The impact of human disturbance on behavior, heart rate, and plasma corticosterone of wild red-tailed hawks (*Buteo jamaicensis*) in captivity

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Abstract

Behavioral assessments provide a non-invasive method for monitoring stress, while most traditional stress monitoring options require restraint and handling. The objective of the present study was to determine if behavioral assessments can be used to identify acute stress in wild red-tailed hawks (*Buteo jamaicensis*) during the wildlife rehabilitation process. Heart rate, plasma corticosterone, and behavior were assessed in red-tailed hawks at a rehabilitation facility when a human disturbance was present and when the disturbance was not present. Findings were compared between undisturbed and disturbed sessions. Behavior and heart rate data were collected remotely by using a remote human heart rate monitor and a video camera. Corticosterone was measured from plasma samples obtained immediately after the observation periods. During preliminary work, results from the human heart rate monitor were compared with a veterinary monitor to ensure accuracy, and no differences were found. Plasma corticosterone and heart rate increased significantly in the presence of a human disturbance, compared to undisturbed levels. Several behaviors were identified as “reactionary” due to an increase during disturbed sessions. Reactionary behaviors included: jumping to a new perch, lateral movement on perch, flight, tail shaking, leaning forwards, standing with wings extended, turning around, and crouching. A wild red-tailed hawk exhibiting these behaviors during wildlife rehabilitation may be displaying signs of an acute physiologic stress response

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Chapter 1: Introduction

Brief Overview of the Stress Response

The avian stress response is mediated by both the nervous system and the endocrine system, which can have widespread physiologic impacts when a stressor is introduced[1]. Stressors can be both external (e.g., temperature extremes, food scarcity, social interactions) and internal (e.g. parasites and other disease-causing pathogens), and an individual's survival depends on their ability to cope with these stressors appropriately[1]. Due to the release of catecholamines such as norepinephrine and epinephrine, activation of the sympathetic nervous system results in rapid increases in blood pressure due to vasoconstriction, as well as increased blood glucose, respiratory rate, muscle tone, and nerve sensitivity[1]. Activation of the hypothalamic-pituitary-adrenal (HPA) axis triggers an endocrine pathway cascade, which results in the release of glucocorticoids from the adrenal glands. In birds, the primary glucocorticoid is corticosterone[2,3]. Increased circulating plasma corticosterone levels in response to acute stress will impact metabolism, behavioral responses, energy mobilization, and immune function[4–6].

Wildlife Rehabilitation of Raptors

Wildlife rehabilitation is the process of providing human care and treatment for injured or sick wild animals, with the goal of returning the animal back to the wild. Based on estimates from annual rehabilitation reports submitted in the upper Midwest in 2011, approximately 7,400 raptors were admitted to rehabilitation centers that year[7], and this number has likely continued to increase over the past decade. The Raptor Center at the University of Minnesota alone admits approximately 1,000 raptor patients per year. Unfortunately, the wildlife rehabilitation process can be very stressful for a raptor being brought into captivity. In addition to the initial debilitating injury or disease, the bird is then subjected to human handling associated with treatments and procedures, while being housed in an unfamiliar captive environment surrounded by other unfamiliar animals. Manual restraint of raptors is required for many procedures including venipuncture, physical exams, and gavage feeding[8,9]. Additionally, the bird may be transported manually to a different area of the facility for treatments before returning to its enclosure again. Chemical restraint can be helpful for minimizing handling-associated stress in some avian patients[8,10], but in the clinical wildlife setting it is not always feasible for shorter procedures, at facilities without veterinarians, or for critical patients where anesthesia and sedation may be contra-indicated.

Wild raptors experience many stressors throughout rehabilitation. During their medical care, raptors at The Raptor Center typically experience an average of 2-4 handling events per week for weight monitoring, physical exams, diagnostics, and medical treatments. Critical patients that are not eating may be handled more than once per day for feeding. Once medical concerns have resolved, the birds are typically handled 1-2 times per week for weight monitoring, physical exams, and flight reconditioning if indicated. Chronic stress can have many negative impacts which may prolong a bird's time in captivity, including delayed wound healing[11,12] and decreased immune function[5]. Stress can also lead to captivity-related injuries like carpal wounds that prolong time to release[13].

Non-invasive stress monitoring in raptor species is limited by the current methods of measuring acute stress in birds, which include plasma corticosterone[6,14–31], heart rate[8,9,32–36], and skin and body temperature[8,9,34,37–39]. Stress-induced hyperglycemia, while common in mammalian species, is less consistent among birds[40–42]. Furthermore, the majority of stress evaluation methods require restraint, which is inherently stressful[8,9,14] Other methods may require confinement to a small area, specialized equipment, or placement of an external or surgical device. These outcomes cannot be obtained easily from visualizing the animal remotely, and the methods have not been validated in raptors.

Physiologic Measures of Stress

Corticosterone

Plasma corticosterone is commonly studied in birds and has been used to assess stress in many domestic and wild species including chickens, psittacines, raptors, pigeons, songbirds, penguins, and ostriches[6,14–31,43]. Plasma corticosterone can increase in birds due to acute stressors such as handling or predators[6,44], but its reliability as an indicator of stress has been questioned in poultry[45,46]. This is because corticosterone levels also fluctuate due to non-stress related factors, including circadian rhythm[47], seasonality[48], migratory status[31], and reproductive status[49]. Additionally, chronic stress can modulate the adrenocortical response to acute stress through downregulation of the HPA axis[50]. The corticosterone response of European starlings that were exposed to acute stressors was significantly reduced in individuals that were already chronically stressed[50]. During migration, acute stress from human handling had minimal impact on corticosterone levels in 3 migratory raptor species[31].

In order to obtain baseline plasma corticosterone values from an individual, venipuncture should be performed shortly after initiating capture, as levels have been shown to start increasing within 3 minutes of stress in birds[51,52]. Due to challenges with rapid sample collection, costs associated with testing, and lag time for receiving results when submitting to a diagnostics lab, plasma corticosterone has limited utility in the clinical setting for immediate stress assessment by wildlife rehabilitators.

Corticosterone can also be measured in fecal[16,53,54] and feather[55,56] samples, which can be obtained non-invasively. However, because of the delayed nature of collecting these samples relative to the occurrence of a stressful event, they are more practical for assessing chronic stress[53,55]. In Northern spotted owls, fecal corticosterone became elevated within two hours after experimental administration of adrenocorticotropic hormone (ACTH), peaked at 12 hours, and returned to baseline within 26 hours post-ACTH injection[57]. These findings highlight that fecal corticosterone is not well-suited for assessing acute stress due to the excretion lag time, and feather samples provide a similar challenge. In a wildlife rehabilitation setting, elevated corticosterone levels in feather samples would be difficult to interpret, as those feathers may have been grown months or years earlier, prior to the bird's admission to the facility.

Heart Rate

Auscultation of the heart is a standard part of the avian physical exam[58]. Heart rate has been evaluated as a measure of stress in several avian species including barred owls and multiple hawk species[8,9,32–36]. However, like plasma corticosterone, heart rate is a non-specific measure of stress in raptors and may also become elevated due to feeding, exercise or increased activity[33], pain, or disease[59]. While heart rate is not specific to stress, tachycardia has been documented in response to human disturbances in several raptor species[8,9,32,33]. In clinical practice, heart rate is most commonly measured via auscultation with a stethoscope or electrocardiogram when the bird is manually restrained or anesthetized. Due to the influence of human presence and restraint, stethoscopes can not be used to obtain resting baseline heart rates in raptors. Remote monitors such as surgical implants[60], externally mounted telemetry units[33,35], and artificial eggs with infrared sensors[36] have been used to document baseline and stressed heart rates in birds in research settings. When continuous telemetry was used to evaluate the heart rate of a captive ferruginous hawk in response to various activities, it documented that the heart rate increased from ranges of 112-235 beats/minute when the bird was undisturbed, to 397-503 beats/minute when a handler approached the cage or the bird was restrained[32]. These levels are likely above the upper limit of what a human can accurately count without a monitoring device.

Temperature

Core body temperature has been documented to increase in many avian species following human handling, including raptors[8,9,34,38,39,44,61], while peripheral body temperature decreases[37,39] due to the vasoconstrictive impacts of stress. Core body temperature in birds can be measured via surgically implanted devices placed under general anesthesia[62] or by a cloacal thermometer[8,9,34,62] which requires manual restraint. Skin temperature monitoring via infrared thermography has been used in wild Eurasian blue tits to measure changes in periorbital temperature in response to the acute stressor of being trapped in a small feeding box[39]. This technology has also been used to detect decreases in temperature of the eye, comb, and wattle areas of chickens in response to handling stress[37]. It has been noted that the periorbital area may be the most applicable area to evaluate for species with minimal exposed skin not insulated by feathers or leg scales[37,39]. In raptors, use of this technology has focused on thermoregluation[64] and use as a diagnostic tool for pododermatitis[63] and electrocution injuries[64], but it has not been evaluated as a method of stress detection. Further research is needed to determine if skin temperature is a good metric for evaluating stress in raptors, but the equipment required may not be attainable or practical for many wildlife rehabilitation settings.

Behavioral Assessments

Behavioral patterns can provide valuable insight into an animal's well-being, and behavioral observations are often used to assess welfare[65–69]. Additionally, behavioral monitoring has been implemented to assess fear responses[70] and stress behaviors[14] in birds. Pain scoring systems focused on behavioral changes have also been developed for assessing pain level in several species including rabbits[71], guinea pigs[72], cattle[73], rodents[74], ferrets[74], and red-tailed hawks[75].

Exposure to acute stress can induce rapid changes in behavior because it activates a series of adaptive mechanisms, including physiologic and behavioral responses[76]. The physiologic responses of increased heart rate, respiratory rate, blood glucose, nerve sensitivity, and muscle tone[1,76] work concurrently with increased alertness, vigilance, and escape behaviors[5,76] helping the animal to survive a potentially dangerous situation. When animals are stressed they may exhibit a dramatic shift in time dedicated to grooming[77], foraging[78], food intake[79], and other maintenance behaviors. Stress can also induce animals to exhibit aggression, immobility, and other coping behaviors[80].

For example, behavioral changes in cockatiels after an acute stressful event have been studied using ethograms[14]. An ethogram is a species-specific catalogue of the behaviors of interest used during behavior research. After stress, these cockatiels had an increase in reactionary behaviors and inactivity, and a decrease in movement around the cage, feeding behaviors, environmental interaction, and aggression towards conspecifics, which correlated with an increase in plasma corticosterone[14]. Although these birds did not show a significant decrease in the frequency of maintenance behaviors, such as preening, preening was less effective after stress due to being displayed in short bursts[14]. Similar findings have been noted in rodents, where grooming behaviors induced by stress may appear chaotic with frequent interruptions[77]. Based on differences in physiologic responses to stress, it has been noted that raptors cope with stress differently than psittacine species[8], and their behavioral stress responses would likely differ as well. “Raising of neck feathers, intense vocalization, and/or swaying of the body or tail feathers” have been reported as behavioral indicators of stress in red-shouldered hawks[33], but it is unknown how these behaviors were determined to be associated with stress.

Study Objectives

Developing strategies to decrease or manage stress in red-tailed hawks depends on having the ability to accurately identify and monitor stress. Welfare improvements can be made for these birds by decreasing stress during the wildlife rehabilitation process but evaluating stress responses in raptors is a limited field of study. The physiologic impacts of stress on heart rate, respiratory rate, and temperature have been studied in raptors[8,9,32,33], and recent studies have focused on responses during manual restraint[8,9].

Behavioral assessments are non-invasive and can be obtained remotely without inducing stress. Behavioral responses to stress have been studied in other avian species[14,25,69], but they have not been evaluated in raptors. The objective of this study is to determine if behavioral assessments can be used to identify acute stress in wild red-tailed hawks during the wildlife rehabilitation process. This will be accomplished by monitoring changes in behavior, heart rate, and plasma corticosterone of wild red-tailed hawks at a rehabilitation facility when exposed to a human disturbance.

Chapter 2: Comparison of Heart Rate Data from Two Heart Rate Monitors

Introduction

A bird's heart rate can provide valuable physiologic information about their response during times of stress, but it can be difficult to obtain a baseline value for comparison since handling and human disturbances induce tachycardia in raptors[8,9,32,33]. Remote monitoring is beneficial because it allows for collection of heart rate data without the need for restraint.

The Medtronic Reveal LINQ™ insertable cardiac monitor (Medtronic, Minneapolis, MN) is a wireless long-term device (measuring 44.8 mm x 7.2 mm x 4.0 mm) designed for subcutaneous implantations in humans to monitor for heart rhythm abnormalities[81]. The device is not reported to have been used in birds, but has been implanted subcutaneously in several domestic and wild animal species including horses[82,83], American black bears[84–86], grizzly bears[87], Eurasian brown bears[86], maned wolves[88], and guinea pigs[89]. Preliminary testing in bald eagles at The Raptor Center showed this device can be applied externally using a conductive hydrogel sheet for several days and still maintain a signal. Given that this device has not been used extensively in smaller patients, and that it has not been tested when applied externally, additional evaluation of the device is required. In order to ensure accurate heart rate data collection in a red-tailed hawk using this external application method, we elected to compare results from the Reveal LINQ™ and the Vetcorder Pro (MAI Health, Elmwood, WI), which is a portable monitor routinely used in exotic veterinary practices for continuous ECG monitoring. Our objective was to determine if heart rate data collected from an externally-applied Reveal LINQ™ (LINQ) were consistent with data collected from the Vetcorder Pro (VET). We hypothesized that there would be no statistical difference between heart rate values obtained concurrently from the two monitors from anesthetized red-tailed hawks.

Materials and Methods

All study procedures received ethical approval from the University of Minnesota Institutional Animal Care and Use Committee under protocol #1906-37122A.

