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ARTICLE

# Length of stay in rehabilitation influences magnitude of the acute stress response in birds of prey

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## Abstract

Wild animals are occasionally injured or become ill as a result of natural causes or human activity. As a way to remediate the damage imposed by humans on wildlife populations, these animals may be taken in for rehabilitation. Federal laws for rehabilitators set limits for the length of time a bird can remain in rehabilitation before a final decision on release or euthanasia is made. Little is known about the consequences of longer stays in captivity within that limited window with regard to stress physiology of the animals. The authors hypothesised that length of stay in rehabilitation would influence the ability of raptors to mount a full stress response upon release from rehabilitation. Blood samples were taken from each bird upon admission for rehabilitation and immediately prior to their release, a process by which the animals are exposed to a significant, acute stressor of capture, handling, and examination. A blood sample was collected for analysis of heterophil to lymphocyte ratios (H:L), which are indicative of long-term baseline stress. The authors found that as length of stay increased, the difference in corticosterone levels reached during an acute stressor from admission samples to release samples decreased. Baseline stress, as indicated by H:L, was not significantly affected by length of stay. The results suggest that birds of prey in rehabilitation habituate to captivity the longer they stay in captivity and may lose some degree of natural responsiveness to stressors.

**Keywords**
Rehabilitation; raptor; corticosterone; heterophil; lymphocyte

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**Abbreviations:**
ANCOVA: Analysis of covariance
CORT: corticosterone
USFWS: United States Fish & Wildlife Service
H:L: heterophil to lymphocyte ratio

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## Introduction

While disease and injury may occur through natural interactions among wildlife, human development, destruction of natural habitats, and release of harmful toxins into the environment are among several factors that pose threats to native wildlife (Willette et al. 2023). These factors can be lethal to wild animals, but they also pose sublethal threats to animals that may become ill, injured, or orphaned, and when they are found in that state, they may be taken in by wildlife rehabilitators. The ultimate goal of wildlife rehabilitation is to return wildlife back to their free-living state with no deficits in performance that would make them less capable of survival and reproduction than other free-living members of the species (Miller 2012; Willette et al. 2023). United States federal rehabilitation laws require that wildlife rehabilitators obtain permits to take wildlife into captivity and provide care, and those permits carry restrictions for how long an animal can be held in a rehabilitation facility before being released, euthanised, or transferred to a different permit such as educational use permits (50 CFR 21.12(b)). Among other purposes, these regulations protect birds from being kept as "pets" and also ensure that rehabilitators work diligently to release the animal back into a free-living condition as soon as possible. For birds, the length of stay in rehabilitation is limited to 180 days, after which a final disposition must be made, or in some cases, an extension or transfer to an education permit may be granted (USFWS permit 3-200-10b). Previous

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studies have demonstrated that there can be negative effects associated with bringing organisms into captivity, or for keeping animals in captivity for a long time prior to release. For example, in a study of chaffinches (*Fringilla coelebs*) brought into captivity for foraging trials, finches tested within two days of capture demonstrated proper foraging over 75% of the time, but for finches held in captivity for more than 12 days, the probability of proper foraging dropped below 50% (Butler et al. 2006). Time in captivity may also influence the post-release behaviour of animals, as was the case with great tits (*Parus major*) that were brought into captivity for behavioural trials, released, and later re-captured for different trials (Kluen et al. 2022). Those with experience in captivity were significantly more exploratory in subsequent captive trials than those who had never been captured (Kluen et al. 2022). Behavioural assays such as the examples described above are important tools in assessing the impact of captivity on the likelihood that an animal is prepared for a return to free-living, but there are also several physiological biomarkers that can aid in that assessment (Black et al. 2011). Giambelluca et al. (2017) found that griffon vultures (*Gyps fulvus*) held in captivity for 15 or 30 days showed no significant deviation from haematological parameters (haematocrit and white blood cell count) compared to free-living birds, but the birds held in captivity for two years showed significant differences in both of the blood measures relative to free-living birds and birds in short-term captivity. In a study of falcons (multiple species) repeatedly handled in captivity and compared to those infrequently handled, birds who were handled frequently showed habituation to handling with significantly reduced heart rates upon handling compared to those handled for the first time (Straub et al. 2003). Taken together, these studies demonstrate that time in captivity can influence behaviour and physiology of birds.

