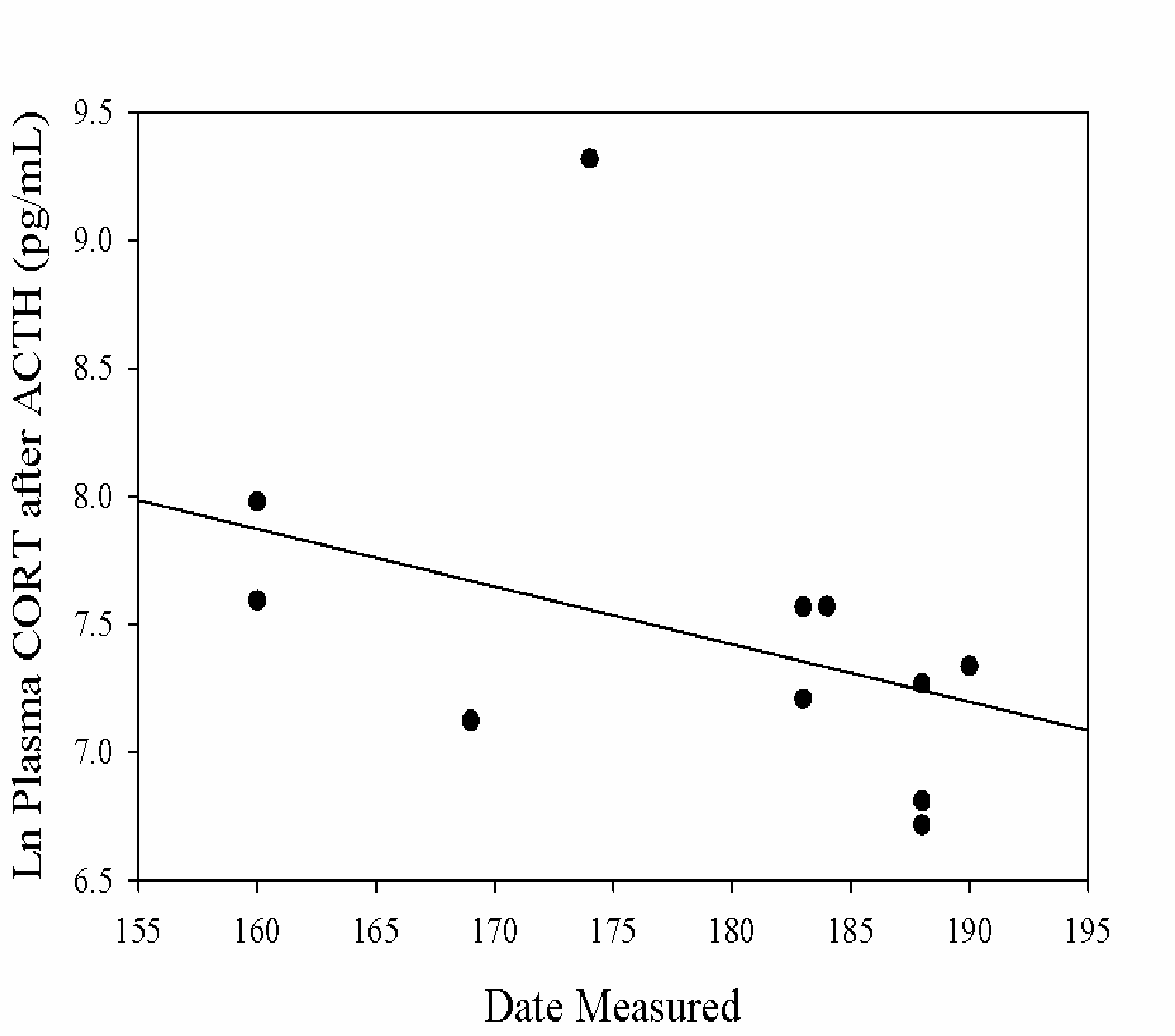

Figure 3.5. Relationship between Date Measured and Plasma CORT Concentrations after ACTH Injections.
Note: Relationship between date measured and plasma CORT concentration 30 minutes after ACTH injections. Date measured was part of the model examining the plasma CORT after ACTH. Other variables of the model included year, condition, age, and stress treatment.

3.5. Discussion

Results suggest red-winged blackbirds respond both behaviorally and physiologically to different stress treatments. Females participate in mobbing and alarm calling behavior more frequently in the presence of the great-horned owl effigy and call than in the presence of the purple martin or female brown-headed cowbird effigies and calls. Females also alter their HPA axis differently in the presence of the three treatment groups.

Mobbing is a particularly interesting behavior, because it is potentially dangerous for the attacker. In fact, the risk of mobbing has been confirmed in other species, where the mobbing bird was killed by the predator (Denson, 1979). However, it has also been suggested there must be some benefit for such a risky behavior to continue. For example, in the American robin (*Turdus migratorius*), most mobbing behavior happens only during the breeding season, suggesting individuals may be more likely to risk injury for the reward of being able to remain on their current territory (Shedd, 1982). Effigies in our study were presented to the blackbirds after territories had mostly been selected, and in some cases nest building had begun. Therefore, female red-winged blackbirds may make a trade-off similar to the one American robins make.

In the presence of a predator, alarm calling may give away a prey's location; however, it may also help individuals signal to others within the colony information about the risk (Miller, 2005). It has been suggested female brown-headed cowbirds use host vocalizations to locate host nests (Clotfelter, 1998). Thus, it would make sense red-winged blackbirds may reduce vocalizations within their territory when a female brown-headed cowbird is present.

Exposure to the threats of predation and nest parasitism have an effect on the HPA axis in females, with lower base line CORT, and decreased CORT levels 30 minutes after ACTH injections. A similar response was seen in European starlings (*Sturnus vulgaris*), after exposure to chronic psychological stress (Rich and Romero, 2005). The down regulation of CORT during repeated exposure to stressors may help to minimize the negative effects of continuously elevated CORT levels (Sapolsky *et al*., 2000).

The breeding season is an energetically taxing time for females, and increased CORT during exposure to stressors can induce glycolysis, and promote the use of energy reserves (Cyr *et al.*, 2007). By suppressing the CORT response via reduced adrenal secretion in the HPA axis and suppressed basal levels, females under chronic stress, from the threat of predation or nest parasitism, may be maximizing energy reserves specifically for breeding rather than responding to the stressors (Astheimer *et al.*, 1995). Previous studies have shown high CORT levels during the breeding season can cause females to abandon their breeding attempt (Love *et al.*, 2004). Females in our study did not reduce clutch size or delay breeding supports the hypothesis that reducing the reactivity of the HPA axis under chronic stress, allowed our females to remain reproductively active, and maintain normal breeding behaviors.

Female response differs between stress treatments. Females have suppressed CORT concentrations at both baseline and after ACTH injections in the nest parasite treatment. Lower CORT levels are observed after ACTH injections, but not in baseline CORT levels in the predator treatment. These responses suggest females are capable of responding at different physiological levels to different types of threats. As females respond behaviorally to the predator stressor through alarm calling and mobbing the effigy, behaviors that are energetically costly (Dugatkin and Godin, 1992), not suppressing baseline CORT levels may allow females to remain prepared to respond to the predator threat quickly (Malische *et al.*, 2007).

The variation in response between the predator and nest parasite effigy may be due to the ability of blackbirds to differentiate the type of threat the different stressors pose for their reproductive success and survival, which has been observed in other species, such as the yellow warbler (*Setophaga petechia)* (Gill and Sealy, 1996). The nest predator effigy used was a Great-horned owl, a predator of adults and juvenile/fledglings (Murphy, 1997). During the early portion of the breeding season (when effigies were presented), the effigy is a threat to the survival of the female more than to her offspring. However, exposure to the brown-headed cowbird effigy is a threat to a female's reproductive success (Payne, 1977). By responding to the predator effigy behaviorally and physiologically, the red-winged blackbirds are protecting themselves, and maintaining lower CORT levels to reserve energy for reproduction. However, with the nest parasite effigy, responding with direct behaviors towards the female brown-headed cowbird may expose her territory and nest, reducing her reproductive success. Thus, females respond physiologically to the stressor.

Female behavioral and physiological responses to predator and nest parasite effigies have the potential to help improve blackbird management. For example, as our study demonstrates under chronic stress females may suppress the reactivity of the HPA axis. This may cause females to be less reactive to acute stressors, such as a real predator attack. If females cannot respond quickly enough in the presence of a predator, it may reduce their chances of surviving the attack. By stressing females during the breeding season, causing more natural takes from predators, we can potentially help reduce the size of fall migrating flocks. Females captured by predators would be not able to finish raising their current brood, or lay a second clutch in late summer. Thus, stress during the breeding season could potentially reduce the number of adult females and the number of just fledged offspring in the fall. In addition, as our results suggest reactivity decreases with season, it may be more important to stress females later in the breeding season or across the entire breeding season rather than just at the start of the season. As we were not able to connect female physiological responses with specific nests in our experiment, future work should focus on examining potential reproductive trade-offs made during the breeding season under stress, specifically how a female's physiology is connected to her reproductive decisions. A comprehensive approach is important to create a more realistic breeding season scenario that can be studied, because no single risk is going to be solely responsible for shaping how a breeding community functions behaviorally and physiologically.

3.6. References

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4. PHYSIOLOGICAL AND REPRODUCTIVE RESPONSES OF FEMALE RED-WINGED BLACKBIRDS UNDER THE PERCEIVED THREATS OF PREDATION AND NEST PARASITISM

4.1. Abstract

Nest construction is one form of parental behavior that has the potential to affect offspring development and survival. The physical structure of the nest protects the eggs and influences the incubation and brooding microclimate. The location of the nest also has important implications for the survival of offspring, providing shelter from the harsh environment and visual coverage from predators. Thus, parental decisions made while building a nest can have long reaching reproductive consequences for the breeding population. Many studies have examined either how nest structure or nest location affect the reproductive success of the parents, but few have examined the combined effects or interactions between the two reproductive decisions. This study will examine how female red-winged blackbirds (*Agelaius phoeniceus*) make reproductive decisions between micro-habitat factors and nest structure components under stress of predation or nest parasitism, and whether female physiological responses are associated with reproductive decisions. In addition, we will examine whether these trade-offs are associated with changes in reproductive success. Our results suggest aversion from nest parasites and stress physiology have the most influence on where and how female red-winged blackbirds build their nests. Females seem capable of making reproductive decisions, without having to change when they start breeding or their clutch size, to optimize their reproductive success each breeding season.

4.2. Introduction

Nest construction is one form of parental behavior that has the potential to affect offspring development and survival. The physical structure of the nest protects the eggs and influences incubation and brooding microclimates (Collias, 1964). The location of the nest also has implications for the survival of offspring, providing shelter from the harsh environment and visual coverage from predators (Johnson and Temple, 1990; Frere et al., 1992; Kolbe and Janzen, 2002; Ardia et al., 2006). Thus, parental decisions made while building a nest can have reproductive consequences that last across generations. Many studies have examined either how nest structure or nest location affect reproductive success of parents, but few have examined the combined effects or interactions between the two reproductive decisions (but see Childress and Gautheir and Thomoas, 1993; Bennun, 2000; Greenwald, 2009).

The decisions a female makes while building a nest are made in the context of stressors or threats in the environment that may be harmful to the parents or offspring. Common stressors include predation risk to self or offspring, and risk of nest parasitism. Studies have shown breeding females have the ability to respond to the threat of nest predators by selecting less conspicuous, but also lower quality locations (Milks and Picman, 1994; Forstmeier and Weiss, 2004; Eggers et al., 2006). Other studies on adult predation risk have shown females can select nesting locations further from a predator's territory (Suhonen *et al*., 1994; Norrdahl and Korpimäki, 1998).

For species that invest in high levels of parental care, the risk of brood parasitism can be a significant threat to reproductive success. For example, when a parasitic bird lays eggs in a host's nest, the host will often raise the parasitic nestling at a cost to their own reproductive success (Payne, 1977). In some cases, the nest parasite will also remove an egg from the host's nest, further lowering the host's reproductive success (Clotfelter and Yasukawa, 1999). Studies have shown in defense to this risk, some females will choose to desert their current nest, and re-nest in another location (Goguen and Mathews, 1996).

These environmental stressors may cause physiological changes in the parents by increasing circulating levels of glucocorticoids. During acute stress situations increased levels of glucocorticoids can be beneficial to an individual, providing them with physiological resources necessary for survival. However chronically elevated glucocorticoid levels can be detrimental to an individual, causing decreases in immune response and decreased cognitive ability (Sapolsky *et al.*, 2000). The female's physiological response to stressors can also affect the concentration of hormones deposited in the yolk of her eggs, which can affect offspring growth and development. Hayward and Wingfield (2004) showed increases in circulating levels of the glucocorticoid, corticosterone (CORT), in female Japanese quail (*Coturnix coturnix japonica*), significantly increased CORT levels in egg yolks. They also found offspring from stressed mothers grew slower in the first week after hatching than those from control mothers (Hayward and Wingfield, 2004).

Another hormone found in egg yolks is testosterone (T). Testosterone concentrations in the yolk can also be influenced by the maternal environment, and can effect offspring growth and development (Navara *et al.*, 2006a; Navara *et al.*, 2006b). For example, in black-headed gull chicks, increased T concentrations in the yolk are correlated with increased begging behavior in chicks (Eising and Groothius, 2003). In house finch chicks, increased yolk T concentrations stimulated growth after hatching compared to chicks with lower yolk T concentrations (Navara *et al.*, 2006b).

Potential decisions females may make under the stress of possible nest parasitism or predation stressor include delaying reproduction or altering clutch size, both of which affect reproductive success (Perrins and McCleery, 1989). Females may also alter parental behaviors, such as reducing feeding rates or reducing time spent incubating her clutch (Lima, 2009). Additionally, it is possible decisions about micro-habitat, and nest structure, interact with female physiological condition in complex ways to affect current and future reproductive success.

Building nests is a time and energy consuming process, and parents must often make trade-offs among nest structure, nest location and their own energy budgets, safety, or reproductive success (Conrad and Robertson, 1993; Martin *et al*., 2000). In the crested tit (*Parus cristatus*), males in poor condition are less likely to help build first nests of the season. Females mated to males in poor condition take an average of five days longer to build their nests, resulting in later egg-laying dates and later fledging and migration dates (Lens *et al.*, 1994). Postponed migration could then potentially have long lasting effects on the female's reproductive success and survival of offspring through the winter (Lens *et al.*, 1994). In the great tit (*Parus major*), one study showed bacterial load on feathers increased significantly during the nest building stage (Kilgas *et al.*, 2012). In another study on the same species, nest success increased as the overall size of the nest increased (Álvarez and Barba, 2008). Others studies have shown brood size can be constrained by nest size (Slagsvold, 1989; Møller *et al.*, 2014), and in many species, nest size may be constrained by predation pressures (Møller, 1990; Lima, 2009). Together, these studies suggest there are costs associated with nest building, and these costs may lead to trade-offs between reproductive success and parental condition.

Micro-habitat (i.e. nest site location) is one potential method for the parents to mediate some of the risks associated with nest building. However, if the wrong nesting location is selected, it can also be detrimental to their reproductive success. Most nest-site location studies have focused on predation risk and avoidance, showing micro-habitat characteristics can affect predation rates (Picman *et al.*, 1993; Albrecht, 2004; Horn *et al.*, 2005; Hoover, 2006), and adults can be flexible in their nest site selection under predation risk (Morosinotto *et al.*, 2010; Latif *et al.*, 2012). Several studies have also examined the importance of micro-habitat for parental and offspring condition (Gauthier and Thomas, 1993; Rolstad *et al.*, 2000; Greenwald, 2009; Ambrosini and Saino, 2010). For example, in the cliff swallow (*Hirundo pyrrhonota*), nest site location affects the type of nest the cliff swallow will build (attached vs. detached) (Gauthier and Thomas, 1993). In addition, the type of nest built significantly affects the energy budget of the individual, with individuals using more energy each day to build a detached nest than an attached nest (Gauthier and Thomas, 1993).

Micro-habitat selection of red-winged blackbirds (*Agelaius phoeniceus*) has been studied extensively, showing vegetation height and density are important environmental factors that regulate habitat selection (Albers, 1978). Other studies have shown water depth at nest site, nest cover, nest height, vegetation type and density, proximity of nest to predators, and proximity to a prominent perch are all correlated with female reproductive success in the red-winged blackbird (Robertson, 1972; Holm, 1973; Weatherhead and Robertson, 1977; Lennington, 1980; Picman, 1980; Yasukawa *et al.*, 1992; Turner and McCarty, 1998).

The purpose of this study was to examine what reproductive decisions female red-winged blackbirds make under the stress of predation and nest parasitism, between micro-habitat factors nest structure components, parental care, and whether the female's physiological response is associated with her nesting decisions. In addition, we also wanted to examine if these decisions were correlated with changes in reproductive success. We hypothesized because all of the factors have been shown to be important to reproductive success, there would be interactions between the three, and females would make adjustments between them to optimize reproductive success.

4.3. Methods

4.3.1. Study Species

Red-winged blackbirds are a polygynous species that breed in large colonies in wetlands across North and Central America. Nests are cup-shaped and are most commonly found woven into the cattails (*Typha spp.*) and tall grasses and emergent wetland plants, mainly canary grass (*Phalaris canariensis*) (Beletsky and Orians, 1996). Within a wetland, red-winged blackbirds use all parts of the marsh for breeding and foraging (Minock and Watson, 1983). Observational studies have shown more first year red-winged males breed in the open marsh areas, and the older males prefer the periphery of the marsh (Beletsky and Orians, 1996).

At our field site, males start arriving for breeding in late April and continue through early May. Females usually start arriving two to three weeks after males, and have been known to wait several weeks before selecting a mate and territory (Beletsky and Orians, 1996). Females usually fledge between one and two clutches over the breeding season; however, we monitored nest activity closely, and although we cannot be sure, we tried to ensure our results were from first nesting attempts only. Clutches range in size between two and six eggs, and the incubation and nesting period is between 22 and 27 days long. The breeding season usually lasts through July.

4.3.2. Study Site

All field observations and data were collected from a coulee which is part of the Sheyenne River watershed located in Mapleton, North Dakota, Cass County (Figure 4.1; 46.818324, -97.000946). The system is surrounded primarily by agricultural fields, with the prominent crops including corn, wheat, and soybeans. The coulee is ephemeral, filled with cattails, and when filled with water, the flow is negligible. The coulee is also habitat for other overwater nesting birds including brown-headed cowbirds, marsh wrens (*Cistothorus palustris*), purple martins (*Progne subis*), and yellow-headed blackbirds (*Xanthocephalus xanthocephalus*). Waterfowl, and other wetland species common to the area have also been spotted at our field site. Many birds of prey, such as the great-horned owl have also been seen (*Bubo virginianus*).