Animals

Four second-year red-tailed hawks were enrolled to compare data obtained from two heart rate monitors. All birds were wildlife rehabilitation patients at The Raptor Center at the University of Minnesota and had been admitted due to musculoskeletal trauma to the wings including soft tissue trauma without a fracture (n=1), ulnar fracture (n=1), humerus fracture (n=1), and metacarpal fracture (n=1), see Table 1 for additional patient demographics. The four birds had all been admitted September-November 2021 and had been over-wintered at the facility (mean 164 days in care; range 127-195 days). All trauma and other medical problems had resolved by the time of study enrollment, and the birds no longer required veterinary care or medications.

Housing and Diet

The birds were co-housed in an indoor flight room (approximately 180"W x 110”D x 87”H). Birds were maintained on a day-night photoperiod of 14 hours of light per day. Diet consisted of frozen-thawed whole prey, including rats and mice (Rodent Pro, Inglefield, IN). The room was furnished with long natural branch perches spanning the length of two walls and several smaller free-standing natural branch perches on the ground, as well as a water pan. All birds were under the care of rehabilitation staff.

Data Collection

Over the course of 8 days in April 2022, birds were individually captured and restrained from their enclosure, manually carried down a hallway to the veterinary treatment room, then placed in dorsal recumbency for the placement of the previously described LINQ and VET devices. All birds were anesthetized with isoflurane (VetOne®, Fluriso, Boise, Idaho) via face mask, induced at 5% and maintained on 2-2.5% with a 1-2 L/minute flow of oxygen. Data collection was performed on restrained animals because it allowed for the placement of needle electrodes for the VET, which would not be possible to maintain in an awake animal moving around in its enclosure. Anesthesia helped to limit movement of the animals, which minimized motion artifacts on the ECG readings, in addition to minimizing any stress induced by manual restraint. Once fully anesthetized, feathers were plucked from the area over the left pectoral muscles, approximately 4 cm x 6 cm. The skin was cleaned gently with 2% chlorhexidine gluconate (VetOne®, Boise, Idaho) diluted 1:10 with tap water and 70% isopropyl alcohol (Swan®, distributed by Vi-Jon Inc., St. Louis, MO). A COVIDIEN™ Uni-Patch™ Conductive Gel Pad (COVIDIEN™, Mansfield, MA) was cut to size, slightly larger than the LINQ. The adhesive conductive gel pad was applied to the skin, and the LINQ was centered on the gel pad (Figure 1, image A). The monitor was pressed firmly in place on the skin, then covered with McKesson Transparent Film Dressing 2 ⅜" x 2 ¾" (McKesson, Richmond, VA) to secure. The LINQ was linked to the programmer (Medtronic 2090 Programmer, Medtronic, Minneapolis, MN) by holding the programming head over the device to maintain a strong connection and allow for continuous monitoring while the bird was anesthetized. The VET leads were placed using the Zumaya™ needle electrodes (MAI Health, Elmwood, WI) in a standard bipolar limb lead II configuration, with electrodes distal to the right axillary and left inguinal region (Figure 1, image B). When the appropriate electrode location had been identified, the feathers were parted and the skin was cleaned with 70% isopropyl alcohol (Swan®, distributed by Vi-Jon Inc., St. Louis, MO) prior to inserting the needles under the skin.

Once both heart rate monitors were in place and providing continuous ECG readings, a timer was started, and the heart rates from both monitors were recorded concurrently every 10 seconds for a minimum of 7 minutes. If the ECG reading from either monitor was disrupted during the recording period, the reading from the other monitor at that time point was excluded from analysis. After obtaining a minimum of 42 reading from both monitors, the monitors were removed, and the bird was recovered from anesthesia. Birds were then returned to their home enclosure.

Statistical Analysis

Medians and interquartile ranges (IQR) were calculated for the heart rate data obtained from both heart rate monitors for all subjects. Paired plots were created to visualize LINQ vs. VET data for each subject. Variability was assessed by creating Bland-Altman plots for the heart rate data to compare each monitor, in addition to calculating the median absolute deviation. A Wilcoxon signed rank test was used to test for significant differences in data between monitors. An alpha level of 0.05 was used for significance for all comparisons. Statistical analyses were performed using RStudio© (version 2022.02.1 Build 461).

Results

No difference was found in median heart rates recorded by either monitor for the four subjects. Median heart rates obtained from the VET were 263, 193, 207, and 250 beats/minute, while those obtained with the LINQ were 267, 194, 207, and 250 beats/minute, respectively (Table 2). Heart rate values from both devices for all subjects are represented in paired data plots (Figure 2). The median absolute deviation values (100.0, 35.6, 20.0, 11.9 for the LINQ; 97.9, 34.8, 22.2, 14.8 for the VET) showed that some birds had more variability in overall heart rates than others during the anesthetized episode, but regardless of the individual's variability, the two monitors were consistent when measuring variable heart rates for the individual.

Conclusion

The lack of difference between the median heart rate results from the two devices supports that heart rate data obtained from the Medtronic Reveal LINQ™ is comparable to heart rate data obtained from the Vetcorder Pro. This suggests that the Medtronic Reveal LINQ™ monitor is sensitive enough to detect heart rates in red-tailed hawks when applied using this novel external method, and it is an appropriate device for collecting heart rate data in this species during general anesthesia.

Chapter 3: The impact of human disturbance on behavior, heart rate, and plasma corticosterone of wild red-tailed hawks (*Buteo jamaicensis*) in captivity

Introduction

Wildlife rehabilitation is the process of providing human care and treatment for injured or sick wild animals, with the goal of returning the animal back to the wild. Red-tailed hawks (*Buteo jamaicensis*) are commonly admitted to wildlife rehabilitation centers in the United States, and approximately 150 of these hawks are admitted to The Raptor Center at the University of Minnesota each year (*unpublished data*). Unfortunately, the rehabilitation process can be very stressful for wild birds due to captivity, manual restraint, and an unfamiliar environment. Stress can be detrimental to the immune response[5] and wound healing[11,12], as well as increasing the risk of captivity-related injuries[13], which can prolong time in captivity.

Plasma corticosterone[90,91], heart rate[8,9,32,33], respiratory rate[8,9], and body temperature[8,9] have previously been investigated as measures of acute stress in raptors, but these methods typically require handling which is inherently stressful. Heart rate and temperature data can be collected remotely, but it requires specialized equipment or placement of an external or surgical device. Behavioral assessment is non-invasive and has been used to evaluate stress in other bird species[14,25,69], but they have not been validated in raptors.

In captivity, wild red-tailed hawks display several behaviors that veterinarians and rehabilitators subjectively interpret as "stress behaviors" but they have not been correlated with physiologic measures of stress. The objective of this study was to determine whether behavioral assessments can be used to identify stress in wild red-tailed hawks during wildlife rehabilitation. This was done by exposing red-tailed hawks at a wildlife rehabilitation facility to a human disturbance and monitoring their behavior, heart rate, and plasma corticosterone. Previous studies have focused on manual restraint as an acute stressor[8,9,14] when studying behavior and physiologic stress, but for this study we wanted to monitor the bird's behavioral response while it was free in its enclosure, which required the introduction of a human disturbance. Humans frequently open and close doors at wildlife rehabilitation facilities during tasks like cleaning, feeding, visual assessments, and handling. At The Raptor Center where this study was performed, lights are routinely turned off before opening the doors to indoor flight rooms to minimize stress and reactivity of patients. For this reason, repeated opening/closing of the door (with the light on) was chosen as the disturbance for this study, because it is a common disturbance that was novel to these birds.

If behavioral assessments can be used as a reliable, non-invasive measure of stress in red-tailed hawks, it would allow facilities to identify stress in these patients more easily without needing a significant amount of specialized training or equipment. Feedback from this stress evaluation method could then be used to develop and modify clinical procedures to minimize the negative impacts of stress during the recovery process, thereby improving animal welfare. It was hypothesized that heart rate and plasma corticosterone of wild red-tailed hawks in captivity would both increase in response to a human disturbance, and these measures of physiologic stress would correspond with measurable changes in behavior.

Materials & Methods

All study procedures received ethical approval from the University of Minnesota Institutional Animal Care and Use Committee under protocol #1906-37122A.

Animals

Fifteen wild red-tailed hawks (9 hatch year and 6 adults; 3 females, 6 males, and 6 unknown sex as determined by weight) were enrolled in the study through convenience sampling (see Table 3 for patient demographics). Birds aged as nestlings or fledglings on admission were excluded to limit potential impact from early human exposure. All birds were wildlife rehabilitation patients at The Raptor Center at the University of Minnesota and had been admitted due to a variety of causes, including trauma and emaciation (see Table 3 for a complete list of admission causes).

Birds were enrolled in the study on a continuous basis from June 2021 through December 2021 based on subject availability. All trauma and other medical problems had resolved by the time of study enrollment, and the birds no longer required veterinary care or medications. Only 1 or 2 birds were enrolled at one time due to space and equipment availability. Study enrollment was closed once the target number of fifteen birds had been enrolled for this pilot study.

Subjects had been at the center for an average of 62 days (range 4 to 274 days) at the time of study enrollment. Throughout this time, they received medical care as clinically indicated by their condition, and they experienced an approximate average of 2-4 handling events per week for weight monitoring, physical exams, diagnostics, and medical treatments in the veterinary treatment room. During their medical care birds were housed in indoor convalescent patient caging (approximately 30”W x 20”D x 29” H, with padded inner walls and a natural branch perch)[92]. All capture events from convalescent cages were performed in the dark as is routinely done at the center. Birds were manually restrained and carried to the clinical treatment room for rechecks. Routine blood work and radiographs were performed as needed during their care. Food was delivered to cages while the room lights were turned off to limit human interactions. Diet consisted of frozen-thawed whole prey, including rats and mice (Rodent Pro, Inglefield, IN).

The experimenter made efforts to avoid all visual contact with the birds within the clinical setting prior to study enrollment, to ensure all birds had had the same level of exposure to the experimenter when birds reached the data collection phase.

Study Timeline

A graphical depiction of the study timeline is provided in Figure 3.

Acclimation to Environment (Days 1-5). On day 1 of study participation, the bird was moved from its convalescent cage to an indoor flight room (room dimensions ~110”W x 90”H x 87” tall, no windows, see Figure 4 for details on flight room setup) with padded walls. Birds were maintained on a day-night photoperiod ranging from 10.5 hours light to 15.5 hours light per day, as lighting in the flight rooms is adjusted seasonally to simulate outdoor light conditions. Lights in the hallways outside the flight room are routinely left off throughout the day and night. Flight rooms were furnished with at least 3 different perch options. Natural branch perches (of various sizes) and perches covered in AstroTurf® (Dalton, Georgia) were used to help minimize the risk of pododermatitis[92]. Based on individual management needs, some birds were provided with a water pan. Additional water was added to whole prey items to ensure adequate water intake[93].

A GoPro Hero9 Black camera (GoPro Inc, San Mateo, USA) was attached to a natural branch perch using a clip mount. The camera perch was placed on the floor in the corner of the flight room closest to the door, but the camera was not turned on. The bird was allowed five days to acclimate to the flight room and the presence of a camera. During the acclimation period, the bird was not handled or examined to limit stressful experiences. Birds were housed individually during the entirety of study participation.

Heart Rate Monitor Placement (Day 6). On day 6, birds were captured and restrained from the flight room approximately between 10 and 11am, then manually carried down a hallway to the veterinary treatment room and placed in dorsal recumbency for the placement of a Medtronic Reveal LINQ™ Insertable Cardiac Monitor (Medtronic, Minneapolis, MN) (LINQ). Device placement was performed under anesthesia as described in Chapter 2 (Figure 1, image A). To prevent birds from picking at and removing the heart rate monitor, the area was covered with a 2” gauze sponge (McKesson, Richmond, VA), which was secured with a strip of 3M Durapore™ 2.5 cm wide tape (3M, St. Paul, MN) wrapped loosely circumferentially around the body at the level of the mid-keel. The body feathers cranial to the monitor were then smoothed down over the taped area to cover the monitor and allow the feathers to lay naturally (Figure 1, image B). Anesthesia was discontinued and the bird was returned to the indoor flight room once fully recovered. Prior to returning the bird to the enclosure, the GoPro Hero 9 camera’s “Scheduled Capture” function was enabled and the camera was scheduled to turn on and start recording (120 frames per second, wide viewing angle) at 7:30am the following day. The camera was turned off to conserve battery. The camera perch was placed in the corner of the room immediately in front of the door so that the entire width of the room was visible, but the door was not visible in the field of view. Videos were recorded to SanDisk Extreme 256 gigabyte microSD cards (Western Digital Technologies Inc, Milpitas, CA, USA).

*Undisturbed Data Collection (**UND**) (Day 7).* On day 7, heart rate data and behavior recordings were collected while the bird was at rest in the flight room, and there were no human disturbances present (Figure 3, Image B). The GoPro Hero 9 Black camera started recording the bird's behavior at 7:30am, prior to the arrival of staff and volunteers at the facility, which occurs at approximately 8:00am. All data collection was scheduled for the same time of day for all study subjects to decrease variability from daily fluctuations in glucocorticoid levels[48]. Between 7:40am and 7:45am, the experimenter and a handler quietly approached the door to the enclosure, turned off the light in the room (via a light switch outside the door), and the bird was captured in the dark using a small flashlight, as routinely done for all rehabilitation patients at the facility. Manual restraint was limited to two staff members (not the experimenter) with similar training and experience levels. A timer was started at 0:00 when the light was turned off to initiate capture, and the time recorded when the bird was fully restrained (mean = 25 seconds, range = 13-41 seconds). Additionally, the capture difficulty was recorded based on criteria in Table 4. No capture scores exceeded a "b" rating. The bird was manually restrained and quickly carried down a hallway to the veterinary treatment room for sample collection. As soon as the bird was restrained in dorsal recumbency on a table (approximately 30 seconds after full restraint) the bird's head was covered with a towel to limit visual stimuli. A Medtronic Patient Assistant (Medtronic, Minneapolis, MN) remote was held immediately next to bird's LINQ device and the button on the remote was pressed. The Patient Assistant is a small hand-held device which retroactively saves the previous 7 minutes and 30 seconds of cardiac data to the LINQ device's memory when the button is pressed and the light flashes green.