One major concern of being held in captivity is that residing in an environment free of normal biotic and abiotic interactions may lead to behavioural or physiological habituation (Davis & Maerz 2011). In permanent and temporary captivity for rehabilitation, animals are typically housed in safe enclosures, fed daily, and freed from the need to compete for resources. In the presence of common stressors, such as foraging pressures, competition, and predator encounters, all of the natural occurrences in the free-living state, animals require physiological or behavioural stress responses to face such challenges (Sapolsky et al. 2000; Wingfield 2003). In the absence of this stress response, an animal may be subject to further injury or even death from an inability to achieve an appropriate fight or flight response (Sapolsky et al. 2000). Likewise, stress responses are adaptive in that they can signal the sense of urgency to seek, and compete, for food or mates (Sapolsky et al. 2000; Wingfield 2003). As time in captivity progresses, birds may be less likely to routinely mount a full stress response; thus, when released as free-living birds, and exposed to stressors in the wild, it would be problematic if the time spent time in captivity for rehabilitation led to a dampened response, a lag in the response to the stimulus, or a failure to respond to the stressor altogether.

Standards for Wildlife Rehabilitation (Miller & Schlieps 2021) provides guidance to ensure measures are taken during the rehabilitation process to avoid imposing undue stress on patients. This starts with stabilisation of the animal using strategies that limit added stress, such as quick evaluation and placement of the patient in a secure, quiet, resting space. Following examination and determination of a course of treatment, treatment and intensive rehabilitation are done with appropriate husbandry and limited human interaction (Miller & Schlieps 2021). As the animal builds strength, increased mental stimulation is incorporated into the animal's rehabilitation routine, culminating in regular exercise and opportunities to eat a more natural diet (Miller & Schlieps 2021). These standards for preparing an animal for release, while limiting human interaction and maximising opportunities to simulate natural stressors from the wild (mental stimulation and natural diet acquisition), still leave rehabilitators searching for context-dependent clues and nuance associated with the unique nature of each individual patient. Indeed, contemporary scientific thinking can greatly improve the outcomes from wildlife rehabilitation, and such approaches are, perhaps, best incorporated via the Five Domains model for animal welfare assessment (Mellor & Beausoleil 2015; Mellor et al. 2020). This model appropriately distinguishes multiple domains that must be considered in order to provide a wholistic assessment of wildlife in rehabilitation and is comprised of: "nutrition," "environment," "health," and "behaviour" as internal, physiological factors; the fifth domain is "mental state" (Mellor & Beausoleil 2015; Mellor et al. 2020). Further, this model provides a framework for the increased promotion of positive states that truly enhance survival probabilities post-release while also considering negative effects of rehabilitation, ultimately leading to a method of scoring the overall welfare of animals. Given the standards set forth by National Wildlife Rehabilitators Association (NWRA) and International Wildlife Rehabilitation Council (IWRC), and the knowledge necessary to fully employ the Five Domains model for animal welfare, it is important to incorporate as much empirical information as possible about how animals cope with stress into rehabilitation practices.