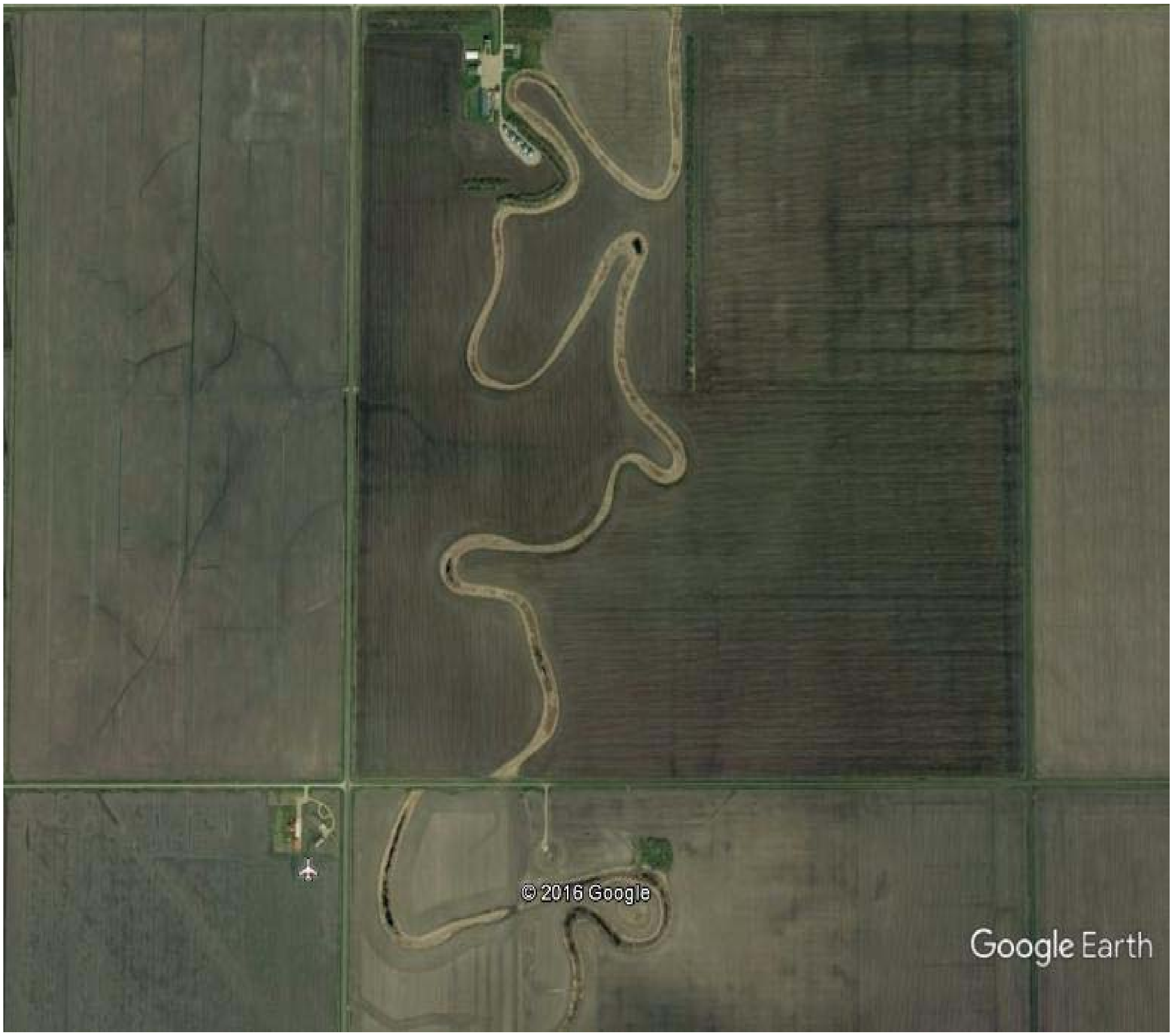

Figure 4.1. Aerial View of a Portion of our Coulee System.
Note: Google (2016) [Google Maps portion of a coulee system located in Mapleton, ND]. Retrieved March 1, 2016, from https://www.google.com/maps/@46.8121661,-97.0017418,1756m/data=!3m1!1e3.

4.3.3. Study Protocol

At the start of the 2015 breeding season, breeding colonies were identified within the coulee by locating males defending territories. The coulee was divided into 18 sites that were 30 meters long and separated by 60 m, a distance greater than previous studies (Olendorf *et al.*, 2004). Multiple males were observed on each site; however, total males were not recorded. Sites were assumed to be physically similar; however, to control for any possible dissimilarities between sites, sites were randomly assigned to one of the three following treatments to examine female response to threats: avian control (purple martin), nest parasite (female brown-headed cowbird), or predator (great-horned owl).

Once females arrived, sites were closely monitored for nest building activities. During the two-week period following the start of nest building, sites were exposed to an effigy and corresponding call of their threat treatment. The exposures lasted for one hour per day for four days. Due to the range in threat species used, sites were randomly exposed twice at sunrise and twice at sunset.

Sites were monitored for nest activity. Active nests were marked using neon orange flagging tape (approximately 30 cm from the vegetation cluster supporting the nest), and location coordinates noted on a Garmin GPS-MAP62sc. Individual nests from each treatment were monitored using a Drift Ghost-S action video camera. Nests were monitored during effigy exposure to evaluate time spent incubating. Cameras were mounted by the nests 30 minutes before sites were exposed to the effigy to ensure females could acclimate to the camera and all behaviors observed during the effigy exposure were due to the effigy. Nests were also monitored when nestlings were 5-8 days old to examine if feeding rates/time spent at the nest differed across treatments. Nestling age was selected to control for differences in feeding rates based on age and yet still young enough so they would be less likely to fledge too early due to human disturbance. Nests were also monitored for clutch initiation (lay date), clutch size, and nest fate throughout the remainder of the breeding season.

The location of the nest within the coulee was recorded by measuring the distance from the center of the nest to the coulee edge (where wetland vegetation and agricultural land meet), and from the center of the nest to the center of the coulee to the nearest centimeter (Figure 4.2). Water depth directly below the nest and height from the bottom of the nest to the top of the water surface was also measured to the nearest centimeter.

Figure 4.2. Aerial View of a Portion of our Coulee Depicting Nest measurements.
Note: Aerial view of a portion of the coulee with a nest (white trapezoid). Measurements were taken from the center of the nest to the edge of the coulee (A), and center of the nest to the center of the coulee (B). Google (2016) [Google Maps portion of a coulee system located in Mapleton, ND]. Retrieved March 1, 2016, from https://www.google.com/maps/@46.8121661,-97.0017418,1756m/data=!3m1!1e3.

To analyze the construction of the nest, we measured inside depth, outside height, inside diameter, and outside diameter to the nearest millimeter. The data was then used to determine the inside and outside volume of the nest using the equation for the volume of a cylinder (Equation 3.1), where V is the volume of the nest, r is the radius of the nest (either inside or outside), and h is the height of the nest (either from the inside depth or outside length).

$$V=\pi r^2 h \qquad \text{(Equation 4.1.)}$$

Whenever we could positively identify the third laid egg, it was removed from the nest within 24 h of laying for hormone analysis of the yolk contents. Eggs were frozen, peeled, and the yolk was separated from the albumin. CORT was extracted from 40 mg of each egg yolk (yolk sample size was validated using a pooled sample of yolk), and hormone concentrations were analyzed using an ELISA (ENZO life science corticosterone ELISA kit, product number ADI-900-097). Testosterone was also extracted from an 8 mg sample of each egg yolk (yolk sample size was validated using a pooled sample of the yolk), and T hormone concentrations were analyzed using a testosterone ELISA (ENZO life science testosterone ELISA kit, product number ADI-900-065).

4.3.4. Data Analysis

Analyses for micro-habitat of nests, nest construction, and female stress levels were performed using JMP® version 11 (SAS Inc.).

Variation within and among the variables describing nest location (distance to edge, distance from center, height off water, and water depth) was analyzed using a principle component analysis. All nest location variables were normally distributed except for “distance from center,” which was log-transformed for further analysis.

4.3.4.1. Treatment, CORT, and T Relationships with Nest Location

Corticosterone and T concentrations were both normally distributed. We examined if there were correlations between yolk CORT and yolk T, yolk CORT and principal components for nest location, and yolk T and principal components for nest location across the sample period. To evaluate the relationships among risk treatments, CORT, and T, we narrowed the time frame to include only the nests initiated after the first exposure to risk treatments. In total, 18 nests were removed from analysis (8 nests from the predator treatment, 7 nests from the nest parasite treatment, and 3 nests from the avian control treatment; 37 nests remained for analysis). Too few nests remained in the nest parasite treatment, thus the relationships among yolk CORT (or yolk T), risk treatment, and nest location were not evaluated. However, two-sampled t-tests evaluating the effects of treatment (avian control and predator treatments) on yolk CORT and yolk T were examined. After the removal of the 18 nests, enough nests still remained to perform an ANOVA to evaluate the relationship between the risk treatments and principal components for nest location. Nests were pooled within treatments.

4.3.4.2. Nest Volume

We identified correlations between the inside and outside volume of a nest. Because of the possibility of parents continuing to add material to nests as the season progressed, we also evaluated whether date measured, or age of nest when measured were correlated with nest volume.

We examined if there were correlations between CORT and volume, or T and volume. We used the Pearson's correlation coefficient to examine if there was a correlation among volume and the principal components for nest location. Next, an ANOVA was done after the removal of the same 18 nests as above to examine the relationship between the treatments and nest volume. Finally, mixed models were completed to study the effects of the principal components for nest location, treatment, and the interaction among treatment and the principal components for nest location on nest volume.

4.3.4.3. Clutch Initiation, Clutch Size, and Parental Behaviors

We examined the relationships between on clutch initiation and our other variables. First, a regression was used to determine if there was a correlation between total width of the coulee and lay date or date measured, because of the possibility the width of the vegetation in the coulee may expand as the season progresses. Next, we evaluated if there were significant correlations between clutch initiation and CORT, clutch initiation and T, clutch initiation and nest volume, and clutch initiation and the principal components for nest location. An ANOVA was completed to look at the effects of treatment on clutch initiation (again, 18 nests were removed).

We analyzed the relationships between clutch size and the various nest and egg characteristics. ANOVAs were performed to examine if there were significant relationships between clutch size and CORT, clutch size and T, clutch size and nest volume, clutch size and principal components for nest location, and clutch size and initiation date. A Bonferroni correction was used to correct for the multiple comparisons ($\alpha = 0.05/6 = 0.0083$). A Chi-square was used to examine if there was a difference in clutch size among stress treatments.

We evaluated whether parental behaviors differed among the stress treatment groups. Incubation time and time at nest (roosting or feeding offspring), were both normally distributed when times were averaged within sites. Incubation time and time at nest were compared among treatments using ANOVAs. Incubation observations occurred during effigy presentations in both the morning and the evening, thus incubation times were compared between morning and evening observations. However, incubation times were not normally distributed across all observations. Thus, a contingency analysis was used to analyze the incubation time between morning and evening observations.

4.3.4.4. Nest Success

The nest survival model available in Program MARK® was used to model the daily survival of red-winged blackbird nests as a function of nest location parameters (PC1 and PC2), treatment groups, nest structure (inside and outside volume), CORT, and nest initiation date (White and Burnham, 1999). We selected Program MARK due to its unique ability to allow for several biological factors of interest to be easily included in nest survival models (Dinsmore *et al.*, 2002).

Encounter occasions, or number of days nest success data was collected in 2015 was 40 days. Two separate sets of models were selected. In the first set of models, nests were divided into three attribute groups by treatment (avian control N=11, nest parasite N=8, predator N=8); however, due to small sample size in CORT data, samples could not be divided by treatment, so all data was left in a single grouping (n=18) in the second set of models. Nest location parameters, nest structure (inside and outside volume), CORT, and nest initiation date were covariates in our models. Models were ranked using the delta Akaike second order information criterion (ΔAICc) and weighted Akaike second order information criterion (ωAICc) calculated for each model, such that a ΔAICc < 2 was considered a significant model based on the parameters of our data, and ωAICc represents the relative likelihood of the model based on the parameters of our data (Dinsmore *et al.*, 2002).

For the first set of models, we started with a null model (Table 4.1), and modeled each effect separately; afterwards, we modeled each effect-treatment interaction. Based on the results of the individual effect models, we examine a variety of other mixed effects models. In the second set of models, we started with a null model (Table 4.2), and modeled each effect separately, followed by a full effect model. We then examined a variety of mixed models based on the results from the individual effect models (Table 4.2).

Table 4.1. Survival Probability Model Set One.

Model	**Notation**
Null Model	$S_{()}$
Effect of Treatment	S_{TRTMNT}
Effect of PC1	S_{PC1}
Effect of PC2	S_{PC2}
Effect of Inside Nest Volume	S_{IVOL}
Effect of Outside Nest Volume	S_{OVOL}
Nest Initiation Date	S_{LDAY}
Effect of Treatment – PC1 Interaction	$S_{TRTMNT*PC1}$
Effect of Treatment – PC2 Interaction	$S_{TRTMNT*PC2}$
Effect of Treatment – Inside Nest Volume Interaction	$S_{TRTMNT*IVOL}$
Effect of Treatment – Outside Nest Volume Interaction	$S_{TRTMNT*OVOL}$
Effect of Treatment – Nest Initiation Date Interaction	$S_{TRTMNT*LDAY}$
Effect of Treatment – 2 groups, Avian Control-Predator and Nest Parasite	$S_{2TRTMNT}$
Effect of Treatment – PC1 Interaction plus Nest Initiation Date	$S_{TRTMNT*PC1 + LDAY}$
Effect of Treatment – PC1 plus Outside Nest Volume	$S_{TRTMNT*PC1 + OVOL}$
Effect of Treatment – PC1 plus Inside Nest Volume	$S_{TRTMNT*PC1 + IVOL}$
Effect of Treatment – PC1 plus Ouftside Nest Volume + Inside Nest Volume	$S_{TRTMNT*PC1 + OVOL + IVOL}$
Effects of Treatment – PC1 plus Nest Initiation Date + Outside Nest Volume	$S_{TRTMNT*PC1 + OVOL + LDAY}$
Effect of 2 Treatment Groups – PC1 Interaction	$S_{2TRTMNT*PC1}$
Effects of 2 Treatment Groups – PC1 Interactions plus Nest Initiation Date	$S_{2TRTMNT*PC1 + LDAY}$

Table 4.2. Survival Probability Model Set Two.

Model	**Notation**
Null	$S_{()}$
Effect of Nest Initiation Date	S_{LDAY}
Effect of Egg Yolk CORT Concentration	S_{CORT}
Effect of Outside Nest Volume	S_{OVOL}
Effect of Inside Nest Volume	S_{IVOL}
Effect of PC1	S_{PC1}
Effect of PC2	S_{PC2}
Full Effect	$S_{LDAY+CORT+OVOL+IVOL+PC1+PC2}$
Effect of PC2 plus Outside Nest Volume	$S_{PC2+OVOL}$
Effect of PC2 plus Inside Nest Volume	$S_{PC2+IVOL}$
Effect of PC2 plus Egg Yolk CORT Concentration	$S_{PC2+CORT}$
Effect of PC2 plus Nest Initiation Date	$S_{PC2+LDAY}$
Effect of PC1 and PC2	$S_{PC2+PC2}$

4.4. Results

4.4.1. Nest Location Principal Component Analysis

More than 73% of the cumulative total variation among water depth, height off the water, distance to the edge, and distance from the center is accounted for by the first two principal components (Table 4.3). The first component (PC1) accounts for 47.77% of the total variation, and describes a positive correlation between water depth and distance to edge (Table 4.3). The second component (PC2) accounts for an additional 22.28% of the total variation, and describes a positive contribution of height off water, and a negative contribution of distance from center (Table 4.3).

Table 4.3. Principal Component Eigenvectors.

Eigenvectors	**Principal Component 1**	**Principal Component 2**
Water Depth	0.56857	-0.13393
Height off Water	-0.23296	0.80345
Distance to Edge	0.64489	-0.00053
Distance from Center	-0.45450	-0.58011
Cumulative Percent	47.945	76.321

4.4.2. Treatment, CORT, and T Relationships with Nest Location

There is no correlation between yolk CORT and yolk T Concentrations ($\rho = 0.2307$, $p = 0.3900$, $N = 16$). There is no significant correlation between PC1 and yolk CORT ($\rho = -0.0036$, $p = 0.9888$, $N = 18$; Figure 4.3); however, there is a significant negative correlation between PC2 and yolk CORT ($\rho = -.5378$, $p = 0.0213$, $N = 18$; Figure 4.4). There is no relationship between T and PC1 ($\rho = 0.2707$, $p = 0.2932$, $N = 17$), or PC2 (0.0863, $p = 0.7418$, $N = 17$).

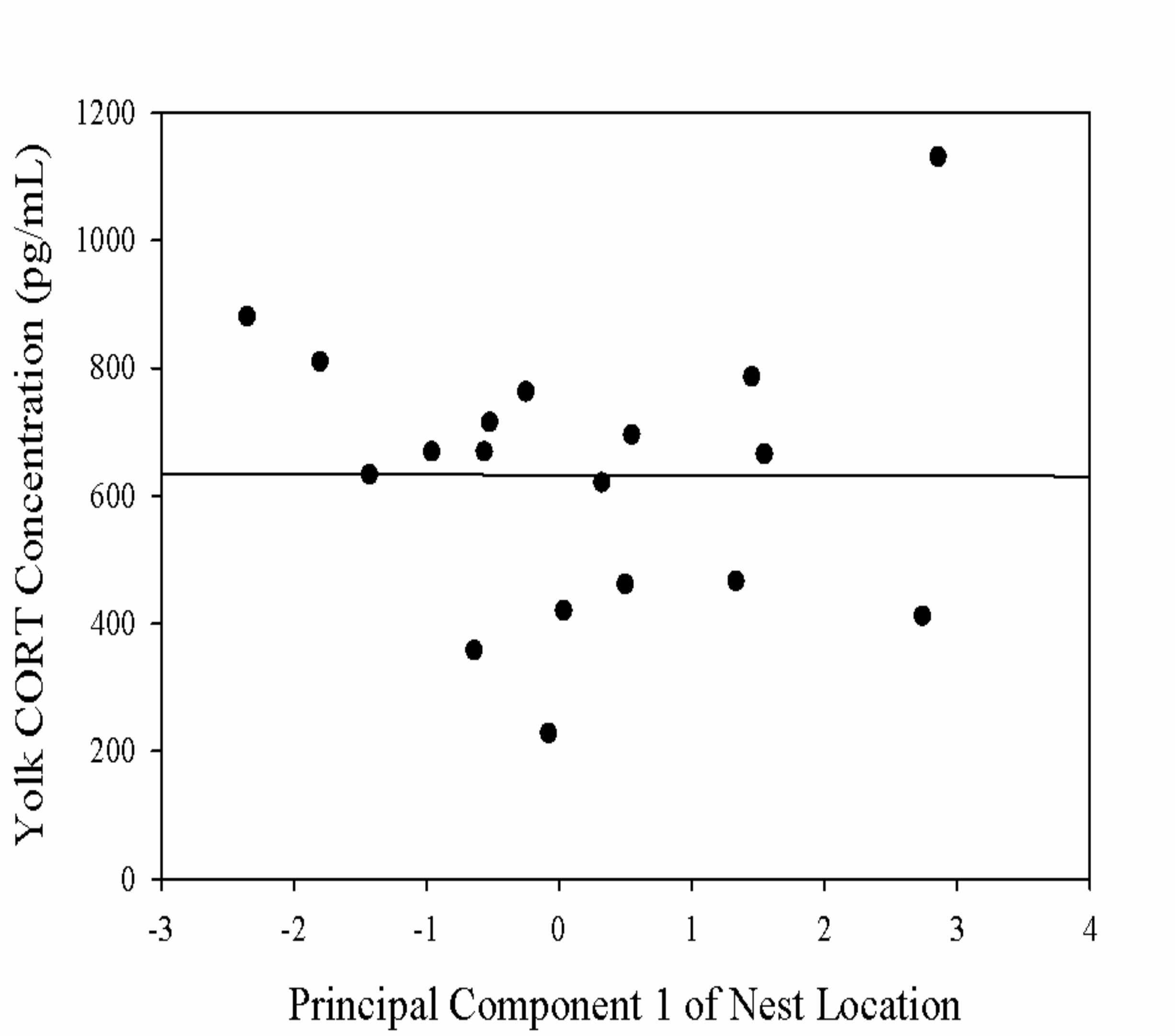

Figure 4.3. Relationship between Principal Component 1 of Nest Location (PC1) and Egg Yolk CORT Concentration.

Note: The relationship between principal component 1 of nest location (PC1) and yolk CORT concentration (pg/mL) is not significant.