Once the heart rate data had been saved to the LINQ device, the bird was re-positioned for venipuncture from the jugular vessel. Venipuncture was performed within 3 minutes of capture in all but two birds (mean = 142 seconds, SD = 26.6 seconds, range = 105-108 seconds). Approximately 1.0 mL of blood was obtained using a 22g needle and 3 mL pre-heparinized syringe. After venipuncture, the LINQ device was interrogated by holding the programming head of the programmer (Medtronic 2090 Programmer, Medtronic, Minneapolis, MN) over the device to maintain a strong connection, then the heart rate data from the episode was saved to a USB drive (SanDisk Cruzer Blade 64GB, Western Digital Technologies Inc, Milpitas, CA, USA) for later analysis. The GoPro camera was checked to ensure video recording had occurred, then the "Scheduled Capture" function was enabled again as described for Day 6, and the camera was turned off to conserve battery. The GoPro camera and bird were both returned to the flight room.

*Disturbed Data Collection (**DIS**) (Day 8).* On day 8, heart rate data and behavior recordings were repeated (Figure 3, Image C) while the bird was exposed to a repeated human disturbance by the door of its enclosure as follows: At approximately 7:35am, the experimenter arrived at the entrance of the enclosure to introduce a stressor. The time was recorded and a timer was started at 0:00 when the experimenter opened the door to the enclosure by approximately 8" for two seconds, then let it close immediately. The opening width and duration were chosen because they allowed the experimenter to quickly glance at the bird and observe if it was displaying any concerning behaviors that may result in injury due to the disturbance. This door-opening procedure was repeated every 30 seconds until the timer reached 7 minutes, as long as the stressor was tolerated by the bird. If at any time, the bird's response was to take flight, the door-opening procedure would be skipped at the next 30 second interval, and at the subsequent 1-2 intervals, the door would only be opened by 1-2" (without the experimenter glancing into the room), before returning to the 8" opening. This was done to minimize the risk of injury to study subjects. Due to the placement of the camera perch in the corner of the room closest to the door, the door disturbance was not visible on the video recording. When the timer reached 7 minutes, the light in the room was turned off and a handler entered the room with a small flashlight to capture and manually restrain the bird. For consistency, the same handler that restrained the bird on day 7 also restrained the bird on day 8. The time from turning the light off until the bird was fully restrained was recorded (mean = 22 seconds, range = 7-46 seconds). Additionally, the capture difficulty was recorded based on criteria in Table 4. No capture scores exceeded a "b" rating. The bird was manually restrained and quickly carried down a hallway to the veterinary treatment room for sample collection. The bird was carried down the hallway to the veterinary treatment room and sample collection was repeated as described on Day 7. Venipuncture was performed within 3 minutes of capture in all but 3 birds (mean = 156 seconds, SD = 39.8 seconds, range = 102-229 seconds). After sample collection, the LINQ was removed from the bird, and the skin was cleaned with dilute chlorhexidine as done prior to placement. The camera perch was removed from the enclosure, and the bird was returned to the flight room under the care of the rehabilitation staff for continued care. All subjects were eventually released back to the wild after study participation and rehabilitation.

Heart Rate Data

For the birds for which we were able to collect these data, a 7 minute 30 second ECG recording was saved from each UND (n=15) and DIS (n=14) episode. The recording was saved to a PDF format with 36 seconds per page and 7.2 seconds per line. Heart rate was calculated for a 7.2-second sample at the beginning of each page by measuring all R-R intervals within the sample and averaging over the sample. These averaged intervals were converted to heart rates based on paper speed. Any areas with sufficient motion artifact to obscure the QRS complexes of the ECG recording were skipped, and an average heart rate was calculated from the 7.2 second sample immediately following the section with motion artifact. Recorded times (time to capture and data collection times) were used to ensure that any heart rate data at the end of the recording that was obtained after the initiation of capture were discarded.

Plasma Corticosterone

Whole blood samples were transferred to 1.5 mL centrifuge tubes after removing the needle from the syringe. All UND and DIS blood samples were centrifuged within 15 minutes of venipuncture for 5 minutes at 3,200 rpm. Plasma was aspirated with a pipette then mixed with dissociation reagent per the manufacturer's protocols. All samples were stored frozen in 0.5 mL PCR tubes (minimum of 4 duplicates) at -20°C per manufacturer protocols until testing. Plasma corticosterone concentrations were determined using an enzyme immunoassay kit (ELISA) (Arbor Assays DetectX® Corticosterone: K014-H1, Arbor Assays, Ann Arbor, MI)[94,95] which is designed for detecting total corticosterone in several sample types, including plasma. The assay has been designed for use with a variety of sample types and validated in birds per the manufacturer. All samples were run in duplicate in a single assay, with coefficient of variation (CV) for optical density values between duplicates <10% for all standards and 29 out of 30 samples. The DIS corticosterone concentration for bird 2 was excluded from analysis due to high variance between duplicates.

Behavioral Analysis

Video editing software (iMovie, Apple, Cupertino, CA) was used to crop and edit the videos to contain only the 7 minutes immediately prior to when the light in the room was turned off for capture. When analyzed, only behaviors from the first 6 minutes and 45 seconds of each video were assessed, in order to exclude any behaviors that may have been induced by noises made by the handler approaching the enclosure during the last 15 seconds of the recording. Once all videos had been cropped to the appropriate length, they were renamed with a number between 1 and 99 from a random number generator, then saved in a separate location from the original videos. Care was taken to ensure that no numbers were repeated for the video titles. A blinded observer was trained to perform the behavioral analysis but was unable to complete the task due to scheduling conflicts, so all behavioral analysis was done by the experimenter. The experimenter was not fully blinded since she had been present during the stressed video captures, but due to the randomly generated video titles, she was not aware of the patient ID or the treatment (UND vs. DIS) while performing the behavioral analysis. All videos were watched with the sound muted so that the door sounds associated with the disturbance could not be used to identify the treatment. Continuous sampling was performed to generate experimental ethograms to describe frequency and duration of all behaviors exhibited by subjects during each 6 minute and 45 second video clip. Prior to video analysis, a behavioral library with detailed behavior definitions was created by modifying a previously established ethogram for cockatiels[14] to suit red-tailed hawk behaviors displayed in a setting without conspecifics. This ethogram contained a total of 28 behavior descriptions (Figure 5). During video analysis, behavior durations were rounded to the nearest whole second and every second of each video was accounted for. Total duration and frequency of each individual behavior were recorded for each of the 15 birds in both scenarios (n=30 total observation events).

Statistical Analysis

Descriptive statistics were calculated for the following variables: heart rate (UND and DIS), duration and frequency of all individual behaviors among birds who performed that behavior (UND and DIS), and plasma corticosterone (UND and DIS). The percentage of birds who performed each behavior under UND and DIS conditions was calculated. Heart rate and plasma corticosterone data were visualized using box plots. The DIS plasma corticosterone value from bird 2 was excluded due to high variance between duplicate samples in the assay. Due to this, the UND plasma corticosterone value for bird 2 was excluded from paired statistical comparisons, but it was left in the data set when calculating summary statistics for UND plasma corticosterone. A Shapiro-Wilk test was used to assess normality for all data. Due to the lack of normal distribution of data for several individuals, a non-parametric test was chosen for comparisons. Wilcoxon signed rank tests were performed to test for differences in the amount of time subjects spent performing individual behaviors when UND or DIS, as well as differences in how frequently subjects performed individual behaviors when UND or DIS. These were calculated from data among birds who performed that particular behavior. This test was also used to test for differences between sampled heart rate averages from UND and DIS sessions for the 14 individuals for which both sets of data were available. Scatterplots were created to assess correlations between plasma corticosterone values and time to venipuncture (time from turning light off for capture until venipuncture successfully performed), as well as plasma corticosterone and time in captivity (days since admission). For heart rate and plasma corticosterone, the percent change from undisturbed to disturbed was calculated for each individual by subtracting the undisturbed value from the disturbed value and dividing this amount by the undisturbed value, then multiplying by 100. The median values were used for this calculation for heart rates, and the concentration values from the ELISA were used for plasma corticosterone calculations. An alpha level of 0.05 was used for significance for all comparisons. Statistical analyses were performed using Rstudio© (version 2022.02.1 Build 461).

A post-hoc power analysis for paired data performed using G*Power version 3.1 software (Heinrich Heine University, Düsseldorf, Germany)[96,97] supported that heart rate data had a statistical power of >80% with a significance level of 0.05, but plasma corticosterone data did not have sufficient statistical power.

Results

Heart Rate

Heart rates in the observation periods were higher in DIS birds compared to UND birds (median = 429 beats per min [115-500] vs. 120 beats per min [79-344], p-value = <0.001), with increases from UND to DIS ranging from 39-400% among the subjects (see Table 5 and Figure 6).

Plasma Corticosterone

Among the 14 birds for which both UND and DIS plasma corticosterone concentrations were obtained, plasma corticosterone increased in 12 out of 14 individuals after the human disturbance relative to their undisturbed value (UND: median = 82.5 pg/mL [range = 48.6-483.3]; DIS: median = 151.2 pg/mL [66.5-1215.0], p-value = 0.01) (see Table 6 and Figure 6). The 12 birds with DIS plasma corticosterone concentrations higher than UND, had corticosterone increases ranging from 13.5% to 281.7%. For the two birds with DIS plasma corticosterone concentrations lower than their UND values, the DIS values decreased by 10.9% and 27.7% .

Behavioral Analysis

Among the 28 individual behaviors described in the red-tailed hawk ethogram developed for this study, only 18 were displayed by subjects during video recordings (Tables 7 & 8). The remaining 10 behaviors (head tucking, laying down, scratching, feaking, bathing, eating, mantling, spinning, stumbling, and casting a pellet) were not included for analysis since they were not performed. For detailed results and a comparison of all behavioral durations and frequencies (among the birds who performed that particular behavior) displayed during the UND and DIS sessions, see Tables 7 & 8.

The percentage of individuals that performed each behavior at least once while UND or DIS are also included in tables 7 & 8. Most behaviors were displayed during both UND and DIS sessions, but with varying durations and frequencies. Only three behaviors were displayed in the presence of the human disturbance but not during UND sessions: standing on the floor (n=2 birds), jumping to a new perch (n=4), and walking on the floor (n=3). The only two behaviors that were displayed during the UND sessions, but not during the DIS sessions, were stretching (n=2) and head shaking (n=1).

The following behaviors increased in either frequency or duration (or both) when birds were DIS compared to when they were UND: jumping to a new perch (*duration*: median = 1 second DIS vs. not performed UND, p-value = 0.04; *frequency*: median =1 time DIS vs. not performed UND, p-value = 0.04), lateral movement along perch (*duration*: median =33.5 seconds DIS vs. 6 seconds UND, p-value = <0.01; *frequency*: median = 10 times DIS vs. 1 time UND, p-value = <0.01), flying (*duration*: median = 8 seconds DIS vs. 3 seconds UND, p-value = 0.01; *frequency*: median = 3.5 times DIS vs. 2.5 times UND, p-value = 0.02), tail shaking (*duration*: median = 6 seconds DIS vs. 1 second UND, p-value =0.01; *frequency*: median = 5 times DIS vs. 1 time UND, p-value =0.01), leaning forward (*duration*: median = 279 seconds DIS vs. 21 seconds UND, p-value = <0.001; *frequency*: median = 19 times DIS vs. 13 times UND, p-value = <0.001), wing extension (*duration*: median = 10 seconds DIS vs. 2 seconds UND, p-value = <0.01; *frequency*: median = 7.5 times DIS vs. 1 time UND, p-value = <0.01), crouching (*duration*: median = 10.5 seconds DIS vs. 3 seconds UND, p-value = 0.01; *frequency*: median = 5 times DIS vs. 2 times UND, p-value = <0.01), and turning around (*frequency*: median = 5 times DIS vs. 1 time UND, p-value = 0.04).

In contrast, perching and preening behaviors decreased in either frequency, duration, or both among DIS vs. UND as follows: perching (*duration*: median = 27 seconds DIS vs. 340.5 seconds UND, p-value = 0.046), preening (*duration*: median = 12 seconds DIS vs. 21 seconds UND, p-value = <0.01; *frequency*: median = 2.5 times DIS vs. 3 times UND, p-value = <0.01). Birds were noted to change behavior more frequently when a human disturbance was present (DIS: median = 44 behaviors performed in 6 minutes and 45 seconds , range = 3-108; UND: median = 11 behaviors, range = 1-44, p-value = 0.002).

Based on the analysis above, the following behaviors were categorized as “reactionary” behaviors: jumping to a new perch, lateral movement on perch, flight, tail shaking (in isolation, not in conjunction with rousing), leaning forwards, standing with wings extended, turning around, and crouching due to an increase during DIS sessions. Perching and preening were categorized as “maintenance” behaviors because they were more common during UND sessions.

Discussion

Developing a non-invasive method for monitoring stress in wild red-tailed hawks during rehabilitation could have a profound impact on their welfare, because it would enable rehabilitators to identify and mitigate stress. The objective of this work was to determine if behavioral assessment can be used to identify acute stress in wild red-tailed hawks during wildlife rehabilitation. Based on the findings of this study, several "reactionary" behaviors were identified, which were associated with increased heart rate and plasma corticosterone. Increased heart rate was found to be a reliable indicator of acute stress during wildlife rehabilitation, but changes in plasma corticosterone were less consistent.

