REPRODUCTIVE FAILURE AND THE STRESS RESPONSE IN AMERICAN KESTRELS NESTING ALONG A HUMAN DISTURBANCE GRADIENT

By

Erin Hennegan Strasser

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Erin Hennegan Strasser

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Julie A. Heath, Ph.D.	Chair, Supervisory Committee
Alfred M. Dufty Jr., Ph.D.	Member, Supervisory Committee
Stephen J. Novak, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Julie A. Heath, Ph.D., Chair of the Supervisory Committee. The thesis was approved for the Graduate College by John R. Pelton, Ph.D., Dean of the Graduate College.

This is dedicated to my dad.

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ABSTRACT

Human disturbance may be an influential environmental stressor that affects birds across life stages. I examined whether external and endogenous factors including habitat type, habitat quality and individual quality (hereafter quality), or human disturbance affect American Kestrel reproductive success in southwestern Idaho. Specifically, I was interested in how these factors lead to nest failure or abandonment. I also investigated whether elevated corticosterone (CORT) concentrations mediate the relationships among explanatory variables and nest failure. As nestling kestrels may respond to stressors differently from adults, I examined whether conditions experienced during the nestling stage affected nestling American Kestrel (*Falco sparverius*) hypothalamic pituitary adrenal (HPA) axis activity. I predicted that nestlings in high disturbance areas and those exposed to experimental stress would be sensitized to acute handling stress (i.e. would exhibit high baseline and stress induced CORT levels).To examine these relationships I monitored 89 nest boxes posted along roads with varying amounts of traffic and surrounding development in Southwest Idaho during the 2008 and 2009 breeding seasons. I captured adult birds during the incubation stages and then followed nest fates by checking the boxes at expected hatch dates, and when nestlings were 10 and 25 days old. Nestling kestrels, raised in high and low human disturbance areas were exposed to a chronic stress protocol (CSP). At 25 days of age nestlings were sampled for baseline and stress induced plasma CORT. Twenty six (36%) of 73 nests failed and most (n = 23, 88%) nests failed during incubation. Human disturbance, but not habitat type or quality measures, was negatively associated with kestrel reproductive success. A disturbance score based on traffic patterns and the distance of the nest from the road best predicted kestrel reproductive success (disturbance: $\beta = -1.08$, 95% CI: -1.87 - -0.28; distance from road: $\beta = 2.44$, 95% CI: 0.54 - 5.42). Adult female kestrels nesting near interstates and busy roads had elevated CORT ($\chi^2 = 6.07$, $P = 0.01$; $\beta = 0.10$, 95% CI: 0.02 - 0.19) and nests of females with elevated CORT were less likely to be successful ($\chi^2 = 4.37$, $P = 0.04$; $\beta = -1.09$, 95% CI: -2.20 - 0). There was no relationship between adult male CORT and disturbance or success. Nestling CORT did not vary with human disturbance levels ($F_{1,\,27} = 1.97$, $P = 0.17$), CSP treatment ($F_{1,\,27} = 0.00$, $P = 0.97$), or with an interaction between human disturbance and CSP ($F_{1,\,27} = 0.00$, $P = 0.97$) suggesting that nestling kestrels do not sense or perceive external conditions related to human disturbance as stressful. Because kestrels are cavity nesters, nestlings may be buffered from external conditions and instead may respond more powerfully to stressors within the nest such as brood size. Brood size at fledging best explained baseline ($\beta = 0.10$, 95% CI: -0.03 - 0.28) and stress induced CORT ($\beta = 0.06$, 95% CI: -0.06 - 0.19). This study provides evidence that adult but not nestling kestrels respond to human activity with a physiological stress response and this can lead to changes in individual behavior resulting in reproductive failure via abandonment. At a larger scale, this study highlights the adverse affects of human disturbance on seemingly “urban-adapted” organisms such as American Kestrels.

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GENERAL INTRODUCTION

Background

Across life stages birds respond to environmental and endogenous challenges. Human disturbance may be an influential environmental stressor that affects reproduction and survival. Birds in areas of high human activity may perceive humans as predators (Beale and Monaghan 2004) or be exposed to high levels of human-associated predators. Responses to these perturbations could increase energy expenditure towards defensive and avoidance behaviors as well as interrupt incubation or brooding. High levels of anthropogenic noise may impair communication between adults and young (e.g. begging vocalizations), potentially reducing parental care and consequently young survival. Human-dominated areas also introduce an array of contaminants, such as pesticides and diesel exhaust fumes, which may alter adult and nestling behavior and physiology. Over an extended period of time, responses to humans may lead to changes in a species abundance or distribution. Such is the case for many songbirds where breeding densities decrease in areas of higher traffic density (Reijnen and Foppen 1994).

How human activities affect wildlife is an increasingly relevant element of physiological ecology and conservation biology (Carey 2005; Walker et al. 2005; Busch and Hayward 2009). The impacts of human activities related to urban development, agriculture, tourism, and roadway use range from: reduced reproductive success (Safina and Burger 1983; Kuitunen et al. 2003; Bouton et al. 2005), interference in avian acoustic signals (Slabbekoorn and Ripmeester 2008) changes in foraging behavior (Burger and Gochfeld, 1991), variation in the timing of reproduction (Partecke et al. 2006), vehicle collisions (Erickson et al. 2005; Litvaitis and Tash 2008) and altered nest site selection (Valkama and Korpimaki 1999; Remacha and Delgado 2009).

Corticosterone (CORT) is a steroid hormone released by the adrenal cortex in response to stressors such as human disturbances. CORT secretion is mediated through the hypothalamic pituitary adrenal (HPA) axis and acts to promote physiological and behavioral changes to enhance survival (Harvey 1984). Under certain conditions, CORT increases may become chronic, shifting animals into an “emergency life-history stage” that alters typical behavior and physiology, notably reproduction (Wingfield et al. 1998). Elevated baseline CORT levels may have adverse consequences on reproductive success by reducing parental investment perhaps to the point of nest abandonment (Wingfield and Sapolsky 2003). In developing young elevated CORT can alter growth (Müller et al. 2009) and have long-term consequences such as reduced cognition (Kitaysky et al. 2003) or direct impacts on fitness such as reduced survival (Blas et al. 2007). CORT may be used as a tool to measure breeding birds’ response to human disturbance. For example, Yellow-eyed Penguins (*Megadyptes antipodes)* exposed to tourists have higher stress induced CORT levels and reduced chick survival compared to penguins exposed to less tourist traffic (Ellenberg et al. 2007). Young birds also respond to disturbance with changes in CORT (Müllner et al. 2004) or may habituate to human activity, both of which may be detrimental to survival.

American Kestrels (*Falco sparverius*) are a widespread falcon that breeds extensively throughout North and South America (Smallwood and Bird 2002). Kestrels often breed in high densities along roadways, in urban and agricultural areas, and readily utilize human-made nest boxes (Bloom and Hawks 1983; Varland and Loughin 1993; Steenhof and Peterson 2009). They favor territories with shorter vegetation and sparse woody cover for hunting insects, small mammals, birds, and reptiles. Nest box projects have been successful in increasing American Kestrel breeding densities (Hamerstrom et al. 1973) however recent evidence suggests that American Kestrels are in decline across North America (Farmer and Smith 2009). Kestrels are common breeders in southwestern Idaho, and have been monitored within my study area from 1986-2006 (Steenhof and Peterson 2009). In this population, American Kestrels display variation in reproductive success with kestrels nesting near high human disturbance (along an interstate, near human developments) exhibiting lower rates of success compared to those nesting in less disturbed areas (Steenhof and Peterson 2009).

I was interested how habitat type, quality of the habitat and quality of individuals, and human disturbance affect reproduction and whether hormones, specifically CORT, mediated relationships among these three factors and reproductive success in kestrels. Because many factors affect kestrel reproductive success, I evaluated multiple research hypotheses to elucidate how disturbance, habitat type, and the quality of a habitat and individual influence reproductive success and CORT (Figure 1; Chapter 1). Nestling kestrels also pose an interesting system for investigating effects of human disturbance on stress biology. Nestlings may perceive or respond to human disturbance differently from adults, thus, I also explored how environmental and endogenous factors as well as experimental chronic stress shape the nestling stress response.

Research Questions and Objectives

The first objective of Chapter 1 was to examine the role of habitat type, quality of habitat and individuals, and human disturbance on adult reproductive success (defined as fledging at least one young). The mechanism by which habitat, quality, and human disturbance affect kestrel reproductive success is likely to be endocrine mediated, specifically via the actions of CORT. I hypothesized that kestrels breeding in areas of high human disturbance would have elevated baseline CORT and lower reproductive success compared to low disturbance areas. Thus my second objective was to determine if breeding kestrel baseline CORT differed along a human disturbance gradient.

Chapter 2 focused on the effects of human disturbance and conditions within the nest on nestling kestrel HPA-axis activity. In nestling kestrels, HPA-axis activity may be related to human disturbance (Müllner et al. 2004) as well as conditions within the nest such as brood size (Lendvai et al. 2009). The objective was to identify conditions experienced during the nestling stage that affect fledgling kestrel HPA-axis activity, as measured by baseline and stress induced CORT patterns. Fledging-age chicks reared in high and low human disturbance areas were exposed to a chronic stress protocol and sampled for baseline and stress induced CORT. I also examined effects of brood size alone, and in combination with, human disturbance on the nestling stress response.

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CHAPTER ONE: THE EFFECTS OF HABITAT, QUALITY, AND HUMAN DISTURBANCE ON AMERICAN KESTREL CORTICOSTERONE AND REPRODUCTIVE SUCCESS

Abstract

Avian reproductive success depends on favorable nesting environment and physiological conditions. Both external and endogenous factors can contribute to egg loss, chick mortality, or poor parental care that results in nest failure. I examined whether habitat type, habitat quality and individual quality (hereafter quality), or human disturbance affect American Kestrel reproductive success in southwestern Idaho. Specifically, I was interested in how these factors lead to nest failure or abandonment. I also investigated whether elevated corticosterone (CORT) concentrations mediate the relationships among explanatory variables and nest failure. To examine these relationships I monitored 89 nest boxes posted along roads with varying amounts of traffic and surrounding development in Southwest Idaho during the 2008 and 2009 breeding seasons. I captured adult birds during the incubation stages and then followed nest fates by checking the boxes at expected hatch dates, and when nestlings were 10 and 25 days old. Twenty six (36%) of 73 nests failed and most (n = 23, 88%) nests failed during incubation. Human disturbance, but not habitat type or quality measures, was negatively associated with kestrel reproductive success. A disturbance score based on traffic patterns and the distance of the nest from the road best predicted kestrel reproductive success (disturbance: $\beta = -1.08$, 95% CI: -1.87 - -0.28; distance from road: β

= 2.44, 95% CI: 0.54 - 5.42). Adult female kestrels nesting near interstates and busy roads had elevated CORT (χ^2 = 6.07, P = 0.01; β = 0.10, 95% CI: 0.02 - 0.19) and nests of females with elevated CORT were less likely to be successful (χ^2 = 4.37, P = 0.04; β = -1.09, 95% CI: -2.20 - 0). There was no relationship between adult male CORT and disturbance or success. In sum, human disturbance negatively affected kestrel reproductive success and results suggest that the relationship between high human disturbance and poor reproductive success in kestrels was mediated by female CORT levels. As females are the primary incubators, they may be more exposed to human disturbance (e.g., traffic noise) that elicits a stress response. High CORT likely leads to abandonment resulting in high rates of failure in high disturbance areas. This study provides evidence that birds respond to human activity with a physiological stress response and this can lead to changes in individual behavior resulting in reproductive failure. At a larger scale, this study highlights the adverse affects of human disturbance on seemingly "urban-adapted" organisms such as American Kestrels.

Introduction

Avian reproductive success (the probability that a breeding attempt will produce at least one young, Thompson et al. 2001) varies in response to environmental and endogenous factors, acting alone or in concert, including habitat type, resource availability, individual quality, and human disturbance. These factors may present a suite of challenges to breeding birds, potentially altering adult behavior and investment in current reproduction. Subsequently, individuals may reduce incubation efforts, nestling care, or even abandon a breeding attempt. How birds cope with these challenges and modulate reproductive investment may depend on physiological mechanisms. For this reason, to begin to understand how and why avian reproduction varies one must identify factors which lead to variation in reproductive success as well as the physiological mechanisms driving these relationships.

Habitats vary in their physical attributes, community structure, and the amount of resources available to breeding birds. Habitat type, here used to refer to the physical and community structure (i.e., vegetative cover), can influence avian reproduction (Breininger and Carter 2003; Bostrom and Ritchison 2006; Murison et al. 2007). For example, songbirds in central Ontario had lower reproductive success in smaller forest fragments, likely due to higher rates of nest parasitism by Brown-Headed Cowbirds (*Molothrus ater*) in these areas (Burke and Nol 2000). Additionally, Northern Spotted Owls (*Strix occidentalis caurina*) produce more offspring per breeding territory in older forests compared with less mature forests (Dugger et al. 2005).

Habitats vary in relative quality as some areas may have higher prey abundance or prey items are more easily accessed in certain habitats. Food availability, a possible component of habitat quality is often cited as an important determinant of reproductive success in raptors (Newton 1989). However quantifying habitat quality based upon a single indicator such as food abundance may not be appropriate in many situations (Johnson 2007). This could be because the ability to acquire food resources, or defend a high quality territory may depend on an individual's capability or quality. Thus the fitness effects of individual or habitat quality can confound one another (Brown et al. 2002; Bety et al. 2004; Sergio et al. 2009). For example lower quality individuals (e.g., younger or inexperienced) are often relegated to poor quality territories and have lower reproductive success (Part 2001; Carrete et al. 2006; Ost and Steele 2009). Further, seasonal declines in avian reproductive success may be associated with seasonal changes in food abundance, territory quality, or parental quality (Arnold et al. 2004; Dawson 2008). Because of this, it can be difficult to empirically distinguish between the effects of habitat quality and individual quality. Thus a number of researchers have used indicators of bird physical condition to represent habitat quality (see Johnson 2007) or have examined both habitat and individual quality in studies of avian fitness (Sergio et al. 2009).