Some of the more commonly used biomarkers of stress in vertebrates are glucocorticoids, which are important steroid hormones for mediating metabolic states and coordinating increased metabolic demands associated with sustained fight-or-flight responses (Sapolsky et al. 2000; Wingfield 2003). Glucocorticoids have strong influences on many other physiological systems, which make them a particularly good metric for stress responsiveness in most species. CORT is the primary hormone secreted by birds in an acute stress response (Sapolsky et al. 2000; Herman et al. 2016). When a stressor is perceived and signalled to the hypothalamus via sympathetic nervous system stimulation, the hypothalamus sends corticotropin releasing hormone to the anterior pituitary. The anterior pituitary, in turn, releases adrenocorticotropic hormone, which activates cells in the cortex of the adrenal glands to synthesise cortisol and release it into the bloodstream (Sapolsky et al. 2000; Wingfield 2003). Cortisol functions to increase gluconeogenesis and optimise energy use in tissues critical for the fight or flight response while also downregulating physiological processes and systems that are not critical for immediate survival, such as long-term immunity and reproduction (Sapolsky et al. 2000). There are two CORT measurements that are commonly used in stress physiology research: baseline CORT and CORT levels during an acute stressor (stress-induced CORT). Baseline CORT is considered an indicator of persistent environmental conditions and, even at low levels, are necessary for daily activities such as the sleep/wake cycle and general motivation to search for food when hungry (Sapolsky et al. 2000). The release of CORT from the adrenal glands during a stress response is known to show a positive, linear relationship with time from 0 to 30 min in birds and following 60 min, the CORT levels steadily return to baseline (Pravosudov 2005; Rich & Romero 2005). Chronic elevation of CORT can also lead to adrenal fatigue, rendering a bird incapable of mounting a robust CORT response to a stressor (Tome et al. 1985; Rich & Romero 2005). In an experiment with wild-caught, and otherwise healthy, chukars (*Alectoris chukar*), birds transferred from the wild into captivity showed significantly reduced CORT-mediated stress responses after three to five days in captivity, showed a rebound to a more robust stress response after nine days, but were not held in captivity for longer than 10 days (Dickens et al. 2008). As demonstrated in American kestrels (*Falco sparverius*) in captivity, repeated restraint and sampling results in reduced CORT levels in developing and adult birds (Love et al. 2003). Conversely, a bird held in captivity for an extended period of time, absent from the stressors of free-living, may face a "use it or lose it" scenario, whereby infrequent generation of a full stress-induced CORT response could leave their maximum CORT levels achieved during a stress response lower than those of free-living birds who were not previously rehabilitated, or they may lack the ability to mount a full CORT response as quickly as other free-living birds.

The majority of research examining physiological responses to captivity has not specifically involved birds in a rehabilitation setting; however, given that glucocorticoid-mediated stress responses are known to follow a stereotyped pattern, with variation in the time course and magnitude among individuals (Schoenle et al. 2018), conclusions drawn from studies where healthy birds are brought into captivity should still provide valuable comparison to stress response dynamics in rehabilitation. Still, it is important to consider the rehabilitation environment and the variation in causes for admittance to rehabilitation. Scheun et al. (2021) found significantly different faecal glucocorticoid metabolite levels throughout rehabilitation for penguins admitted with different ailments (emaciation, injury, or oiling).

Upon activation of the sympathetic nervous system, long-term immunity is a low priority as the fight or flight response, responsible for emergency life-or-death situations, takes over. The avian heterophil is an integral part of dealing with foreign pathogens in the body and is the first white blood cell to respond to infection (Genovese et al. 2013). Heterophils are designed for general defence against pathogens and are more energetically efficient to produce compared to other white blood cells, such as lymphocytes (Davis & Maney 2018). The lymphocyte has evolved for more specialised defence and requires far more energy to produce, given that only 1% of all lymphocytes are permitted into circulation after undergoing selection in the bone marrow (B cells) or thymus (T cells). As a result, there is a downregulation of lymphocytes during stress responses, leading to an elevated ratio of heterophils to lymphocytes in circulating blood (Grasman 2002; Hing et al. 2016; Davis & Maney 2018). In rehabilitation settings, birds have already been exposed to natural physiological stress as well as the stress associated with their illness or injury; therefore the change in their ratio of heterophils to lymphocytes (H:L) could be a telling metric to understand how baseline stress changes during time in rehabilitation (Grasman 2002; Davis & Maney 2018). In a study of roadside hawks (*Rupornis magnirostris*) undergoing rehabilitation (Guerra et al. 2018), birds held in the rehabilitation facility for three months had significantly greater H;L than birds held for six months, suggesting that habituation was occurring over that time period.

To assess potential influences of the length of time in rehabilitation on the acute stress response, CORT levels were measured in raptors admitted to the Illinois Raptor Center from the initial admission exam and also their second exam prior to release. The goal was to gain a comprehensive understanding of the stress profile of birds with variable lengths of stay in rehabilitation. To assess changes in baseline stress, the H:L from blood smears made during the admission exam was compared to those from the release exam. We hypothesised that as length of stay in rehabilitation increased, the difference in the magnitude of the CORT response to handling would decrease relative to the same response upon admission. We also hypothesised that as length of stay increased, H:L would decrease as struggles to obtain food, threats from predators, and competition are all eliminated in rehabilitation, reducing CORT-mediated reductions in lymphocyte production.