Heart Rate

In the present study, wild red-tailed hawks in captivity developed significant tachycardia in response to a human disturbance, which consisted of a human experimenter opening and closing the door to their enclosure repeatedly. The heart rates noted in this study are comparable to ranges previously measured via radio-telemetry in a Ferruginous hawk monitored under several different undisturbed and disturbed conditions, where heart rate was noted to increase approximately three-fold when a handler approached the enclosure[32]. The undisturbed heart rate range for the Ferruginous hawk (112-235 beats/minute) and maximal heart rates in response to a stressor (502 beat/minute)[32] were also similar to those obtained in this study. Radio telemetry was also used to remotely monitor heart rates in response to disturbances in two captive red-shouldered hawks, with similar ranges noted[33]. In addition to tachycardia secondary to disturbance stress, the heart rates of two captive red-shouldered hawks also became elevated during exercise and feeding[33], neither of which were evaluated during the present study, aside from short incidental flights within the enclosure. A physiologic response to increased activity levels and exercise during DIS episodes may also have played a role in the tachycardia noted in the present study, and changes in heart rate were likely due to both activity and stress.

Plasma Corticosterone

Overall, there was significant variability in the plasma corticosterone values obtained in this study. While most birds did have an increase in plasma corticosterone between undisturbed and disturbed sessions, there was significant crossover between the ranges and wide variability in the percent change from the undisturbed value. Throughout avian literature, corticosterone is often reported in units of ng/mL[14,17,91], with a review reporting ranges of 0.25 ng/mL to ~150 ng/mL in stressed chickens[45]. When converted to pg/mL, the lower end of this spectrum exceeds the corticosterone values found in the present study. Fecal corticosterone results evaluated in raptors using the same assay kit as the present study reported values in pg/mL in the same order of magnitude as found here[94]. Many earlier studies report corticosterone values obtained using radioimmunoassay (RIA) techniques. While RIA was previously the most common method used to quantify corticosterone, its use is decreasing due to the human health risks associated with using radioactive isotopes[98]. Commercially-available enzyme-linked immunosorbent assays (ELISA) are now widely used and accepted[98]. A study comparing corticosterone results from RIA and ELISA methods in condors found that RIA results had greater precision and accuracy, and results were not comparable between the two methods[99]. Commercial ELISA kits may not provide exact corticosterone values, but they can be used to evaluate "relative differences" between subjects in the same study[98]. Among other variables, assays can vary in specificity for corticosterone and cross-reactivity with other glucocorticoids, accuracy, reliability, and whether they detect free or total corticosterone[45].

There are several possible factors that may have influenced the variability in plasma corticosterone values found in this study, and several that have been ruled out as less likely causes. It has been noted that the adrenocortical response can vary based on the severity of the stressor a bird is subjected to[6,45], but all birds in this study were subjected to the same disturbance performed by the same person. Daily fluctuation in plasma corticosterone[47,48] is also an unlikely cause since all samples were collected at the same time of day, but seasonal variation in corticosterone cannot be ruled out[48] since data collection occurred over the course of 6 months. Due to the convenience sampling design of this study, with enrollment of patients at a rehabilitation facility when they became eligible (recovered from injury or disease) and space was available, it was not possible to avoid collecting data over several months. Additional factors may include the individual's previous experiences, individual variation in how stress is experienced, resiliency, cause of admission, frequency of restraint and medical care prior to study enrollment, age, sex[100], cross-reactivity of the assay[45], body condition[90], previous habitat and human exposure[101], and small sample size.

The timing of the adrenocortical response of red-tailed hawks has not been studied, but it is widely accepted that blood samples obtained within three minutes of initiating stress in birds can be considered close enough to baseline[51,52]. Additional evaluations have shown that while corticosterone may start increasing sooner in some species, samples obtained within 2-3 minutes should still be sufficient to represent basal values[51]. However, in previous studies the significant difference in plasma corticosterone was noted between the early samples (collected within 2-3 minutes) and samples obtained after 30 minutes of restraint stress[51]. In the present study, disturbed samples were collected within three minutes of initiating capture, following a 7-minute disturbance period. This means that samples were being collected between approximately 8 ½ to 10 minutes (no samples collected sooner than 1 ½ minutes after initiating capture) after the start of the disturbance, which may not have been sufficient time for a complete adrenocortical response. This time frame was chosen due to the desire to collect plasma corticosterone samples immediately after a time period where both heart rate and behavior were monitored concurrently, and the remote heart rate monitor only allowed capture of a 7 ½ minute ECG recording. However, based on findings in screech owls that there was a significant difference between plasma corticosterone values obtained at 1 minute and 5 minute time periods after handling stress, and a significant difference between 5 minute and 10 minute samples[91], the sampling window should have been sufficient for an adrenocortical response to occur. If the variation in undisturbed plasma corticosterone concentrations was due to the adrenocortical response taking significantly longer or shorter time in red-tailed hawks than in previously studied species, a more consistent bias in one direction for the undisturbed samples would have been anticipated, rather than the wide range that was noted. A few samples did take slightly longer than three minutes to obtain, but no correlation was noted between undisturbed plasma corticosterone values and time-to-venipuncture.

Another potential factor could be the recent handling (within approximately 24 hours) event for placement of the heart rate monitor. However, plasma corticosterone values in juvenile, captive screech owls have been noted to decline significantly within 30-60 minutes after stress[91], and plasma corticosterone values in captive starlings returned to baseline (or near baseline) values within 60 minutes of being exposed to a variety of 30 minute stressors[50]. For this reason, it is unlikely that handling on the previous day affected plasma corticosterone results.

Although the goal of this study was to evaluate the physiologic response to an acute stressor in red-tailed hawks, it is reasonable to expect that these birds were already under the influence of chronic stress due to captivity associated with wildlife rehabilitation. For this reason, the undisturbed data obtained in this study is not representative of true baseline values. Chronic stress has been documented to modulate the adrenocortical response in raptors during stressors like migration[31], and this is likely a contributing factor to the variability in the plasma corticosterone results.

Behavioral Changes

The red-tailed hawks had significant behavioral shifts, with perching and preening displayed more commonly by birds when undisturbed. In contrast, in previous work cockatiels displayed increased resting behaviors like "standing still on a perch" after stress from manual restraint[14]. However, similar to the cockatiels, the preening behavior of the red-tailed hawks in this study was noted to be lower quality during the disturbed sessions, with short bursts of activity and frequent interruptions, rather than a sustained behavior for feather maintenance. Cockatiels had decreased locomotion behaviors after manual restraint[14], while in our study the red-tailed hawks displayed lateral movement along a perch or flight more commonly during the disturbed sessions. One possible explanation for these differences is a difference in stress response between psittacines and raptors. Physiologic differences based on changes in heart rate after manual restraint between red-tailed hawks and Hispaniolan Amazon parrots have previously been documented[9,10]. The present study supported that there is a difference in the behavioral stress responses between raptors and psittacines as well, which may be secondary to the prey and predator roles of these species in the ecosystem. Care should be taken when comparing stress responses between species, particularly if they have differing ecological roles.

There is limited previous research describing specific behavioral changes in stressed hawks, but a previous study does make reference to “raising of neck feathers, intense vocalization, and/or swaying of the body or tail feathers,” as behavioral clues indicating a stress response in red-shouldered hawks[33]. The only similar behavior noted to increase during disturbed sessions in this study was tail shaking, which several birds displayed in association with postural adjustments immediately after moving along the perch or landing after flight. Vocalizations were not evaluated because videos were muted for analysis, but based on the author’s clinical experience, red-tailed hawks are less vocal than red-shouldered hawks. Food was not present in the enclosures during video recordings for this study, so it was not possible to assess a change in feeding behavior, although this has been documented to decrease in other species with stress[14].

For behavioral analysis in this study, a continuous sampling (or all-occurrences sampling) approach was selected. Although this method is more time-consuming and labor-intensive than other commonly used methods, it has the benefit of providing both duration and frequency of individual behaviors[102]. Analyzing duration and frequency of behaviors provided a more complete picture of the birds’ behavioral responses. Long-sustained behaviors such as perching were better represented by duration, while quick behaviors that only took a few seconds to complete were better represented by frequency.

Several different behavioral stress response patterns were noted during analysis of the video recordings. Some birds appeared to repeat the same behaviors for several minutes when the disturbance was present, with their behaviors becoming very predictable. The most prominent patterns noted included a combination of two or more of the following behaviors on repeat: leaning forwards, wing extension, crouching, flight, and walking back and forth along the main perch. The majority of these patterns also included intermittent episodes of tail shaking when settling into a new position after movement. Overall, the birds tended to fidget, move, and change between behaviors more frequently during the disturbed sessions, as noted by the difference in total number of behaviors displayed between undisturbed and disturbed sessions.

While leaning behavior was displayed by a few birds during undisturbed sessions, the vast majority would default to either perching (or foot-tucking) when they were at rest and not performing active behaviors. However, when the disturbance was present, birds more frequently adopted a leaning forward position when they were at rest between other behaviors. While the two behaviors are very similar in appearance, there is a lot more tension throughout the bird's body with the leaning position, indicating they are on alert and more actively engaged with the surrounding environment. It is important to consider that behavior must always be interpreted in context, and that seeing a single isolated "reactionary" behavior displayed by an otherwise calm bird is likely not of concern. However, if a hawk at a rehabilitation facility is cycling through reactionary behaviors repeatedly as was noted by some of the birds in this study, this could be an indication of a physiologic stress response, which warrants further investigation of causes.

Limitations

Potential limitations of the study include video analysis being performed by the experimenter who was not fully blinded. Efforts were made to minimize potential bias by ensuring that behavior descriptions in the ethogram were as objective as possible, renaming all videos with a randomized number so patient ID and treatment were unknown while viewing the video, watching all videos with muted sound so the disturbed sessions could not be identified based on sounds of the door opening, and delaying video analysis for 3 months after the last video had been recorded. Additionally, viewing a 6 minute 45 second video recording reflects less than 0.5% of the duration of the day, and therefore is not representative of the entire behavioral repertoire of these birds. Many influences could not be observed, such as impacts on feeding behavior, because birds were all fed in the evening.

The ethogram was specifically tailored to the environment and enclosure in this study, and it may not be all-inclusive for birds in another setting. The ethogram also does not contain any behaviors describing interactions with conspecifics, because all birds in this study were individually housed. Typically, avian stress studies focus on the physiologic response to manual restraint, as this has been well-established in the literature[8,9,14,19,44,48,52]. This study focused on a human disturbance that has not previously been established as sufficient to induce a stress response. The reason for choosing this alternative stressor was to allow for behavioral observation while the bird was not being handled, in addition to collecting heart rate data remotely during this time.

Ideally the birds in this study would have been allowed several days in the enclosure to acclimate without handling after heart rate monitor placement, but due to the external application of the monitors, there was concern they may become dislodged or be removed by the bird prior to data collection being completed. Additionally, if there was a carryover effect from the stress response of the monitor placement to data collection on the following day, then there would likely also have been a carryover effect from the stress associated with restraint and data collection after the undisturbed session, to results from the disturbed session the following day. While possible, this is thought to be unlikely, as plasma corticosterone has been shown to return to basal values after many different types of stressors[50]. A crossover design may have been beneficial to negate any potential carryover effects, though that would require a larger number of study participants.

For this study we elected to focus on trends and changes in behavior as they related to plasma corticosterone and heart rate over the entirety of UND and DIS sessions. Additional knowledge may have been gained if behavior and heart rate data had been time-synced to evaluate instantaneous heart rates associated with individual behaviors. This would be an interesting direction for future study. In several birds, short episodes of artifact were noted on the ECG recordings, which appeared to correlate with times of high motion and activity. Motion artifact could limit the utility of the LINQ device when applied externally in this species, if investigating episodes of flight or other high levels of exercise.

Conclusion

The increased heart rate and increased plasma corticosterone values noted in most birds in this study suggest that the human disturbance was sufficient to elicit a physiologic stress response in these red-tailed hawks. The behaviors that increased when the disturbance was present included leaning forward, turning around, flying, wing extension, crouching, tail shaking, lateral movement along the perch, and jumping to a new perch. If a wild red-tailed hawk in a captive setting displays these reactionary behaviors in response to a disturbance, it may be suggestive of an acute physiologic stress response. Although plasma corticosterone increased after the introduction of a human disturbance in most of these individuals, the undisturbed range of corticosterone values had significant variability and crossover with the disturbed range of corticosterone values. Plasma corticosterone is unlikely to be a reliable indicator of stress for red-tailed hawks that are already under chronic stress from wildlife rehabilitation if a single sample is obtained in isolation. Heart rate was found to be a reliable indicator of acute stress in these birds during rehabilitation; however, remote heart rate monitoring is not practical at most facilities.

Tables

Table 1: Summary of demographics, admission cause, and housing for heart rate monitor comparison study subjects. Age determined by plumage, all subjects aged as second-years (SY). Subjects were co-housed in the same flight room.

Bird ID	Age	Sex	Enclosure	Days in captivity at enrollment	Admission cause
21-977	SY	F	E	127	Found in back yard, soft tissue injury of right wing
21-947	SY	F	E	149	Found on sidewalk, left ulna fracture secondary to projectile
21-833	SY	M	E	195	Left metacarpal fracture occurred during banding incident
21-862	SY	M	E	185	Found in yard, right humerus fracture

Table 2: Comparison of heart rate data collected from four anesthetized red-tailed hawks via the Medtronic Reveal LINQ™ and Vetcorder Pro. Median and interquartile range (IQR) of heart rate values calculated from data collected every 10 seconds over a 7-8 minute time period are reported. P value calculated from paired t-test (alpha = 0.05), no significant differences noted.