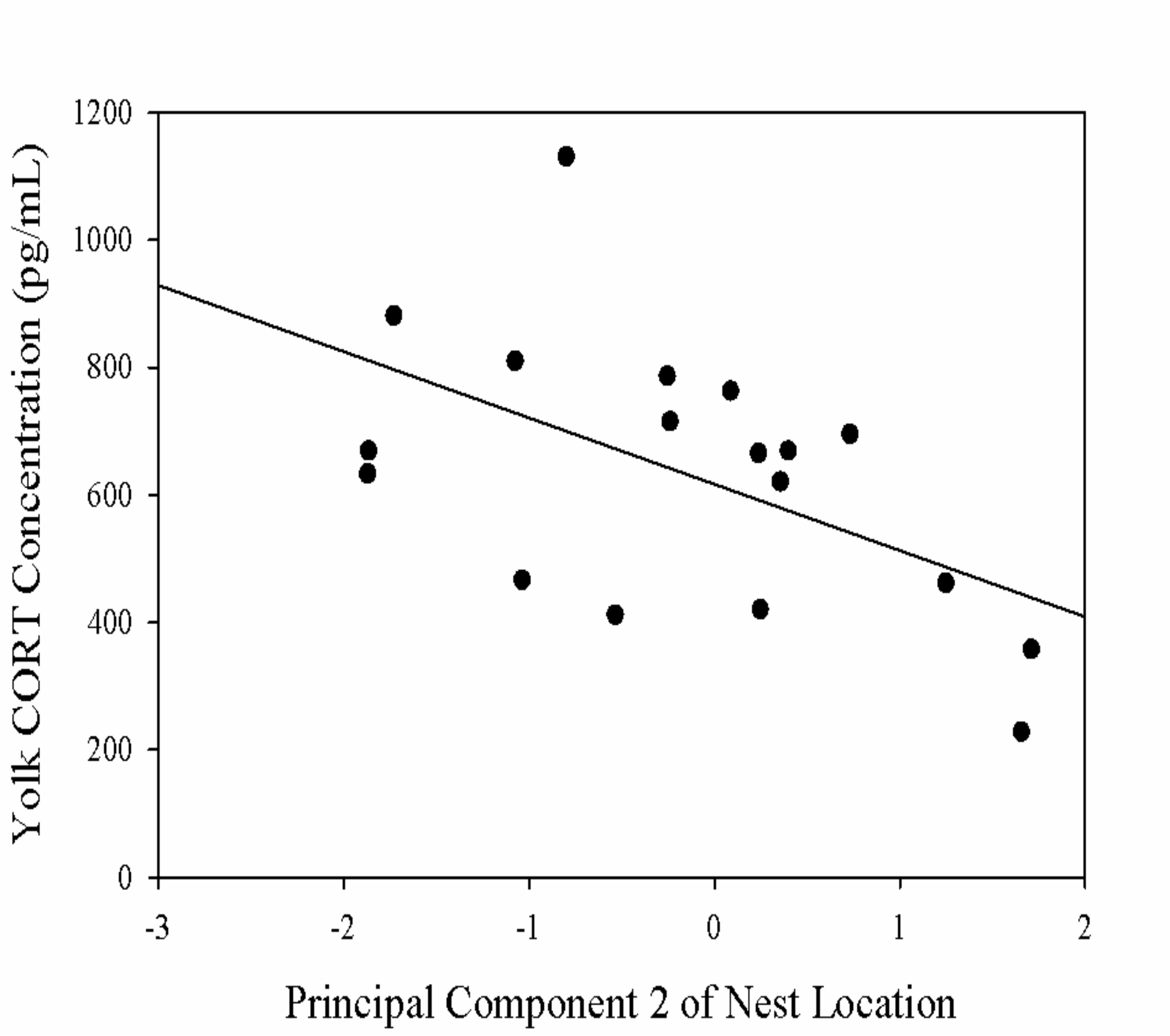

Figure 4.4. Relationship between Principal Component 2 of Nest Location (PC2) and Egg Yolk CORT Concentration
Note: The relationship between principal component 2 of nest location (PC2) and yolk CORT concentration (pg/mL). There is a significant negative correlation.

There is no significant difference between mean yolk CORT of the predator treatment ($\bar{x}$ = 598.390 pg/mL, SE = 63.345) and mean yolk CORT of the avian control treatment ($\bar{x}$ = 733.819 SE = 70.822; t = -1.4253, p = 0.1971, N = 9). Testosterone does not differ between the avian control treatment ($\bar{x}$ = 98.807, SE = 78.7328) and the predator treatment ($\bar{x}$ = 122.481, SE = 85.9846; t = 0.3973, p = 0.6476, N = 8).

Treatment has a significant effect on nest location, but only on PC2 ($F_{2,34} = 7.4864$, $p = 0.0020$, $N = 37$; Figure 4.5). A Tukey-Kramer Post-hoc reveals the nest parasite treatment has significantly lower mean PC2 ($\bar{x} = -0.9262415$, SE = 0.21870), than the predator treatment ($\bar{x} = 0.17095$, SE = 0.21012, $p = 0.0027$) or avian control treatment ($\bar{x} = 0.00954$, SE = 0.21870, $p = 0.0127$). There is no significant difference in mean PC2 between the predator treatment and the avian control treatment ($p = 0.8561$). Treatment does not have a significant effect on PC1 (PC1: $F_{2,34} = 0.0148$, $p = 0.9853$, $N = 37$; avian control: $\bar{x} = -0.242$, SE = 0.404; nest parasite: $\bar{x} = -0.170$, SE = 0.404; predator: $\bar{x} = -0.263$, SE = 0.388).

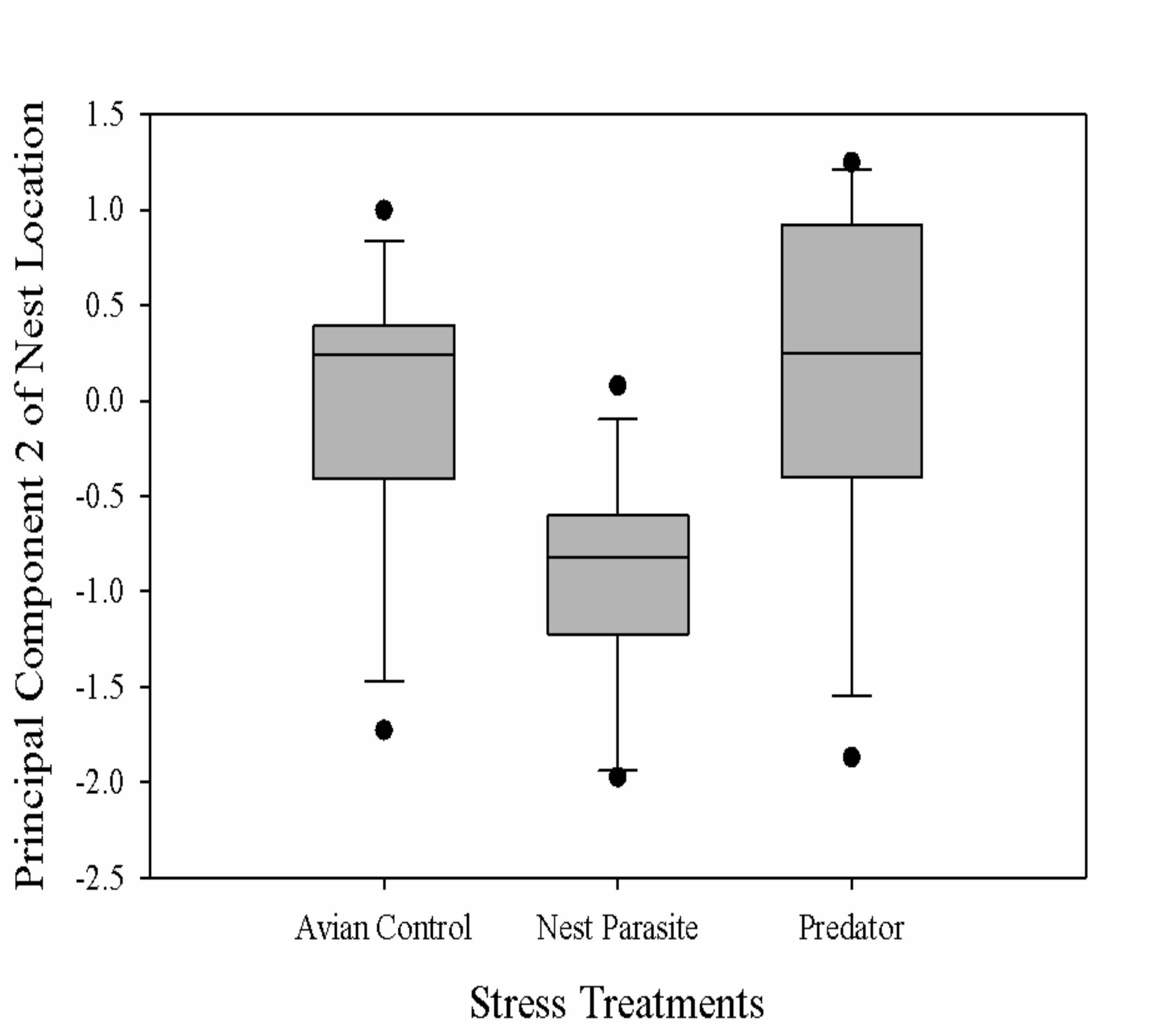

Figure 4.5. Effects of Stress Treatments on Principal Component 2 of Nest Location (PC2). Note: Difference in principal component 2 of nest location (PC2) in avian control, nest parasite, and predator stress treatments.

4.4.3. Nest Volume

There is a significant correlation between inside and outside volume of the nests ($\rho = 0.5567$, $p < 0.0001$, N = 55). The date the nest was measured and the age of the nest are not significant predictors of the outside or inside volume of the nest (outside volume: $\rho = -0.2195$, $p = 0.1427$, N = 46; inside volume: $\rho = 0.1103$, $p = 0.4654$, N = 46). Neither was removed from analysis because they may potentially represent two separate behaviors of the red-winged blackbird. Outside volume may represent continued modification on the nest, and inside volume may represent initial conditions of the nest, and actual available space to raise offspring.

Yolk CORT and outside or inside volume of the nest are not significantly related (outside volume: $\rho = 0.1664$, $p = 0.5093$, $N = 18$; inside volume: $\rho = 0.1095$, $p = 0.6652$, $N = 18$). There is no significant relationship between yolk T and outside or inside volume of the nest (outside volume: $\rho = -0.3894$, $p = 0.1223$, $N = 17$; inside volume: 0.1968, $p = 0.4489$, $N = 17$).

Nest location is, however, significantly related to inside and outside volume of the nest. Specifically, PC1 is positively correlated to inside and outside volume (outside volume: $\rho = 0.3385$, $p = 0.0115$, $N = 55$; inside volume: $\rho = 0.2898$, $p = 0.0115$, $N = 55$; Figure 4.6). PC2 is not significantly correlated to inside or outside volume (inside volume: $\rho = -0.1101$, $p = 0.4234$, $N = 55$; outside volume: $\rho = 0.0558$, $p = 0.6859$, $N = 55$; Figure 4.7). Inside and outside volume also do not differ significantly among treatments [(inside volume: $F_{2,34} = 0.8390$, $p = 0.4409$, $N = 37$; avian control: $\bar{x} = 359882$ mm^3, SE = 39148; nest parasite: $\bar{x} = 421626$ mm^3, SE = 39148; predator: $\bar{x} = 421530$ mm^3, SE = 37612); (outside volume: $F_{2,34} = 0.8942$, $p = 0.4183$, $N = 37$; avian control: $\bar{x} = 1212913$ mm^3, SE 104666; nest parasite: $\bar{x} = 1022646$ mm^3, SE = 104666, predator: $\bar{x} = 1163845$ mm^3, SE = 100560)].

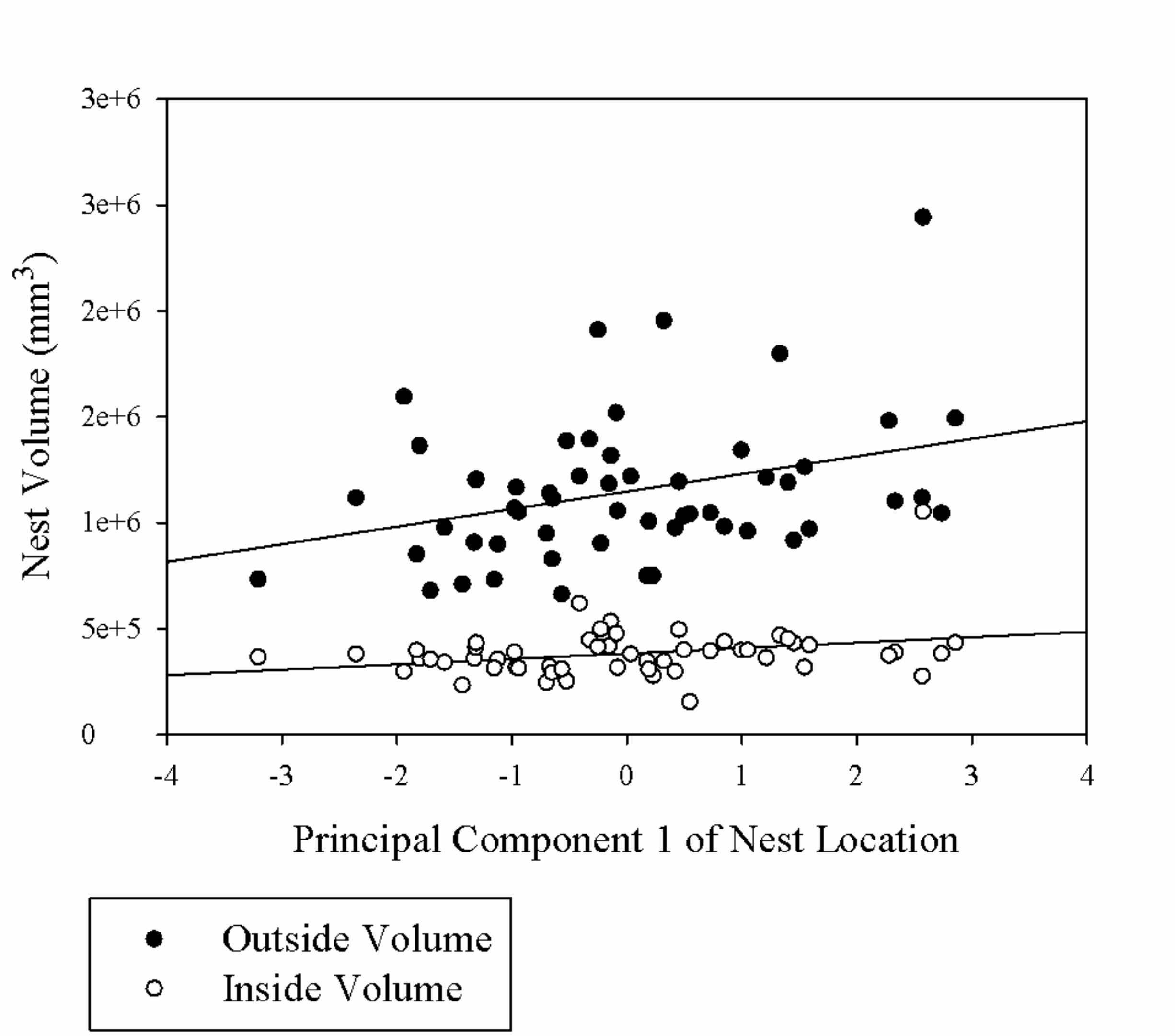

Figure 4.6. Relationship between Nest Volume and Principal Component 1 of Nest Location (PC1).
Note: Relationship between principal component 1 of nest location (PC1) and both inside and outside nest volume (mm^3). Inside and outside volume are significantly positively correlated to principal component 1 of nest location.

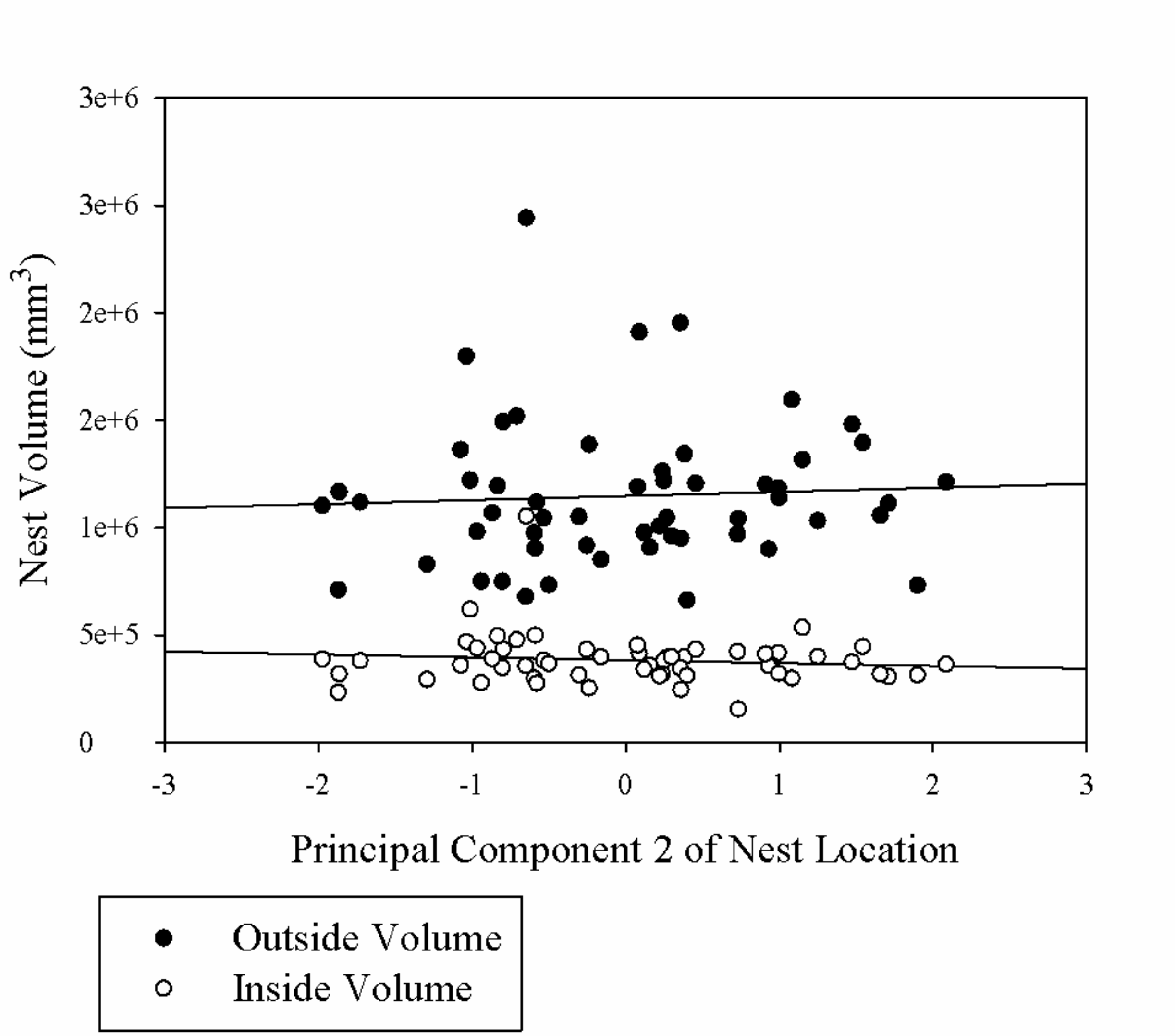

Figure 4.7. Relationship between Nest Volume and Nest Site Location (PC2).
Note: Relationship between principal component 2 of nest location and both inside and outside nest volume (mm^3). Inside and outside volume are not significantly correlated to principal component 1 of nest location.

The mixed model including treatment, PC1, and PC2 is not a significant predictor of inside or outside volume (inside volume: $F_{8,28} = 2.1821$, $p = 0.0607$, $R^2 = 0.384$, N = 37; outside volume: $F_{8,28} = 1.5356$, $p = 0.1899$, $R^2 = 0.305$, N = 37). However, the mixed model including treatment, PC1, and the interaction between them is a significant predictor of inside volume ($F_{5,31} = 3.2914$, $p = 0.0169$, $R^2 = 0.347$, N = 37; Figure 4.8). The first principal component of nest location is a significant factor in the model ($F_{1,1} = 4.1849$, $p=0.0493$). The interaction between treatment and PC1 is also a significant factor ($F_{2,2} = 4.3639$, $p = 0.0214$), with the parameter avian control treatment – principal component interaction being significant ($p = 0.0481$). The mixed model including treatment, PC1, and the interaction between the two is not a significant predictor of outside volume ($F_{5,36} = 2.35$, $p = 0.0641$, $R^2 = 0.275$, $N = 37$).