## Methods and materials

### Blood sample collection

We collected blood samples from 274 raptors, among 7 species (Table 1), admitted, rehabilitated, and released from the Illinois Raptor Center from 2016 to 2023. The Illinois Raptor Center is a wildlife rehabilitation organisation in Decatur, Illinois. A blood sample was taken upon the admission exam and the release exam via venipuncture of the brachial vein with a 22 gauge needle, and blood was collected into a 75 mm microhematocrit capillary tube. The range of the time it took to obtain the blood sample from the raptor from removal from the holding cage, mew, or flight enclosure was 400 to 1114 s, the mean time was 720.16 (± 4.06 SE) s, and the median was 714 s. The mean sample time for admission samples was 725.32 ± 5.51 SE) and for release samples it was 731.16 ± 6.31 SE). With each sample, a drop of blood was placed on a microscope slide, smeared across the slide, fixed with methanol, and stained with modified Wright's stain for use in assessing H:L (Campo & Davila 2002). The remaining blood in capillary tubes was centrifuged at 5000 rpm for 5 min. The plasma was drawn from the capillary tube with a Hamilton® syringe, transferred to a microcentrifuge tube, and stored at -20° C until utilisation in immunoassay. Given the time-sensitive nature of the release of CORT, we restricted our analysis to birds for whom admission and release samples were collected between 600 and 850 s (± 125 s of the mean). This reduced our sample size for analysis to 182 birds.

Within the chosen inclusion window, there were no significant correlations between length of stay and either the admission sample time ($r$ = -0.059), nor the release sample time ($r$ = -0.048); therefore, no further adjustments were made to the response variable (CORT), and any error introduced by variation in sample time within that window was considered to be randomly distributed among the different length of stay durations. The mean length of stay was 78.01 days (± 3.06 SE).

**Table 1** Species information and sample size for corticosterone analysis and H:L analysis for each raptor included in this study.

| Species | *Corticosterone n* | *H:L n* |
|---|---|---|
| American Kestrel (*Falco sparverius*) | 33 | 18 |
| Bald Eagle (*Hailaeetus leucocephalus*) | 20 | 7 |
| Barred Owl (*Strix varia*) | 49 | 26 |
| Cooper's Hawk (*Accipiter cooperii*) | 24 | 12 |
| Eastern Screech Owl (*Megascops asio*) | 32 | 18 |
| Great-horned Owl (*Bubo virginianus*) | 59 | 28 |
| Red-tailed Hawk (Buteo jamaicensis) | 57 | 24 |

### Corticosterone enzyme immunoassay

DetectX® CORT Enzyme Immunoassay Kits were used (Arbor Assays, Inc.), and manufacturer's instructions were followed, to complete the assay for plasma samples. The CORT value from the admission sample was subtracted from the CORT value from the release sample, giving us a measure of differences in the magnitude of CORT-mediated during rehabilitation. Given the trauma that led to admission for rehabilitation, it is likely that birds may have a dampened CORT response to handling and sampling as a result of adrenal fatigue (Tome et al. 1985), or a strong response if they were only recently injured prior to admission. Still, a rehabilitated bird ready for release should have the most robust stress response.

**Heterophil to lymphocyte ratio.** A total of 133 blood smears were analysed for H:L ratio (Table 1). Slides were viewed using a Zeiss® binocular compound light microscope at 400x magnification with an oil immersion lens. To determine H:L, we either counted to a combined 50 heterophils and lymphocytes or scanned 50 fields of view, counting all heterophils and lymphocytes in those fields, whichever came first (Wilcoxen et al. 2015). This count was completed for each admission and release sample. H:L from the admission sample was subtracted from H:L from the release sample, giving us a measure of the H:L difference during rehabilitation.

### Statistical analysis

The CORT difference (release value – admission value) was tested for normal distribution using a K-S test and was found to be normally distributed ($p$ = 0.139). The H:L difference was tested for normal distribution using a K-S test and was found to be normally distributed ($p$ = 0.081).

An analysis of covariance (ANCOVA) was used for two analyses. One with difference in CORT (release – admission) and the other with difference in H:L (release – admission) as the dependent variable. For each analysis, species and age (adult or juvenile) were fixed factors, length of stay was the covariate, and each species*length of stay and age*length of stay interaction terms were included in each analysis. The three-way interaction of species*age*length of stay was also included in the initial analysis for each dependent variable; however, it was removed from the analysis when not statistically significant. All statistical analyses were completed using Statistical Package for the Social Sciences (SPSS) 25.0 (IBM Corp. 2017).