	Vetcorder Pro (beats/min)		**Reveal LINQ (beats/min)**		
Bird ID	**Median**	**(IQR)**	**Median**	**(IQR)**	**P-Value**
21-977	263	(194-326)	267	(200-333)	0.081
21-947	193	(168-213)	194	(167-207)	0.196
21-833	207	(187-217)	207	(178-214)	0.476
21-862	250	(241-258)	250	(240-261)	0.233

Table 3: Summary of demographics, admission cause, and housing for behavioral response study subjects. Sex determined as male (M), female (F), or undetermined (U) based on weight. Age determined by plumage, all subjects aged as hatch years (HY) or adults (AD)

Subject	Bird ID	Age	Sex	Enclosure	Days in captivity at enrollment	Admission cause
1	20-619	AD	U	E	274	Found at landfill with severe feather damage from gas flare
2	21-330	AD	F	B	4	Fell from sky onto patio
3	21-271	AD	M	D	69	Found by road, right metacarpal fracture
4	21-455	HY	M	H	38	Found in garage, emaciated, left major metacarpal fracture
5	21-505	HY	M	D	36	Hanging around yard, not flying far
6	21-484	HY	M	H	42	In yard for a few days, unable to fly, left coracoid fracture
7	21-478	HY	M	B	48	Suspect hit-by-car, neck wound with fly strike, distal diaphyseal closed spiral right ulnar fracture
8	21-405	AD	U	H	64	Witnessed hit-by-car, bilateral mild ocular trauma, radiographic evidence of internal trauma, mild soft tissue injuries
9	21-659	HY	M	C	32	Found by road, right ulnar fracture
10	21-631	AD	U	C	45	Found on ground unable to fly, left coracoid fracture
11	21-614	HY	F	B	60	Found in parking lot, emaciated
12	21-726	HY	F	B	53	Found on side of highway, with evidence of internal trauma
13	21-567	HY	U	B	90	Found in Mississippi River, suspected aspiration pneumonia
14	21-895	HY	U	B	30	Found in yard with poor flight, incomplete fracture left proximal humerus
15	21-897	AD	U	B	38	Found on side of road, emaciated, chronic right ulnar fracture, several shotgun projectiles

Table 4: Capture difficulty score. All birds were captured by a human handler in a dark room, with the use of a small flashlight.

Score	Characteristics of capture
a	Captured directly from the perch, minimal to no struggling or flapping, normal respiratory rate and effort after the grab, minimal struggling in hand once restrained.
b	Jumped off of perch prior to capture or flew/ran 1-2 lengths of the enclosure, moderate flapping (>10 flaps) prior to full restraint of wings, might be breathing quickly bun not panting, intermittent struggling in hand.
c	Flew/ran 5+ lengths of the enclosure (from one end to the other), very challenging grab, panting/heavy breathing when in hand, warm to the touch, constantly struggling while in hand, escapes the hold and has to be gathered up again

Table 5: Summary of red-tailed hawk heart rate data from undisturbed (UND) and disturbed (DIS) sessions. Heart rates calculated from representative samples taken throughout the ECG recording. Percent change from UND to DIS calculated by subtracting UND from DIS, dividing by DIS, and multiplying by 100. P value calculated from Wilcoxon Signed Rank test (alpha = 0.05), significant values marked with asterisk.

Subject	**UND heart rate (beats/min)**		**DIS heart rate (beats/min)**		**Percent change from UND to DIS**	**P value**
	median	**(IQR)**	**median**	**(IQR)**		
1	120	(115-125)	--	--	--	--
2	150	(136-158)	500	(429-500)	233	<0.001*
3	150	(143-150)	500	(500-500)	233	<0.001*
4	120	(107-130)	167	(136-214)	39	0.02*
5	100	(100-103)	500	(500-500)	400	<0.001*
6	100	(96-122)	354	(333-429)	254	<0.001*
7	167	(167-168)	429	(429-500)	156	<0.001*
8	94	(91-100)	375	(375-375)	298	<0.001*
9	81	(81-88)	375	(333-375)	362	<0.001*
10	143	(143-150)	375	(333-375)	162	<0.001*
11	100	(94-103)	429	(375-429)	329	<0.001*
12	102	(84-103)	429	(425-437)	321	<0.001*
13	91	(89-97)	438	(429-444)	381	<0.001*
14	240.5	(199-251)	435	(415-451)	81	<0.001*
15	141	(132-157)	380	(374-400)	170	<0.001*

Table 6: Plasma corticosterone (CORT) values for undisturbed (UND) and disturbed (DIS) sessions. Any values not obtained within 3 minutes of initiating capture marked with * or **. See notes below table for details on collection time. Percent change from UND to DIS calculated by subtracting UND from DIS, dividing by DIS, and multiplying by 100.

Subject	UND CORT (pg/mL)	DIS CORT (pg/mL)	Percent change UND to DIS
1	130.5	210.8	61.5
2	405.3	NA	NA
3	82.45	127	54.0
4	57.56	95.45	65.8
5	76.56	91.81	19.9
6	58.61	66.5**	13.5
7	89.36	173	93.6
8	299.8	216.7**	-27.7
9	229.3	379.5	65.5
10	49.41*	188.6**	281.7
11	48.61	81.11	66.9
12	80.84*	93.7	15.9
13	63.04	234.8	272.5
14	145.1	129.3	-10.9
15	483.4	1215	151.3

*venipuncture >3 minutes after initiating capture, but obtained within 3 minutes 10 seconds

**venipuncture >3 minutes after initiating capture, but obtained within 3 minutes and 45 seconds

Table 7: Summary of duration of displayed behaviors within the analyzed 6 minute and 45 second time period (total of 405 seconds), during undisturbed and disturbed sessions. Median and interquartile (IQR) reported only from data of those birds who performed that particular behavior, with percent of birds perfoming that behavior also reported. P value calculated from Wilcoxon Signed Rank test (alpha = 0.05) using data from all subjects, significant values marked with asterisk.

	Undisturbed			**Disturbed**			
	% of Birds (n=15)	Duration of Behavior Median	(IQR) (sec)	% of Birds (n=15)	Duration of Behavior Median	(IQR) (sec)	P-value
Perching	80%	340.5	(228-384.75)	87%	27	(6-68)	0.046*
Standing on floor	0%	--		13%	4.5	(2.75-6.25)	0.16
Foot-tucking	47%	191	(162-405)	27%	38.5	(12.5-116.75)	0.12
Standing on one foot	27%	4	(2.75-6.5)	27%	6.5	(3.75-9.25)	0.96
Jumping to new perch	0%	--		27%	1	(1-1)	0.04*
Lateral movement on perch	20%	6	(4-15)	67%	33.5	(21.25-41)	<0.01*
Flying	13%	3	(2-4)	53%	8	(5.5-15.5)	0.01*
Turning around	20%	3	(2.5-9)	53%	8	(3.75-12.5)	0.06
Walking on floor	0%	--		20%	2	(1.5-2)	0.08
Preening	60%	21	(13-67)	13%	12	(7-17)	<0.01*
Rousing	33%	2	(2-5)	27%	2.5	(2-3.25)	0.72
Stretching	13%	6.5	(4.75-8.25)	0%	--		0.16
Tail shaking	47%	1	(1-2.5)	73%	6	(4-10.5)	0.01*
Head shaking	13%	1.5	(1.25-1.75)	0%	--		0.16
Leaning forwards	33%	21	(10-27)	87%	279	(248-306)	<0.001*
Wing extension	20%	2	(1.5-9.5)	67%	10	(7-14.75)	<0.01*
Crouching	7%	3	(3-3)	53%	10.5	(4.75-18.25)	<0.01*
Muting	27%	2	(1-3)	53%	2	(1-3)	0.15

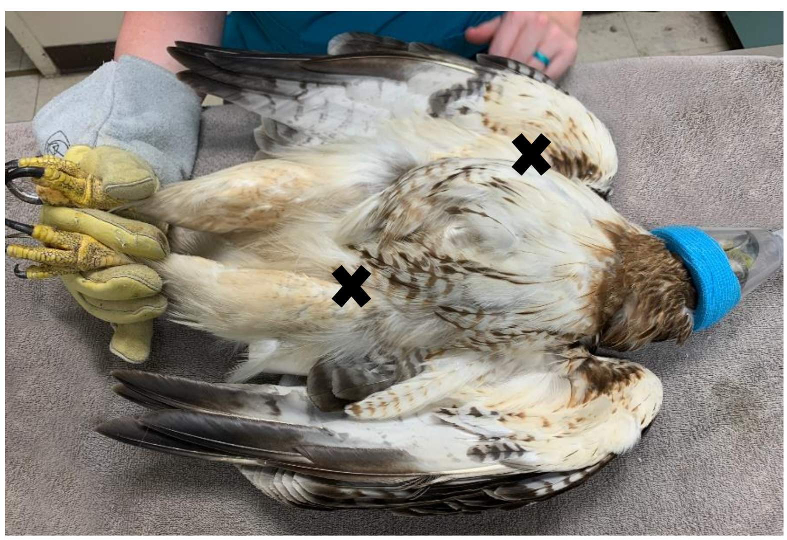
Table 8: Summary of frequency of displayed behaviors within the analyzed 6 minute and 45 second time period (total of 405 seconds), during undisturbed and disturbed sessions. Median and interquartile (IQR) reported only from data of those birds who performed that particular behavior, with percent of birds perfoming that behavior also reported. P value calculated from Wilcoxon Signed Rank test (alpha = 0.05) using data from all subjects, significant values marked with asterisk.

	Undisturbed			***Disturbed***			
	% of Birds (n=15)	Frequency of Behavior Median	(IQR)	% of Birds (n=15)	Frequency of Behavior Median	(IQR)	P value
Perching	80%	4.5	(1.75-8.25)	87%	1	(1-3)	0.17
Standing on floor	0%	--		13%	1.5	(1.25-1.75)	0.16
Foot-tucking	47%	1	(1-2)	27%	1.5	(1-2)	0.31
Standing on one foot	27%	2	(1.75-2)	27%	2	(1-3)	0.98
Jumping to new perch	0%	--		27%	1	(1-1)	0.04*
Lateral movement on perch	20%	1	(1-4)	67%	10	(6.5-16.25)	<0.01*
Flying	13%	2.5	(1.75-3.25)	53%	3.5	(1.75-5.5)	0.02*
Turning around	20%	1	(1-3.5)	53%	5	(1-7.5)	0.04*
Walking on floor	0%	--		20%	1	(1-1)	0.08
Preening	60%	3	(2-11)	13%	2.5	(1.75-3.25)	<0.01*
Rousing	33%	1	(1-1)	27%	1	(1-1)	0.64
Stretching	13%	1.5	(1.25-1.75)	0%	--		0.16
Tail shaking	47%	1	(1-2)	73%	5	(3-6.5)	0.01*
Head shaking	13%	1.5	(1.25-1.75)	0%	--		0.16
Leaning forwards	33%	3	(2-4)	87%	19	(13-25)	<0.001*
Wing extension	20%	1	(1-4.5)	67%	7.5	(5-9.5)	<0.01*
Crouching	7%	2	(2-2)	53%	5	(3.75-6)	<0.01*
Muting	27%	1	(1-1)	53%	1	(1-1.25)	0.11

Figures

Figure 1: Heart rate monitor placement on red-tailed hawks under general anesthesia. Image A shows a Medtronic Reveal LINQ™ monitor adhered to the skin with a conductive hydrogel patch after plucking the left pectoral area. Image B shows how the body feathers are replaced to cover the monitor after placement. The X's in image B indicate the approximate locations used for needle electrodes (standard bipolar limb lead II placement) when collecting data with the Vetcorder Pro.

A



B

Figure 2: Paired data plots of heart rate data from four anesthetized red-tailed hawks (21-977, 21-947, 21-833, and 21-862) collected concurrently from the Medtronic Reveal LINQ™ (applied externally to the left pectoral area with a conductive hydrogel sheet) and Vetcorder Pro (using needling electrodes in standard bipolar limb lead II placement)

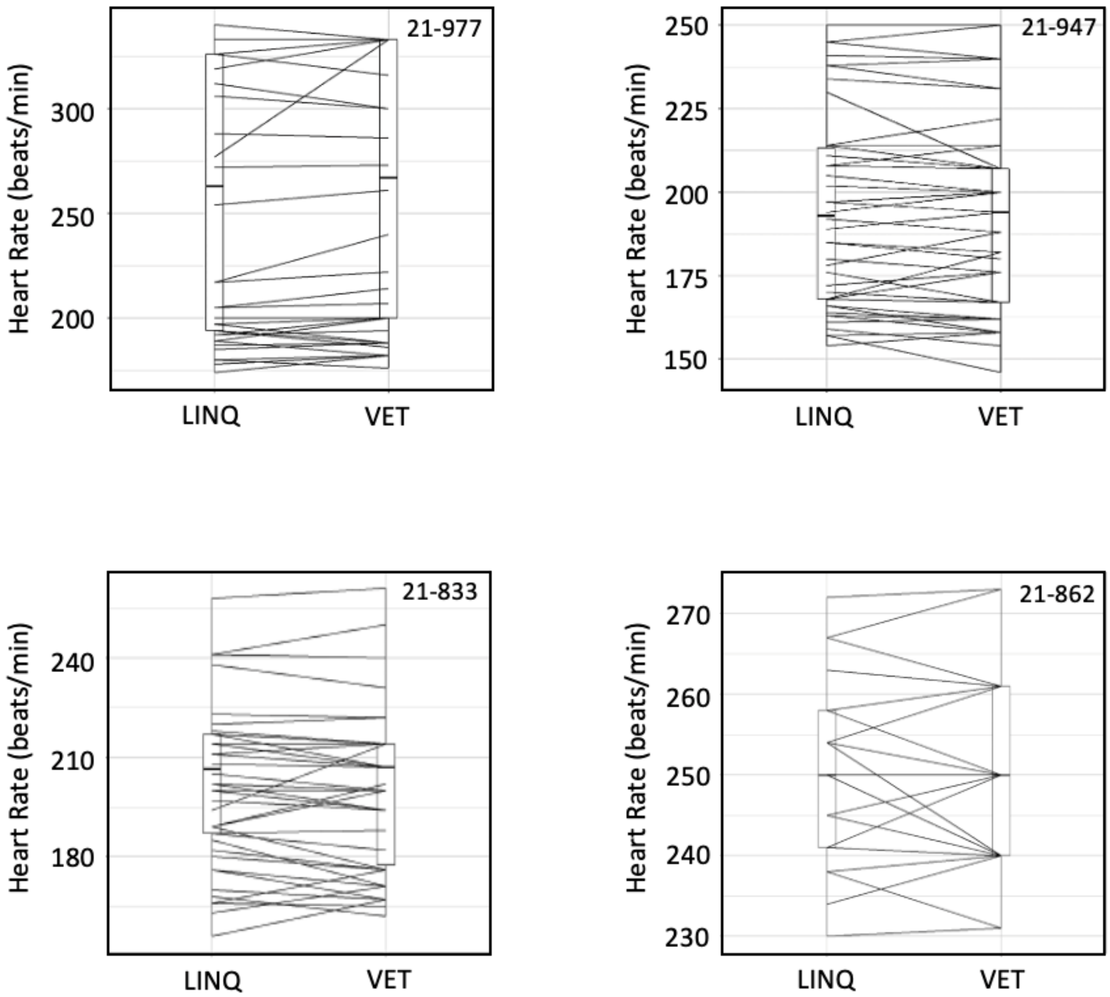


Figure 3. Graphical depiction of overall study timeline (image A) including heart rate monitor placement and sampling. The black triangles indicate data collection events. Data collection included venipuncture for plasma corticosterone, heart rate monitoring, and video recording of behavior outlined further in images B & C.