Recently, much attention has been paid to the relationship between birds and human disturbance. Human perturbations related to urban development, agriculture and roadway present a suite of challenges which can negatively impact birds. Exposure to human activity may lead to behavioral and physiological changes such as decreased nest attentiveness (Baudains and Lloyd 2007), poor body condition (Liker et al. 2008), nest abandonment (Safina and Burger 1983), and habitat avoidance (Thiel et al. 2008; Speziale et al. 2008). Birds may be negatively affected by human disturbance because they perceive humans as potential predators (Beale and Monaghan 2004), human activity increases exposure to contaminants (Eens et al. 1999) or human associated predators (Haskell et al. 2001), or anthropogenic noise may impair communication (Habib et al. 2007; Slabbekoorn and Ripmeester 2008). Consequently, where levels of human disturbance are high, reproductive success may be reduced.

Endocrine systems, specifically the hypothalamic-pituitary-adrenal (HPA) axis may play a role in orchestrating the relationships among environmental and endogenous factors and a bird’s reproductive success. Activation of the HPA-axis in response to environmental and endogenous challenges results in the release of corticosterone (CORT) (Harvey et al. 1984). Elevated CORT facilitates behavioral and physiological changes (e.g., escape behavior, increased foraging, gluconeogenesis) aimed at coping with stressful conditions while suppressing nonessential activities such as reproduction (Wingfield et al. 1998; Sapolsky et al. 2000). Chronic stress can alter baseline CORT (McEwen and Wingfield 2003), causing reduced parental effort (Miller et al. 2009) or nest abandonment (Silverin 1998). Thus, reproductive success may decline with exposure to stressors (e.g., high levels of human or vehicle traffic, poor body condition, low food availability) that cause an increase in baseline CORT.

American Kestrels (*Falco sparverius*) are a widespread, cavity-nesting species that breed in a variety of areas including urbanized and agricultural habitats. Kestrels readily utilize human-made nest boxes that are often erected along roads and interstates where they are easy to monitor and where suitable short grass (roadside) habitats exist (Bloom and Hawks 1983; Varland and Loughin 1993; Steenhof and Peterson 2009). Despite the popularity of nest box programs and their ubiquitous distribution, American Kestrel populations have been declining in the past several decades (Farmer and Smith 2009). Steenhof and Peterson (2009) observed that adult American Kestrels utilizing nest boxes in Southwest Idaho show variation in reproductive success depending upon nest box locations. Kestrels nesting near areas of presumably high human activity (along interstates and busy secondary roads, near human developments) had lower reproductive success compared to those nesting near areas low of human activity. Unfortunately the cause of reproductive failure was unclear.

My objective was to better understand the causes of American Kestrel reproductive success; specifically, I was interested in factors associated with nest failure. I examined environmental and endogenous factors associated with nest failure and whether individual CORT concentrations mediated relationships among environmental and endogenous conditions and reproductive success. I hypothesized that variation in American Kestrel reproductive success was related to: 1) habitat type, as defined by vegetation cover within the breeding territory, 2) variation in quality reflecting access to food resources, energetic condition, and parental provisioning (hereafter quality) or 3) exposure to human disturbance reflecting potential predation risks as well as acoustic or visual perturbations that may result in coping strategies. These hypotheses are not mutually exclusive and I evaluated whether there were relationships among habitat type, quality, and disturbance level. I also examined whether habitat type, quality, or disturbance was related to individual baseline CORT concentrations and if CORT was predictive of reproductive success (Figure 1). I also explored the effects of vehicular mortality on adult kestrels, an alternative explanation for high rates of reproductive failure along the interstate and busy roads.

Materials and Methods

I studied American Kestrels breeding in nest boxes located in southwestern Idaho within Ada, Canyon, Elmore and Payette Counties (43º N 116 º W) (Figure 2). The study area encompassed an approximately 500 km^2 area, 100 km in length. Wooden nest boxes were mounted 2.5-10 m high on wooden posts along secondary roads, on trees in rural residential areas, and on signs along Interstate 84. Twenty-five interstate boxes were mounted in February 2008 to replace boxes from a previous study (Steenhof and Peterson 2009) that were removed in 2006. Additionally, I mounted several new boxes in historic locations along secondary roads and near homes. Nest box locations in all areas (along interstates, on posts, and on trees in rural areas) were established as early as 1986 (Steenhof and Peterson 2009). Nearest neighbor distances between nest boxes ranged from 178-2574 m. In 2008, I monitored 89 nest boxes and in 2009 I monitored 87 boxes.

American Kestrel Reproduction

Each year prior to the breeding season boxes were cleaned, repaired or replaced if necessary and lined with pine shavings. Beginning in early March, I visited boxes once a week to determine occupancy, clutch initiation (hereafter lay date), and clutch size. If a nest was discovered with more than one egg I backdated to estimate lay date following the assumption that kestrels lay one egg every other day. To adjust for annual variation in breeding, I calculated an adjusted lay date for each nest by dividing the Julian lay date by the median Julian lay date for that year (Steenhof and Heath 2009). Once clutches were complete I revisited as often as necessary (every 1- 2 days) to capture adults during the incubation stage. I then returned at the estimated hatch date (approximately 30 days post clutch initiation). Average time (± SD) between adult capture date and the date of expected hatch was 11.4 ± 8.9 days. Nests were checked again when the oldest nestling was approximately 10 and 25 days of age to determine hatch success and fledging success, respectively. If a pair produced at least one 25 day old (approximately 85% of fledging age) nestling, that pair was considered successful. Causes of failure were determined by the presence or absence of eggs, nestlings, or adult birds as well as the presence of European Starlings (*Sturnus vulgaris*) and their nests. Starlings readily occupy nest boxes after kestrel abandonment, but do not appear to remove kestrel eggs. A nest was considered abandoned if a nest contained intact, cold eggs with or without a starling nest. Boxes with kestrel eggs and no sign of starlings were checked repeatedly throughout the day to assess whether adults were present. A nest was considered depredated if a nest contained no signs of nestlings or eggs. A nest at a low disturbance site was abandoned after construction took place nearby and was not included in analyses of outcome. Another nest failed after the box was destroyed during a storm and was not included in analyses.

I sampled adult male and female kestrels at the beginning of the breeding season, 1 - 4 weeks after clutch completion and prior to eggs hatching. Adults were captured by plugging the nest box entrance with a padded PVC pipe. To reduce the effects of handling stress on baseline CORT, I collected blood samples within five minutes of opening the nest box (Romero and Reed 2005). Blood samples were collected between 0900 and 1400 hours to minimize time-of-day effects on circulating plasma CORT (Wingfield et al. 1992). Approximately, 0.1 - 0.4 ml of blood was collected from the jugular vein with a 26½ gauge needle and syringe. Samples were stored in heparinized vials, placed on ice, and returned to the lab within five hours of sampling. In the lab, blood was centrifuged at 10,000 rpm for 15 minutes. Plasma was drawn off and stored at -80ºC until analyses. After bleeding, I banded individuals with federal aluminum bands, measured mass, and scored fat. Mass was determined to the nearest gram using a digital scale. Birds were assigned a fat score (0-3) based upon the size and shape of fat deposits in the right subalar region. A score of 0 indicated no fat present, 1 represented a trace of fat, 2 indicated a wide streak, and 3 was assigned to a pad that visibly protruded above the level of the muscle (DeLong and Gessaman 2001). As a possible indicator of male kestrel quality, in 2009 I measured subterminal band width to the nearest 1mm on male kestrels at the central retrix using a ruler (Wiehn 1997). All procedures were conducted with approval from Boise State University's Institutional Animal Care and Use Committee (protocol #006-08-007).

Habitat Type

To determine habitat types I used a geographical information system (GIS ArcGIS 9.2 software) to create a 900 m radius buffer approximating a kestrel breeding territory around Universal Transverse Mercator (UTM) points (NAD 1983 zone 11) for each nest box. American Kestrel breeding territory size most likely varies with region, population density and resource availability (Bird and Palmer 1988) but kestrels in southwest Idaho typically occupy nest boxes that are approximately 900 ± 43 meters apart (Steenhof pers. comm.). I examined Northwest Gap Analysis Program (NWGAP) satellite imagery of southwest Idaho (http://www.gap.uidaho.edu/landcoverviewer.html) to quantify habitat based on Landsat imagery. Imagery data was compiled by NWGAP in 2001. From available land cover classes I created 22 general land cover polygons. I then quantified the proportion (in m^2) of each of the 22 land cover polygons within nest territories and determined which made up the greatest overall proportion of total nest box habitat (Table 1). Land cover classes such as open water and intermountain greasewood flat were removed from the analysis as they made up small proportions of overall area. The most prevalent land covers were consolidated into 4 general habitat classes:

agriculture, introduced vegetation, sagebrush/shrub steppe, and human development. The agriculture class consisted of the land cover types cultivated cropland and pasture or hay fields. The habitat class introduced vegetation included introduced upland forbland and grassland. Sagebrush/shrub steppe consisted of big sagebrush shrubland, semi-desert shrub steppe, and big sagebrush steppe. The human development class consisted of developed open space, developed low, medium and high intensity (reflects presence or absence of structures and roads) and strip/gravel mines. I then determined the proportion (% acreage) of each general habitat class for each nest territory.

Quality

Because features that govern habitat quality in kestrels may reflect variation in individual quality I addressed predictions involving habitat and individual quality simultaneously. A common method to quantify habitat quality is to measure bird breeding performance (Johnson 2007). This method is utilized when one cannot directly link habitat quality to a habitat feature or food resources. Because the goal of this research was to explain patterns of reproductive success, this approach was inappropriate. Thus I evaluated several measures of resource availability and accessibility that may reflect both habitat quality within a breeding territory and the quality of breeding kestrels. For most pairs I had information on lay date, and indices of body condition including male and female mass and fat scores. For some pairs I had information on male subterminal band width and plasma carotenoids. All of these variables have been associated with resource availability or signals of bird quality. To verify that these relationships were consistent in my population, and that habitat and individual quality co-varied, I examined Spearman correlation matrices among these variables.

Human Disturbance

To quantify human disturbance I measured several traffic, road and building variables at each occupied nest box. Variables included posted roadway speed (mph), number of lanes, daily average traffic volume, nest box distance from center of roadway (median if interstate box), nearest building, and the presence or absence of human-made structures. Data on daily average traffic volume was compiled from Ada and Canyon County Highway Districts (ACHD), and Idaho Department of Transportation (IDT) websites (http://achd.ada.id.us/; http://www.itd. idaho.gov/ highways/). This data was collected within the past 10 years. Road, traffic, and human development variables were entered into a principal components analysis (PCA) to create a disturbance index. The final principal component (PC1) included the variables average daily traffic volume, posted roadway speed, number of lanes and proportion of human development within a nest territory (NWGAP imagery, see habitat analysis)(Table 2). The habitat class human development was used in the analysis of disturbance instead of habitat type as it was highly correlated with traffic, road, and structure variables.

I removed duplicate nest boxes from the PCA if traffic rates did not vary across years (based upon ACHD and IDT data). PC1 accounted for more than 80% of the total variation among occupied nest boxes therefore each box was assigned individual PC1 scores for disturbance (Table 2). Higher PC1 scores indicated higher disturbance areas (i.e. the interstate, busy secondary roads, or highly developed areas) while lower scores indicated lower disturbance areas such as smaller secondary roads with few or no buildings.

Distance of box from road center and nearest building was determined using a laser rangefinder (Bushnell Yardage Pro) to the nearest 0.5 m. If a nest box was at the intersection of more than one road all values were averaged. Each nest was assigned to an ordinal category representing the nest's distance from the road. Categories were based on natural breaks in the distance-from-road distribution. Nest boxes that were less than or equal to 17.5 m from the road were assigned a 1, boxes > 17.5 m but < 34 m were assigned a 2, and if a nest box was greater than or equal to 34 m from the road it was assigned a 3.

Blood chemistry

Corticosterone

Total CORT concentrations were determined by enzyme-linked immunosorbent assay (ELISA, Caymen Chemicals). Samples were run in duplicate when possible. Briefly, CORT was twice extracted from 10-30 µl of plasma with 5 ml diethyl ether. The lipophilic supernatant was poured off and dried under a stream of nitrogen gas in a warm water bath. Extracted samples were reconstituted with 100 µl of EIA buffer, vortexed and 50 µl aliquots were added to 96-well plates coated with mouse monoclonal antibody. I added corticosterone-specific acetylcholinesterase tracer and rabbit corticosterone antiserum and placed plates on an orbital shaker for two hours. Plates were developed and read at 405 nm with a Biotek EL800 plate reader. The concentration of CORT was calculated by comparing results to a standard curve and adjusted for extraction efficiency and plasma sample size. Extraction efficiency was determined by analyzing a standard CORT sample. Inter-assay variation was calculated from repeated values of a pooled sample. All values were corrected for assay extraction efficiency (mean ± SD) 83 ± 7.6%. Inter-assay variation averaged 8.63% and average intra-assay variation was 2.02%.

Carotenoids

Plasma carotenoids were quantified using blood samples collected during adult sampling. Variable amounts of plasma (40 µl, 30 µl, 25 µl) were mixed with 360 µl, 370 µl, or 385 µl acetone for a total sample volume of 400 µl (dilutions 1:10, 1:13, 1:16). Samples were centrifuged at 1,500 g for 10 minutes. The supernatant was drawn off and examined with a spectrophotometer at 476 nm (Bortolotti et al. 1996). Total carotenoid concentration within a sample was estimated using standard curves for the carotenoid lutein (alpha-carotene-3-3-diol, Sigma Aldrich). Standard curves were run at each dilution (1:10, 1:13, 1:16).