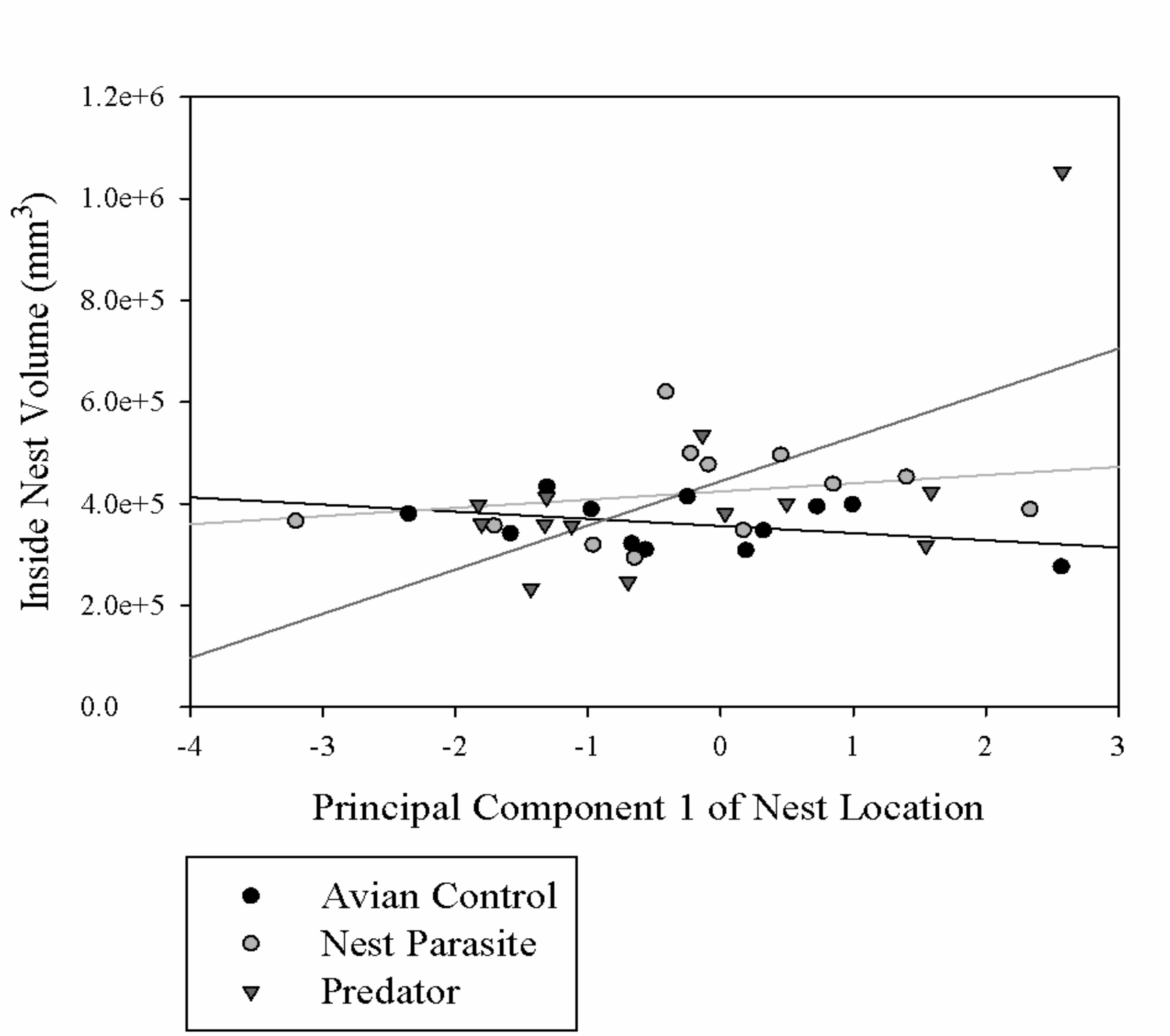

Figure 4.8. Effect of Stress Treatments on the Relationship between Inside Nest Volume and Principal Component 1 of Nest Location (PC1).
Note: A model examining the effects of the different stress treatments (avian control, nest parasite, and predator) on the relationship between inside nest volume and the principal component 1 of nest location (PC1) was performed. The relationship between principal component 1 of nest location and the avian control treatment is a significant predictor of inside nest volume.

4.4.4. Clutch Initiation and Clutch Size

Clutch initiation and the date the nest was measured are not significantly correlated to the full width of the coulee (clutch initiation: $\rho = -0.0041$, $p = 0.9782$, $N = 46$; date measured: $\rho = 0.2554$, $p = 0.0599$, $N = 55$).

Clutch initiation is not correlated to yolk CORT ($\rho = 0.3229$, $p = 0.1912$, $N = 18$), yolk T ($\rho = 0.2851$, $p = 0.2673$, $N = 17$), outside volume ($\rho = -0.1573$, $p = 0.2964$, $N = 46$), inside volume ($\rho = 0.2230$, $p = 0.1363$, $N = 46$), PC1 ($\rho = -0.1942$, $p = 0.1959$, $N = 46$), or PC2 ($\rho = -0.1460$, $p = 0.3329$, $N = 46$). Clutch initiation does not differ among treatments ($F_{2,26} = 0.9719$, $p = 0.3917$, $N = 29$).

There is not variation in clutch initiation between clutch sizes ($F_{2,43} = 3.2268$, $p = 0.0495$, $N = 46$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = 151.75$, SE = 1.3634; clutch = 4: $\bar{x} = 146.647$, SE = 1.4788; clutch = 5: $\bar{x} = 149.111$, SE = 2.0324). Egg yolk CORT concentrations does not differ between clutch sizes ($F_{2,15} = 0.6719$, $p = 0.5255$, $N = 18$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = 683.098$ pg/mL, SE = 73.12; clutch = 4: $\bar{x} = 615.095$ pg/mL, SE = 89.56; clutch = 5: $\bar{x} = 517.310$ pg/mL, SE = 126.65). Egg yolk T concentrations ($F_{2,14} = 2.1420$, $p = 0.1542$, $N = 17$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = 81.705$ pg/mL, SE = 20.594; clutch = 4: $\bar{x} = 105.964$ pg/mL, SE = 20.594; clutch = 5: $\bar{x} = 159.517$ pg/mL, SE = 31.458), outside volume ($F_{2,51} = 1.3496$, $p = 0.2685$, $N = 54$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = 1230069$ mm^3, SE = 69208; clutch = 4: $\bar{x} = 1092759$ mm^3, SE = 73987; clutch = 5: $\bar{x} = 1051035$ mm^2, SE = 113017), and inside volume ($F_{2,51} = 0.2248$, $p = 0.7994$, $N = 54$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = 382949$ mm^3, SE = 24542; clutch = 4: $\bar{x} = 386055$ mm^3, SE = 26236; clutch = 5: $\bar{x} = 355205$ mm^3, SE = 40077), PC1 ($F_{2,51} = 0.2475$, $p = 0.7817$, $N = 54$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = -0.12606$, SE = 0.28870; clutch = 4: $\bar{x} = 0.05877$, SE = 0.30864; clutch = 5: $\bar{x} = 0.24481$, SE = 0.47145), PC2 ($F_{2,51} = 0.4862$, $p = 0.6178$, $N = 54$, $\alpha = 0.0083$; clutch ≤ 3: $\bar{x} = -$

0.12878, SE = 0.20720; clutch = 4: $\bar{x}$ = 0.16649, SE = 0.22151; clutch = 5: $\bar{x}$ = 0.06740, SE = 0.33836) do not vary among clutch sizes. Clutch size also does not differ among treatments (χ^2_4 = 1.707, p = 0.7895, N = 36).

The occurrence of incubating females during the presence of the effigy presentations does not differ between morning and evening observations (χ^2_2 = 2.702, p = 0.2590, N = 16). Incubation time does differ among treatments ($F_{2,4}$ = 10.6448, p = 0.0250, N = 7). A Tukey-Kramer Post-hoc analysis reveals females exposed to the predator treatment spend significantly less time incubating her eggs ($\bar{x}$ = 0.08 sec. incubating/ total time observed, SE = 0.0488) compared to the avian control ($\bar{x}$ = 0.40 sec. incubating/ total time observed, SE = 0.0598, p = 0.0309). Predator treatment is not significantly different from the nest parasite treatment ($\bar{x}$ = 0.35 sec. incubating/ total time observed, SE = 0.0598; p = 0.0521). Avian control is not significantly different from the nest parasite treatment (p = 0.8408). Time spent on the nest (feeding and roosting) when chicks were between 5-8 days old does not differ among treatments ($F_{2,4}$ = 0.0161, p = 0.9841, N = 7; avian control: $\bar{x}$ = 0.24 sec. on nest/ total time observed, SE = 0.1093; nest parasite: $\bar{x}$ = 0.28 sec. on nest/ total time observed, SE = 0.1339; predator: $\bar{x}$ = 0.26 sec. on nest/ total time observed, SE = 0.1339).

4.4.5. Nest Success

Under the specific parameters of this study, the daily survival of red-winged blackbird nests is a function of multiple effects in both sets of models (Table 4.4 and 4.5). In the first set of models, the location parameters of PC1 have the greatest individual effect on nest survival, with a slope estimate from the model of β = 0.31 (1SE = 0.19, 95%CI = -0.05, 0.68) on a logit scale. By examining the interactions between treatment and PC1, we substantially improved our best model (an increase of 1.1076 ΔAICc units). However, the predator treatment is the only parameter with an effect of PC1 on a logit scale to not include zero in the confidence interval [Predator β = 1.32, 1SE = 0.49, 95% CI = 0.35, 2.28; Avian Control β = 0.24, 1SE = 0.31, 95% CI = -0.36, 0.85; Nest Parasite β = 0.03, 1SE = 0.26, 95% CI = -0.51, 0.57]. The logistic regression equation for our best model has a y-intercept of β = 3.03 (1SE = 0.29, 95% CI = 2.46, 3.60). Thus, the logistic regression equation for our best model was

$$\text{logit } S=3.03+0.24(\text{PC1-AC})+0.03(\text{PC1-NP})+1.32(\text{PC1-P}) \qquad \text{(Equation 4.2.)}$$

Where, AC is the avian control treatment, NP is the nest parasite treatment, and P is the predator treatment. This can then be back transformed where

$$S=1/(1+\exp\{-[3.03+0.24(\text{PC1-AC})+0.03(\text{PC1-NP})+1.32(\text{PC1-P})]\}) \text{ (Equation 4.3.)}$$

In our second set of models, PC2 has the greatest effect on daily nest survival with an estimate slope of β = 0.5 (1SE = 0.29, 95% CI = -0.08, 1.08). The model examining the effects of egg yolk CORT concentrations had little support (β = -0.13, 1SE = 0.25, 95% CE = -0.62, 0.37; Table 4.5).

Table 4.4. Survival Probability Model Set One Output.

Model	**Deviance**	**K**	**AICc**	**ΔAICc**	**ωAICc**
$S_{TRTMNT*PC1}$	72.1802	4	80.3167	0	0.16374
$S_{TRTMNT*PC1 + LDAY}$	71.1286	5	81.3341	1.0174	0.09845
$S_{TRTMNT*PC1 + OVOL}$	71.1894	5	81.3949	1.0782	0.09551
S_{PC1}	77.3836	2	81.4243	1.1076	0.09411
$S_{2TRTMNT*PC1}$	75.9013	3	81.9829	1.6662	0.07118
S_{LDAY}	78.0164	2	82.0571	1.7404	0.06859
$S_{2TRTMNT*PC1 + LDAY}$	73.9337	4	82.0702	1.7535	0.06814
$S_{TRTMNT*PC1 + IVOL}$	72.1546	5	82.3601	2.0434	0.05894
$S_{TRTMNT*PC1 + OVOL + LDAY}$	70.3821	6	82.6708	2.3541	0.05046
$S_{TRTMNT*PC1 + OVOL + IVOL}$	70.8444	6	83.133	2.8163	0.04005
S_{PC2}	79.7677	2	83.8084	3.4917	0.02857
S_{IVOL}	79.8103	2	83.8509	3.5342	0.02797
S_{OVOL}	80.0431	2	84.0838	3.7671	0.0249
$S_{2TRTMNT}$	80.1433	2	84.184	3.8673	0.02368
$S_{()}$	80.2581	2	84.2987	3.982	0.02236
$S_{TRTMNT*OVOL}$	76.7256	4	84.8621	4.5454	0.01687
$S_{TRTMNT*PC2}$	76.9074	4	85.044	4.7273	0.0154
$S_{TRTMNT*LDAY}$	76.9164	4	85.0529	4.7362	0.01534
S_{TRTMNT}	80.1355	3	86.2171	5.9004	0.00857
$S_{TRTMNT*IVOL}$	78.4397	4	86.5762	6.2595	0.00716

Table 4.5. Survival Probability Model Set Two Output.

Notation	**Deviance**	**K**	**AIC_c**	**ΔAIC_c**	**ωAICc**
S_{PC2}	58.6769	2	62.7282	0	0.17991
$S_{()}$	61.625	1	63.642	0.9138	0.11393
S_{OVOL}	60.0858	2	64.1371	1.4089	0.08895
$S_{PC2+OVOL}$	58.1098	3	64.2128	1.4846	0.08564
$S_{PC2+PC2}$	58.241	3	64.344	1.6158	0.0802
$S_{PC2+IVOL}$	58.2533	3	64.3563	1.6281	0.07871
$S_{PC2+CORT}$	58.3882	3	64.4912	1.763	0.07451
S_{IVOL}	60.5124	2	64.5636	1.8354	0.07186
$S_{PC2+LDAY}$	58.6768	3	64.7798	2.0516	0.0645
S_{PC1}	60.8393	2	64.8905	2.1623	0.06103
S_{LDAY}	61.2445	2	65.2958	2.5676	0.04983
S_{CORT}	61.3851	2	65.4364	2.7082	0.04645
$S_{LDAY+CORT+OVOL+IVOL+PC1+PC2}$	56.1398	7	70.6289	7.9007	0.00346

4.5. Discussion

Parental decisions made at the start of the breeding season, especially right before or during the building of a nest, can have long reaching reproductive consequences for the breeding population. Our results suggest the interaction between decisions made about nest location, nest structure, survival risk, parental care, and female CORT response are complex, to say the least. Our results also suggest that aversion of nest parasites and stress physiology have the most influence on where and how female red-winged blackbirds build their nests. The threat of predation also reduces the amount of time a female incubates her clutch. Females seem capable of making decisions about and among these different factors, without having to change when they start breeding or their clutch size, to optimize their reproductive success each breeding season.

4.5.1. Nest Location and Principal Components

Nest micro-habitat parameters can be described using the first two principal components from an analysis of the variables water depth, nest height off of water, nest distance from edge, and nest distance from center (Table 4.3). The first principal component primarily describes the location of the nest within the coulee, with a larger PC1 describing nests located closer to the center of the coulee (Figure 4.1), and over deeper water. The second principal component primarily describes nest positioning on the emergent vegetation (cattail and grasses), with a larger PC2 describing nests built higher in the reeds. To a lesser degree, principal component two also describes nest location within the coulee, with larger values describing nests located closer to the center of the coulee.

The coulee located within our field site can be described as having an open channel in the center of the coulee with dense cattails and grasses on both sides; thus, a nest with a large PC1 and PC2 would be located within the clear portion of the channel and high in the emergent vegetation (Figure 4.1).

4.5.2. Relationships with Nest Location

The location of avian control nests suggests under normal breeding circumstances females select nesting locations closer to the center of the coulee and higher in the cattails. Selecting nest locations high in the cattails and in less densely covered areas may leave nests more vulnerable to avian predators (Albrecht *et al*., 2006). Previous studies have shown scarlet rosefinches (*Carpodacus erythrinus*) have higher nest success in nests that are more concealed (Albrecht, 2004); and, in reed warblers (*Acrocephalus scirpaceus*), increases in vegetation density also increased nest success (Darolová *et al*., 2014). However, in the red-winged blackbird, it is possible females are more concerned with flooding issues (building nests lower on the water may leave nests vulnerable to flooding early in the season), or mammalian predators that may access nests closer to land and require minimal climbing, than they are with avian predators (Albrecht *et al*., 2006; Sawin *et al*., 2003). The phenomena of female birds positioning their nests based on flooding concerns has been seen in riparian bird communities, where nest height is positively correlated with nest success (Best and Stauffer, 1980).

Our results support the hypothesis that risk of avian predators is not driving female nest site selection. Our avian predator treatment did not affect female choice on where to build her nest. Our results do suggest, however, females are spending less time incubating their offspring when exposed to the threat of predation by birds of prey. This time may be spent mobbing the avian predator (Lima, 2009). As female and male red-winged blackbirds actively participate in cooperative mobbing of avian predators (Olendorf *et al*., 2004), avian predators may be a relatively low risk to blackbird nests.

Risk treatment has a significant effect on the second principal component. Females exposed to a nest parasite select nest locations deeper in the cattails and grasses and lower on the water. The threat of nest parasitism by the brown-headed cowbirds is an issue for female red-winged blackbirds, and affects decisions made about where to build a nest. Our results suggest a female may leave her nest more vulnerable to factors like flooding or depredation by mammals in order to conceal her nest from a female brown-headed cowbird looking for a host nest. In some species females will behave inconspicuously when nest parasites are spotted near their territory by reducing visits to the nest and time spent calling while near the nest (Neudorf and Sealy, 1992; Banks and Martin, 2001). Although we did not see a difference in time incubating between the avian control and nest parasite treatment, it is possible if a female has not already invested much in nest building she may select a new, more cryptic, nesting location if she has spotted a female brown-headed cowbird in the area.

Egg yolk CORT concentrations are also negatively correlated with the second principle component, indicating that females depositing high levels of CORT in their eggs, are also selecting nest locations in dense vegetation, and low on the water. Several hypotheses may explain the similarities observed in the nest locations selected by females with high CORT levels and nest locations selected by females exposed to the threat of nest parasitism. The first hypothesis is under the increased risk of nest parasitism, female plasma CORT concentrations may increase, which increases the likelihood that females move their nests to more hidden locations. The second is females with similar CORT levels naturally associate with particular nest habitats based on some ecological or environmental factor. For example, females with naturally lower CORT levels associate with the middle of the coulee, however when faced with the threat of nest parasitism, these females move their nests to more hidden locations. In either hypothesis, egg CORT concentrations are just a proxy of the female's CORT concentrations. Previous studies have shown increased CORT levels in adult females corresponds to increased CORT concentrations in her eggs (Hayward *et al*., 2005)

Under the first hypothesis, as a response to the physiological changes caused by the stress of potential nest parasitism, females are moving their nests to more hidden locations. Previous studies have shown increases in environmental stressors increase CORT concentrations in a female's eggs (Saino *et al*., 2005). Further support for this hypothesis comes from research that has shown increases in plasma corticosterone in adults can cause behavioral changes such as increased locomotor activity (Breuner *et al*., 1998; Pitk *et al*., 2012). For example in white-crowned sparrows (*Zootrichia leucophrys*), males with artificially increased CORT concentrations abandon breeding territories for longer periods of time than control males (Breuner and Hahn, 2003). In another study with the same species, increases in CORT concentrations increased perch hopping behavior (Breuner *et al*., 1998). This demonstrates during stressful situations, elevated CORT concentrations cause instinctive increases in movement to help an individual remove itself from the stressor. Thus, it is possible under the increased stress of seeing a female brown-headed cowbird in her territory, a female red-winged blackbird's CORT levels elevate, increasing the likelihood of her instinctively move her nesting location.

Under the second hypothesis, the variation in plasma base line CORT concentrations among females, which may be partially due to phenotypic variation in physiology (Hayward *et al*., 2005) is naturally associated with nest site selection. These similar females may be segregating their nesting locations in response to some other biological or environmental cue besides the threat of nest parasitism (i.e. female age and experience, distance from other females, etc.), such that females with high basal CORT concentrations are found lower on the water and in more dense reeds. This corresponds with other studies, which have shown both female age and nesting density can effect where a female breeds (Brown *et al*., 1990; Janiszewski *et al*., 2017). Once exposed to the threat of nest parasitism, however, all females (irrelevant of CORT concentrations) are more likely to move their nests to more hidden locations. This hypothesis is partially supported by the fact we did not see a physiological difference between the avian control and predator treatment groups, suggesting the threats we have manipulated are not effecting female CORT concentrations. Our data also show yolk testosterone concentrations did not differ between treatments, or vary by location, which further supports the idea females are not responding physiologically to the threats, but rather are responding behaviorally. Our finding is opposite of what has been observed in other species, where the threat of predation has caused an increase in basal CORT levels (in adults and in eggs yolks), and increased yolk T concentrations (Cockrem and Silverin, 2002; Navara *et al*., 2006a; Saino *et al*., 2005).

4.5.3. Nest Volume

Inside and outside volume are both positively correlated with the first principal component, such that nests located closer to the center of the coulee and over deeper water are overall larger. This pattern suggests females nesting in the middle of the coulee are making larger investments in nest building than females on the edge of the coulee. However, there is no interaction among treatments, suggesting under increased threat females do not change the volume of their nests but do make other trade-offs. As females in the center of the coulee generally also have lower CORT levels, the data on nest volume suggests higher quality females (i.e. females with lower CORT levels) invest more in nest building by building bigger nests. This is supported by a previous study showing female blue tits (*Cyanistes caeruleus*) infected with Trypanosoma avium build smaller nests (Tomás *et al*., 2005).

4.5.4. Conclusions

The results from our study support the idea females are making decisions about where and how to build their nests at the start of the breeding season. Both the threat of nest parasitism by the brown-headed cowbird and CORT concentrations are correlated with where a female builds her nest; and, nest location (and potentially CORT concentrations) is correlated with the size of the nest. Further, neither nest initiation nor clutch size were affected by treatment, egg yolk CORT concentrations (female physiology), nest location, or nest volume in the study, even though both can have an important impact on the reproductive success of a female (Perrins and McCleery, 1989).This suggests the combination of the other reproductive decisions females are making during the breeding season allow females to not have to adjust when to start their breeding season or how large of a clutch to have. In addition, as all of the daily survival probability models suggest, females are able to make important reproductive decisions about the parameters studies in this paper so as not to have significant variation in reproductive success.