Image A: Summary of overall study timeline

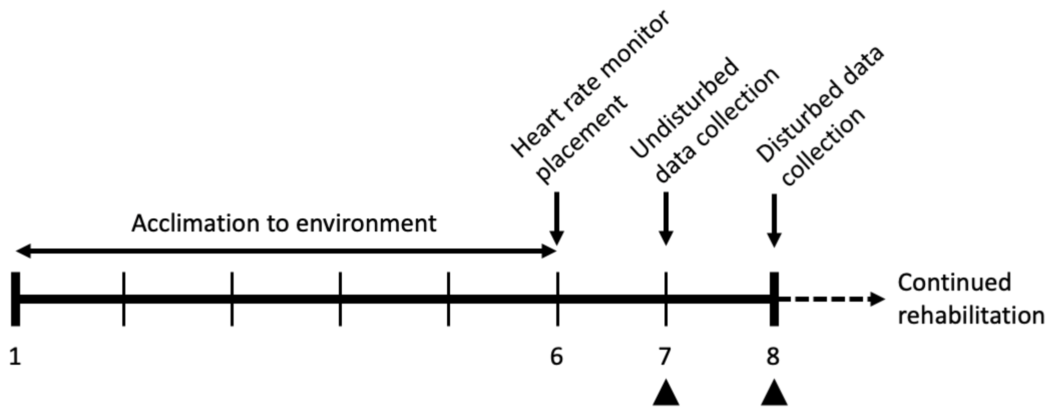


Image B: Detailed timeline of undisturbed (UND) data collection on day 7 of study

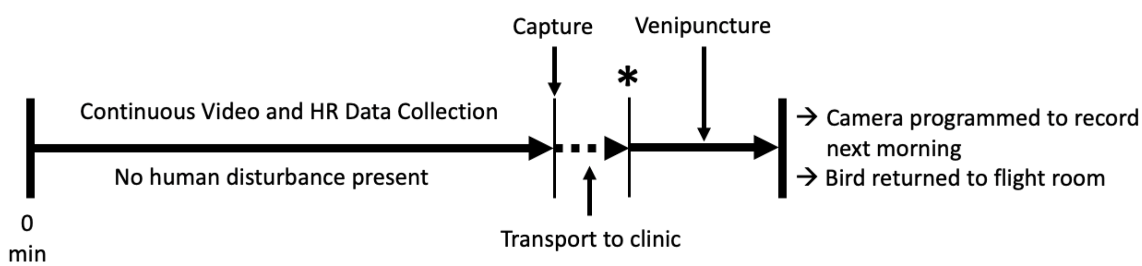


Image C: Detailed timeline of disturbed (DIS) data collection on day 8 of study

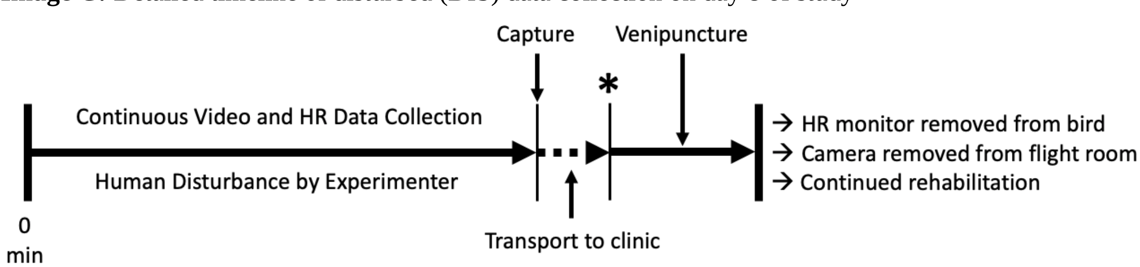


Figure 4. Representative schematic of a flight room setup provided for study subjects. Setup varied slightly based on perch availability. Birds were provided with a variety of perch locations and textures, and some birds were provided with a water pan. A GoPro Hero 9 camera was located in the corner of the room by the door, attached to a branch perch with a clip mount. Field-of-view indicated by dashed arrows. Room dimensions ~110"W x 90"D x 87"H, with padded inner walls.

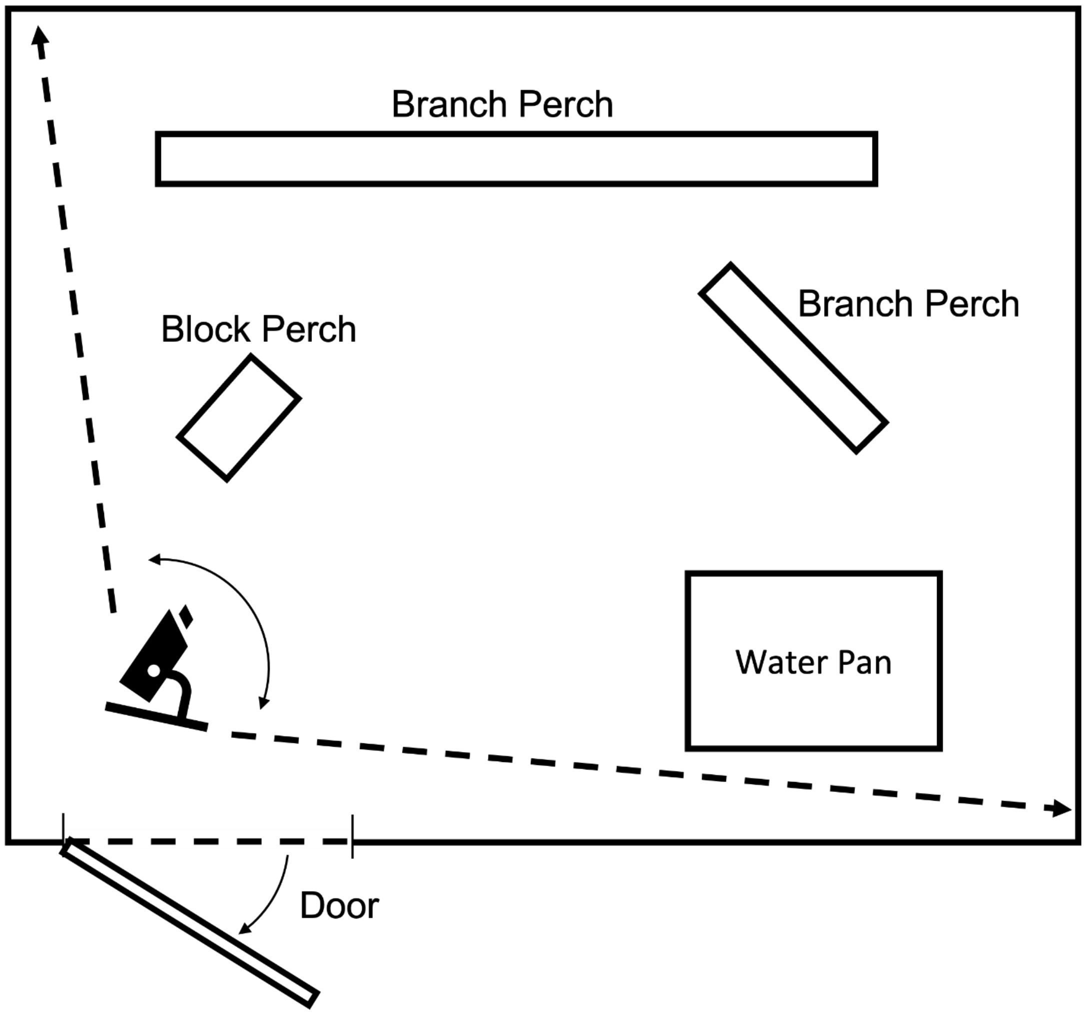
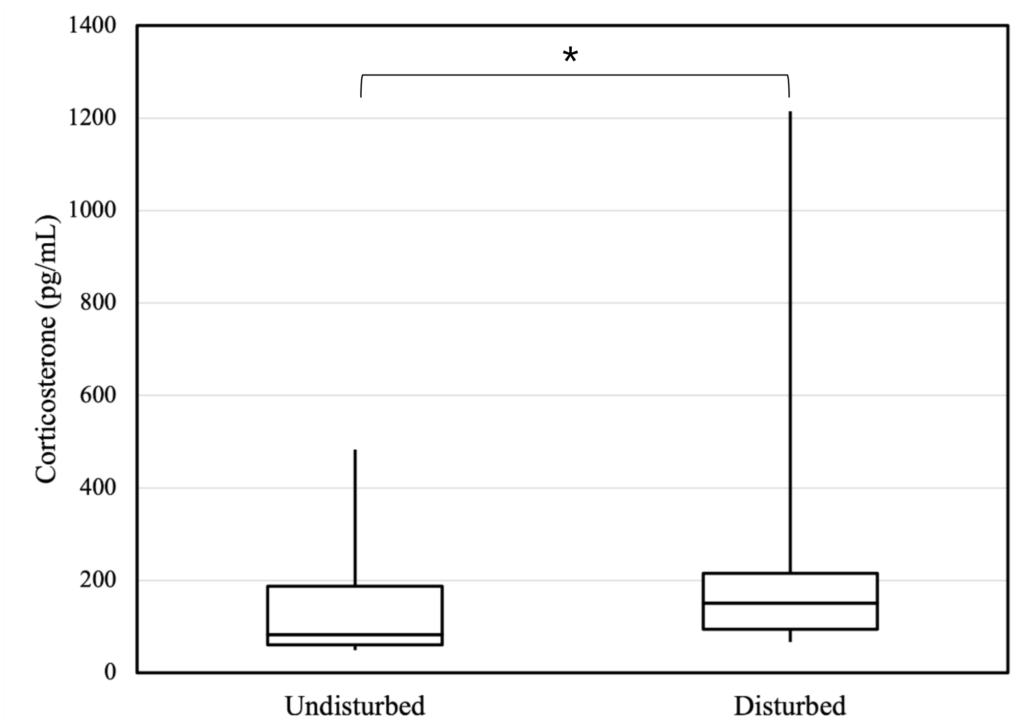


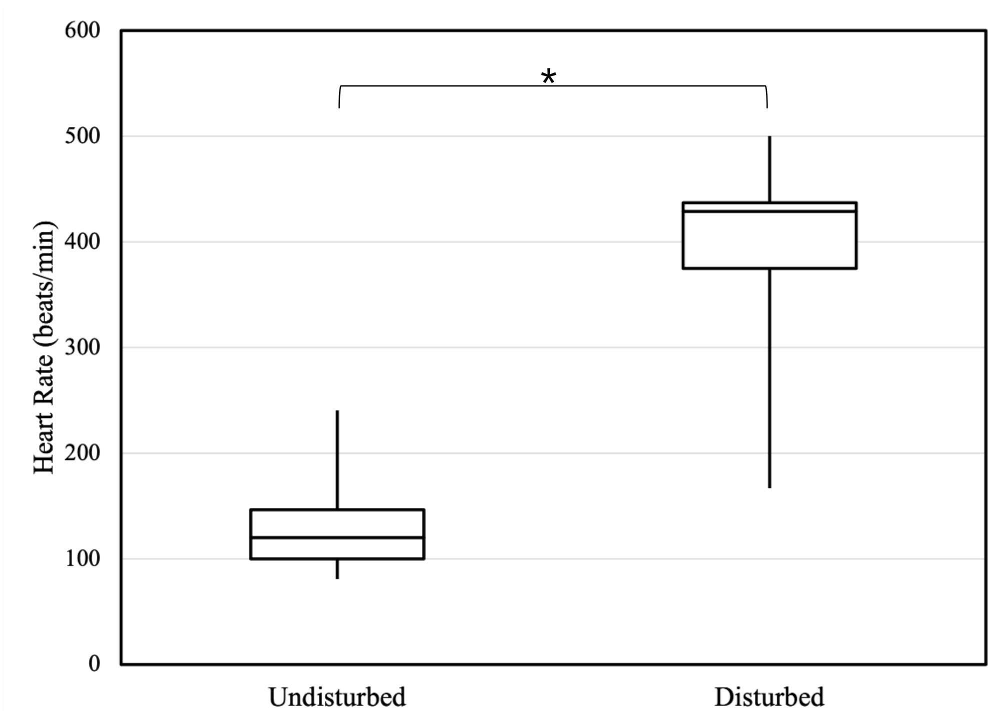
Figure 5: Behavioral ethogram for wild-red-tailed hawks housed individually in an indoor flight room during wildlife rehabilitation.