Road Mortality

To examine whether nest failure along the interstate and busy roads was a result of adult mortality caused by vehicle collisions I conducted mortality surveys one day after a nest appeared abandoned. Boxes were discovered abandoned an average (± SD) of 11.43 ± 8.95 days after the previous box check. Surveys consisted of transects 900 m in length and approximately 15 m in width on either side of the nest box on both sides of the road. In addition the roadside shoulder was visually monitored during regular nest box checks. To estimate probability of carcass detection during regular visual monitoring (not walking transects), an assistant placed 12 marked Coturnix Quail (*Coturnix coturnix*) carcasses along the interstate. Quail were randomly placed with respect to direction (east or west) side of road (left or right), and distance from shoulder (1 - 2 m or 3 - 4 m). Approximately 2 hours after quail placement, I drove my normal survey route and collected detected carcasses. To account for scavenger bias I placed 5 quail and one female kestrel carcass along roadsides throughout the 2009 study season. During routine (every 3-7 days) nest box checks, I noted presence or absence of carcasses at a location.

Statistical Analyses

I used an information-theoretic approach to evaluate the roles of habitat, quality, and human disturbance in explaining kestrel reproductive success (1) or failure (0). I fit generalized linear models with a logit-link function (PROC gen mod, SAS 9.1, SAS Institute, 2002) to the binomial response variable of reproductive success or failure and used Akaike Information Criterion adjusted for small sample size (AIC_c) to compare models (Burnham and Anderson 2002). The model with the lowest AIC_c was considered the most parsimonious or best fit model. The difference between the model with the lowest AIC_c value and all other models (including the intercept only model) was represented by ΔAIC_c. Models < 2 ΔAIC_c were considered equally plausible in explaining reproductive success and I also assessed the relative support of candidate models using Akaike weights (w_i). I examined residuals to test model assumptions and log transformed variables if necessary. Data were pooled across years as year did not have an effect on CORT or reproductive success.

For each research hypothesis (i.e., habitat type, quality, and disturbance) I had several possible explanatory variables and I did not have *a priori* models to compare. To find the best predictive variables for each major hypothesis I compared all possible models within each hypothesis category. Before creating models, I examined Spearman correlation coefficients among variables and eliminated correlated variables ($r_s \geq 0.30$) from models. Once constructed, the best model for each hypothesis competed against one another to explain kestrel reproductive success. Beta estimates (ß) and 95% confidence intervals (CI) are presented. Additionally, I evaluated relationships among disturbance scores, habitat type, and quality indicators using Spearman's correlations.

I investigated whether capture date, time of day, or bled time influenced baseline CORT using linear regressions. To evaluate the effects of human disturbance on adult baseline CORT, I used a generalized linear model (GLM, Proc Genmod in SAS) with a normal distribution with baseline CORT as the response variable and disturbance PCA score and distance from road index? as the independent factors. Males and females were analyzed separately because the genders were exposed to different environmental and endogenous conditions as well as have different costs and benefits associated with reproductive effort. I examined the effects of CORT on kestrel reproductive success using a generalized linear model with a binomial distribution and logit-link function. Nests that failed as a result of adult road mortality as well as a single box which failed after nearby construction and another destroyed by weather were removed from analyses of outcome but were included in the analysis of disturbance on CORT when samples were available. All analyses were conducted in SAS 9.1 (SAS Institute, Cary NC).

Results

Reproductive Summary

During the 2008 breeding season there were 36 nest attempts, and in 2009 I documented 37 attempts (Table 3). No kestrels were observed initiating a second nesting attempt after successfully fledging young. In 2008, I sampled 33 adult females and 27 adult males. In 2009, 32 adult female and 27 adult male kestrels were sampled. Occasionally I was unable to trap either the male or female from a nest. In five cases I was unable to trap either adult from an interstate nest box due to early failure.

Earliest clutch initiations were 29 March, 2008 and 19 March, 2009 and latest were 3 June, 2008 and 11 July, 2009. Median clutch initiation dates were 16 April and 12 April for 2008 and 2009, respectively. Clutch size (not including nests with 1 egg, n = 3) differed between years (clutch: Wilcoxon Z = -2.09, P = 0.04). Mean clutch size (± SD) in 2008 was 4.8 ± 0.8 eggs, and was 5.2 ± 0.6 eggs in 2009. The number of eggs which successfully hatched did not differ between years and averaged 4.3 ± 1.2 hatchlings per successful brood. Productivity did not differ between years and averaged 3.9 ± 1.3 chicks per successful pair. Similarly, nest outcome (fail or success) did not differ between years with 26 of 73 (35.6%) nests failing. Of nests that failed, 23 (88.4%) did so during the incubation stage and three failed during the nestling stage (Table 3). Of the 21 failed nests included in analyses of outcome, 16 (76.2%) appeared to have been abandoned (nests had intact clutches and no predator sign) (Table 3). In 2009, one pair abandoned a nest midway through incubation and initiated a second unsuccessful breeding attempt at a different nest box. In addition to abandonment, two pairs failed to hatch eggs after adults were observed incubating for more than 35 days. Three nests had no sign of eggs or nestlings suggesting predation or infanticide.

Effects of Habitat Type on Reproductive Success

The intercept-only model best predicted the effects of habitat on reproductive success (Table 4). Thus support for the effect of habitat on reproductive success was not strong as no model explained reproductive success better than the null model (intercept only).

Effects of Quality on Reproductive Success

I did not include plasma carotenoids or male subterminal band width in models as sample sizes were limited. Male and female plasma carotenoids were correlated with one another and male carotenoids were correlated with indices of body condition (Table 5) which were included in quality models. Adjusted lay date and adult mass had strong relationships to other measures of individual and habitat quality (Table 5). Adjusted lay date was the best quality predictor of reproductive success (Table 6). Birds that initiated nests later had a lower probability of success ($\beta = -4.32$, 95% CI: -8.14 - -0.50). All other measures of quality had higher AIC_c values than the null model and were not considered.

Effects of Human Disturbance on Reproductive Success

The model including human disturbance score as well as distance from road best explained the effect of human disturbance on kestrel reproductive success (Table 7).

Overall Model of Predictor Variables on Outcome

Human disturbance best explained reproductive outcome in American Kestrels (Table 8). As disturbance indices increased the probability of success declined (β = -1.08, 95% CI: -1.87- -0.28, Figure 3). The probability of success increased as categorical nest distance from road increased (β = 2.44, 95% CI: 0.54 - 5.42, Figure 3). The probability of success declined more rapidly when nest box distance from road was less than or equal to 17.5 m from the center of road when compared to those greater than 17.5 m but less than 34 m from a road. The effect of nest box distance from road on success was less pronounced when nests were greater than or equal to 34 m from a road.

There was no relationship between disturbance PC score and percentage of agriculture (r_s = -0.17, P = 0.15) or sagebrush/shrub steppe (r_s = -0.12, P = 0.30) within a territory. There was a significant inverse relationship between disturbance score and proportion of introduced vegetation within a nest territory (r_s = -0.35, P = 0.003, Figure 4). Adjusted lay date (r_s = 0.03, P = 0.82, Figure 5), adult male (r_s = 0.13, P = 0.32) and female mass (r_s = -0.09, P = 0.47) showed no relationship with disturbance. Additionally, lay date did not vary with habitat type (agriculture: r_s = -0.20, P = 0.08; sagebrush/shrub steppe: r_s = 0.05, P = 0.65; introduced vegetation: r_s = 0.06, P = 0.60).

Road Mortality

Of the 16 nests that failed in 2009, I conducted road mortality surveys for 10 territories. I did not conduct mortality survey for one pair because both adults were subsequently recaptured during a second nesting attempt. One nest failed after a storm destroyed the nest box and another two were abandoned after extended incubation (both adults observed at box with un-hatched eggs). At one site a breeding female was found dead (presumably from an auto collision). I did not conduct a mortality survey for this box. Prior to performing a mortality survey, I located an unmarked female kestrel near a recently abandoned nest. In 2008, I discovered a banded female carcass in the road near her nest with nestlings. Thus, I documented 3 cases of nest failure resulting from adult vehicle mortality (Table 3). During mortality surveys I also noted several other road-killed birds and small mammals including American Robins (*Turdus migratorius)*, European Starlings (*Sturnus vulgaris)*, Rock Pigeon (*Columbia livia)*, Mourning Doves (*Zenaida macroura)*, Barn Owls (*Tyto alba)*, and Great Horned Owls (*Bubo virginianus).* The amount of time from placement to scavenger removal ranged from 14 - 49 days (mean = 21). I discovered 9 of 12 (75%) observer bias carcasses.

Corticosterone Parameters

I analyzed samples from 54 males and 64 females for baseline CORT. I found no relationship between baseline CORT and bleed time (males: $r^2 = 0.02$, $P = 0.27$, females: $r^2 = 0.05$, $P = 0.08$), Julian bleed date (males: $r^2 = 0.02$, $P = 0.26$, females: $r^2 = 0.02$, $P = 0.22$), or time of day (males: $r^2 = 0.007$, $P = 0.55$, females: $r^2 = 0.001$, $P = 0.79$). Male and female CORT were not related ($r^2 = 0.00$, $P = 0.97$).

Disturbance index affected female ($\chi^2 = 6.07$, $P = 0.01$; $\beta = 0.10$, 95% CI 0.02 - 0.19, Figure 6) but not male ($\chi^2 = 0.03$, $P = 0.86$; $\beta = -0.01$, 95% CI: -0.08 - 0.07, Figure 7) baseline CORT. Additionally, of nests that did not fail as a result of road mortality, construction, or weather (n = 61) the probability of reproductive success declined as female CORT increased ($\chi^2 = 4.37$, $P = 0.04$; $\beta = -1.09$, 95% CI: -2.20 - 0.00, Figure 8) however there was no effect of male CORT on reproductive success ($\chi^2 = 0.41$, P = 0.52; β = 0.43, 95% CI: = -0.89 - 1.75). Further, female CORT and male CORT were not related to adjusted lay date (female: $\chi^2 = 0.92$, P = 0.34; β = -0.37, 95% CI: -1.14 - 0.38; male: $\chi^2 = 2.63$, P = 0.10; β = -0.62, 95% CI: -1.36 - 0.12) or the proportion of introduced vegetation in a breeding territory (female: $\chi^2 = 0.34$, P = 0.56; β = 0.00, 95% CI: -0.01 - 0.01; male: $\chi^2 = 0.00$, P = 0.96; β = 0.00, 95% CI: -0.01 - 0.01).

Discussion

Reproductive Success

Kestrels nesting near busy roads and development had lower reproductive success resulting from high rates of abandonment. Additionally, female kestrels in high human disturbance areas had elevated baseline CORT and had a lower probability of successfully fledging young. In 2008 and 2009 approximately 35% of American Kestrel nests failed. This failure rate is similar to nest box populations in Florida (25-45%; Smallwood and Collopy 2009), Central Iowa (22-48%; Varland and Loughin 1993), and California (7-32%; Bloom and Hawks 1983). Most nests that failed had intact eggs suggesting nest abandonment during incubation was the greatest cause of American Kestrel nest failure. Additional failures appear to have occurred as a result of predation, infanticide, or hatch failure.

Human disturbance was a strong predictor of kestrel reproductive success. Kestrels were more likely to successfully fledge nestlings when human disturbance was lower. The effects of development and road and traffic variables on success were less pronounced when nests were farther from roads. These results indicate that nest boxes in areas of reduced human development, those near lower traffic roads, and those away from roads were most likely to succeed. Other studies have indicated that human effects on birds are linked to distance from development and roads (Slabbekoorn and Ripmeester 2008; Kuitunen et al. 2003; Valkama and Korpimaki 1999). Kestrels nesting farther from roads may be less exposed to visual and acoustic perturbations or contaminants such as diesel exhaust fumes which have been implicated in impaired reproductive function (Li et al. 2007).

Another interpretation could be that kestrels nesting farther from roads are less likely to be hit by cars. Collisions with artificial structures such as vehicles, buildings, and power lines are a significant cause of avian mortality (Erickson et al. 2005). However there are several sources of bias associated with estimating rates of avian collision fatalities (Morrison 2002). Observers searching for carcasses may fail to detect a body even when it is present. In addition carcass removal by scavengers or vehicles as well as decay over time may confound the detection of vehicle killed kestrels (Antworth et al. 2005). Lastly, kestrels hit by cars may not die immediately and attempt to move away from the site of collision. As a result these individuals may die outside of the transect area. I was unable to account for this last source of bias. Additionally, I did not account for road mortality in 2008 nor did I account for carcass detectability during walking transects. I did not find kestrel carcasses next to roads during road mortality surveys conducted after a pair apparently abandoned a nest. However, I did discover one carcass before I became aware of abandonment and another carcass was discovered by a citizen. Additionally, in 2008 I located a road-killed female kestrel whose nest failed shortly thereafter. Estimates of scavenging bias were low and I located 75% of observer bias carcasses. These observations suggest that vehicular collisions do occur with kestrels nesting near roads and may account for some cases of nest failure. However if I account for the 75% carcass detection probability, at most 4 of 26 cases of failure were because of adult road mortality. Further, if vehicle mortality did account for high rates of nest failure I would expect mortality and thus failure to occur randomly throughout the breeding season. This was not the case as most nests failed during the incubation stage. As investment in a current reproductive effort is most likely not as high during incubation compared with chick rearing (Bonier et al. 2009), this indicates adults were abandoning nests more often than failing as a result of adult mortality.

The most parsimonious habitat type model included the intercept-only term therefore my analyses did not support a relationship between habitat type and kestrel reproductive success. There was a significant negative relationship between disturbance and proportion of introduced vegetation within a nest territory, interesting as invasive plants are often associated with areas of human use (Borgmann and Rodewald 2005; Thuiller et al. 2006). One possible explanation for this trend is that higher disturbance sites had more developed land (e.g. parking lots, roads, and houses) with few open areas to support introduced vegetation. Introduced vegetation, which made up a large proportion of my study area, provides an important resource for breeding kestrels. Kestrels in Florida prefer to hunt in habitats composed of short grasses and forbs with a lack of woody canopy cover, characteristic of the introduced vegetation habitat class (Smallwood 1987). However, I found no support for the effect of introduced vegetation on success. A multi-scale approach which examines territory characteristics at multiple spatial scales or habitat heterogeneity may be needed to fully understand the importance of habitat on kestrel reproduction.