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5. STRESS PHYSIOLOGY IN MALE RED-WINGED BLACKBIRDS (AGELAIUS PHOENICEUS): A COMPARISON OF WILD-CAPTIVE AND FREE-LIVING INDIVIDUALS

5.1. Abstract

One limitation to studying avian species in captivity is captive individuals are exposed to different stressors than natural populations, and may have different coping mechanisms. These coping mechanisms may include changes to an individual's physiological stress response, which is modulated by the hypothalamus-pituitary-adrenal (HPA) axis, and the glucocorticoid, corticosterone (CORT). The human-animal conflict that has arisen with red-winged blackbirds (*Agelaius phoeniceus*), due to the immense amount of damage they cause to grain crops each year, has resulted in over forty years of collaborative research projects, many of which involve initial laboratory studies. Thus, it is important to examine if captivity of wild red-winged blackbirds causes changes to their HPA axis, and hormone stress response. We exposed wild-captive and free-living male red-winged blackbirds to an acute stress protocol and collected blood samples every fifteen minutes for an hour to measure if the stress of captivity causes physiological changes to their CORT stress response. We found captivity does alter the overall stress response of red-winged blackbirds, such that captive males have a delayed CORT response to acute stressors. In light of these findings, we suggest caution when trying to extrapolate captive data to natural populations of this species.

5.2. Introduction

There is a human-animal conflict that has arisen with red-winged blackbirds (*Agelaius phoeniceus*) due to the severe damage this species can cause to grain and crops – with $70 million worth of damage nationally each year (United States Department of Agriculture *et al.*, 2015). This conflict has made the red-winged blackbird a species of interest in the scientific community for over forty years, and has resulted in a forty year collaborative project with USDA-APHIS-WS National Wildlife Research Center scientists, agricultural producers, commodity groups, research boards, universities, and local, State and Federal agencies to develop safer and more effective management techniques (United States Department of Agriculture, 2015). The main areas of research for blackbird management include, or have included in the past, developing new scare techniques, improving evasion methods, application of repellents, and developing new methods to control populations (Linz *et al*., 2011).

Many of the areas of research on red-winged blackbirds involve initial behavioral and physiological studies in laboratory settings. A common practice in avian research is to bring wild individuals into captivity for study. Captive studies can be useful because test animals are easily accessible and environmental factors can be controlled to help focus research questions (Bateson and Feenders, 2010). Although captive studies can provide valuable information about a species, they also have limitations that can affect their usefulness. In particular, captive individuals are exposed to a completely different suite of stressors than natural populations, and likewise, have a unique set of coping mechanisms (Archard and Braithwaite, 2010). For example, in natural populations, when an individual perceives a threat, one coping mechanism is to move away from the situation. However, in captivity an individual is limited in the space they have to move, and are limited to coping more physiologically rather than behaviorally to threats.

Physiological coping mechanisms include stress responses, which are modulated by the hypothalamus-pituitary-adrenal (HPA) axis. During a stressful situation an animal's adrenal gland secretes glucocorticoids via the HPA axis (Rich and Romero, 2005). In birds, this process starts with the stimulation of the hypothalamus, which secretes corticotrophin-releasing factor to stimulate the pituitary. Once stimulated, the pituitary secretes adrenocorticotropin hormone (ACTH) which causes the adrenal gland to release corticosterone (CORT) (Rich and Romero, 2005). CORT acts on the pituitary in a negative feedback loop to suppress further CORT release once the stressful situation disappears or is lessened (Dickens *et al*., 2009a). The HPA axis and CORT are important for maintaining and restoring homeostasis and helping animals survive stressful episodes by increasing energy in muscle tissue through facilitation of metabolic changes that activate glucose stores and inhibit additional glucose storage (Cyr *et al*., 2007; Monaghan and Spencer, 2014). These physiological changes promote and support escape behaviors (Sapolsky *et al*., 2000; Cyr *et al*., 2007). However, maintaining high levels of glucocorticoids for extended periods of time has been linked with physiological consequences that endanger fitness, including hyperglycemia, neuronal cell death, and suppression of the immune and reproductive systems (Cyr *et al*., 2007). Chronic stress is one potential reason for maintaining elevated CORT levels for an extended period of time (Cyr *et al*., 2007).

There are many sources of stress for wild-caught individuals kept in captivity, such as confinement and reduced retreat space, abnormal social groups, and aversive sounds and odors (Morgan and Trombrog, 2007). When individuals are chronically stressed their physiological response may be modified to help them better cope with external stressors (Koolhaas *et al*., 1999). The ability to suppress physiological sensitivity is beneficial to helping wild individuals cope with the stresses of captivity (Angelier *et al*., 2016). For example, in rock pigeons (*Columbia livia*), individuals with a greater CORT stress response lost more body weight and were less successful in adjusting to captivity than individuals with suppressed CORT responses (Angelier *et al*., 2016).

Few studies have compared the difference in physiological responses of captive and free-living populations. However, the studies that have been conducted show mixed results. In white-throated sparrows (*Xonotrichia albicollis*) and white-crowned sparrows (*Zonotrichia leucophrys*) mean CORT levels were two to three times higher in captive individuals than in free-living individuals (Marra *et al*., 1995). In another study comparing acute stress responses in 11 species of first generation captive bred and wild-caught parrots, researchers found a prolonged CORT response in wild-caught birds compared to the first generation captive bred individuals (Cabezas *et al*., 2013). Additionally, in a study comparing the cortisol response in captive bred and wild cavy (*Cavia porcellus*), results indicated there was no difference in cortisol response between the populations (Künzl *et al*., 2003). These studies suggest extrapolating results from captive studies to those in free-living conditions should be done with caution.

Understanding how red-winged blackbirds respond to stressors in captive and natural environments is an important step if researchers what to continue using controlled laboratory settings to develop scare devices and other methods for managing this species' damage crops. Most hormone studies with red-winged blackbirds have focused on correlations between testosterone and breeding behaviors (Harding *et al*., 1988; Beletsky *et al*., 1989; Beletsky *et al*., 1992). One study did examine the seasonal variation of pre-stressed, or baseline CORT concentrations during the breeding season and found plasma CORT levels peak for males at the start of nest building – when females are most receptive to mating (Johnsen, 1996).

This study compares the physiological response of male wild-captive and free-living red-winged blackbirds to acute stressors during the breeding season in North Dakota, USA. To our knowledge it is the first to examine complete stress profiles, or the change in CORT concentrations over a period of time rather than measuring baseline CORT concentrations, in this species. Additionally, it is the first to compare CORT responses of captive and free-living populations of red-winged blackbirds.

5.3. Methods

5.3.1. Study Site

In 2016 all free-living (hereafter referred to as wild) males were caught in a drainage ditch located on the edge of North Dakota State University (NDSU) main campus in Fargo, North Dakota. The drainage ditch is part of the Southeast Cass County Water Resource District. The captive males were one year old males that were caught in August and September of their hatch-year in 2015, and housed over-winter at the NDSU – Conservation Sciences Research Center, a roofed outdoor aviary, located at the Red River Zoo in Fargo, ND. Birds were housed in cages with a maximum of 10 males. They were provided food (suet cakes and a mixture of sunflower seed, mealworms, raisins, non-medicated chick starter feed, and peanuts), *ad libitum* access to water and allowed access to a heated roost protected from the wind.

5.3.2. Experimental Procedure

At the start of the breeding season (May) mist nets were placed parallel and perpendicular to the drainage ditch after sunrise. We baited our mist nets by playing female red-winged blackbird calls near the nets. Nets were observed from approximately 30 m distance. Once a male was caught, he was removed and bled within three minutes of capture. Birds were placed in an opaque breathable bag, and bled every 15 minutes after capture for one hour, for a total of five samples per male. Males were then banded with USFWS silver bands, and released.

The first six wild males were caught between May 7, 2016 and May 20, 2016, ten captive males were sampled between May 23, 2016 and June 1, 2016, and the remaining four wild males between June 9, 2016 and June 23, 2016. Only two captive male were sampled each day. They were caught from a single cage and at the same time to maintain similar stress levels between individuals. Males were bled within three minutes of first seeing a human, and then every 15 minutes for one hour, for a total of five samples per male. When males were not being bled, they were held in opaque bags.

All blood samples were taken from the brachial vein, and samples were collected from alternating veins for each sample. Samples were collected using Microvette® CB 300 LH, containing lithium heparin, and approximately 50 ul of blood were taken for each sample for a total of 250 ul of blood per bird. Blackbirds weigh on average between 60 and 88 g, and the sample accounts for between 4% and 6% of their total blood volume (Beletsky, 1996). Blood samples were kept on ice until they could be spun down at 4000 g for 5 min. to separate the blood cells from plasma. The plasma was extracted, and frozen for future analysis.

Corticosterone was extracted from plasma from each sample, and concentrations were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (ENZO Life Science, ADI-900-097). Corticosterone was extracted from plasma samples by diluting 12 ul (or however much was available if less than 12 ul) of plasma in 200 ul of double distilled water, and extracted with 1.5 ml diethyl ether. After quickly freezing the water phase, the ether phase was decanted, and the process was repeated three times. The ether phases were then dried down on a heating block set at 20ºC, and a multi-probe drying rack with nitrogen gas. Once samples were dry, they were suspended in 338 ul of assay buffer from the ELISA kit and kit directions were followed. Dilution factors were calculated for any sample with less than 12 ul of plasma. Males were randomly assigned to one of five ELISA plates, and samples were plated in triplicate.

The absorbance readings from the standards from each of the five ELISA plates were then combined to create one standard curve, using the following equation:

Concentration=EXP(-(LOG(Abs/(1-Abs))-2.25026)/0.455707) (Equation 5.1.)

Where Abs is the absorbance reading of each sample.

5.3.3. Data Analysis

Hormone data for wild and captive males was analyzed using JMP® version 11 (SAS Inc.). All data was normally distributed except basal CORT concentrations, which were natural log transformed for further analysis. We were not able to bleed some males within the first three minutes of capture, therefore, we analyzed if basal CORT concentrations were correlated with first bleed time using linear regression. Basal CORT concentrations were not significantly correlated with initial bleed time ($F_{1,17} = 1.7611$, $p = 0.2020$, $R^2 = 0.09$), thus no males were removed from analysis.

We used separate two-sample t-tests to examine whether mean basal CORT, peak (maximum) CORT concentrations, or the time to reach peak CORT concentration differed between the wild and captive males. Also, total CORT concentration over the 60 min. sample period and the average CORT concentration during the 60 min. sample period were compared for wild and captive populations using two-sample t-tests. Male CORT profiles over a 60 min. period were categorized.

5.4. Results

There is not a significant difference in basal CORT concentrations between wild ($\bar{x}$ = 2.26 pg/mL, SE = 0.07) and captive ($\bar{x}$ = 2.22 pg/mL, SE = 0.06) males (t = 1.50, p = 0.15; Figure 5.1). There is also not a significant difference in peak CORT concentrations between wild

($\bar{x}$ = 529.11pg/mL, SE = 48.83) and captive ($\bar{x}$= 490.36 pg/mL, SE = 46.33) males (t = 0.58, p = 0.57; Figure 5.2).

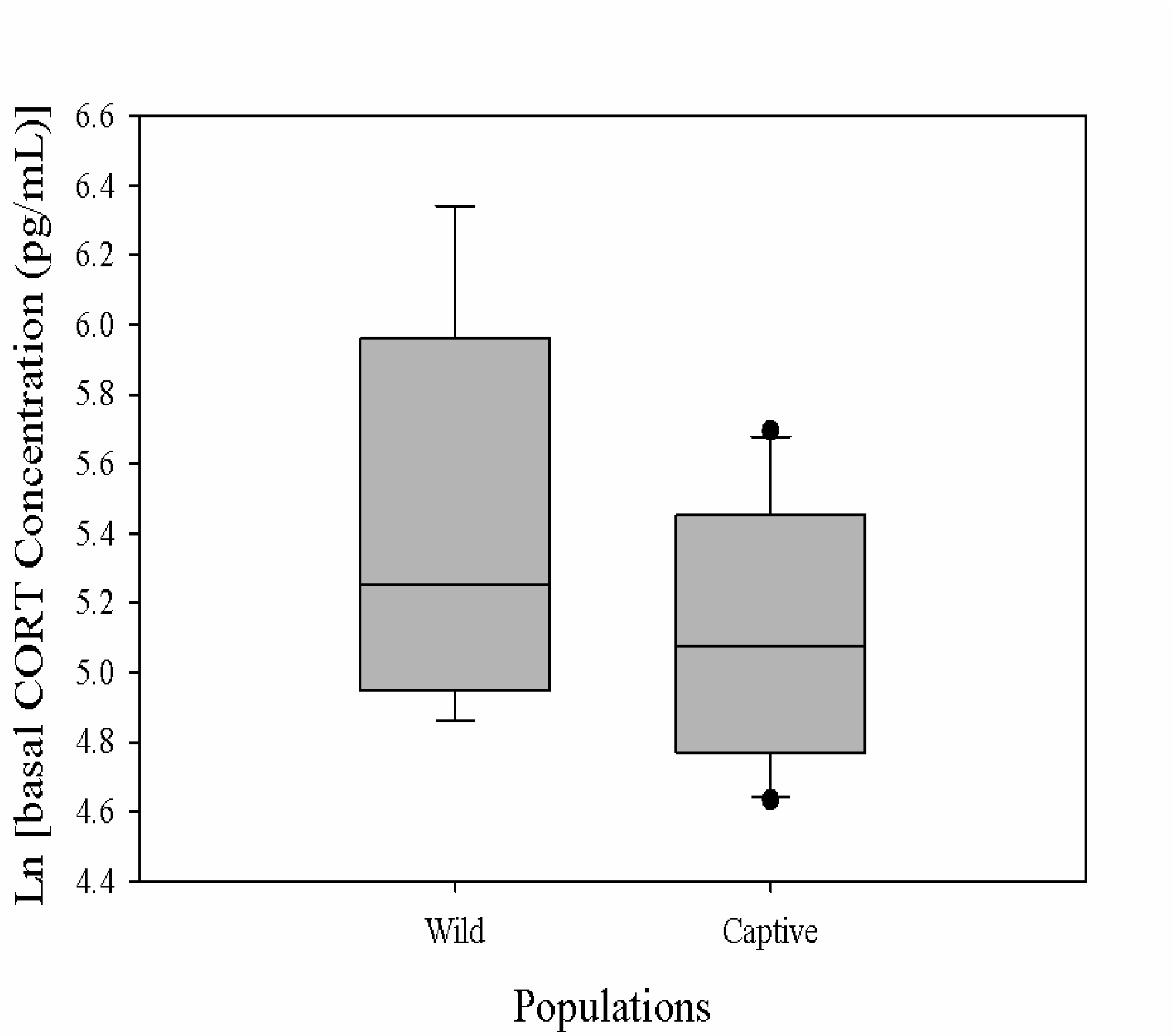

Figure 5.1. Effects of Captivity Treatments on Basal CORT Concentration.
Note: Baseline CORT concentrations were compared between captivity populations. There is no difference in baseline CORT between captive and wild males.

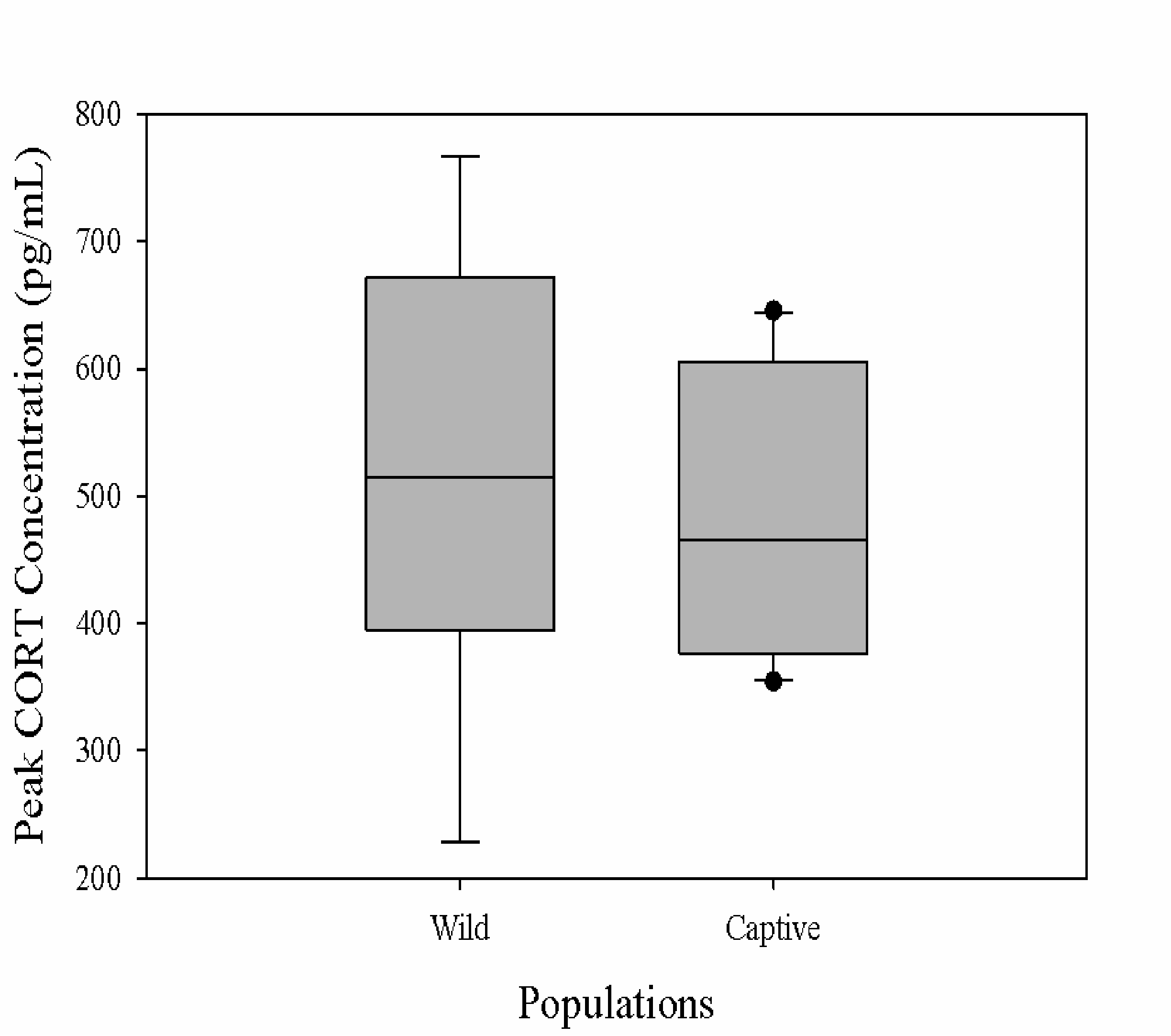

Figure 5.2. Average Peak CORT Concentration.
Note: Peak CORT concentrations were compared between captivity populations. There is no difference in peak CORT between captive and wild males.

The rate at which males reach peak CORT concentrations is not significantly different between wild ($\bar{x}$ = 7.65 pg/mL/min., SE = 1.22) and captive ($\bar{x}$ = 6.36 pg/mL/min., SE = 1.16) individuals (t = 0.77, p = 0.45; Figure 5.3). However, the time it takes males to reach peak CORT concentrations is significantly different between wild ($\bar{x}$ = 32.48 min., SE = 4.93) and captive ($\bar{x}$ = 52.76 min., SE = 4.68) males, such that wild males reach peak CORT concentrations sooner than captive males (t = -2.98, p = 0.0042; Figure 5.4).