Behavior	Behavior description
Perching	The bird is standing upright with both feet in contact with a perch. It may move its head intermittently to look around the room. The angle of the tail feathers is nearly perpendicular to the floor.
Standing on floor	The bird is standing upright with both feet in contact with the floor.
Head tucking	The bird has its head tucked under either wing while standing or laying down. It may be on the ground or on a perch.
Foot tucking	The bird is standing with one foot tucked up towards the body and is only weight-bearing on the other foot. The foot that is tucked is fully or nearly fully obscured by the body feathers
Laying down	The bird is laying with its legs tucked under its body, and the front of the body and keel are resting on the floor. Wings are tucked next to the. body
Standing on one foot	The birds is standing on one foot, but the non-weight-bearing foot and leg remain extended or only partially flexed. The elevated foot is not tucked up under the body feathers
Changing perches	The bird moves from one perch to another in a controlled manner, either by walking, hopping, or flying. The bird may turn and look at the perch momentarily before moving to it.
Lateral perch movement	The bird is moving laterally from one area of a perch to another area along the same perch, either by walking or hopping, and the movement appears controlled (no stumbling). It may flap or extend its wings for balance, but it does not take flight.
Flying	The bird flaps both wings and takes flight.
Turning around	The bird performs a single 180º turn while perched and pauses or switches to a different behavior. It does not repeatedly turn around without pauses.
Preening	The bird is running its beak along and/or lightly biting its feathers.
Rousing	The bird erects its feathers momentarily, briefly shakes its whole body, then relaxes the feathers down to a normal position. The bird may also flick, or shake their head back and forth immediately following this behavior, or may shake their tail as well as the body.
Stretching	The bird is stretching out a leg, wing, or both. Bird may stretch both wings above head, with wrists forward and sometimes touching as the bird leans forward
Scratching	The bird is using one foot to scratch along its face, neck, or elsewhere on its body.
Feaking	The bird is leaning down and rubbing its beak on a perch. It is a behavior usually associated with cleaning the beak after eating.
Bathing	The bird is taking a bath in its bath pan (if available). This usually involves fluffing the feathers along the body and flicking its head. The bird may also flap its wings during this behavior or rub head/head feathers on the wings or back in a rhythmic motion
Tail shake	The bird rustles its tail feathers from side to side, but it does not fully rouse or shake its whole body.

Eating	The bird is ingesting food. It may be standing and pulling on food with its beak in an effort to rip smaller pieces off to then swallow.
Mantling	The bird is hunched and spreading/fanning its wings and tail over food or an object to conceal it. The bird is not considered to be mantling if there is no food present, even if a similar posture is being displayed.
Leaning forwards	The bird is standing with its weight shifted forward, or with its head carriage shifted forward compared to a relaxed perching position which appears upright. The wings are still tucked along the body and there is no visible gap between the side of the body and the carpus, but there may be some tension visible in the shoulders and wings. The angle of the tail feathers is affected due to the forward shift of the body, which elevates the tail slightly relative to the perpendicular angle noted when perching.
Wing extension	The bird extends the wings out so that the carpi and ventral surface of the wings are no longer in contact with the sides of the body, and the wings may be only slightly or fully extended. The bird may extend the wings out and then immediately tuck them back along the body in a normal perching position immediately, it may extend and tuck the wings back along the body several times, or it may leave the wings extended out for a prolonged period of time. The bird's body weight may also be shifted forward.
Crouching	The bird is hunched forward with the wings partially or mostly spread and appears to be about to take flight. The bird's weight is shifted forward significantly (more dramatic than seen with leaning forward or wing extension), and the angle of the body is nearly horizontal (parallel to the floor). The bird may or may not take flight after this behavior. It may also be looking around the room mechanically while performing this behavior.
Flight	The bird takes flight by flapping both wings and both feet lose contact with the perch. It may land on a perch or the floor immediately or may circle the room before landing.
Spinning	The bird is standing on a perch and rapidly turns around 360 degrees or more, so that it appears to be spinning in place. It may also spread or flap its wings for balance, but it does not take flight. There is very minimal lateral movement along the perch with this behavior.
Muting	The bird is eliminating waste, typically by leaning forward slightly prior to lifting tail its tail and expelling mutes
Casting pellet	The bird expels a pellet from its beak. It may appear like the bird is regurgitating.

Figure 6**:** Box plots of heart rate (HR) data and plasma corticosterone (CORT) concentrations collected from undisturbed and disturbed red-tailed hawks. P value calculated from Wilcoxon Signed Rank test (alpha = 0.05), significant comparisons marked with asterisk.





Bibliography

1. Siegel HS. Physiological Stress in Birds. *Bioscience*. 1980;30(8):529-534. doi:10.2307/1307973
2. Schoech SJ, Rensel MA, Heiss RS. Short- and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: A review. *Curr Zool*. 2011;57(4):514-530. doi:10.1093/czoolo/57.4.514
3. Romero LM. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol*. 2004;19(5):249-255. doi:10.1016/j.tree.2004.03.008
4. Martin LB. Stress and immunity in wild vertebrates: Timing is everything. *Gen Comp Endocrinol*. 2009;163(1-2):70-76. doi:10.1016/j.ygcen.2009.03.008
5. Sapolsky RM, Romero LM, Munck AU. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. *Endocr Rev*. 2000;21(1):55-89. doi:10.1210/edrv.21.1.0389
6. Pakkala JJ, Ryan Norris D, Newman AEM. An experimental test of the capture-restraint protocol for estimating the acute stress response. *Physiol Biochem Zool*. 2013;86(2):279-284. doi:10.1086/668893
7. Arent LR, Willette M, Buhl G. Raptors as Victims and Ambassadors: Raptor Rehabilitation, Education, and Outreach. In: *Urban Raptors*. Washington, DC: Island Press/Center for Resource Economics; 2018:229-245. doi:10.5822/978-1-61091-841-1_16
8. Doss GA, Mans C. The Effect of Manual Restraint on Physiological Parameters in Barred Owls (*Strix varia*). *J Avian Med Surg*. 2017;31(1):1-5. doi:10.1647/2016-167
9. Doss GA, Mans C. Changes in Physiologic Parameters and Effects of Hooding in Red-tailed Hawks (*Buteo jamaicensis*) During Manual Restraint. *J Avian Med Surg*. 2016;30(2):127-132. doi:10.1647/2015-096
10. Mans C, Guzman DS-M, Lahner LL, Paul-Murphy J, Sladky KK. Sedation and Physiologic Response to Manual Restraint After Intranasal Administration of Midazolam in Hispaniolan Amazon Parrots (Amazona ventralis). *J Avian Med Surg*. 2012;26(3):130-139. doi:10.1647/2011-037R.1
11. Padgett DA, Marucha PT, Sheridan JF. Restraint Stress Slows Cutaneous Wound Healing in Mice. *Brain Behav Immun*. 1998;12(1):64-73. doi:10.1006/brbi.1997.0512
12. French SS, Matt KS, Moore MC. The effects of stress on wound healing in male tree lizards (Urosaurus ornatus). *Gen Comp Endocrinol*. 2006;145(2):128-132. doi:10.1016/j.ygcen.2005.08.005
13. Scott D. Natural History and Medical Management of Raptors. In: *Medical Management of Wildlife Species*. Wiley; 2019:215-228. doi:10.1002/9781119036708.ch17
14. Turpen KK, Welle KR, Trail JL, Patel SD, Allender MD. Establishing Stress Behaviors in Response to Manual Restraint in Cockatiels (Nymphicus hollandicus).
15. Bebus SE, Jones BC, Schoech SJ. Link between past threatening experience and future neophobic behaviour depends on physiological stress responsiveness. *Anim Behav*. 2020;167:233-241. doi:10.1016/j.anbehav.2020.07.017

16. Fraisse F, Cockrem JF. Corticosterone and fear behaviour in white and brown caged laying hens. *Br Poult Sci*. 2006;47(2):110-119. doi:10.1080/00071660600610534
17. Cockrem JF, Silverin B. Sight of a Predator Can Stimulate a Corticosterone Response in the Great Tit (Parus major). *Gen Comp Endocrinol*. 2002;125(2):248-255. doi:10.1006/gcen.2001.7749
18. Cockrem JF, Potter MA, Barrett DP, Candy EJ. Corticosterone Responses to Capture and Restraint in Emperor and Adelie Penguins in Antarctica. *Zoolog Sci*. 2008;25(3):291-298. doi:10.2108/zsj.25.291
19. Edwards LE, Botheras NA, Coleman GJ, Hemsworth PH. Behavioural and physiological responses of laying hens to humans. *Anim Prod Sci*. 2010;50(6):557. doi:10.1071/AN09227
20. Wein Y, Bar Shira E, Friedman A. Avoiding handling-induced stress in poultry: use of uniform parameters to accurately determine physiological stress. *Poult Sci*. 2017;96(1):65-73. doi:10.3382/ps/pew245
21. Bejaei M, Cheng KM. Effects of pretransport handling stress on physiological and behavioral response of ostriches. *Poult Sci*. 2014;93(5):1137-1148. doi:10.3382/ps.2013-03478
22. Barnett JL, Hemsworth PH. Fear of humans by laying hens in different tiers of a battery: Behavioural and physiological responses. *Br Poult Sci*. 1989;30(3):497-504. doi:10.1080/00071668908417174
23. Barnett JL, Hemsworth PH, Hennessy DP, McCallum TH, Newman EA. The effects of modifying the amount of human contact on behavioural, physiological and production responses of laying hens. *Appl Anim Behav Sci*. 1994;41(1-2):87-100. doi:10.1016/0168-1591(94)90054-X
24. Lattin CR, Merullo DP, Riters L V., Carson RE. In vivo imaging of D2 receptors and corticosteroids predict behavioural responses to captivity stress in a wild bird. *Sci Rep*. 2019;9(1):10407. doi:10.1038/s41598-019-46845-x
25. Carroll G, Turner E, Dann P, Harcourt R. Prior exposure to capture heightens the corticosterone and behavioural responses of little penguins (Eudyptula minor) to acute stress. *Conserv Physiol*. 2016;4(1):cov061. doi:10.1093/conphys/cov061
26. Adams NJ, Farnworth MJ, Rickett J, Parker KA, Cockrem JF. Behavioural and corticosterone responses to capture and confinement of wild blackbirds (Turdus merula). *Appl Anim Behav Sci*. 2011;134(3-4):246-255. doi:10.1016/j.applanim.2011.07.001
27. Müller C, Jenni-Eiermann S, Blondel J, et al. Effect of human presence and handling on circulating corticosterone levels in breeding blue tits (Parus caeruleus). *Gen Comp Endocrinol*. 2006;148(2):163-171. doi:10.1016/j.ygcen.2006.02.012
28. Carleton RE, Caldwell RM. It's in the bag: Corticosterone levels and behavioral responses of Eastern Bluebirds (Sialia sialis) vary with type of holding bag. *Wilson J Ornithol*. 2019;131(3):701. doi:10.1676/18-0017
29. Gormally BMG, Ramos S, Yin H, Romero LM. Recovery periods during repeated stress impact corticosterone and behavioral responses differently in house sparrows. *Horm Behav*. 2019;112:81-88. doi:10.1016/j.yhbeh.2019.04.009
30. Small TW, Bebus SE, Bridge ES, et al. Stress-responsiveness influences baseline

glucocorticoid levels: Revisiting the under 3 min sampling rule. *Gen Comp Endocrinol*. 2017;247:152-165. doi:10.1016/j.ygcen.2017.01.028
31. Rogers HM, Bechard MJ, Kaltenecker GS, Dufty AM. The Adrenocortical Stress Response in Three North American Accipiters During Fall Migration. *J Raptor Res*. 2010;44(2):113-119. doi:10.3356/JRR-09-59.1
32. Busch D, DeGraw W, Clampitt N. Effects of handling-disturbance stress on heart rate in the ferruginous hawk (Buteo regalis). *J Raptor Res*. 1978;12(3/4):122-125. http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=1&SID=2Fd5MH4NaeYngXumc5h&page=1&doc=1.
33. Patton KT, Croonquist MJ, Crawford WC. Management-related stress in the red-shouldered hawk. *Zoo Biol*. 1985;4(3):235-243. doi:10.1002/zoo.1430040304
34. Cabanac AJ, Guillemette M. Temperature and heart rate as stress indicators of handled common eider. *Physiol Behav*. 2001;74(4-5):475-479. doi:10.1016/S0031-9384(01)00586-8
35. Viblanc VA, Smith AD, Gineste B, Groscolas R. Coping with continuous human disturbance in the wild: insights from penguin heart rate response to various stressors. *BMC Ecol*. 2012;12(1):10. doi:10.1186/1472-6785-12-10
36. Nimon AJ, Schroter RC, Oxenham RKC. Artificial eggs: Measuring heart rate and effects of disturbance in nesting penguins. *Physiol Behav*. 1996;60(3):1019-1022. doi:10.1016/0031-9384(96)00079-0
37. Herborn KA, Graves JL, Jerem P, et al. Skin temperature reveals the intensity of acute stress. *Physiol Behav*. 2015;152:225-230. doi:10.1016/j.physbeh.2015.09.032
38. Bittencourt M de A, Melleu FF, Marino-Neto J. Stress-induced core temperature changes in pigeons (Columba livia). *Physiol Behav*. 2015;139:449-458. doi:10.1016/j.physbeh.2014.11.067
39. Jerem P, Herborn K, McCafferty D, McKeegan D, Nager R. Thermal Imaging to Study Stress Non-invasively in Unrestrained Birds. *J Vis Exp*. 2015;(105). doi:10.3791/53184
40. Remage-Healey L, Romero LM. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. *Am J Physiol Integr Comp Physiol*. 2001;281(3):R994-R1003. doi:10.1152/ajpregu.2001.281.3.R994
41. Malisch JL, Bennett DJ, Davidson BA, Wenker EE, Suzich RN, Johnson EE. Stress-Induced Hyperglycemia in White-Throated and White-Crowned Sparrows: A New Technique for Rapid Glucose Measurement in the Field. *Physiol Biochem Zool*. 2018;91(4):943-949. doi:10.1086/698536
42. Jeronen E, Isometsä P, Hissa R, Pyörnilä A. Effect of acute temperature stress on the plasma catecholamine, corticosterone and metabolite levels in the pigeon. *Comp Biochem Physiol Part C Comp Pharmacol*. 1976;55(1):17-22. doi:10.1016/0306-4492(76)90005-8
43. Huber N, Fusani L, Ferretti A, Mahr K, Canoine V. Measuring short-term stress in birds: Comparing different endpoints of the endocrine-immune interface. *Physiol Behav*. 2017;182:46-53. doi:10.1016/j.physbeh.2017.09.017
44. Gray DA, Maloney SK, Kamerman PR. Restraint increases afebrile body temperature but attenuates fever in Pekin ducks (Anas platyrhynchos). *Am J Physiol Integr Comp Physiol*. 2008;294(5):R1666-R1671.