In addition to the effects of human disturbance on reproductive success, the second ranked model suggests that successful kestrels initiated breeding earlier; however quality was not the best predictor of reproductive success. In support of using lay date as an indicator of habitat and individual quality, lay date or correlates of lay date (date of nest initiation, arrival date at nest site) are well documented to affect reproductive success and other aspects of fitness in birds (Perrins 1970). For example, Steenhof and Heath (2009) found that earlier laying female kestrels within my study area tended to return to the study site in subsequent years. Whether early breeding reflects the quality of the habitat within the breeding territory or individual birds remains to be seen. Lay date did correlate positively with female mass but did not differ across habitat types. It is difficult to assess habitat quality because no condition indexes correlated with habitat type and because I did not gather direct information on resource availability (e.g. prey abundance) in a breeding territory. Thus lay date may not be a comprehensive indicator of quality, and may be a more appropriate indicator of individual quality alone.

Lay date and male plasma carotenoids had a weak but non-significant inverse relationship. As carotenoids are acquired through diet, carotenoid levels can be indicative of food availability (Bortolotti et al. 2000), territory quality (Arriero and Fargallo 2006; Bostrom and Ritchison, 2006; Casagrande et al. 2006) and male quality (Hill et al.1999; Faivre et al. 2001; Helfenstein et al. 2008). Unfortunately my sample size for carotenoids was limited and I could not directly test for the relationship between carotenoids on reproductive success. Similarly, sample size for male subterminal band was limited and future research should explore the role of both plasma carotenoids and subterminal band in kestrel reproductive success.

Although human disturbance levels best predicted success, habitat type and quality (e.g., lay date, carotenoids) may play a role in other aspects of reproduction such as productivity, chick growth, or propensity for subsequent breeding attempts. Further analyses should explore these relationships. Additionally, NWGAP imagery and traffic count data were collected within the past decade, but information on other disturbance and quality parameters reflected current conditions. Care should be taken in interpreting my results and trends should be verified when updated data is available.

Baseline Corticosterone

Female kestrels had elevated baseline CORT in areas of high human disturbance and were less likely to succeed in fledging young. Female CORT did not vary with lay date, adult mass, or the proportion of introduced grassland. Therefore, stress caused by human disturbance appears to have led to reduced reproductive success. Whether elevated CORT led to changes in reproductive physiology (e.g. reduced egg fertility) or behavior cannot be determined. As the majority of failure occurred within the incubation stage, failure likely occurred because high CORT led females to abandon.

At some point in time, elevated CORT in female kestrels may have been an adaptive response, allowing females to cope with human disturbance, the current demands of reproduction, and prepare for nestling care. At some point in time, the nature of the relationship between CORT and reproductive success may have changed (see Busch and Hayward 2009); female baseline CORT reached chronic stress levels and abandonment occurred. This could have occurred after exposure to numerous types of human disturbance, even investigator disturbance. Although capture and handling was minimized (20 minutes maximum), it may have contributed to the cumulative negative effects of chronically elevated CORT, leading to abandonment.

Evidence suggests that elevated CORT can redirect animals towards survival and away from reproduction (Wingfield et al. 1998; Sapolsky et al. 2000). Kestrels may make the tradeoff to reduce current reproductive investment and focus on survival if there are future breeding opportunities. In this kestrel population, the average number of years a female kestrel breeds is 1.2 years (Steenhof and Heath 2009). Based on this limited number of breeding opportunities, kestrels should attempt to down-regulate the stress response and focus on current reproduction. Female kestrels may perceive humans and associated perturbations as predation risk (Beale and Monaghan 2004) eliciting elevated CORT (Cockrem and Silverin 2001) and possible abandonment (Silverin 1998). But humans likely do not present a direct threat to adult, egg, or nestling mortality (with the exception of road mortality) and elevated CORT and subsequent abandonment because of human disturbance may be maladaptive.

Although female kestrels appear to incur reduced fitness associated with elevated CORT, male kestrels did not. This is similar to results found in White-crowned Sparrows (*Zonotrichia leucophrys*), where females but not males had higher baseline CORT and fledged fewer offspring (Bonier et al. 2007). However, contrary to my study, male but not female sparrows had higher CORT in urban compared to rural areas. In kestrels, the sex specific relationship between CORT, disturbance, and success could reflect variation in parental effort or investment (O'Reilly and Wingfield 2001; Bokony et al. 2009).

Although male and female kestrels both invest time in incubation and hunting and territory defense there is (Smallwood and Bird 2002) To deal with these energetic demands, males may down-regulate the stress response, resulting in lower baseline levels. Another possible explanation is that females who make the final nest site choice (Balgooyen 1976) also make the decision to abandon and a sensitized stress response mediates this.

Future work should attempt to incorporate a greater number of lower disturbance boxes to validate the trend of elevated CORT and increased frequency of failure in high disturbance areas. Although my results suggest that CORT is a mechanistic link between human disturbances and low reproductive success this is purely correlative; more work is needed to definitively make this link. This investigation would also benefit from analyses of prolactin, a hormone driving incubation and brooding behavior that play a role in mediating the effect of perturbations on reproductive effort (Chastel et al. 2005; Angelier and Chastel 2009).

In conclusion, I found that human disturbance predicted kestrel reproductive success; females in high disturbance areas had higher baseline CORT, and the probability of success declined as female baseline CORT increased. Although numerous studies have examined the relationship between CORT and conservation relevant factors or CORT and reproductive success, few have directly linked changes in baseline CORT with human disturbance and subsequent variation in reproductive success (but see Bonier et al. 2007; Cyr and Romero 2007; Ellenberg et al. 2007). This study provides conclusive support for this relationship and may elucidate a specific cause of kestrel declines in North America (Farmer and Smith 2009). Further, kestrels are assumed to tolerate human perturbations as they often nest in human dominated areas. This research indicates otherwise as kestrels abandon breeding attempts when human disturbance is high. Thus information on a species' physiological state, relative fitness, as well as presence or absence are necessary to fully understand how organisms respond to humans.

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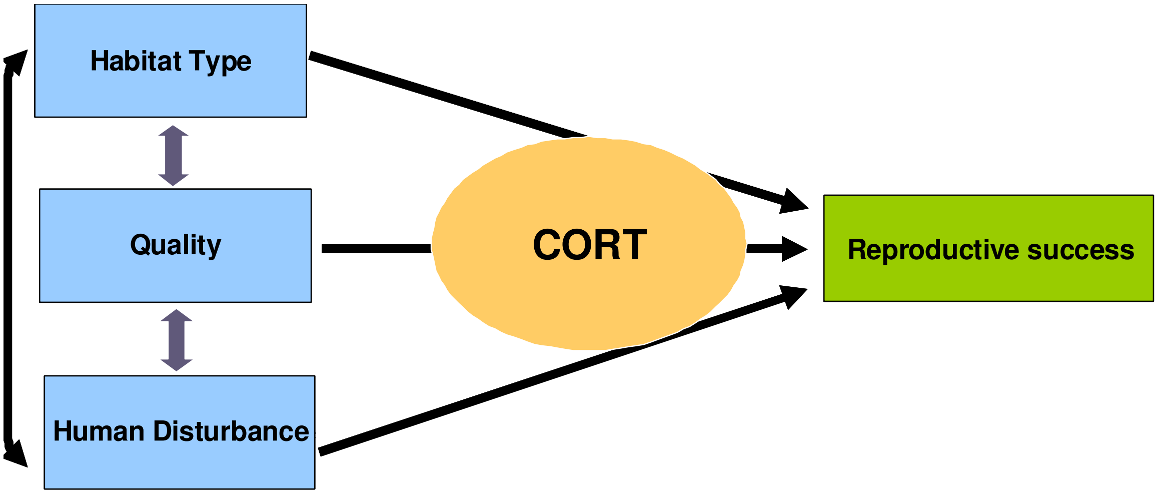

Figure 1: Possible relationship between habitat, quality, and human disturbance, and reproductive success in American Kestrels. I hypothesized that variation in kestrel reproductive success was related to habitat, quality, or exposure to human disturbance. These hypotheses were not mutually exclusive and I evaluated interactions among predictor variables. I also examined whether baseline corticosterone (CORT) mediates the relationship between habitat, quality, and human disturbance and nest failure.

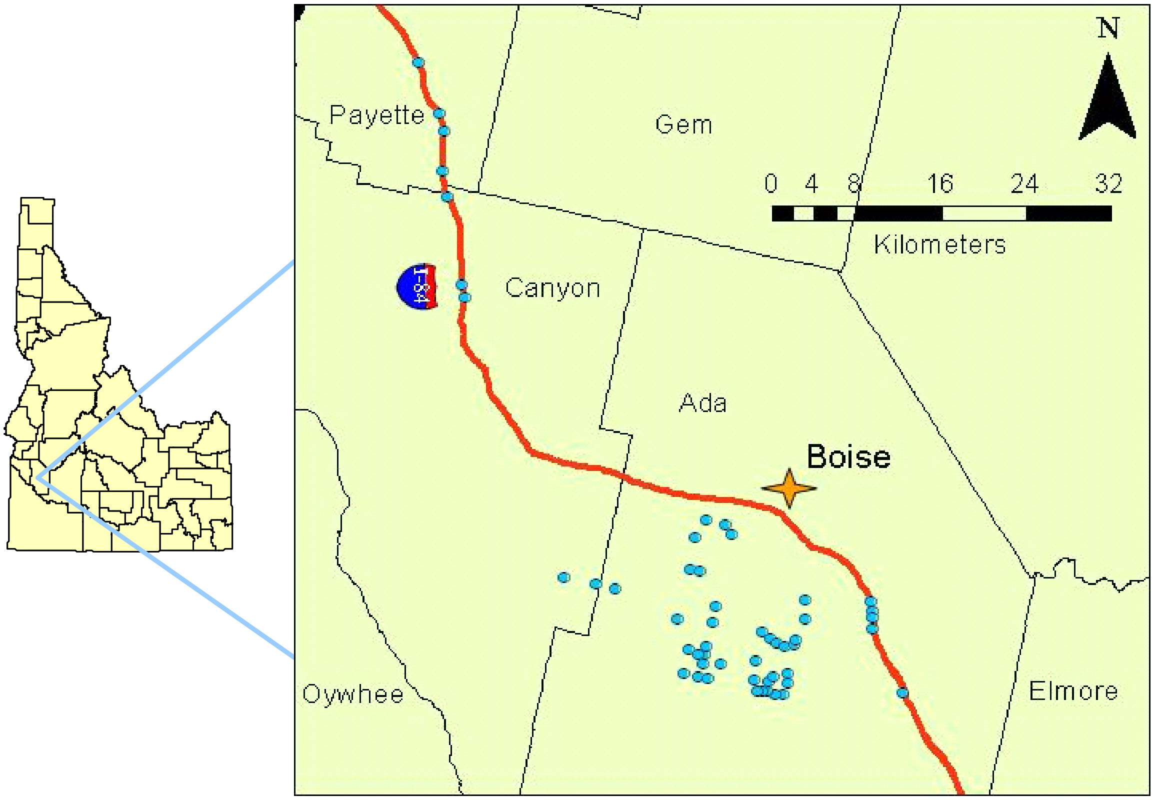

Figure 2: Map of study area and American Kestrel nest box locations used to study effects of human disturbance on reproductive success in southwest Idaho. Blue dots represent nest boxes that were occupied in 2008 or 2009. Unoccupied boxes are not displayed.

Table 1: Land cover types that made up the greatest proportion of habitat types in kestrel breeding territories in southwest Idaho used to generate classes of habitat type. Shown are mean ± SD meters^2.

Land cover	Final habitat category	Mean	Standard deviation
Developed open space	Human development	10.33	11.14
Developed low intensity		2.74	4.91
Developed medium intensity		1.13	4.03
Developed high intensity		0.29	1.06
Quarries/strip mines		0.10	0.55
Pasture or hay fields	Agriculture	9.27	10.24
Cultivated cropland		38.74	26.11
Introduced upland forbland	Introduced vegetation	0.03	0.08
Introduced upland grassland		20.92	20.41
Big sagebrush shrubland	Sagebrush / shrub steppe	11.01	12.89
Semi-desert shrub steppe		2.71	7.67
Big sagebrush steppe		0.27	0.57

Table 2: Eigenvalues and eigenvectors for principal components created from the variables average daily traffic volume, posted roadway speed (mph), number of lanes and proportion of human developed land cover in a breeding territory.

Principal component	Eigenvalue	Proportion of variability	Cumulative proportion of variability
PC1	3.21	0.80	0.80
PC2	0.65	0.16	0.96

Eigenvectors

Variable	PRIN1	PRIN2
Average daily traffic	0.55	-0.13
Speed (mph)	0.53	-0.22
Number of lanes	0.53	-0.30
Proportion of human development	0.37	0.92

Table 3: Number of nest boxes, number of nesting attempts, frequency and percentage of reproductive success and failure and causes of failure for American Kestrels in Southwestern Idaho 2008-2009. Causes of failure included abandonment, road mortality and other. Other included probable cases of predation or infanticide, hatch failure after extended incubation, as well as weather or construction caused failure.

Year	Nest boxes	Nest attempts	Successful	Failed	Failed egg stage			Failed nestling stage	
					Abandoned	Road mortality	Other	Road mortality	Other
2008	88	36	26(72.2%)	10(27.8%)	8	0	0	1	1
2009	87	37	21(55.8%)	16(43.2%)	8	2	5	0	1
Combined years	175	73	47(64.4%)	26(35.6%)	16	2	5	1	2
Used in analyses of outcome*		68	47(69.1%)	21(30.9%)	16	0	3	0	2
Used in analyses of CORT and disturbance**		64	44(68.7%)	20(31.2%)	12	1	4	1	2

*Nests which failed as a result of adult road mortality, a nest destroyed by weather, as well as a nest where adults abandoned and re-nested after nearby construction were not included in analyses of nest outcome (n = 5).