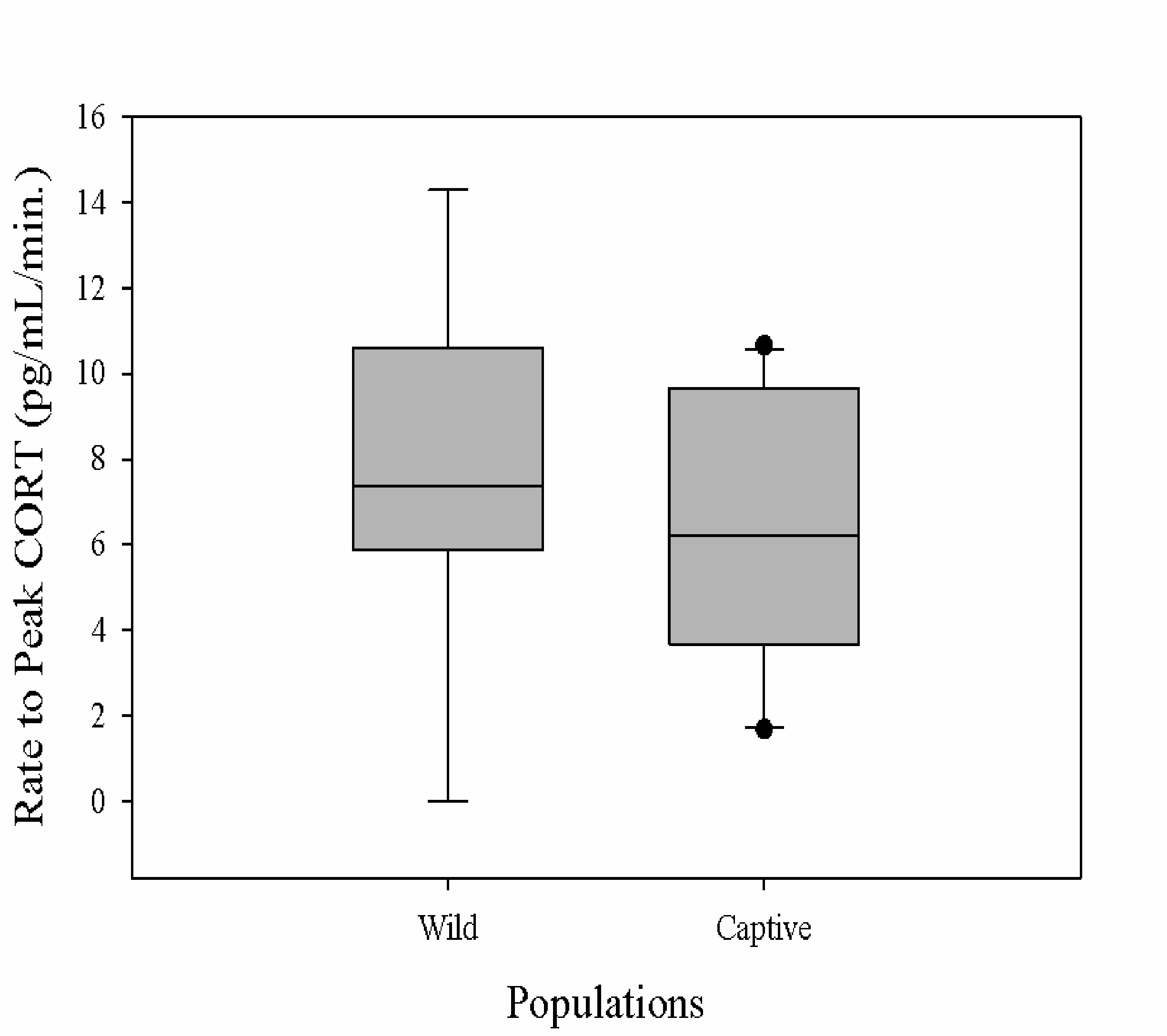

Figure 5.3. Average Rate to Peak CORT Concentration.
Note: Rate to peak CORT concentrations were compared between captivity populations. There is no difference in the rate it took males to reach peak CORT concentrations between captive and wild males.

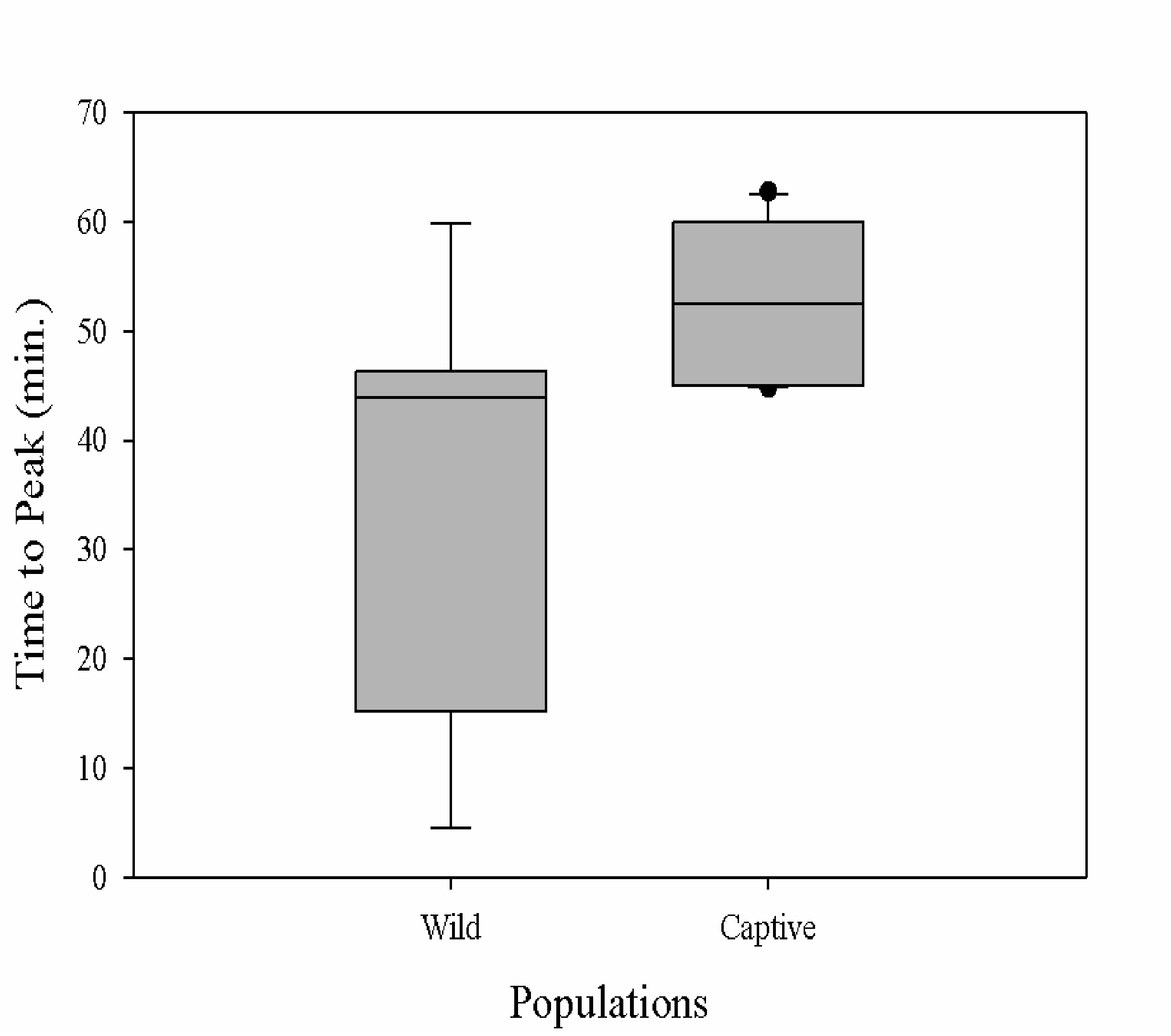

Figure 5.4. Average Time to Peak CORT Concentration.
Note: The time to peak CORT concentrations were compared between captivity populations. There is a significant difference in the time males reached peak CORT concentrations, such that captive males take longer to reach peak CORT concentrations than wild males.

The total CORT concentration over the 60 minutes did not differ significantly between wild ($\bar{x}$ = 21309.8 pg/mL, SE = 1783.6) and captive ($\bar{x}$ = 19300.2 pg/mL, SE = 1692.1) males (t = 0.82, p= 0.43; Figure 5.5). Average CORT concentration over the 60 minutes also did not differ significantly between wild ($\bar{x}$ = 355.16 pg/mL, SE = 29.73) and captive ($\bar{x}$ = 321.67 pg/mL, SE = 28.20) males (t = 0.82, P = 0.43; Figure 5.6).

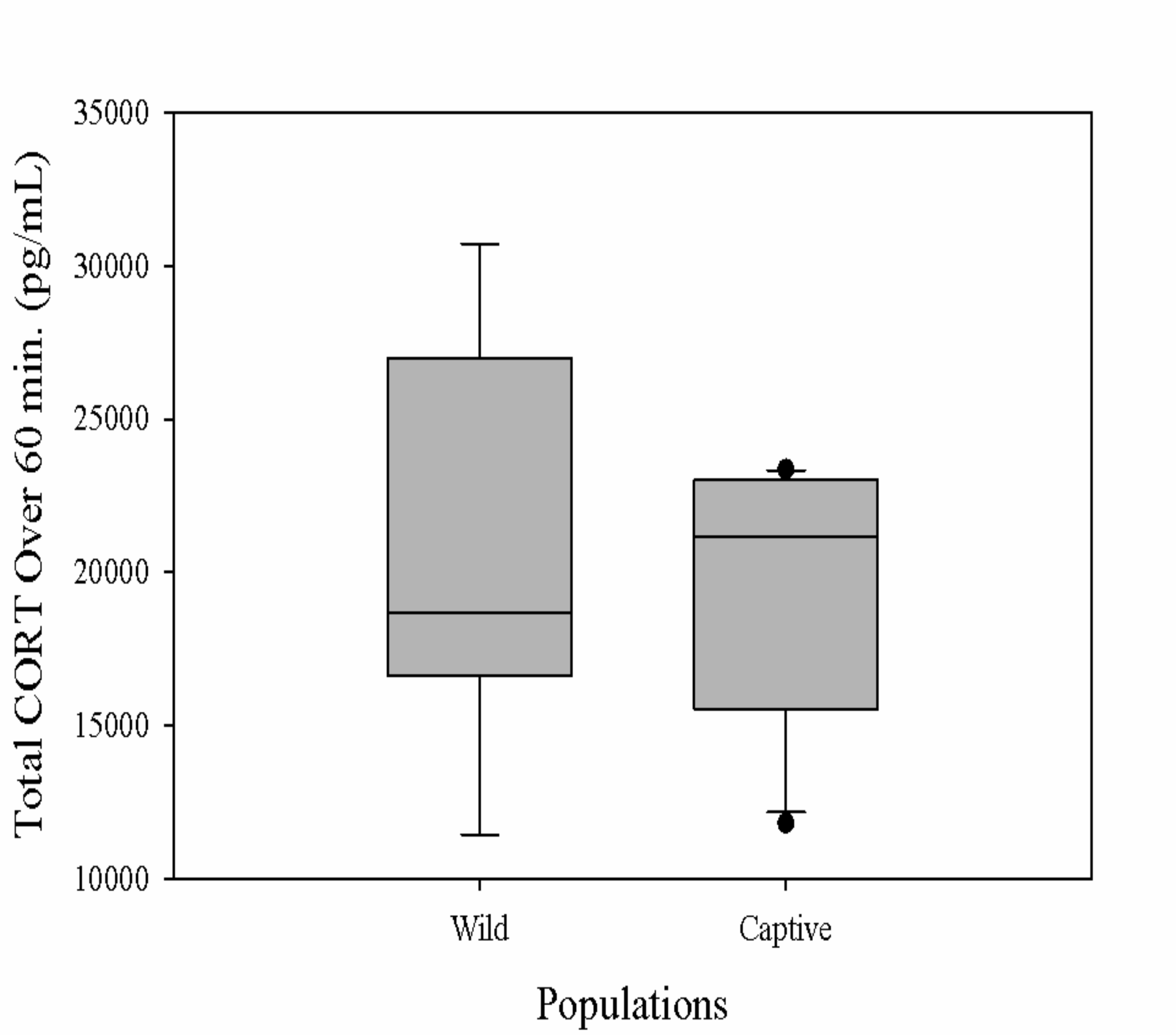

Figure 5.5. Total CORT over the 60 Minute Sampling Period.
Note: The total CORT concentrations of males over the 60 min. sampling period were compared between captive and wild males. There is not a significant difference in average or total CORT between populations.

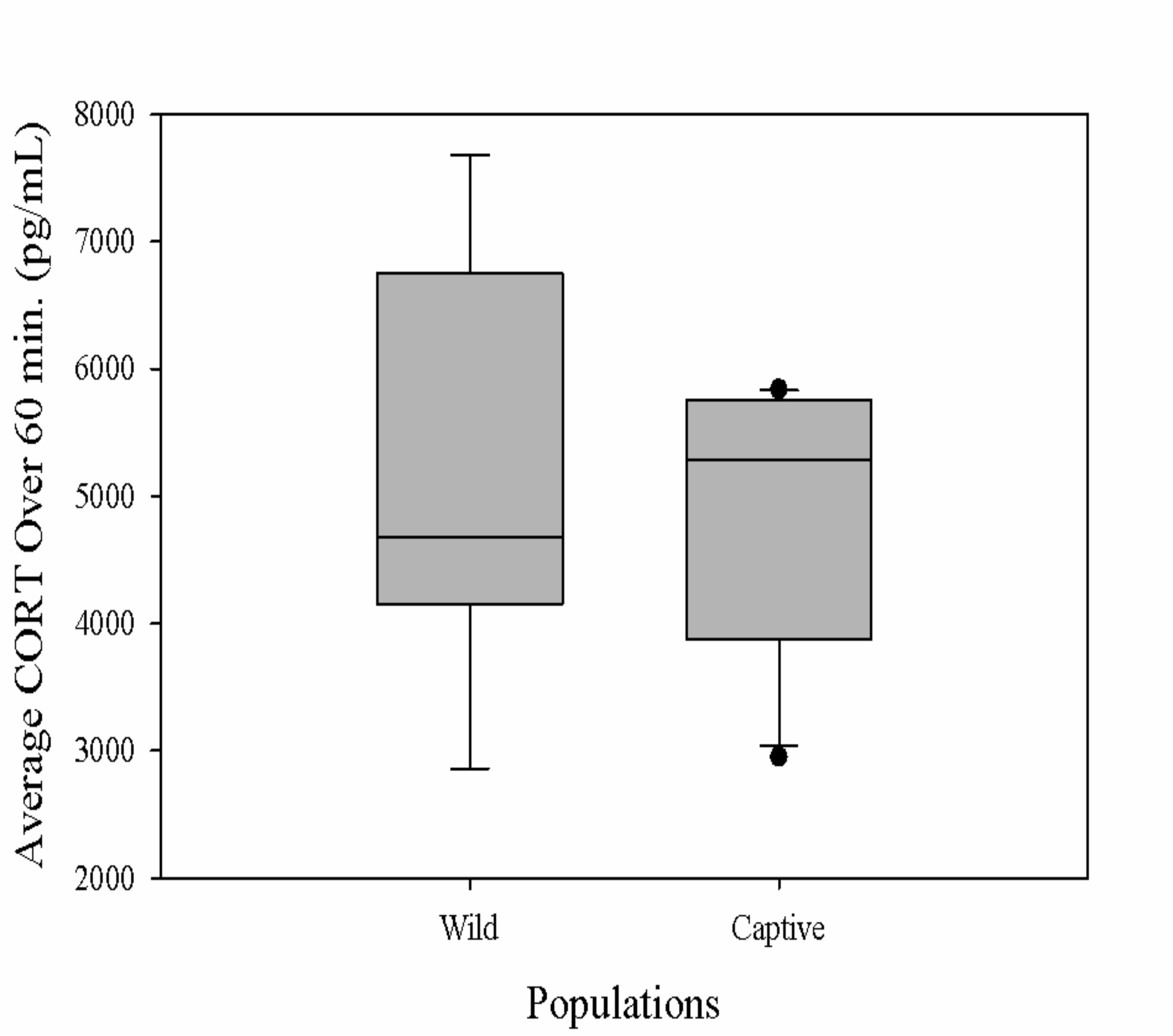

Figure 5.6. Average CORT over the 60 Minute Sampling Period.
Note: The average CORT concentrations of males over the 60 min. sampling period were compared between captive and wild males. There is not a significant difference in average or total CORT between populations.

When comparing male response to stress for both wild and captive males, we determined individuals have one of three unique 60 minute stress response profiles (Figure 5.7). The first profile is what is normally expected, where CORT levels increase in response to an acute stressor, peak after the stressor, and then concentrations recover to around basal levels (Rich and Romero, 2005). In the second profile male CORT levels increase in response to an acute stressor, peak and start to recover around 15 minutes, but peak and recover again. In the third profile male

CORT levels continue to increase during the 60 minutes without any sign of reaching a peak or recovering.

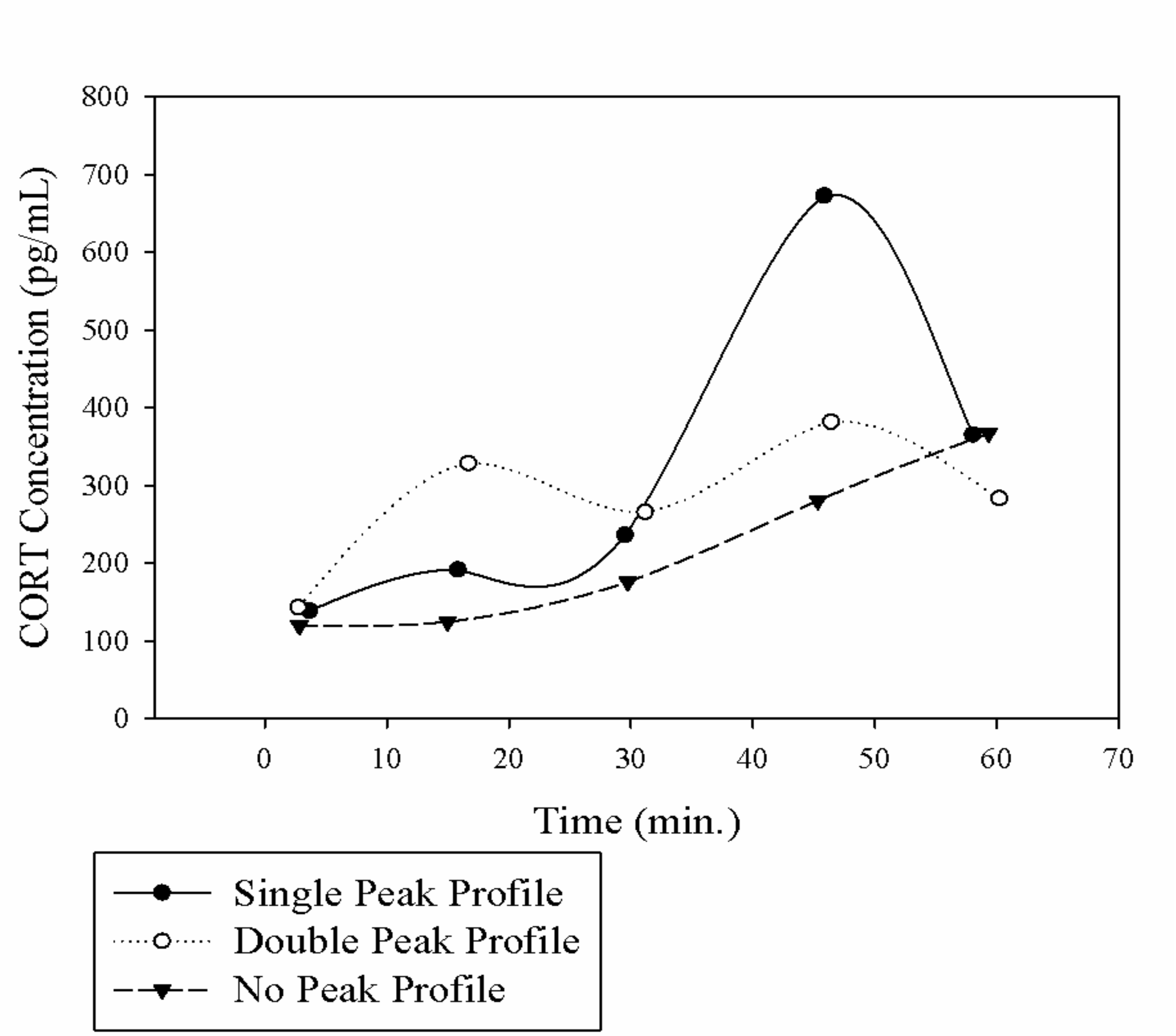

Figure 5.7. Examples of the Three Stress Response Profiles.
Note: A simple regression plotting time vs. CORT concentration across the 60 minute sample period. The three lines represent an example of what the three stress response profiles look like across time.

5.5. Discussion

In the current study we compared the physiological stress response of wild individuals in their natural and captive environments, and characterized the stress hormone profile for red-winged blackbirds. Our results indicate the chronic stress of being in captivity does affect how male red-winged blackbirds respond physiologically to acute stress in at least one aspect of their stress response profile. Our results also suggest males have one of three unique profiles for CORT response to an acute stressor over a 60 minute period.

There is not an observable difference in mean basal or mean peak CORT concentrations between our captive and wild-caught populations. This is in contrast to previous studies on chronically stressed birds, which have shown a negative relationship between chronic stress and both peak and basal CORT concentrations (Rich and Romero, 2005; Dickens *et al.*, 2009b). For example, after the chronic stress experienced from being captured, held in captivity, and then translocated to another site, researchers saw chukars (*Alectoris chukar*) had decreased basal CORT concentrations (Dickens *et al.*, 2009a). Likewise, in European starlings, researchers found basal and peak CORT levels were lower in psychologically stressed birds than in control birds (Rich and Romero, 2005). The overall down regulation of CORT concentrations seen in these previous studies suggest a controlled physiological change to the HPA axis, aimed at minimizing the effects of CORT to the individual over extended periods of time (Rich and Romero, 2005). In our study, stressed individuals may have been able to habituate to their environments, or may have adjusted other aspects of the HPA axis, such as response time (Dickens *et al.*, 2009b).