doi:10.1152/ajpregu.00865.2007
45. Scanes CG. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poult Sci*. 2016;95(9):2208-2215. doi:10.3382/ps/pew137
46. Greene ES, Rajaei-Sharifabadi H, Dridi S. Feather HSP70: a novel non-invasive molecular marker for monitoring stress induced by heat exposure in broilers. *Poult Sci*. 2019;98(9):3400-3404. doi:10.3382/ps/pez120
47. de Jong IC, van Voorst AS, Erkens JH., Ehlhardt DA, Blokhuis HJ. Determination of the circadian rhythm in plasma corticosterone and catecholamine concentrations in growing broiler breeders using intravenous cannulation. *Physiol Behav*. 2001;74(3):299-304. doi:10.1016/S0031-9384(01)00562-5
48. Romero LM, Remage-Healey L. Daily and Seasonal Variation in Response to Stress in Captive Starlings (Sturnus vulgaris): Corticosterone. *Gen Comp Endocrinol*. 2000;119(1):52-59. doi:10.1006/gcen.2000.7491
49. WILSON SC, CUNNINGHAM FJ. EFFECT OF PHOTOPERIOD ON THE CONCENTRATIONS OF CORTICOSTERONE AND LUTEINIZING HORMONE IN THE PLASMA OF THE DOMESTIC HEN. *J Endocrinol*. 1981;91(1):135-143. doi:10.1677/joe.0.0910135
50. Rich EL, Romero LM. Exposure to chronic stress downregulates corticosterone responses to acute stressors. *Am J Physiol Integr Comp Physiol*. 2005;288(6):R1628-R1636. doi:10.1152/ajpregu.00484.2004
51. Romero LM, Reed JM. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp Biochem Physiol Part A Mol Integr Physiol*. 2005;140(1):73-79. doi:10.1016/j.cbpb.2004.11.004
52. Romero LM, Romero RC. Corticosterone Responses in Wild Birds: The Importance of Rapid Initial Sampling. *Condor*. 2002;104(1):129-135. doi:10.1093/condor/104.1.129
53. Millspaugh JJ, Washburn BE. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *Gen Comp Endocrinol*. 2004;138(3):189-199. doi:10.1016/j.ygcen.2004.07.002
54. Tarjuelo R, Barja I, Morales MB, et al. Effects of human activity on physiological and behavioral responses of an endangered steppe bird. *Behav Ecol*. 2015;26(3):828-838. doi:10.1093/beheco/arv016
55. Romero LM, Fairhurst GD. Measuring corticosterone in feathers: Strengths, limitations, and suggestions for the future. *Comp Biochem Physiol Part A Mol Integr Physiol*. 2016;202:112-122. doi:10.1016/j.cbpa.2016.05.002
56. EXPÓSITO-GRANADOS M, PAREJO D, CHASTEL O, AVILÉS JM. Physiological stress and behavioural responses of European Rollers and Eurasian Scops Owls to human disturbance differ in farming habitats in the south of Spain. *Bird Conserv Int*. 2020;30(2):220-235. doi:10.1017/S0959270919000388
57. Wasser S, Bevis K, King G, Hanson E. Noninvasive Physiological Measures of Disturbance in Northern Spotted Owl. *Conserv Biol*. 1997;11(4):1019-1022.
58. Rich GA. Basic History Taking and the Avian Physical Examination. *Vet Clin North Am Small Anim Pract*. 1991;21(6):1135-1145. doi:10.1016/S0195-5616(91)50128-5
59. Heatley JJ, Marks S, Mitchell M. Raptor emergency and critical care: therapy and

techniques. *Compendium*. 2001;23:561-570.
60. Kettlewell PJ, Mitchell MA, Meeks IR. An implantable radio-telemetry system for remote monitoring of heart rate and deep body temperature in poultry. *Comput Electron Agric*. 1997;17(2):161-175. doi:10.1016/S0168-1699(96)01302-6
61. Cabanac M, Aizawa S. Fever and tachycardia in a bird (Gallus domesticus) after simple handling. *Physiol Behav*. 2000;69(4-5):541-545. doi:10.1016/S0031-9384(00)00227-4
62. Moreira KF, Neves CQ, Borges SC, et al. Acute thermal stress promotes morphological and molecular changes in the heart of broiler chickens. *Res Soc Dev*. 2020;9(8):e63985059. doi:10.33448/rsd-v9i8.5059
63. Porter-Blackwell R, Paul-Murphy JR, le Jeune SS, Thermography CV, Martínez-López B, Seibert BA. Pilot Study: Correlation of the Surface Skin Temperature Between the Leg and Foot Using Thermographic Imaging in Captive Hawks. *J Avian Med Surg*. 2020;34(2):164. doi:10.1647/1082-6742-34.2.164
64. Melero M, González F, Nicolás O, et al. Detection and assessment of electrocution in endangered raptors by infrared thermography. *BMC Vet Res*. 2013;9(1):149. doi:10.1186/1746-6148-9-149
65. Hausberger M, Lerch N, Guilbaud E, et al. On-Farm Welfare Assessment of Horses: The Risks of Putting the Cart before the Horse. *Animals*. 2020;10(3):371. doi:10.3390/ani10030371
66. Asher L, Collins LM, Ortiz-Pelaez A, Drewe JA, Nicol CJ, Pfeiffer DU. Recent advances in the analysis of behavioural organization and interpretation as indicators of animal welfare. *J R Soc Interface*. 2009;6(41):1103-1119. doi:10.1098/rsif.2009.0221
67. Auer U, Kelemen Z, Engl V, Jenner F. Activity Time Budgets—A Potential Tool to Monitor Equine Welfare? *Animals*. 2021;11(3):850. doi:10.3390/ani11030850
68. Miller LJ, Vicino GA, Sheftel J, Lauderdale LK. Behavioral Diversity as a Potential Indicator of Positive Animal Welfare. *Animals*. 2020;10(7):1211. doi:10.3390/ani10071211
69. Huth JC, Archer GS. Comparison of Two LED Light Bulbs to a Dimmable CFL and their Effects on Broiler Chicken Growth, Stress, and Fear. *Poult Sci*. 2015;94(9):2027-2036. doi:10.3382/ps/pev215
70. Son J-H. The Effect of Stocking Density on the Behaviour and Welfare Indexes of Broiler Chickens. *Journla Agric Sci Technol*. 2013;A(3):307-311.
71. Hampshire V, Robertson S. Using the facial grimace scale to evaluate rabbit wellness in post-procedural monitoring. *Lab Anim (NY)*. 2015;44(7):259-260. doi:10.1038/laban.806
72. Oliver VL, Athavale S, Simon KE, Kendall L V, Nemzek JA, Lofgren JL. Evaluation of Pain Assessment Techniques and Analgesia Efficacy in a Female Guinea Pig (Cavia porcellus) Model of Surgical Pain. *J Am Assoc Lab Anim Sci*. 2017;56(4):425-435. http://www.ncbi.nlm.nih.gov/pubmed/28724492.
73. Gleerup KB, Andersen PH, Munksgaard L, Forkman B. Pain evaluation in dairy cattle. *Appl Anim Behav Sci*. 2015;171:25-32. doi:10.1016/j.applanim.2015.08.023
74. Mayer J. Use of behavior analysis to recognize pain in small mammals. *Lab Anim (NY)*. 2007;36(6):43-48. doi:10.1038/laban0607-43
75. Mazor-Thomas JE, Mann PE, Karas AZ, Tseng F. Pain-suppressed behaviors in

the red-tailed hawk (Buteo jamaicensis). *Appl Anim Behav Sci*. 2014;152:83-91. doi:10.1016/j.applanim.2013.12.011

76. Morgan KN, Tromborg CT. Sources of stress in captivity. *Appl Anim Behav Sci*. 2007;102(3-4):262-302. doi:10.1016/j.applanim.2006.05.032
77. Smolinsky AN, Bergner CL, LaPorte JL, Kalueff A V. Analysis of Grooming Behavior and Its Utility in Studying Animal Stress, Anxiety, and Depression. In: ; 2009:21-36. doi:10.1007/978-1-60761-303-9_2
78. Tu B-X, Wang L-F, Zhong X-L, et al. Acute restraint stress alters food-foraging behavior in rats: Taking the easier Way while suffered. *Brain Res Bull*. 2019;149:184-193. doi:10.1016/j.brainresbull.2019.04.021
79. Bazhan N, Zelena D. Food-intake regulation during stress by the hypothalamo-pituitary-adrenal axis. *Brain Res Bull*. 2013;95:46-53. doi:10.1016/j.brainresbull.2013.04.002
80. Koolhaas J., Korte S., De Boer S., et al. Coping styles in animals: current status in behavior and stress-physiology. *Neurosci Biobehav Rev*. 1999;23(7):925-935. doi:10.1016/S0149-7634(99)00026-3
81. Tomson TT, Passman R. The Reveal LINQ insertable cardiac monitor. *Expert Rev Med Devices*. 2015;12(1):7-18. doi:10.1586/17434440.2014.953059
82. Buhl R, Hesselkilde EM, Carstensen H, et al. Detection of atrial fibrillation with implantable loop recorders in horses. *Equine Vet J*. 2021;53(2):397-403. doi:10.1111/evj.13301
83. Buhl R, Nissen SD, Winther MLK, et al. Implantable loop recorders can detect paroxysmal atrial fibrillation in Standardbred racehorses with intermittent poor performance. *Equine Vet J*. 2021;53(5):955-963. doi:10.1111/evj.13372
84. Iles TL, Laske TG, Garshelis DL, et al. Medtronic Reveal LINQ™ Devices Provide Better Understanding of Hibernation Physiology in the American Black Bear (Ursus Americanus). In: *2017 Design of Medical Devices Conference*. American Society of Mechanical Engineers; 2017. doi:10.1115/DMD2017-3498
85. Laske TG, Garshelis DL, Iaizzo PA. Big data in wildlife research: remote web-based monitoring of hibernating black bears. *BMC Physiol*. 2014;14(1):13. doi:10.1186/s12899-014-0013-1
86. Laske TG, Evans AL, Arnemo JM, et al. Development and utilization of implantable cardiac monitors in free-ranging American black and Eurasian brown bears: system evolution and lessons learned. *Anim Biotelemetry*. 2018;6(1):13. doi:10.1186/s40317-018-0157-z
87. Jansen HT, Evans Hutzenbiler B, Hapner HR, et al. Can offsetting the energetic cost of hibernation restore an active season phenotype in grizzly bears (Ursus arctos horribilis)? *J Exp Biol*. 2021;224(12). doi:10.1242/jeb.242560
88. Moraes RN, Laske TG, Leimgruber P, et al. Inside out: heart rate monitoring to advance the welfare and conservation of maned wolves (Chrysocyon brachyurus). Cooke S, ed. *Conserv Physiol*. 2021;9(1). doi:10.1093/conphys/coab044
89. Woulfe KC, Wilson CE, Nau S, et al. Acute isoproterenol leads to age-dependent arrhythmogenesis in guinea pigs. *Am J Physiol Circ Physiol*. 2018;315(4):H1051-H1062. doi:10.1152/ajpheart.00061.2018
90. Heath JA, Dufty, Jr. AM. Body Condition and the Adrenal Stress Response in Captive American Kestrel Juveniles. *Physiol Zool*. 1998;71(1):67-73.

doi:10.1086/515888
91. Dufty AM, Belthoff JR. Corticosterone and the Stress Response in Young Western Screech-Owls: Effects of Captivity, Gender, and Activity Period. *Physiol Zool*. 1997;70(2):143-149. doi:10.1086/639564
92. Scott DE. Housing and Husbandry. In: *Raptor Medicine, Surgery, and Rehabilitation*. ; 2016:270-272.
93. Miller E, Schlieps J, eds. *Standards for Wildlife Rehabilitation*. National Wildlife Rehabilitators Association: Bloomington, MN; 2021.
94. Haman MA. Using Biomarkers to Optimize the Rehabilitation of Wild Raptors. 2018.
95. Taff CC, Zimmer C, Vitousek MN. Efficacy of negative feedback in the HPA axis predicts recovery from acute challenges. *Biol Lett*. 2018;14(7):20180131. doi:10.1098/rsbl.2018.0131
96. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175-191. doi:10.3758/BF03193146
97. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149-1160. doi:10.3758/BRM.41.4.1149
98. Kinn Rød AM, Harkestad N, Jellestad FK, Murison R. Comparison of commercial ELISA assays for quantification of corticosterone in serum. *Sci Rep*. 2017;7(1):6748. doi:10.1038/s41598-017-06006-4
99. Glucs ZE, Smith DR, Tubbs CW, et al. Glucocorticoid measurement in plasma, urates, and feathers from California condors (Gymnogyps californianus) in response to a human-induced stressor. Vaudry H, ed. *PLoS One*. 2018;13(10):e0205565. doi:10.1371/journal.pone.0205565
100. Tetel V, Tonissen S, Fraley GS. Sex differences in serum glucocorticoid levels and heterophil:lymphocyte ratios in adult pekin ducks (Anas platyrhynchos domesticus). *Gen Comp Endocrinol*. 2022;317:113975. doi:10.1016/j.ygcen.2021.113975
101. Ditchkoff SS, Saalfeld ST, Gibson CJ. Animal behavior in urban ecosystems: Modifications due to human-induced stress. *Urban Ecosyst*. 2006;9(1):5-12. doi:10.1007/s11252-006-3262-3
102. LEHNER PN. Sampling Methods in Behavior Research. *Poult Sci*. 1992;71(4):643-649. doi:10.3382/ps.0710643