**If CORT values were available, the aforementioned nests were included in the analysis of disturbance and CORT.

Table 4: Habitat types within a nesting territory evaluated as potential predictors of kestrel reproductive success. Top models based upon log-likelihood (LL), number of parameters (*K*), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i) were chosen to represent habitat type in overall model selection.

Model	AIC_c	ΔAIC_c	w_i	LL	K
Intercept	86.26	0.00	0.28	-42.03	1
Sagebrush/shrub steppe[a]	86.30	0.04	0.27	-40.85	2
Introduced vegetation[b]	86.46	0.20	0.25	-40.93	2
Sagebrush/shrub steppe + introduced vegetation	88.04	1.78	0.11	-40.39	3
Agriculture[c]	88.48	2.22	0.09	-41.94	2

[a] Sagebrush/shrub steppe = Proportion sagebrush and shrubs within 900m radius around nest box.
[b] Introduced vegetation = Proportion introduced vegetation (grass and forbs) within 900m radius around nest box.
[c] Agriculture = Proportion agriculture (irrigated cropland and pasture) within 900m radius around nest box.

Table 5: Spearman rank-correlation matrix of relationships between estimates of American Kestrel pair and resource quality.

Values in bold indicate significance at $P \leq 0.05$.

Variable measured	Adjusted lay date		Female fat		Female mass		Female carotenoids		Male fat		Male mass		Male carotenoids		Male subterminal band	
	r_s	n	r_s	n	r_s	n	r_s	n	r_s	n	r_s	n	r_s	n	r_s	n
Adjusted lay date	1	68														
Female fat	-0.14	67	1	67												
Female mass	**-0.45**	**67**	**0.39**	**67**	1	67										
Female carotenoids	-0.21	44	-0.23	44	-0.15	44	1	44								
Male fat	-0.05	55	**0.30**	**55**	0.13	55	-0.19	35	1	55						
Male mass	-0.19	55	0.19	55	0.11	55	-0.14	35	**0.35**	**55**	1	55				
Male carotenoids	-0.28	45	-0.16	45	0.11	44	**0.40**	**30**	-0.27	44	**-0.36**	**44**	1	45		
Male subterminal band	-0.06	28	**0.40**	**28**	**0.34**	**28**	0.08	17	-0.05	27	0.07	27	-0.01	23	1	28

Table 6: Quality variables evaluated as potential predictors of kestrel reproductive success. Top models based upon log-likelihood (LL), number of parameters (K), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i) were chosen to represent quality in overall model selection.

Model	AIC_c	ΔAIC_c	w_i	LL	K
Lay[a]	53.95	0.00	0.58	-24.23	2
Intercept	56.11	2.16	0.20	-26.83	1
Lay + male mass	57.33	3.38	0.12	-23.95	3
Female mass	58.56	4.61	0.06	-26.53	2
Male mass	58.80	6.85	0.05	-26.65	2
Male mass + female mass	62.04	8.09	0.01	-26.30	3

[a] Lay = adjusted lay date

Table 7: Human disturbance variables evaluated as potential predictors of kestrel reproductive success. Top models based upon log-likelihood (LL), number of parameters (K), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i) were chosen to represent disturbance in overall model selection.

Model	AIC_c	ΔAIC_c	w_i	LL	K
Disturbance[a] + distance[b]	64.23	0.00	0.96	-28.41	3
Disturbance	70.72	6.49	0.04	-33.03	2
Distance	86.39	22.15	0.00	-40.86	2
Intercept	86.28	22.05	0.00	-42.03	1

[a] Disturbance = disturbance PCA score generated from variables # lanes, avg. daily traffic, speed (mph), and % developed land cover within 900m buffer around nest.

[b] Distance = nest box distance from center of roadway (m) ranked as 1, 2, 3.

Table 8: Overall model used to explain kestrel reproductive success displaying log-likelihood (LL), number of parameters (*K*), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i).

Model[a]	AIC_c	ΔAIC_c	w_i	LL	K
Disturbance + distance	64.23	0.00	0.78	-28.41	3
Disturbance + distance + lay	66.79	2.56	0.22	-28.15	4
Intercept	86.28	22.04	0.00	-42.03	1
Lay	86.90	22.67	0.00	-41.12	2

[a] Model represents the top variable or combination of variables within each model category (habitat type, quality, human disturbance).

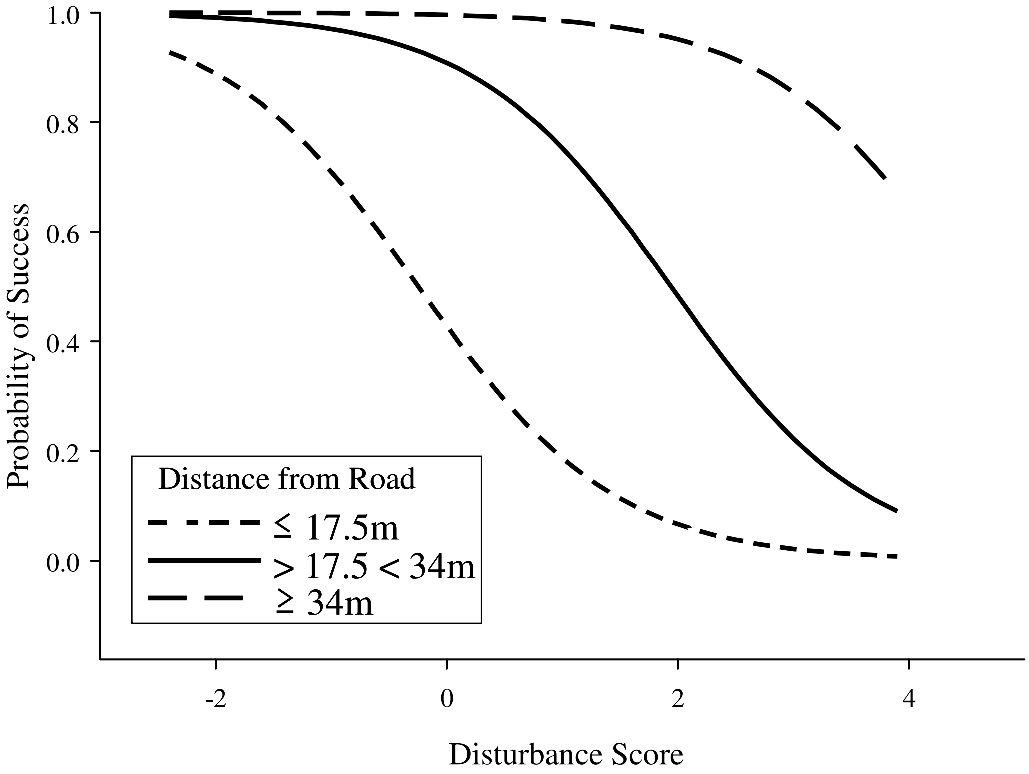

Figure 3: Probability of American Kestrel reproductive success in relation to human disturbance score (β = -1.08, 95% CI: -1.87- -0.28, n = 68) and nest box distance from road (β = 2.44, 95% CI: 0.54-5.42).

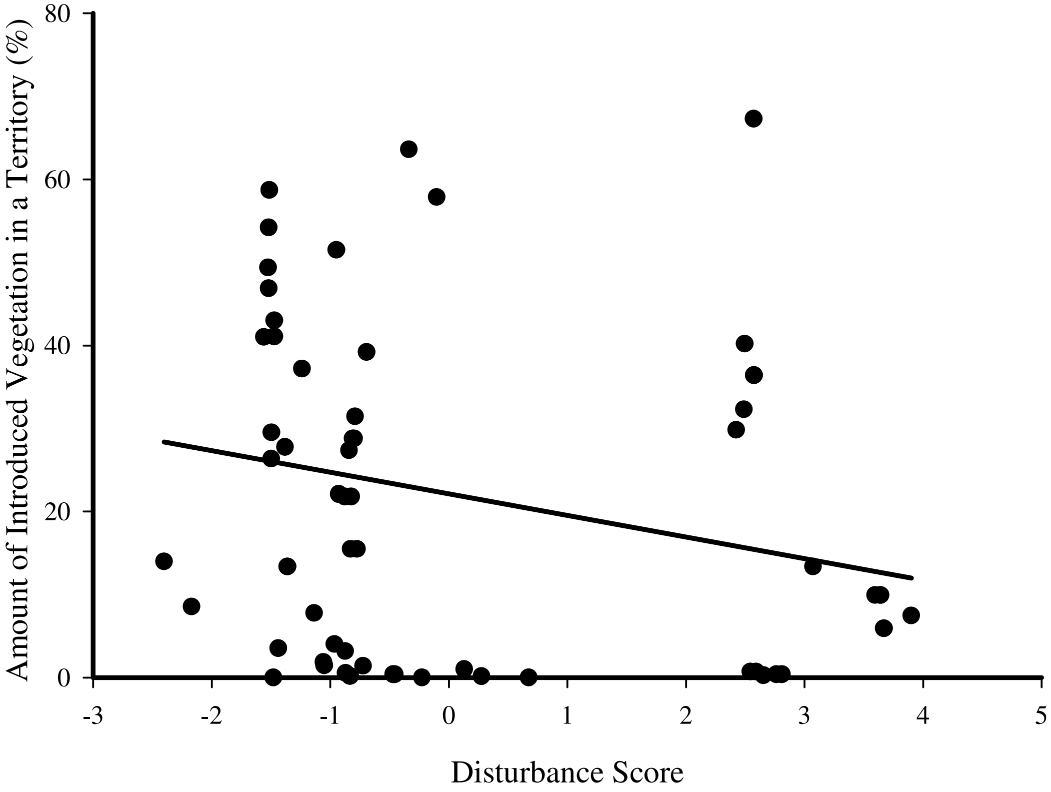

Figure 4: Relationship between human disturbance score and proportion of introduced vegetation within a nest box territory (r_s = -0.35, P = 0.003, n = 73).

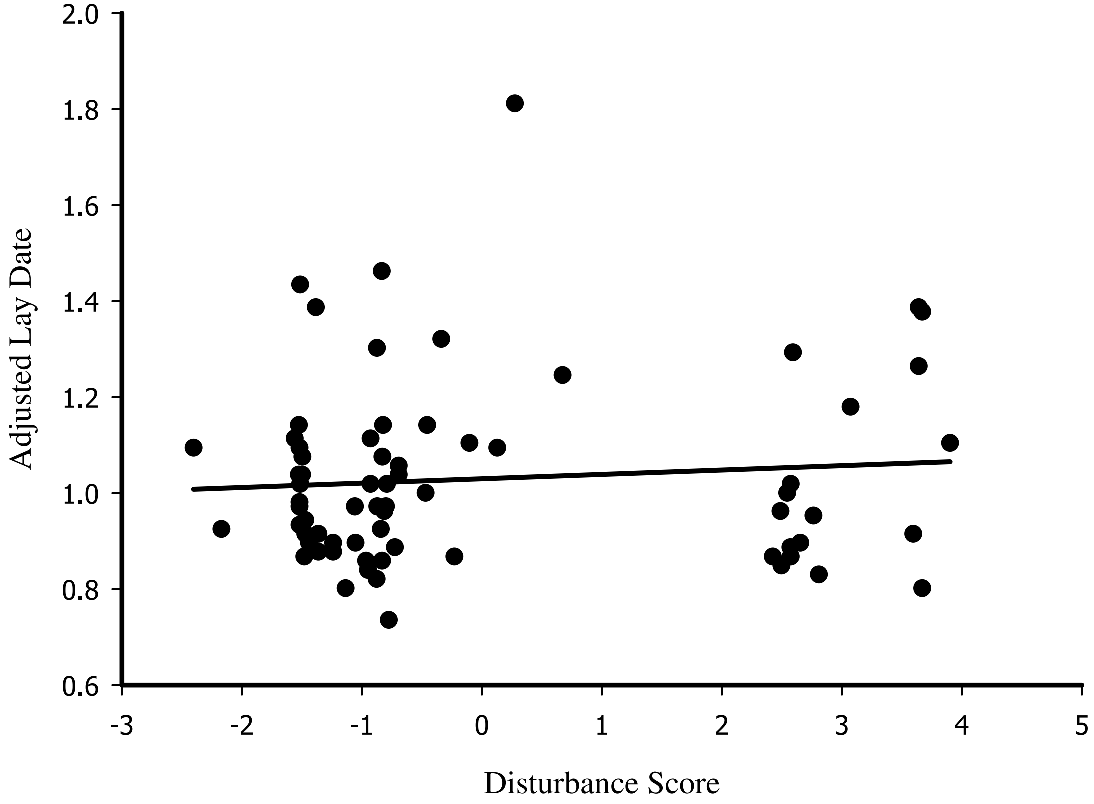

Figure 5: Relationship between human disturbance score and lay date adjusted for year differences ($r_s = 0.03$, $P = 0.82$, $n = 73$).