Total CORT concentrations does not differ between captive and wild males; although, the time to reach peak is significantly longer for captive individuals than for wild individuals. Increased time to reach peak CORT levels suggests captive males have been exposed to chronic stress. Previous studies on other species have shown under stress, the HPA axis can adjust to reduce CORT levels in the system (Holberton and Wingfield, 2003). However, the response seen in our study is opposite of what has been seen in some species. For example, in chronically stressed rats, response to an acute stressor causes a rapid increase in CORT, followed by a rapid suppression of CORT (Mizoguchi *et al.*, 2001). Researchers identified this response as a partial habituation to the chronic stressor (Mizoguchi *et al.*, 2001). Our results may also show a partial habituation, but in the opposite direction. Males that are constantly being bombarded with stressors may habituate by only reacting to a stressful situation if the acute stressor does not quickly dissipate. A similar, but less intense response was found in chukar, where long-term captive individuals showed a delay in peak CORT concentrations compared to newly caught individuals (Dickens *et al.*, 2009b). Thus, it is possible captive males compensate for the stressors of captivity by altering the function of the HPA axis so they are less likely to respond physiologically to stress, and when they do, they respond much slower than wild individuals.

Interestingly, we identified three very unique CORT profiles over a 60 minute period (Figure 5.7). Males with a double peak seem to have a rapid physiological response to stressors followed by a rapid down regulation of the HPA axis. In situations where the stressor does not diminish quickly, however, their HPA axis releases additional CORT into the system, causing a second peak. Males in the wild population varied in age, and some of the variation seen in profiles may be due to variation in age. For example, in one study on house sparrows (*Passer domesticus*), researchers found a negative correlation with age and stress response (Lendvai *et al.*, 2015).

Frequently, researchers bring wild animals into captivity to conduct studies in controlled environments (Dickens and Romero, 2009). Although, not all of our measures of the stress response indicate differences between captive and free-living males, our results do suggest males are coping with the stress of captivity by making limited alteration to their HPA axes, such that their physiological response to acute stressors is delayed. As the development of scare devices and other management techniques for the red-winged blackbird involve initial behavioral and physiological studies in laboratory settings, our findings suggest some physiological data collected on blackbirds in captivity may differ from data collected on wild individuals (Marra *et al.*, 1995; Romero and Wingfield, 1999; Künzl *et al.*, 2003; Dickens and Romero, 2009; Cabezas *et al.*, 2013). Captive studies may therefore confound research efforts if researchers are not conscious of the coping mechanisms of captive individuals, and do not take into account the possibility of a delayed stress response from captive males.

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6. CONCLUSIONS

6.1. Introduction

Physiology, in its broadest sense, has the potential to play a very important role in conservation and management plans. Where traditionally, conservation and management plans have focused their monitoring efforts at the community or population levels (Cooke *et al.*, 2013), physiology can provide the knowledge and tools to examine the underlying mechanisms driving population responses to environmental and anthropogenic perturbations (Coristine *et al.*, 2014). In addition to providing insight into individual and population responses, physiological tools and knowledge can also be directly applied to a conservation or management plan to help monitor or directly manipulate a population (Cooke, 2014). Thus, the two main purposes of this disquisition were to: (1) examine if and how physiology is being used in conservation planning, and provide suggestions on how to strengthen the interface between physiology and conservation and management planning; and (2) provide an example of the types of physiological research that can be useful for conservation and management planning, using the red-winged blackbird (*Agelaius phoeniceus*) as our study species.

6.2. Examination of the use of Physiology

Upon examination of the 146 endangered species recovery plans written between 2005 and 2016, we determined although physiology is being used within the plans, it is primarily being used in the form of discussing the natural history of the species, rather than being applied to help monitor or directly manipulate individuals within a population. We hypothesize the absence of physiological tools and knowledge in conservation planning is primarily due to a deficit of physiological knowledge passing between physiologists and the cohort of federal agency recovery plan writers. We provided three main recommendations to further guide conservation scientists, managers, and physiologists to work synergistically to solve conservation problems: (1) the breadth of knowledge within a recovery plan writing team should be increased via increased training of federal agency employees and the inclusion of authors with academic affiliations; (2) physiologists should make their research more available to conservation scientists and federal agencies by clearly linking their research to conservation; and, (3) communication should be enhanced between government conservation scientists and physiologists.

Prior studies examining the efficacy of endangered species recovery plans support our suggestions (Clark *et al*., 2002). For example, in one study examining the use of biology in recovery plans, the authors noted by including at least one author with an academic research affiliation, the use of direct biological links increased when developing recovery criteria and monitoring strategies (Gerber and Schultz, 2001). In another study, researchers discussed the underuse of modern conservation biology tools (Clark *et al*., 2002). They suggested this underuse was partially because many of the modern tools have been developed by biologists outside of the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS), and the federal employees charged with writing the endangered species recovery plans had not received current training to know how to use the tools (Clark *et al*., 2002). Finally, a prior bibliometric analysis of the interface between conservation and physiology suggested only approximately 2% of the physiological research conducted between 2006 and 2012 had been integrated into conservation research projects (Lennox and Cooke, 2014).

To help improve the transfer of knowledge between physiologists, conservation biologists, and managers, we believe physiologists need to take a more active role in developing tools, methodologies, and knowledge that are directed towards conservation and management purposes. To help physiologists identify potential important areas of research, we also analyzed what types of physiological knowledge and tools have been used most often in recovery plans. Of the 12 sub-disciples of physiology that had previously been identified (Wikelski and Cooke, 2006; Cooke *et al*., 2013; Madlinger and Love, 2015), the most commonly used disciplines included: immunology and sensory physiology, reproductive physiology, comparative physiology and biochemistry, environmental toxicology, and environmental and ecological physiology.

6.3. Stress Physiology in Female Red-winged Blackbirds

To illustrate how physiology can be useful for conservation and management plans, we provided an example of the types of research that may produce important information for conservation biologists and managers using the red-winged blackbird as our research species. The red-winged blackbird is an excellent candidate for physiological research aimed at population management for several reasons. The blackbird is often considered a pest species, due to the immense amount of crop damage they cause each year (United States Department of Agriculture *et al*., 2015). There has been some success with previous management approaches aimed at reducing the blackbirds' impact on crops. However, many of these approaches include population suppression, chemical repellents, and destruction of natural habitat (Linz *et al*., 2011). With today's consumers worried about the environment, animal welfare, and how their produce is grown, managers and farmers may need to shift their focus to management techniques that are nonlethal and environmentally and organic production friendly (Oh *et al*., 2015; Herrnstadt *et al*., 2016).

One potential nonlethal and environmentally friendly management technique that has not been thoroughly explored is to exploit a prey species (red-winged blackbird) natural fear of predators. The use of scare devices is not a new method for protecting crops, and it has had some success (Linz *et al*., 2011). There is potential, however, to improve the use of scare devices by having a thorough understanding of the physiological mechanisms and responses blackbirds have to the threat of predation. As the threat of predation can be stressful for individuals, one potentially important physiological piece of the puzzle is stress physiology. When an individual is exposed to a stressful situation the hypothalamus-pituitary-adrenal (HPA) axis acts to modulate the stress response (Rich and Romero, 2005). As part of this axis, the adrenal gland secretes the steroid hormone, corticosterone (CORT), which helps an individual escape the stressor by converting glucose stores into usable energy (Cyr *et al*., 2007). Continuously elevated levels of CORT can be detrimental for individuals, causing decreased immune efficiency, neurological deficits, and reduced reproductive success (Cyr *et al*., 2007). Thus, one potential way to exploit the blackbirds' physiological stress response to the threat of predation is by causing individuals to maintain elevated levels of CORT via various predation stressors.

Although most of the damage blackbirds cause to crops happens in the fall when their diet shifts from insects to seeds and grains, we chose to focus our research to during the breeding season (Linz *et al.*, 2017). Each year, the surviving offspring from the breeding season provide the new recruitment for the large flocks seen in the fall that cause the majority of the crop damage. By manipulating female reproductive success by increasing their physiological stress response to predation or nest parasitism during the breeding season, we can potentially reduce recruitment numbers for the fall.

By exposing females to predator and nest parasitism effigies (threats), thus causing additional stress, during the breeding season, we were able to examine how females respond behaviorally and physiologically to stress, and how these responses alter their reproductive decisions. In general, we found females are responding both behaviorally and physiologically to the increased threat of predation and nest parasitism, but depending on the threat, their responses differed.

Under the threat of predation from a great-horned owl (*Bubo virginianus*), females responded with anti-predatory behaviors (increased alarm calling and attacking of the predator), and with a physiological response (female plasma CORT levels or yolk hormone levels). Females also made alterations to their reproductive decisions/behaviors by decreasing the amount of time they incubated their eggs. However, they did not make changes in their nest location, structure, lay date, nest success, or offspring feeding rate. The predator used in our experiment is considered a predation risk for adults (Murphy, 1997) more than a risk for eggs and nestlings, thus it may be useful for future studies to examine how females respond to nest predators as well. Another criticism is the possibility that the individuals in our experiment habituated to our predator effigy (Mizoguchi *et al*., 2001) even though they continued to attack the effigy the entire time it was presented to them. To increase the stress level for the blackbirds, future researchers may want to vary the types of predators, or use multiple predators at once.

The threat of nest parasitism by a brown-headed cowbird (*Molothrus ater*) caused females to respond with changes to their reproductive decisions and their stress physiology. However, our results do not indicate a change in lay date, nest success, incubation time, feeding rate, or nest volume. Our results do suggest if female red-winged blackbirds are exposed to the threat of nest parasitism early enough during the breeding season, they will move their nests from the center of the coulee and high up in the vegetation to the more dense vegetation on the edges of the coulee and lower on the water (i.e. areas well hidden from an aerial view, but more exposed to potential mammalian predators and flooding). As females with high CORT levels also selected nesting locations in more dense vegetation and low on the water, one possible hypothesis is the stress of potential nest parasitism is enough to cause increased CORT levels, which then cause females to move their nests in response to the physiological changes. However, as our female plasma data suggests the threat of nest parasitism, actually suppresses CORT concentrations, this may not be the case. Rather, females of similar physiological condition may be selecting specific areas to nest in, and when exposed to the threat of nest parasitism, regardless of physiological condition, females are relocating their nests to areas of high vegetation density and low on the water. As we had limited sample size, were not able to directly examine the relationship between yolk CORT concentrations and the threat of nest parasitism, and were not able to connect specific females with specific nests, we suggest additional studies that address these issues to help further tease apart the two competing hypotheses. Other important findings from our research indicate nests built in the center of the coulee are larger, and females in poor physiological condition are possibly building smaller nests.

Although not all of our results have clear implications for future blackbird management, we believe understanding the physiological responses of these birds to stress can provide useful information for managers. One potential management technique that should be examined further is how to exploit nest placement decisions to reduce population size. Our results suggest females with elevated CORT levels are placing nests low in the reeds and closer to the edge of the coulee. Such placement may leave nests more vulnerable to mammalian nest predation and flooding (Sawin *et al*., 2003; Albrecht *et al*., 2006). If managers can find consistent techniques for manipulating stress levels in females (i.e. different predator stressors, potentially a brown-headed cowbird effigy, etc.) more females may move their nests to these vulnerable locations, potentially causing nesting failure, and reduced fall recruitment. The females in dense and low vegetation also had elevated CORT levels in their egg yolks. As elevated CORT levels in egg yolk have been connected to slower growth in offspring (Hayward and Wingfield, 2004), increasing nests located in the dense and low reeds may also reduce fledging and recruitment success even without total nest failure. However, to determine true survival rates of offspring, a study should be conducted that follows nests from egg through fledging and fall recruitment.

Another potential management technique is to exploit how under chronic stress females may suppress the reactivity of the HPA axis. This may cause females to be less reactive to acute stressors, such as a real predator attack (Dickens and Romero, 2009). By stressing females during the breeding season, causing more natural takes from predators, we can potentially help reduce the size of fall migrating flocks. We could potentially reduce the number of adult females and the number of just fledged offspring in the fall if females become prey before they are able to successfully fledge their offspring. In addition, as our results suggest reactivity decreases with season, it may be more important to stress females later in the breeding season or across the entire breeding season rather than just at the start of the season.

6.4. Physiological Stress Response in Captivity

As many red-winged blackbird studies have an initial stage involving the study of wild-caught blackbirds in captive settings, we believe it is important to understand how an individual’s stress response in captivity may differ from an individual’s response in their natural setting. Previous studies have shown captivity has its own unique suite of stressors that can cause chronic stress in for an individual (Morgan and Trombrog, 2007). In addition, as individuals in captivity are not physically capable to removing themselves from a stressful situation (Cockrem and Silverin, 2002) they may have to cope with the stressor at the physiological level by altering the function of their HPA axis (Koolhaas *et al*., 1999; Cockrem and Silverin, 2002).

By comparing the physiological response, to an acute stressor, of wild males in captivity and wild males in their natural environment we were able to examine the affects of captivity on the HPA axis of the red-winged blackbird. We were also able to develop a stress response profile of male red-winged blackbirds that can be used as a standard for future physiological studies of the species. In summary, our results suggest the chronic stress of captivity does minimally alter the function of the HPA axis of male red-winged blackbirds, and there are three different male stress response profiles.

We discovered three different response profiles in male red-winged blackbirds. The first profile is what may be normally expected, where CORT levels increase in response to an acute stressor, peak after the stressor, and then concentrations recover to around basal levels (Rich and Romero, 2005). In the second profile male CORT levels increase in response to an acute stressor, peak and start to recover around 15 minutes, but peak and recover again. The third profile has no peak or recovery, such that male CORT levels continue to increase during the entire 60 minute test.

It took captive males longer to reach peak CORT levels than wild males, suggesting captivity causes a change in the reactivity of the HPA axis. Captive males have a delayed response to stress, which may be their way of coping with the chronic stress of captivity. If captive males are constantly being exposed to acute stressors, they may modify their stress response to only responding if the acute stressor does not quickly dissipate (Dickens *et al*., 2009). This modification may help captive males limit their exposure to the detrimental effects of elevated CORT levels (Cyr *et al*., 2007).

The results of our study suggest researches need to be conscious of how captivity alters the physiology of this species. If natural studies are not possible, then captive studies should be conducted using multiple physiological measurements to provide a fuller picture of the physiological responses of the captive individuals. Also, caution should be taken when trying to extrapolate captive data to natural conditions. These results may also have implications beyond studying the physiology of this species. An individual's stress response can also alter their behavioral response. For example, in the white-crowned sparrow (*Zonotrichia leucophrys*), increases in CORT concentrations are correlated with increased perch hopping behavior (Breuner *et al*., 1998). Thus, behavioral data collected on captive males of this species should also be interpreted conditionally.

6.5. Conclusions

The physiology of a species is a very important piece to the puzzle when trying to understand how and why an individual or population responds to environmental and anthropogenic changes. Physiological knowledge, tools, and methodologies have important implications for conservation and management plans. However, our results from our review of the conservation recovery plans suggest physiology is not being used to its full potential. It is the combined responsibility of physiologists, conservation biologists, and managers to work together to broaden the use of physiology in conservation and management plans.

We conducted three research studies on the red-winged blackbird with the aim of providing physiological data that could be applied to help improve population management techniques for this species. An individual's physiological stress response is a complex suite of mechanisms that help an individual cope with a stressor (Wikelski and Cooke, 2006). Thus, it should be no surprise our results indicate an individual's behavioral, reproductive, and physiological responses to the stress caused by captivity or an increased threat of predation or nest parasitism, is highly complex. Although our results do not provide all of the answers, or suggest a single all-encompassing management technique, we believe our study does begin to provide the foundational physiological knowledge required to help manage the red-winged blackbird population.

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APPENDIX. SUPPLEMENTARY TABLE

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans.

Class	Species (Common Name)	Scientific Name	Year	Example of Terms Used	Use Category	Sub-Discipline	Literature Citation
Amphibians	Austin blind Salamander	Eurycea waterlooensis	2016	physiology pollutants temperature	NH	environmental toxicology (NH) immunology and epidemiology (NH)	USFWS 2005a, Amended with Addendum 2016a
	Austin blind Salamander	Eurycea waterlooensis	2005	physiology pollutants temperature	NH	environmental and ecological physiology (NH) environmental toxicology (NH) immunology and epidemiology (NH)	USFWS 2005a
	Barton Springs salamander	Eurycea sosorum	2005	physiology pollutants temperature	NH	environmental and ecological physiology (NH) environmental toxicology (NH) immunology and epidemiology (NH)	USFWS 2005a
	California tiger Salamander – Location: Central California Distinct Population Segment	Ambystoma californiense	2016	disease physiology	NH NRA	immunology and epidemiology (NRA) locomotor performance physiology (NH)	USFWS 2015b
	California tiger Salamander Location: Santa Barbara County, California	Ambystoma californiense	2015	hormone	NH RBA	Chemical communication (NH) environmental toxicology (NH) immunology and epidemiology (RBA)	USFWS 2015d

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Amphibians Continued	Chiricahua leopard frog	Rana chiricahuensis	2007	hormone disease physiology temperature	NH	chemical communication (NH) environmental and ecological physiology (NH) environmental toxicology (NH, RBA) immunology and epidemiology (NH, RBA) locomotor performance physiology (NH) reproductive physiology (NH)	USFWS 2007a
	Copperbelly water snake Location: Indiana north of 40 degrees north latitude, Michigan, Ohio	Nerodia erythrogaster neglecta	2008	metabolic	NH	cardiorespiratory physiology (NH)	USFWS 2008f
	Dusky gopher frog	Rana sevosa	2015	disease physiology	NH	immunology and epidemiology (NH) locomotor performance physiology (NH)	USFWS 2015e
	Giant garter snake	Thamnophis gigas	2015	chemical cues disease thermal regulation	NH	chemical communication (NH) immunology and epidemiology (NH)	USFWS 2015n
	Wyoming Toad	Bufo hemiophrys baxteri	2015	disease hormone nutrients toxicity	NH RBA NRA	bioenergetics and nutritional physiology (RBA) chemical communication (NRA) environmental toxicology (NH) immunology and epidemiology (NH) reproductive physiology (NH, NRA)	USFWS 2015q

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Arachnids	Braken Bat Cave Meshweaver	Cicurina venii	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Cokendolpher Cave Harvestman	Texella cokendolpheri	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Government Canyon Bat Cave Meshweaver	Cicurina vespera	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Kauai cave wolf or pe'e pe'e maka 'ole spider	Adelocosa anops	2006	physiology temperature	NH	environmental and ecological physiology (NH)	USFWS 2006e
	Government Canyon Bat Cave Spider	Neoleptoneta microps	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Madla's Cave Meshweaver	Cicurina madla	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Robber Baron Cave Meshweaver	Cicurina baronia	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
Aves	Akiapolaau	Hemmingathus wilsoni	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Akikiki	Oreomystis bairdi	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Attwater's greater prairie-chicken	Tympanuchus cupido attwateri	2010	epidemiology physiology	NH NRA	comparative physiology and biochemistry (NH) immunology and epidemiology (NH, NRA)	USFWS 2010a
	California clapper rail	Rallus longirostris obsoletus	2014	physiology	NH	environmental and ecological physiology (NH)	USFWS 2013d

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Aves Continued	Crested honeycreeper	Palmeria dolei	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2005h
	Guam kingfisher	Todiramphus cinnamominus	2008	hormone pathology	NH RBA	bioenergetics and nutritional physiology (NH, RBA) chemical communication (RBA) comparative physiology (NH) epidemiology and immunology (NH, RBA) reproductive physiology (NH, RBA)	USFWS 2008d
	Hawaii akepa	Loxops coccineus	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Hawaiian common gallinule	Gallinula chloropus sandvicensis	2012	disease	NH RBA	immunology and epidemiology (NH, NRA)	USFWS 2011c
	Hawaiian coot	Fulica americana alai	2012	disease	NH NRA	immunology and epidemiology (NH, NRA)	USFWS 2011c
	Hawaii creeper	Oreomystis mana	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Hawaiian Crow	Corvus hawaiiensis	2009	disease hormone	NH RBA NRA	chemical communication (NRA) immunology and epidemiology (NH, RBA) reproductive physiology (RBA, NRA)	USFWS 2009d
	Hawaiian Duck	Anas wyvilliana	2012	disease	NH NRA	immunology and epidemiology (NH, NRA)	USFWS 2011c
	Ivory-billed woodpecker	Campephilus principalis	2010	N/a	N/a	N/a	USFWS 2010b