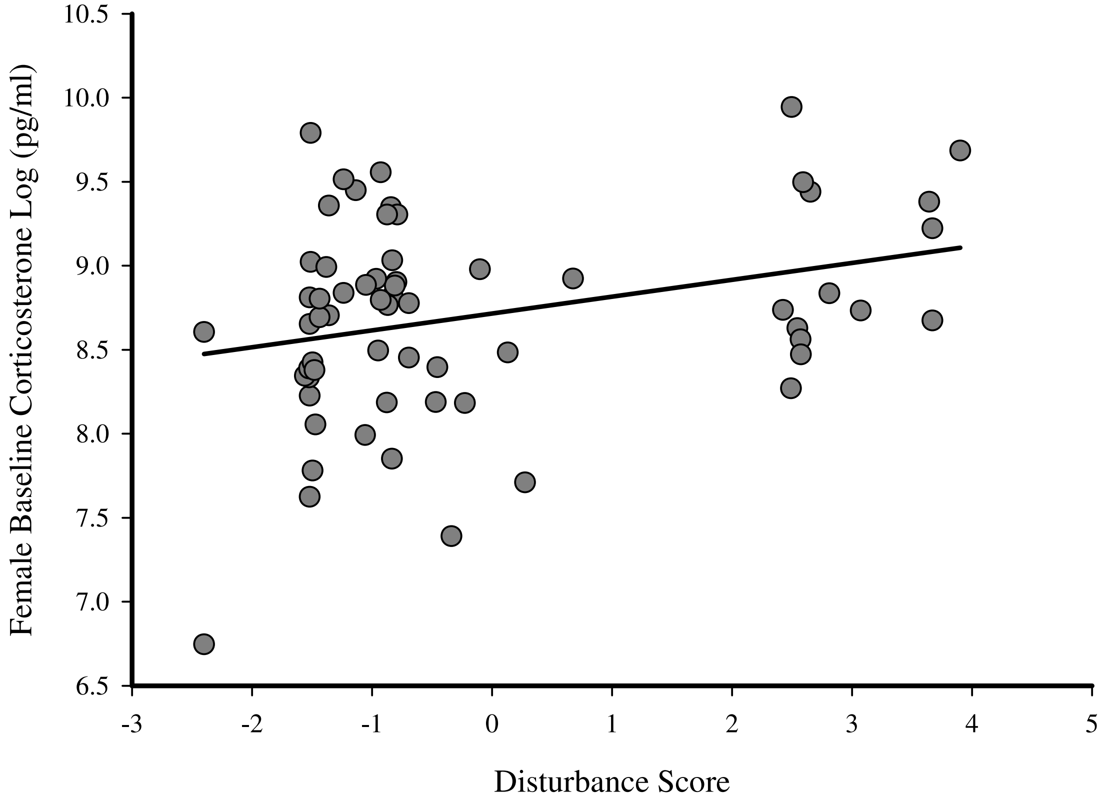

Figure 6: Relationship between human disturbance score and adult female baseline corticosterone log (pg/ml). The relationship was significant $\chi^2 = 6.07$, P = 0.01; β = 0.10, 95% CI: 0.02 - 0.19, n = 64) and displayed a positive trend where as disturbance increased, baseline CORT increased (Line equation: $y = 8.70 + 0.10*x$).

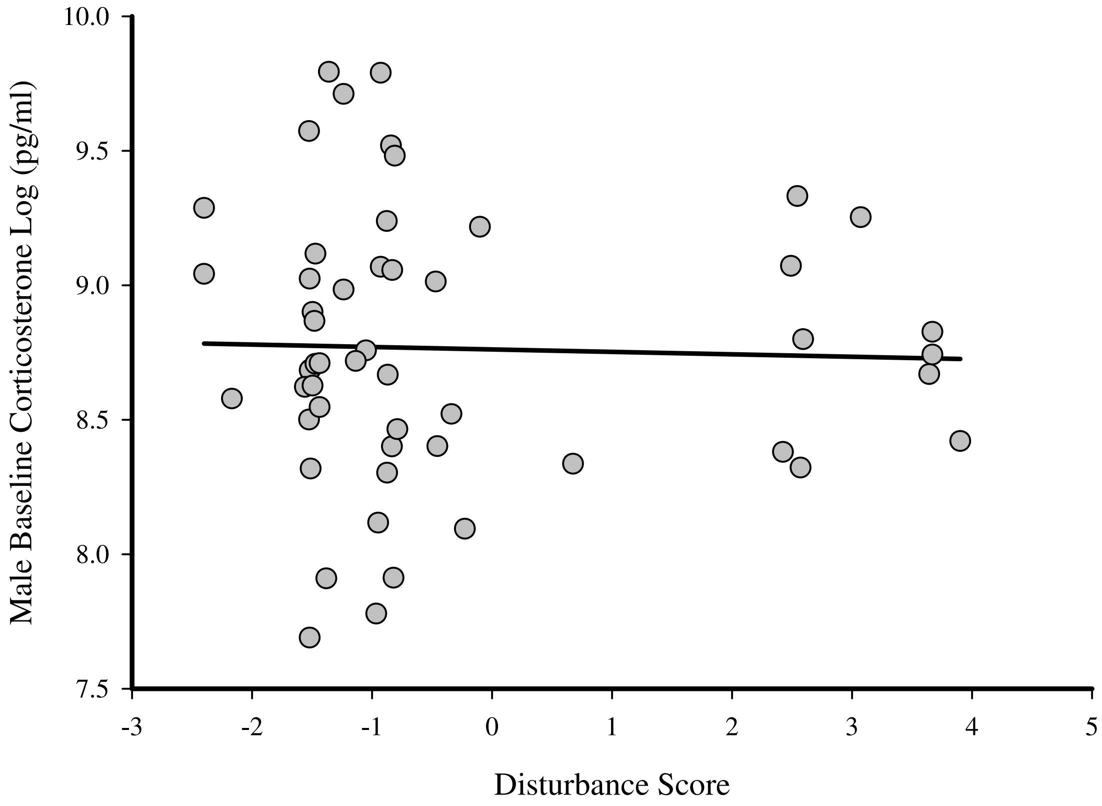

Figure 7: Relationship between disturbance PCA score and adult male baseline corticosterone log (pg/ml). The relationship was non significant ($\chi^2 = 0.03$, $P = 0.86$; $\beta = -0.01$, 95% CI: -0.08 - 0.07, n = 54; Line equation: $y = 8.74 + - 0.003*x$).

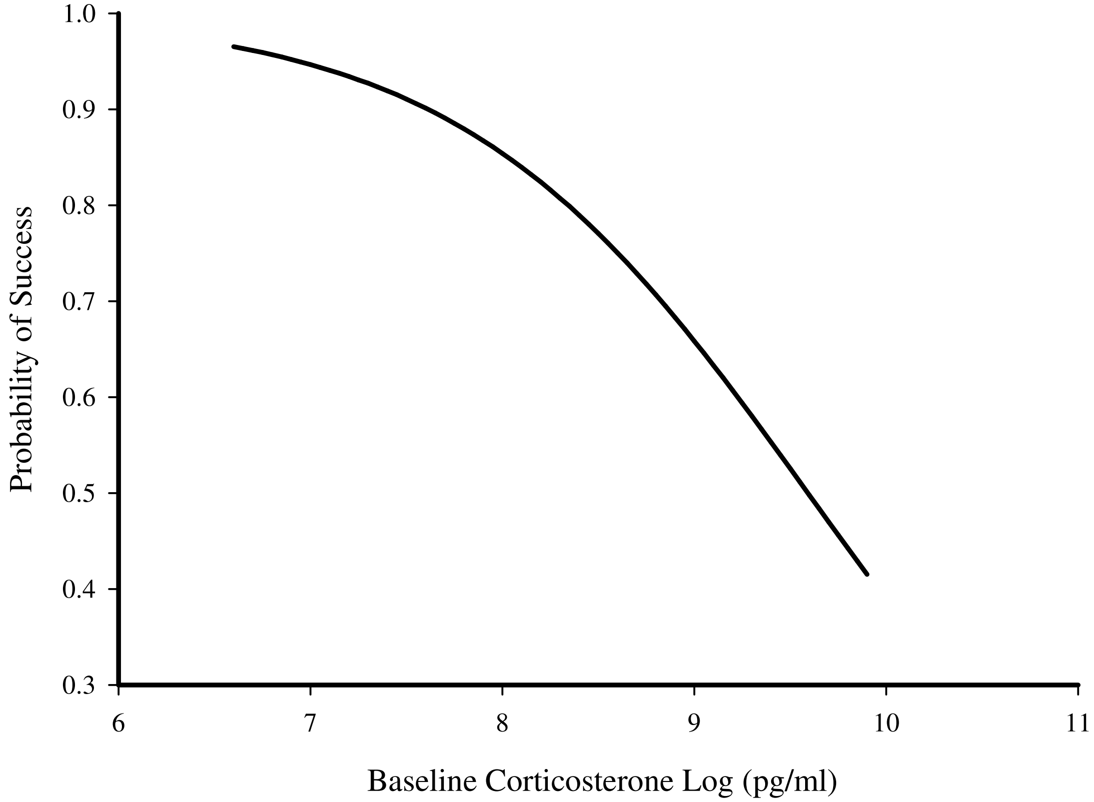

Figure 8: Relationship between female baseline corticosterone log (pg/ml) and the probability of reproductive success. This relationship was significant ($\chi^2 = 4.37$, P = 0.04; β = -1.09, 95% CI: -2.20 - 0.00, n = 61) where as female baseline corticosterone increased the probability of success declined (Line equation: y = 10.57 + -1.09*x).

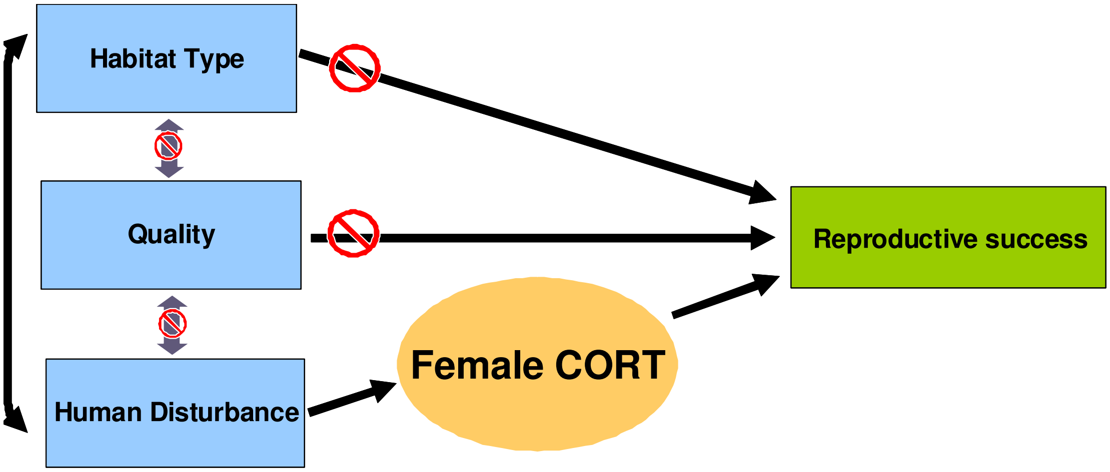

Figure 9: Diagram of hypotheses and relationships among predictor and response variables. High human disturbance best predicted kestrel reproductive success and females in high disturbance boxes had elevated CORT and higher rates of nest failure. There was no relationship among habitat type and quality and CORT however habitat type did differ across disturbance.

CHAPTER 2: EFFECTS OF EARLY DEVELOPMENTAL CONDITIONS AND CHRONIC STRESS ON NESTLING AMERICAN KESTREL CORTICOSTERONE CONCENTRATIONS

Abstract

Nestling birds' ability to respond to stressful situations may be a component of fitness and developmental conditions can lead to significant variation in the stress response. In this study I examined whether conditions experienced during the nestling stage affected nestling American Kestrel (*Falco sparverius*) hypothalamic pituitary adrenal (HPA) axis activity. Nestling kestrels, raised in high and low human disturbance areas were exposed to a chronic stress protocol (CSP). At 25 days of age nestlings were sampled for baseline and stress induced plasma corticosterone (CORT). I predicted that nestlings in high disturbance areas and those exposed to the CSP would be sensitized to acute handling stress (i.e. would exhibit high baseline and stress induced CORT levels). Nestling CORT did not vary with human disturbance levels ($F_{1, 27} = 1.97$, $P = 0.17$), CSP treatment ($F_{1, 27} = 0.00$, $P = 0.97$), or with an interaction between human disturbance and CSP ($F_{1, 27} = 0.00$, $P = 0.97$) suggesting that nestling kestrels do not sense or perceive external conditions related to human disturbance as stressful. Because kestrels are cavity nesters, nestlings may be buffered from external conditions and instead may respond more powerfully to stressors within the nest. I also examined effects of brood size alone and in combination with disturbance, on nestling stress responses. Brood size at fledging best explained baseline (β = 0.10, 95% CI: -0.03 - 0.28) and stress induced CORT (β = 0.06, 95% CI: -0.06 - 0.19). These results indicate that nestlings in large broods are more sensitive to stressors within their nest cavity compared to disturbance outside the cavity.

Introduction

Developmental conditions can have significant effects on adult morphology, behavior, and physiology (Maccari et al. 2003; Blas et al. 2007; Monaghan 2008; Butler et al. 2010). Ultimately, conditions experienced during development may impact the components of fitness: growth, recruitment, reproduction, and survival (Sedinger et al. 1995; Burness et al. 2000; McClung et al. 2004; Blas et al. 2007; Lendvai et al. 2009). Developmental conditions, particularly those that cause chronic stress can contribute to variation in the stress response, effects that may persist into adulthood (Spencer et al. 2009).

In nestling birds, the ability to respond to stressful events through secretion of corticosterone (CORT) may have both costs and benefits to fitness. In response to unfavorable or challenging conditions CORT is secreted as part of the hypothalamic-pituitary-adrenal (HPA) axis stress response. Elevated CORT allows individuals to cope with short term or acute challenges by suppressing non-essential behaviors and physiological activities while promoting those required for immediate survival (reviewed in Wingfield et al. 1998; Saplosky et al. 2000). In nestling birds, elevated CORT promotes begging behavior (Loiseau et al. 2008), food intake (Bray 1993; Kitaysky et al. 2003) and increased locomotor activity (Corbel and Groscolas 2008).

Although adaptive during periods of acute stress, chronically elevated CORT (chronic stress) can be detrimental to behavioral and physiological systems (Siegel 1980). In developing animals chronic stress can have detrimental effects that persist through life stages. For example, elevated nestling CORT has been linked to compromised cognitive abilities (Kitaysky et al. 2003), neophobia (Spencer and Verhulst 2007), changes in baseline CORT or HPA axis reactivity (Pravosudov and Kitaysky 2006; Cyr and Romero 2007; Lendvai et al. 2009), and reduced survival (Blas et al. 2007). Despite its relevance, little is known about how developmental conditions affect HPA-axis activity in wild nestling birds.

Human disturbance related to urban environments, roads, and tourism has been correlated with nestling or fledgling baseline and stress induced CORT levels (Müllner et al. 2004; Walker et al. 2005a; Partecke et al. 2006). Human disturbance may elicit a stress response as human noise may impair communication between adults and nestlings (e.g. begging vocalizations), potentially affecting parental care. Or because adult kestrels respond negatively to human disturbance (see Chapter One), nestlings may perceive human perturbations as a predation threat. Nestlings may respond to human activity with a down-regulated or dampened stress response (Rich and Romero 2005; Whitman 2006) presumably to minimize the long-term negative effects CORT has during development (Wada et al. 2007). Alternatively, sensitization to stress can occur which may facilitate coping or cause repercussions associated with chronically elevated CORT (Cyr and Romero 2007; Busch and Hayward, 2009).It is also possible that some nestlings do not respond to human disturbance with a physiological stress response (Brewer et al. 2008). How nestlings respond to potential stressors likely depends upon the species, age, and type or duration of stressor. For example, newly hatched Magellanic penguins (*Spheniscus magellanicus*) in tourist visited sites have significantly elevated maximum CORT levels as compared to unvisited chicks, however at fledging, this difference in stress responsiveness disappears (Walker et al 2005a; Walker et al 2005b).

In addition to human disturbance, conditions within the nest such as variation in brood size or social hierarchies have the potential to influence nestling stress responses. This may occur as a consequence of food restriction as well as sibling age or size hierarchies (Saino et al. 2003; Love et al. 2003a; Lendvai et al. 2009). Elevated nestling CORT in large broods may reflect a response to the stress of con-specific interactions, overcrowding, or the increased risk of parasite infestation (Saino et al. 2002).

The objective of this study was to identify conditions experienced during the nestling stage that affect free-living nestling American Kestrel (*Falco sparverius*) HPA-axis activity. American Kestrels are cavity nesting raptors that readily utilize nest boxes. Kestrels are often found in human-dominated areas near roads, agriculture, and urban developments. Brood size ranges from 3-6 semi-altricial nestlings, which can create competitive sibling hierarchies with potential for brood reduction (Smallwood and Bird 2002). Kestrels as young as 10 days post hatch respond to acute handling stress and near fledging are able to mount an adult-type CORT response (Love et al. 2003b). I examined how fledgling age chicks reared in high and low human disturbance areas exposed to a chronic stress protocol (CSP) vary in baseline (BL) and stress induced (SI) CORT. I predicted that if human disturbance affects HPA axis development in nestling kestrels then nestlings inhabiting high disturbance areas will show elevated BL and SI CORT patterns. Repeated exposure to chronic stress may lead to a sensitized HPA-axis response; therefore, I expected nestlings exposed to the CSP as well as control high disturbance nestlings to display high BL and SI CORT in response to acute handling stress. I also examined the relative role of brood size on nestling BL and SI CORT compared with exposure to disturbance.

Materials and Methods

To test the roles of human disturbance and brood size in nestling kestrel HPA-activity I monitored 89 nest boxes in 2008 and 87 nest boxes in 2009 in southwestern Idaho (Chapter 1, Figure 3). Wooden nest boxes were mounted on wooden posts along secondary roads, on trees in rural residential areas, and on signs along Interstate 84. Each year prior to the breeding season boxes were cleaned, repaired or replaced if necessary and lined with pine shavings. Nest boxes were checked weekly from approximately mid-March through mid-August to determine occupancy, clutch initiation, and hatch date. If nestlings were discovered as hatchlings they were aged following Griggs and Steenhof (1993) and I backdated to estimate hatch date (the date the first chick hatched). At day 10 post hatch, nestlings were banded with federal aluminum bands if their tarsi were sufficiently wide. Nests were visited again at day 25 post hatch to obtain BL and SI CORT blood samples (see Chronic Stress Protocol below) and to band unmarked nestlings. All procedures were conducted with approval from Boise State University's Institutional Animal Care and Use Committee (protocol #006-08-007).

Human Disturbance Analysis

To quantify human disturbance, I measured several traffic, road and building variables at each occupied nest box. Variables included posted roadway speed (mph), number of lanes, daily average traffic volume, nest box distance from center of roadway (median if interstate box), nearest building, and I noted the presence or absence of structures. The proportion of human development within a 900 m radius buffer of the nest box was quantified using Northwest Gap Analysis Program (NWGAP) satellite imagery of southwest Idaho (http://www.gap.uidaho.edu/landcoverviewer.html). Human development land cover types consisted of developed open space, developed low, medium and high intensity (reflects presence or absence of structures and roads) and strip/gravel mines. Data on daily average traffic volume was compiled from Ada and Canyon County Highway Districts, and Idaho Department of Transportation websites (http://achd.ada.id.us/; http: //www.itd. idaho.gov/ highways/). Distance of box from road center and nearest building was determined using a laser rangefinder (Bushnell Yardage Pro) to the nearest 0.5 meter. If a nest box was at the intersection of more than one road all values were averaged. I assigned ordinal categories for each nests distance from road based on natural breaks in the distance from road distribution. Nest boxes that were less that or equal to 17.5 m from the road were assigned a 1, boxes > 17.5 m but < 34 m were assigned a 2, and if a nest box was greater than or equal to 34 m from the road it was assigned a 3.

Road, traffic, and human development variables were entered into a principal components analysis (PCA) to create a disturbance index. The first principal component

(PC1) included the variables average daily traffic volume, posted roadway speed, number of lanes and proportion of human developed area within a nest territory.

Some experimental units may not be independent of one another as some nest boxes were used in both 2008 and 2009 or twice in one year. To account for this I removed nest boxes from the PCA if traffic did not vary at those nests between years. PC1 accounted for more than 80% of the total variation among occupied nest boxes therefore each box was assigned individual PC1 scores for disturbance (Table 2; Chapter 2). Disturbance score ranged from -2.40 to 2.57 with higher PC1 scores indicating higher disturbance areas (i.e. the interstate, busy secondary roads, or highly developed areas) while lower scores indicated lower disturbance areas such as smaller secondary roads with few or no buildings. Disturbance scores were centered by year to account for the greater number of high disturbance boxes occupied in 2009.

Chronic Stress Protocol

Broods stratified by high or low disturbance were randomly assigned to a control or chronic stress protocol (CSP) treatment adapted from Cyr and Romero (2007). Boxes were considered low disturbance if they had a PC score of less than -0.78. The CSP began when nestlings were approximately 17 days of age and ended at 23-24 days of age. Controls were not visited, except for when young were 10 days old, until nestlings were 25 days of age. CSP broods were exposed to a rotation of six stressors that included loud radio noise, novel objects placed in or on top of the nest box, a Great-Horned Owl (*Bubo virginianus*) decoy positioned near the nest, a human talking, and a human and dog near the nest box. The CSP consisted of three randomly selected stressors administered for 30 minutes, spaced 30 minutes to one hour apart for seven days. One day out of the 7 day period CSP broods did not receive the protocol. This protocol was designed to be unpredictable, a key component of a stressor (Rich and Romero 2005; Cyr and Romero 2007). Additionally, I expected adult kestrels to reduce visitation rates during the protocol which could also elicit a nestling stress response.

Following completion of the CSP, I followed a standardized capture and restraint stress protocol to assess the affects of acute stress on BL and SI CORT (Wingfield et al.1992). From every CSP or control brood one or two 25 day old nestlings were bled within 256 seconds of nest box contact to reflect BL CORT. If there were three or more nestlings in a brood I sampled the largest and smallest nestling for BL CORT. If there were only two nestlings, I randomly bled the largest or smallest of the two. After baseline sampling, nestlings were placed in a covered, compartmentalized plastic bucket. I collected SI samples 15 min after arriving at the nest box from two (or one if limited by brood size) nestlings. I did not bleed the same birds twice to avoid underestimating CORT levels (Dufty 2008). Because both small and large nestlings were sampled, possible effects of hatch order on CORT were reduced. If a brood consisted of one nestling, I collected only BL from this individual. For all samples, I collected 0.1 - 0.4 ml of blood using a 26 ½ gauge needle and syringe via the jugular vein. Samples were stored in heparinized vials, placed on ice and returned to the lab within 5 hours of sampling.

Corticosterone Determination

Upon return to the lab I centrifuged all samples at 10,000 rpm for 15 minutes. Plasma was extracted and stored in 1.5 ml microcentrifuge tubes at -80°C until analysis.

Total CORT concentrations were determined by enzyme-linked immunosorbent assay (ELISA Cayman Chemicals). Samples were run in duplicate when possible. Briefly, CORT was twice extracted from 10-30 µl of plasma with 5 ml diethyl ether. The lipophilic supernatant was poured off and dried under a stream of nitrogen gas in a warm water bath. Extracted samples were reconstituted with 100 µl of EIA buffer, vortexed and 50 µl aliquots were added to 96-well plates coated with mouse monoclonal antibody. I added corticosterone-specific acetylcholinesterase tracer and rabbit corticosterone antiserum and then placed plates on an orbital shaker for two hours. Plates were then developed and read at 405 nm with a Biotek EL800 plate reader. The concentration of CORT was calculated by comparing results to a standard curve and adjusted for extraction efficiency and plasma sample size. Extraction efficiency was determined by analyzing a standard CORT sample. Inter-assay variation was calculated from repeated values of a pooled sample. All values were corrected for assay extraction efficiency (mean ± SD) 83 ± 7.6%. Inter-assay variation averaged 8.63 % and average intra-assay variation was 2.02%.

Statistical Analyses

CSP Analysis

To examine the effects of human disturbance and the CSP on nestling CORT I ran a three factor repeated measures ANOVA with a block for year. Treatment (control or CSP) and disturbance (high or low) were between-group factors and bleed time (0-15 minutes) was a within-subjects factor. Both BL and SI CORT were log transformed to meet the assumptions of a parametric test. Nests with only one nestling were removed from the CSP analysis (Tarlow et al. 2001; Blas et al. 2005). Data from males and females were combined because there was no difference in BL or SI CORT between sexes. As elevated CORT in laying birds may be transferred to egg yolk and can potentially alter the nestling stress response (Hayward and Wingfield 2004), I used generalized linear models to analyze the relationship between adult female and nestling CORT.

Analyses of Brood Size and Human Disturbance

I used an information-theoretic approach to evaluate the roles of brood size and human disturbance in explaining nestling kestrel HPA-axis activity (BL or SI CORT). This method attempts to find the model that best explains my data while incorporating a minimal number of parameters (Burnham and Anderson 2002). I used generalized linear models with normal distribution for the response variables BL and SI CORT, a block for year, and a repeated statement to account for sibling effects (PROC gen mod, SAS 9.1) to generate log-likelihood values. Likelihoods were converted to Akaike's Information Criterion adjusted for small sample size (AIC_c) (Burnham and Anderson 2002). The model with the lowest AIC_c value was considered the most parsimonious model. The difference between the model with the lowest AIC_c value and all other models (including the intercept only model) was represented by ΔAIC_c where models $< 2\ \Delta AIC_c$ were considered equally plausible in explaining nestling BL or SI CORT. The relative support of candidate models was also determined by constructing Akaike weights (w_i). I report parameter estimates (β) as well as 95% confidence intervals (CI).

Results

I sampled 106 nestlings from 42 nest boxes. Baseline CORT (n = 57) ranged from 1,187 – 17,281 pg/ml with a mean ± SD of 5,328 ± 3,705pg/ml and SI CORT (n = 49) ranged from 4,893-46,698 pg/ml with a mean ± SD of 17,022 ± 8,608 pg/ml. There was a significant year affect on CORT ($F_{1, 53}$= 14.54, P = 0.0001). Brood size ranged from 1-6 nestlings and averaged 3.9 ± 1.3 chicks per nest. Brood size did not differ between years nor was there a relationship between disturbance score and brood size. Additionally I found no relationship between adult female and nestling CORT levels indicating that maternal effects do not explain nestling kestrel CORT patterns.

Chronic Stress Protocol

Thirty-three nest boxes with 93 nestlings were used in the CSP study. Nestlings of all disturbance and treatment groups responded to acute handling stress as indicated by an increase in total CORT from 0 -15 minutes (Figure 1). However, nestlings did not display a significant difference in BL or SI CORT across disturbance levels ($F_{1, 27}$ = 1.97, P = 0.17), treatment ($F_{1, 27}$ = 0.00, P = 0.97), or the interaction between disturbance and CSP treatment ($F_{1, 27}$ = 0.00, P = 0.97) (Figure 1).

Nest Box and Environmental Conditions

The most parsimonious model describing nestling BL CORT included the effect of brood size indicating that nestlings of larger broods have higher BL CORT (β = 0.10, 95% CI: -0.03 - 0.28). All additional competing models had ΔAIC_c values greater than 2 (Table 1).

Brood size best explained variation in nestling SI CORT (Table 2). Nestlings in larger broods (β = 0.06, 95% CI: -0.06 - 0.19) had elevated SI CORT. As with BL CORT confidence intervals overlapped zero. There was also evidence that human disturbance score (β = 0.01, 95% CI: -0.03 - 0.06) and nest box distance from road (β = -0.07, 95% CI: -0.20 - 0.06) explained variation in SI CORT (Table 2).

Discussion

Chronic Stress Protocol

Nestling kestrel total CORT did not vary with human disturbance category or CSP treatment. These results suggest that nestling kestrels did not perceive human disturbance or my CSP as a threat, or nestlings exposed to the CSP and human disturbance may acclimate and not react with a physiological stress response. Similarly, fledgling Magellanic Penguins (*Spheniscus magellanicus*) showed no difference in BL or SI CORT across tourist and non-tourist visited areas (Walker et al. 2005b) and Black-legged Kittiwake chicks (*Rissa tridactyla*) did not differ in their BL or SI CORT across levels of investigator disturbance (Brewer et al. 2008). Chronic stress can have severe negative consequences on developing young such as suppression of growth (Müller et al. 2009) and reduced cognition (Kitaysky et al. 2003). The ability to maintain low BL and SI CORT in the face of environmental perturbations could minimize the deleterious effects of CORT on developing young. It is also possible that the consequences of disturbance manifest later in life. This has been documented in hoatzins (*Opisthocomus* *hoazin*) where nestling stress response does not differ between undisturbed and tourist exposed nests, however juveniles raised in tourist exposed sites are sensitized to humans (Müllner et al. 2004). This appears to be a possible strategy to avoid the deleterious effects of stress during periods of crucial development (i.e., during the nestling stage prior to fledging). However, there are differences between hoatzin and kestrel nest structures that may affect exposure to environmental stressors. Hoatzins utilize open stick nests while kestrels nest in cavities and nestlings may be buffered from the external environment.