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Aves Continued	Kauai akialoa (honeycreeper)	Akialoa stejnegeri	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Kauai nukupuu	Hemignathus Hanapepe	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Kauai \`o\`o (honeyeater)	Moho braccatus	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Large Kauai Thrush	Myadestes myadestinus	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Laysan duck	Anas laysanensis	2009	disease toxins	NH RBA	environmental toxicology (NH) immunology and epidemiology (NH, RBA)	USFWS 2009e
	Mariana Crow	Corvus kubaryi	2006	hormone physiology	NH RBA	chemical communication (NH) environmental toxicology (NH) immunology and epidemiology (NH, RBA) reproductive physiology (NH)	USFWS 2005c
	Maui akepa	Loxops ochraceus	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Maui nukupuu	Hemignathus affinis	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Aves Continued	Maui parrotbill (honeycreeper)	Pseudonestor xanthophrys	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Mexican spotted owl	Strix occidentalis lucida	2012	disease physiology reproduction	NH NRA	bioenergetics and nutritional physiology (NH) environmental and ecological physiology (NH) immunology and epidemiology (NH, NRA) reproductive physiology (NH)	USFWS 2012a
	Molokai creeper	Paroreomyza flammea	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Molokai thrush	Myadestes lanaiensis rutha	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Northern spotted owl	Strix occidentalis caurina	2011	heat stress physiology	NH	environmental and ecological physiology (NH)	USFWS 2011e
	Oahu creeper	Paroreomyza maculate	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Oahu elepaio	Chasiempis ibidis	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	\`O\`u (honeycreeper)	Psittirostra psittacea	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Aves Continued	Palila (honeycreeper)	Loxioides bailleui	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Piping Plover Location: Except Great Lakes watershed	Charadrius melodus	2016	toxicity	NH	environmental toxicology (NH)	USFWS 2015m
	Po`ouli (honeycreeper)	Melamprosops phaeosoma	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Puerto Rican parrot	Amazona vittata	2009	N/a	N/a	N/a	USFWS 2009c
	Rota bridled White-eye	Zosterops rotensis	2007	disease	NH	immunology and epidemiology (NH, NRA)	USFWS 2007c
	Short-tailed albatross	Phoebastria (=Diomedea) albatrus	2009	contaminants physiology	NH RBA	environmental toxicology (NH, RBA) reproductive physiology (NH)	USFWS 2008c
	Small Kauai Thrush	Myadestes palmeri	2006	disease thermal	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2006f
	Spectacled eider Location: Wherever found	Somateria fischeri	2008	disease	RBA	immunology and epidemiology (RBA)	USFWS 2008a
	Steller's Eider Location: Arkansas breeding population	Polysticta stelleri	2008	physiology toxins	RBA	comparative physiology (RBA) environmental toxicology (RBA)	USFWS 2008b
	Thick-billed parrot	Rhynchopsitta pachyrhyncha	2013	disease temperature stress	NH RBA	environmental and ecological physiology (NH) immunology and epidemiology (NH, RBA)	USFWS 2013h

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Aves Continued	Western snowy plover Location: Pacific Coast population DPS-U.S.A. (CA, OR, WA), Mexico (within 50 miles of Pacific coast)	Charadrius alexandrinus nivosus	2007	disease toxins	NH	environmental toxicology (NH) immunology and epidemiology (NH, RBA)	USFWS 2007d
	Whooping crane	Grus americana	2007	physiology reproduction disease nutrition	NH RBA	bioenergetics and nutritional physiology (NH, RBA) environmental and ecological physiology (NH RBA) immunology and epidemiology (NH, RBA, NRA) reproductive physiology (NH, RBA)	Canadian Wildlife Service and USFWS 2007
	Yuma clapper rail	Rallus longirostris yumanensis	2010	disease toxicity	NH RBA	environmental toxicology (NH, RBA) immunology and epidemiology (NH)	USFWS 2009f
Bivalvia	Georgia pigtoe mussel	Pleyrobema hanleyianum	2014	biochemical genetic oxygen stress temperature stress	NH RBA	comparative physiology and biochemistry (RBA) environmental and ecological physiology (NH, RBA) physiological genomics (RBA)	USFWS 2014c
	Scaleshell mussel	Leptodea leptodon	2010	physiology toxicology	RBA	comparative physiology and biochemistry (RBA) environmental and ecology physiology (RBA) environmental toxicology (RBA)	USFWS 2010f

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Branchiopoda	Conservancy fairy shrimp	Branchinecta conservation	2006	hormone temperature toxic	NH	chemical communication (NH) environmental and ecology physiology (NH) environmental toxicology (NH)	USFWS 2005h
	Longhorn fairy shrimp	Branchinecta longiantenna	2006	hormone temperature toxic	NH	chemical communication (NH) environmental and ecology physiology (NH) environmental toxicology (NH)	USFWS 2005h
	Vernal pool fairy shrimp	Branchinecta lynchi	2006	hormone temperature toxic	NH	chemical communication (NH) environmental and ecology physiology (NH) environmental toxicology (NH)	USFWS 2005h
	Vernal pool tadpole shrimp	Lepidurus packardi	2006	hormone temperature toxic	NH	chemical communication (NH) environmental and ecology physiology (NH) environmental toxicology (NH)	USFWS 2005h
Gastropoda	Chittenango ovate amber snail	Succinea chittenangoensis	2006	parasite temperature	NH RBA	environmental and ecological physiology (NH) immunology and epidemiology (NH, RBA)	USFWS 2006a
	Cylindrical lioplax	Lioplax cyclostomaformis	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	Flat pebblesnail	Lepyrium showalteri	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	Interrupted Rocksnail – Georgia	Leptoxis foremani	2014	biochemical genetic oxygen stress temperature stress	NH RBA	comparative physiology and biochemistry (RBA) environmental and ecological physiology (NH, RBA) physiological genomics (RBA)	USFWS 2014c

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Gastropoda Continued	Lacy elimia	Elimia crenatella	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	Newcomb's snail	Erinna newcombi	2006	disease	RBA	immunology and epidemiology (RBA)	USFWS 2006d
	Painted rocksnail	Leptoxis taeniata	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	Plicate rocksnail	Leptoxis plicata	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	Rough hornsnail	Pleurocera foremani	2014	biochemical genetic oxygen stress temperature stress	NH RBA	comparative physiology and biochemistry (RBA) environmental and ecological physiology (NH, RBA) physiological genomics (RBA)	USFWS 2014c
	Round rocksnail	Leptoxis ampla	2005	oxygen	NH	environmental and ecological physiology (NH)	USFWS 2005e
	White Abalone Location: North America (West Coast from Point Conception, CA, U.S.A., to Punta Abreojos, Baja California, Mexico)	Haliotis sorenseni	2009	disease temperature	NH RBA NRA	environmental and ecological physiology (NH, RBA) immunology and epidemiology (NH, NRA)	NMFS 2008c
Insecta	Behren's silverspot butterfly	Speyeria zerene behrensii	2016	N/a	N/a	N/a	USFWS 2015i
	Blackburn's sphinx moth	Manduca blackburni	2005	physiology temperature	NH	comparative physiology and biochemistry (NH) environmental and ecology physiology (NH)	USFWS 2005f
	Carson wandering skipper	Pseudocopaeodes eunus obscurus	2007	temperature	NH	environmental and ecological physiology (NH)	USFWS 2006c
	Casey's June Beetle	Dinacoma caseyi	2013	N/a	N/a	N/a	USFWS 2013f

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Insecta Continued	Delta green ground beetle	Elaphrus viridis	2006	hormone temperature toxic	NH	chemical communication (NH) environmental and ecology physiology (NH) environmental toxicology (NH)	USFWS 2005h
	Fender's blue butterfly	Icaricia icarioides fender	2010	pesticides temperature	NH	environmental toxicology (NH)	USFWS 2010c
	Helotes mold beetle	Batrisodes venyivi	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Hungerford's crawling water Beetle	Brychius hungerfordi	2006	oxygen pH temperature,	NH RBA	environmental and ecological physiology (NH, RBA)	USFWS 2006b
	Laguna Mountains skipper	Pyrgus ruralis lagunae	2016	N/a	N/a	N/a	USFWS 2015a
	[no common name] Beetle	Rhadine exilis	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	[no common name] Beetle	Rhadine infernalis	2011	physiology	NH	comparative physiology and biochemistry (NH)	USFWS 2011a
	Salt Creek Tiger beetle	Cicindela nevadica lincolniana	2015	pesticide thermal	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015c
Malacostraca	Kauai cave amphipod	Spelaeorchestia koloana	2006	physiology temperature	NH	environmental and ecological physiology (NH)	USFWS 2006e
Mammalian	Black-footed ferret	Mustela nigripes	2013	disease poison	NH RBA NRA	environmental toxicology (NH, RBA) immunology and epidemiology (NH, RBA, NRA)	USFWS 2013d
	Canada Lynx Location: Contiguous U.S. Distinct Population Segment	Lynx canadensis	2005	N/a	N/a	N/a	USFWS 2005d

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Mammalian Continued	Columbia Basin Pygmy Rabbit Location: Columbia Basin Distinct Population Segment	Brachylagus idahoensis	2013	physiology	NH	comparative physiology (NH)	USFWS 2012b
	Florida panther	Puma (=Felis) concolor coryi	2008	physiology	NH RBA NRA	comparative physiology and biochemistry (RBA) immunology and epidemiology (RBA, NRA) reproductive physiology (NH, RBA, NRA)	USFWS 2008e
	Grizzly bear Location: Northern Continental Divide Ecosystem	Ursus arctos horribilis	2013	physiology	NH NRA	bioenergetics and nutritional physiology (NH, NRA)	USFWS 2013a
	Grizzly bear Location: Yellowstone	Ursus arctos horribilis	2016	N/a	N/a	N/a	USFWS 1993, Amended 2007g and 2016b
	Gulf Coast jaguarundi	Herpailurus (=Felis) yagouaroundi cacomitli	2013	disease	NH RBA	immunology and epidemiology (NH, RBA)	USFWS 2013b
	Hawaiian monk seal	Monachus schauinslandi	2007	disease nutrition physiology reproduction, thermoregulation toxins	NH RBA	bioenergetics and nutritional physiology (NH) environmental and ecological physiology (NH) environmental toxicology (RBA) immunology and epidemiology (NH) reproductive physiology (NH)	NMFS 2007

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Mammalian Continued	Indiana bat	Myotis sodalist	2007	physiology temperature thermoregulation toxins	NH RBA	bioenergetics and nutritional physiology (NH, RBA) environmental and ecological physiology (NH, RBA) environmental toxicology (NH, RBA)	USFWS 2007b
	Killer whale Location: Southern Resident Distinct Population Segment	Orcinus orca	2008	physiology toxins	NH NRA	bioenergetics and nutritional physiology (NRA) chemical communication (RBA) comparative physiology and biochemistry (NRA) environmental and ecological physiology (NH) environmental toxicology (NH, RBA) reproductive physiology (NH)	NMFS 2008a
	Mount Graham red squirrel	Tamiasciurus hudsonicus grahamensis	2011	disease physiology	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2011b
	Mariana fruit Bat	Pteropus mariannus mariannus	2010	N/a	N/a	N/a	USFWS 2009b
	New Mexico meadow jumping mouse	Zapus hudsonius luteus	2014	N/a	N/a	N/a	USFWS 2014b
	North Atlantic Right Whale	Eubalaena glacialis	2010	contaminant physiology reproduction	RBA NRA	comparative physiology and biochemistry (RBA) environmental toxicology (NRA) reproductive physiology (RBA)	NMFS 2005

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Mammalian Continued	Northern Sea Otter Location: Southwest Alaska Distinct Population Segment	Enhydra lutris kenyoni	2013	disease hormone metabolic oxygen physiology temperature	NH RBA NRA	bioenergetics and nutritional physiology (NH) cardiorespiratory physiology (NH) chemical communication (NRA) immunology and epidemiology (RBA) reproductive physiology (NRA)	USFWS 2013g
	Ocelot	Leopardus pardalis	2016	hormone physiology	RBA NRA	chemical communication (NRA) comparative physiology and biochemistry (RBA) immunology and epidemiology (NRA) environmental toxicology (NRA) reproductive physiology(NRA)	USFWS 2016e
	Polar bear	Ursus maritimus	2015	disease physiology	NH RBA	environmental and ecological physiology (RBA) immunology and epidemiology (NH)	USFWS 2015g
	Preble's meadow jumping mouse	Zapus hudsonius preblei	2016	physiology	NH RBA	environmental and ecological physiology (RBA) nutritional physiology (NH)	USFWS 2016c
	Salt marsh harvest mouse	Reithrodontomys raviventris	2014	physiology	NH	environmental and ecological physiology (NH)	USFWS 2013e
	San Miguel Island Fox	Urocyon littoralis littoralis	2015	physiology toxic	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015j

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Mammalian Continued	Santa Catalina Island Fox	Urocyon littoralis catalinae	2015	disease physiology reproduction	NH RBA NRA	immunology and epidemiology (RBA, NRA) reproductive physiology (NH, RBA)	USFWS 2015j
	Santa Cruz Island Fox	Urocyon littoralis santacruzae	2015	physiology toxic	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015j
	Santa Rosa Island Fox	Urocyon littoralis santarosae	2015	physiology toxic	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015j
	Sei whale	Balaenoptera borealis	2012	epidemiology physiology	NH RBA	environmental and ecological physiology (NH) immunology and epidemiology (NH) neurophysiology and sensory biology (RBA)	NMFS 2011
	Sierra Nevada bighorn sheep Location: Sierra Nevada	Ovis canadensis sierra	2008	Disease physiology	NH	comparative physiology and biochemistry (NH) immunology and epidemiology (NH)	USFWS 2007e
	Sonoran pronghorn	Antilocapra americana sonoriensis	2016	physiological stress physiology thermal stress	NH	N/a	USFWS 2016f
	Sperm whale	Physeter catodon (=macrocephalus)	2010	physiology disease	NH RBA	environmental and ecological physiology (NH, RBA) immunology and epidemiology (NH)	NMFS 2010
	St. Andrew beach mouse	Peromyscus polionotus peninsularis	2010	N/a	N/a	N/a	USFWS 2010d

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Mammalian Continued	Steller sea lion Location: Western Distinct Population Segment	Eumetopias jubatus	2008	Disease nutrition toxins	NH RBA NRA	bioenergetics and nutritional physiology (NH) comparative physiology and biochemistry (RBA, NRA) environmental toxicology (NH) immunology and epidemiology (NH, RBA, NRA)	NMFS 2008b
	Utah prairie dog	Cynomys parvidens	2012	physiology	NH RBA	environmental and ecological physiology (NH) environmental toxicology (NH) immunology and epidemiology (NH, RBA)	USFWS 2012d
Osteichthyes	Alabama sturgeon	Scaphirhynchus suttkusi	2013	Oxygen	NH	cardiorespiratory physiology (NH)	USFWS 2013c
	Apache trout	Oncorhynchus apache	2009	Disease temperature	NH NRA	environmental and ecological physiology (NH) immunology and epidemiology (NH and NRA)	USFWS 2009a
	Atlantic salmon Location: Gulf of Maine Distinct Population Segment	Salmo salar	2016	oxidative stress	NH	cardiorespiratory (NH)	USFWS and NOAA Fisheries 2016
	Bull Trout Location: Coastal Recovery Unit	Salvelinus confluentus	2015	Thermal toxins	NH NRA	environmental and ecological physiology (NH) environmental toxicology (NRA)	USFWS and Oregon Department of Fish and Wildlife 2015a
	Bull Trout Location: Columbia Headwaters Recover Unit.	Salvelinus confluentus	2015	physiology thermal	NH	environmental and ecological physiology (NH)	USFWS and Montana Ecological Service 2015
	Bull Trout Location: Kalamath Recovery Unit.	Salvelinus confluentus	2015	physiological stress thermal stress	NH	environmental and ecological physiology (NH)	USFWS 2015f

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Osteichthyes Continued	Bull Trout Location; Mid-Columbia Recovery Unit	Salvelinus confluentus	2015	thermal stress	NH	environmental and ecological physiology (NH)	USFWS and Oregon Department of Fish and Wildlife 2015b
	Bull Trout Location: Saint Mary Recovery Unit	Salvelinus confluentus	2015	N/a	N/a	N/a	USFWS 2015o
	Bull Trout Location: Upper Snake Recovery Unit	Salvelinus confluentus	2015	thermal	NH	environmental and ecological physiology (NH)	USFWS 2015p
	Bull Trout Location: U.S.A., conterminous, lower 48 states	Salvelinus confluentus	2015	reproduction	NH	environmental toxicology (NH) reproductive physiology (NH)	USFWS 2015k
	Devils River minnow	Dionda diaboli	2005	physiology	NH RBA	environmental and ecological physiology (RBA) immunology and epidemiology (RBA) reproductive physiology (NH, RBA)	USFWS 2005b
	Laurel dace	Chrosomus saylori	2015	metabolic rate oxygen physiology temperature	NH RBA	bioenergetics and nutritional physiology (NH) comparative physiology and biochemistry (RBA)	USFWS 2016d
	Lost River sucker	Deltistes luxatus	2013	Oxygen temperature toxins	NH RBA	environmental and ecological physiology (NH) environmental toxicology (RBA)	USFWS 2012c
	Pallid sturgeon	Scaphirhynchus albus	2014	Oxygen physiology reproductive physiology	NH RBA	comparative physiology and biochemistry (NH, RBA) environmental and ecological physiology (RBA) reproductive physiology (RBA)	USFWS 2014d

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Osteichthyes Continued	Rio Grande Silvery Minnow	Hybognathus amarus	2010	physiology disease reproduction toxins	NH RBA	comparative physiology and biochemistry (RBA) environmental and ecological physiology (NH) environmental toxicology (NH, RBA) immunology and epidemiology (NH) reproductive physiology (NH)	USFWS 2010e
	Santa Ana sucker Location: 3 California river basins	Catostomus santaanae	2014	oxygen stress physiology temperature stress toxins	NH	cardiorespiratory physiology (NH) environmental and ecological physiology (NH, RBA) environmental toxicology (NH, RBA)	USFWS 2014a
	Sharpnose Shiner	Notropis oxyrhynchus	2015	physiology toxic	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015h
	Shortnose Sucker	Chasmistes brevirostris	2013	Oxygen temperature toxins	NH RBA	environmental and ecological physiology (NH) environmental toxicology (RBA)	USFWS 2012c
	Smalleye Shiner	Notropis buccula	2015	physiology toxic	NH	environmental and ecological physiology (NH) environmental toxicology (NH)	USFWS 2015h
	Smalltooth sawfish Location: United States Distinct Population Segment	Pristis pectinata	2009	physiology	NH	environmental and ecological physiology (NH)	NMFS 2009
	Tidewater goby	Eucyclogobius newberryi	2005	physiology temperature	NH	environmental and ecological physiology (NH) immunology and epidemiology (NH)	USFWS 2005g

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Osteichthyes Continued	Vermilion darter	Etheostoma chermocki	2007	physiology reproduction	NH	environmental and ecological physiology (NH) reproductive physiology (NH)	USFWS 2007f
	Warm Springs dace	Rhinichthys osculus thermalis	2015	Disease physiology reproduction toxins	NH NRA	environmental toxicology (NH) immunology and epidemiology (LH) reproductive physiology (NRA)	USFWS 2015l
Reptilia	Desert tortoise Location: Wherever found, except AZ south and east of Colorado R., and Mexico	Gopherus agassizii	2011	physiology reproduction toxicity nutrition disease	NH RBA	bioenergetics and nutritional physiology (RBA) comparative physiology and biochemistry (NH) environmental and ecological physiology (NH) environmental toxicology (NH) immunology and epidemiology (NH, RBA) reproductive physiology (NH, RBA)	USFWS 2011d
	Kemp's ridley sea turtle	Lepidochelys kempii	2011	Disease physiology pollutants reproduction	NH RBA NRA	environmental and ecological physiology (NH) environmental toxicology (NH, RBA) immunology and epidemiology (NH) comparative physiology and biochemistry (RBA) reproductive physiology (NH, NRA)	NMFS et al., 2011

Table A1. USFWS and NMFS Endangered Species Act Recovery Plans (continued).

Reptilia Continued	Loggerhead sea turtle Location: Northwest Atlantic Ocean Distinct Population Segment	Caretta caretta	2009	metabolic physiology reproduction toxicology	NH RBA	bioenergetic and nutritional physiology (NH) comparative physiology and biochemistry (RBA) environmental toxicology (NH) reproductive physiology (NH, RBA)	NMFS and USFWS 2008

Note: A table with all ESA recovery plans finished between 2005 and 2016. Reports are organized by the class of the species. Each species includes examples of how physiological terms were used, what categories were used (Natural History – NH, Research Based Action – RBA, and Non-research Action – NRA), and the sub-disciplines used within the report.

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