It is also possible that the levels of environmental and experimental human disturbance in this study were not high enough to elicit a stress response. Adult female American Kestrels in high disturbance areas have elevated BL CORT and readily abandon their nests during the egg stage (Chapter 2); thus, the nestlings in this study were from nests that persisted. Nestlings may respond to a CSP directly or indirectly as adults may limit nest visits during the protocol, a trend suggested by Cyr and Romero (2007). However, many adult kestrels appeared unperturbed by the CSP and continued to provision nestlings. Unfortunately I did not collect quantitative data on adult provisioning during the CSP for all nest boxes or during the entire protocol.

Nest Box and Environmental Conditions

As cavity nesters, kestrel nestlings may be buffered from external conditions such as human disturbance. Consequently kestrel HPA-axis development and activity may be better explained by conditions within the nest, such as brood size. My results imply that nestling BL CORT varies with brood size but not disturbance. Although brood size accounted for the greatest proportion of variation in BL CORT 95% CI overlapped zero and the positive effect size for brood size was small. Nonetheless brood size is a likely factor driving HPA activity and has been shown to be important in previous studies. For example, Barn Swallows (*Hirundo rustica*) from experimentally enlarged broods had significantly higher CORT suggesting behavioral or food deprivation stress (Saino et al. 2003). Nestling kestrels may respond to agonistic interactions between nestlings with a stress response as competition for food is more intense in large broods. Over time, chronic stress from large broods may have led to changes in BL CORT. Elevated levels of CORT are responsible for mobilizing energy during stress through increases in gluconeogenesis, liponeogenesis, and protein catabolism. Therefore, another possible explanation for the pattern of high CORT in nestlings of larger broods is that high CORT acts to mobilize energy stores when food is scarce, as may be the case in larger broods.

Similarly to BL CORT, brood size best predicted SI CORT and had a positive relationship with nestling SI CORT. Birds in larger broods may encounter challenges within the nest such as sibling competition for food, or increased parasite infestation causing variation in adrenocortical function. This is consistent with results from studies of nestling House Sparrows (*Passer domesticus*) where birds reared in experimentally enlarged broods had elevated SI CORT (Lendvai et al. 2009). This effect was most pronounced in younger broods becoming gradually smaller towards fledging. The second best SI CORT model reveals a role of disturbance score and nest box distance from road. Beta estimates indicated that nestlings in higher disturbance areas and closer to roads have higher SI CORT. Contrary to this, CSP results suggest that disturbance does not drive nestling HPA activity. This inconsistency could be explained by the presence of a weak relationship between disturbance and SI CORT that is noticeable only when disturbance has reached a certain threshold. It is possible that this disturbance threshold was reached in a small proportion of nests (possibly because of adult abandonment of high disturbance nests) and differences in statistical methods (model selection vs. null hypothesis testing) interpreted the data differently. As a result, it is difficult to definitively link human disturbance to nestling CORT and may require alternative assessments of stress.

Unfortunately, my analyses of nestling HPA-axis activity were restricted to total CORT. Corticosteroid-binding globulin (CBG) can regulate the amount of free CORT available for uptake into tissues (Siegel 1980). In comparison to total CORT, free CORT is not bound to CBG. Variation in free CORT and CBG is not accounted for by measuring total CORT, and both respond differently to stressors (Breuner and Orchinick 2002). For example, although unstressed and chronically stressed starling chicks did not differ in their BL or SI total CORT, stressed nestlings had higher SI free CORT (Cyr and Romero 2007). Future research should address nestling kestrel CBG levels or free CORT in response to human disturbance to better understand these relationships.

In summary, nestlings of larger broods tended to have higher BL and SI CORT. This presents a tradeoff for adult birds such that larger broods may lead to relatively higher fitness however the quality of nestlings from larger broods may be reduced because elevated CORT during development can be detrimental to nestling surviorship. As conditions within the nest box, but not human disturbance affected nestling CORT, this suggests that cavity nests act as important buffers from external perturbations by reducing the negative consequences of CORT on developing young. Further research is necessary to determine if elevated CORT in nestlings from larger broods has long term fitness implications.

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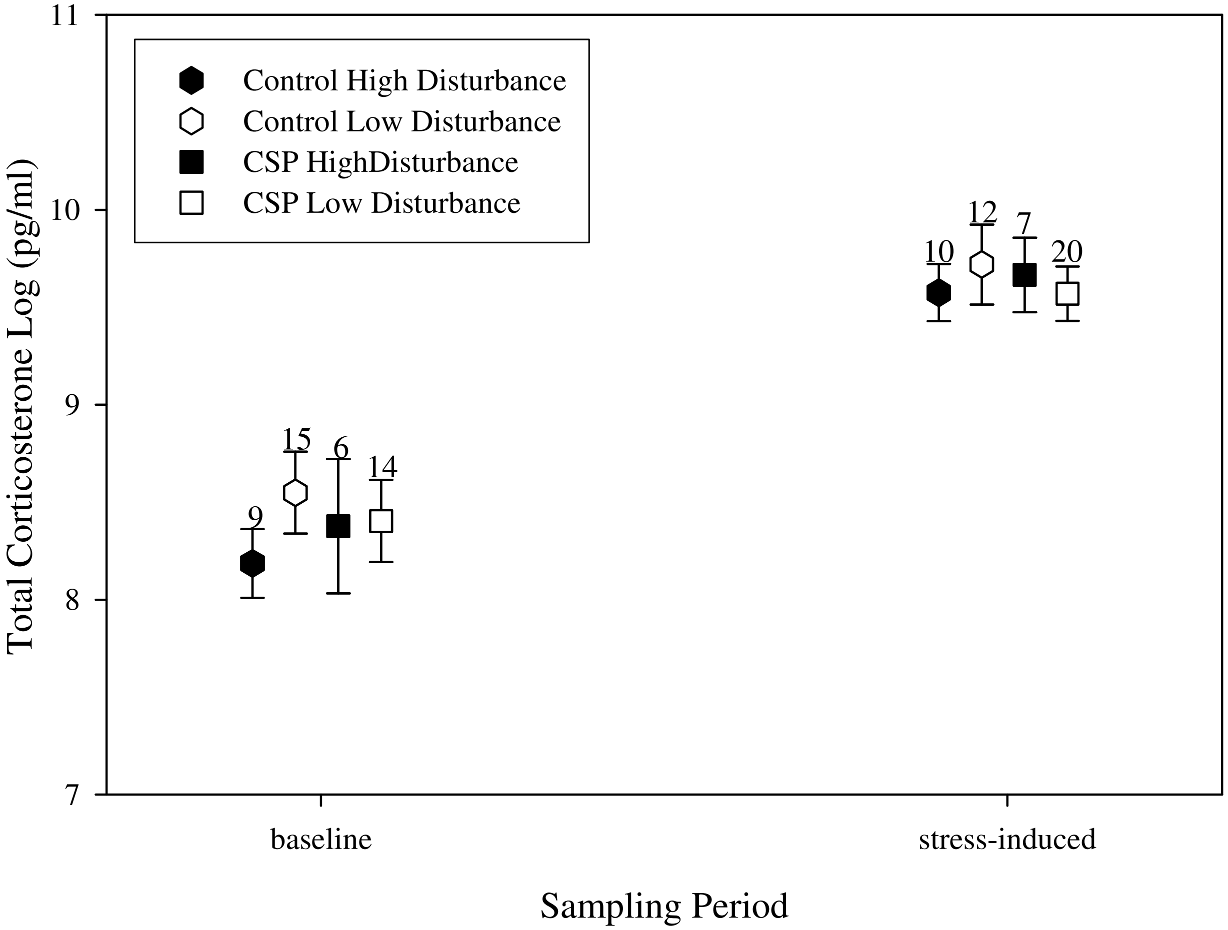

Figure 1: Changes in log transformed plasma corticosterone levels (mean ± SE) over time between nestlings of high or low human disturbance category and chronic stress treatment (control or CSP). Nestlings did not display a significant difference in BL or SI cort across disturbance levels ($F_{1,27} = 1.97$, $P = 0.17$), treatment ($F_{1,27} = 0.00$, $P = 0.97$), or the interaction between disturbance and CSP treatment ($F_{1,27} = 0.00$, $P = 0.97$).

Table 1: Log-likelihood (LL), number of parameters (*K*), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i) of models used to explain nestling American Kestrel baseline corticosterone.

Model	AIC_c	ΔAIC_c	w_i	LL	*K*
Brood size [a] + year	99.30	0.00	0.83	-45.26	4
Disturbance [b] + distance [c] + year	103.66	4.36	0.09	-46.23	5
Brood size + disturbance + distance + year	103.99	4.69	0.08	-45.14	6
Intercept	112.53	13.23	0.00	-55.23	1

[a] Brood size = number of nestlings in a brood at fledging

[b] Disturbance = disturbance PCA score generated from variables # lanes, avg. daily traffic, speed (mph), and % developed land cover within 900 m buffer around nest.

[c] Distance = nest box distance from center of roadway in meters, ranked as 1, 2, 3.

Table 2: Log-likelihood (LL), number of parameters (*K*), Akaike's Information Criterion value adjusted for small sample size (AIC_c), change in AIC_c value from the top model (ΔAIC_c), and Akaike weight (w_i) of models used to explain nestling American Kestrel stress-induced corticosterone.

Model	AIC_c	ΔAIC_c	w_i	LL	*K*
Brood size [a] + year	54.47	0.00	0.58	-22.77	4
Disturbance [b] + distance [c] + year	56.07	1.60	0.26	-22.32	5
Brood size + disturbance [b] + distance [c] + year	57.15	2.68	0.15	-21.55	6
Intercept	72.97	18.50	0.00	-35.44	1

[a] Brood size = number of nestlings in a brood at fledging

[b] Disturbance = disturbance PCA score generated from variables # lanes, avg. daily traffic, speed (mph), and % developed land cover within 900 m buffer around nest.

[c] Distance = nest box distance from center of roadway in meters, ranked as 1, 2, 3.

GENERAL CONCLUSIONS

The preceding chapters describe studies investigating the effects of environmental and endogenous factors on American Kestrel reproductive success, baseline corticosterone (CORT), and nestling hypothalamic-pituitary-adrenal (HPA) axis stress response. Specifically, I explored how human disturbance, which poses an increasingly relevant challenge to organisms, 1) plays a role in kestrel reproductive failure and baseline CORT patterns and 2) whether human disturbance, as well as brood size, influenced nestling HPA-axis activity. I found that kestrels were negatively affected by human disturbance; however, responses to disturbance appear to be both sex and age specific.

The hypothesis that variation in kestrel reproductive success is related to human disturbance near a nest was supported; in Chapter One, I found that human disturbance, but not habitat type or the quality of habitat or individuals' best explained nest failure. Furthermore female kestrels, but not males, in high disturbance areas had elevated baseline CORT, and high female CORT was predictive of lower reproductive success primarily through abandonment. This finding implies that CORT provides a mechanistic link between human disturbance and reproductive failure and is consistent with a number of recent studies demonstrating the deleterious effects of CORT as well as human disturbance on reproduction.

High rates of nest failure during the incubation stage as well as low detections of road-killed kestrels suggest that failure resulted after females abandoned a breeding attempt. In any event, death from vehicle collisions may be another deleterious consequence of living near human dominated areas. Loud noise created by high traffic volumes or perceived predation risks associated with human activities are two possible scenarios that triggered a stress response and elevated CORT. Female kestrels are the primary incubators and may have been more exposed to disturbance and thus experienced elevated CORT. As has been documented in a number of species, elevated CORT likely affected the decision to abandon the nest. Further research is needed to definitively state that this relationship is mechanistic and not just correlative.

Evidence from Chapter Two suggests that 25 day old nestling kestrels do not respond to human disturbance with an elevated stress response. Nestlings along a disturbance gradient as well as those exposed to a chronic stress protocol (CSP) did not differ in baseline (BL) or stress-induced (SI) CORT. Instead, brood size, reflective of conditions within the nest, best explained nestling BL and SI CORT patterns and nestlings of larger broods had higher BL and SI CORT. Competition for food, the stress of conspecific interactions, maintenance of energy stores, or increased parasite infestations could explain this finding. Human disturbance may influence nestling SI CORT; however, this relationship was not conclusive. Most high disturbance nests were abandoned in the incubation stage and I was limited in samples of high disturbance nestlings. Thus, disturbance may not have been high enough to cause stress in nestlings. Also it is possible that human disturbance does not pose a direct threat to nestlings, as the nest box buffers nestlings from external conditions. It should be emphasized that there are additional measures of stress such as corticosteroid binding globulins (CBG) that could contribute valuable information to this study.

This study illustrates that there are adverse effects of human disturbance on kestrels. Costs are manifested as a reduction in successful breeding attempts and female CORT appears to be a mechanistic link between disturbance and abandonment. Although nestlings did not respond to human disturbance, in the future they may attempt to breed in similarly disturbed areas and suffer high rates of nest failure. Adult mortality from vehicle collisions appeared minimal, but I did not examine rates of fledgling-vehicle mortality, which are likely higher than that of adults. Results from this study are significant because kestrels are often assumed to be tolerant of human activities. My results may provide insight into causes of the documented decline in kestrel populations as well as inform the establishment of nest box programs. Additionally, this research adds to a growing body of empirical evidence demonstrating a possibly mechanistic relationship between baseline CORT levels and fitness.