## Results

The three-way interaction of species*age*length of stay was not significant for either the CORT ($F_{7,\ 173}$ = 1.021, $p$ = 0.547) or the H:L data ($F_{7,\ 123}$ = 0.942, $p$ = 0.688); therefore, the three-way interactions were not further considered in the analysis.

There were no species-dependent relationships (no significant statistical interaction) between the CORT release-admission difference ($F_{6,\ 174}$ = 1.607, $p$ = 0.141) or H:L ratio release-admission difference ($F_{6,\ 124}$= 1.180, $p$ = 0.301) and length of stay. Furthermore, there were no species-dependent differences in CORT release-admission ($F_{6,\ 174}$ = 1.373, $p$ = 0.206) or H:L release-admission ($F_{6,\ 124}$= 0.481, $p$ = 0.826), independent of length of stay. Given the results of these statistical assessments for each CORT and H:L, we grouped all species together for final assessment of the impacts of length of stay on stress physiology.

There were no age-dependent relationships (no significant statistical interaction) between the CORT release-admission difference ($F_{6,\ 174}$= 2.401, $p$ = 0.078) or the H:L ratio release-admission difference ($F_{6,\ 123}$= 0.991, $p$ = 0.715) and length of stay. Despite the lack of a significant interaction between age and length of stay with regard to the CORT release-admission difference (which demonstrates that the pattern was the same for adult and immature birds), the relationship was stronger for immature birds ($r^2$ = 0.482; Fig. 2) than for adults ($r^2$ = 0.233; Fig. 3).

We found no statistically significant relationship between the difference in release-admission H:L ratios and length of stay ($F_{1,\ 132}$= 2.964, $p$ = 0.101; Fig. 1).

There was a statistically significant relationship between length of stay and the difference between release and admission CORT ($F_{1,\ 160}$ = 20.904, $p$ < 0.001). The $r^2$ was 0.374, indicating that 37.4% of the variance in the difference in release-admission CORT levels can be explained by variance in length of stay.

## Discussion

The authors' first hypothesis was supported. As length of stay increased, the difference in the magnitude of CORT release from admission to release decreased. However, the second hypothesis was not supported as we found no significant relationship between the difference between H:L at admission and release, and length of stay. While the two measurements do not align, it is important to remember that the CORT measurements in this study are indicative of a physiological response during an acute stressor (in this case capture and hold), while H:L ratios are more of a chronic measurement of how animals are coping with

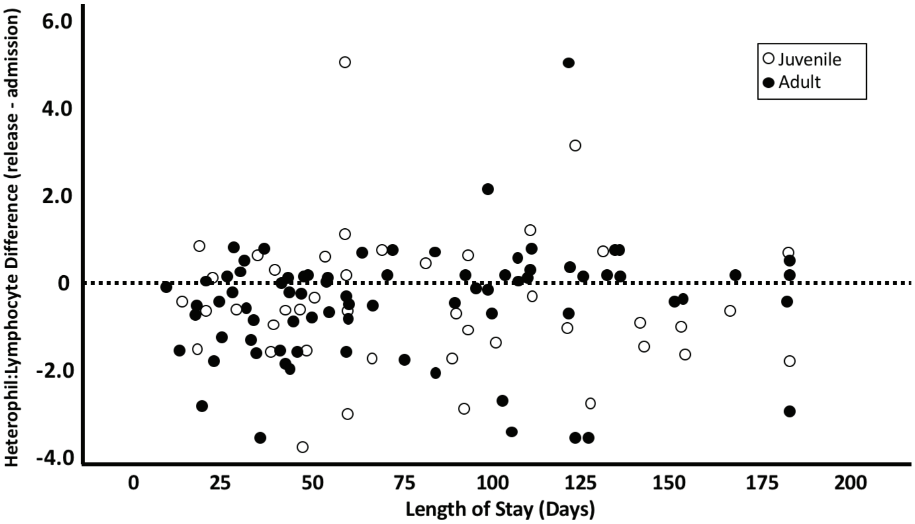

**Figure 1** Difference in H:L observed between admission blood smears and release blood smears collected from the Illinois Raptor Center. *Note*: The dashed line represents "no change" with birds above the line having higher ratios upon release and birds below the line having lower ratios upon release.

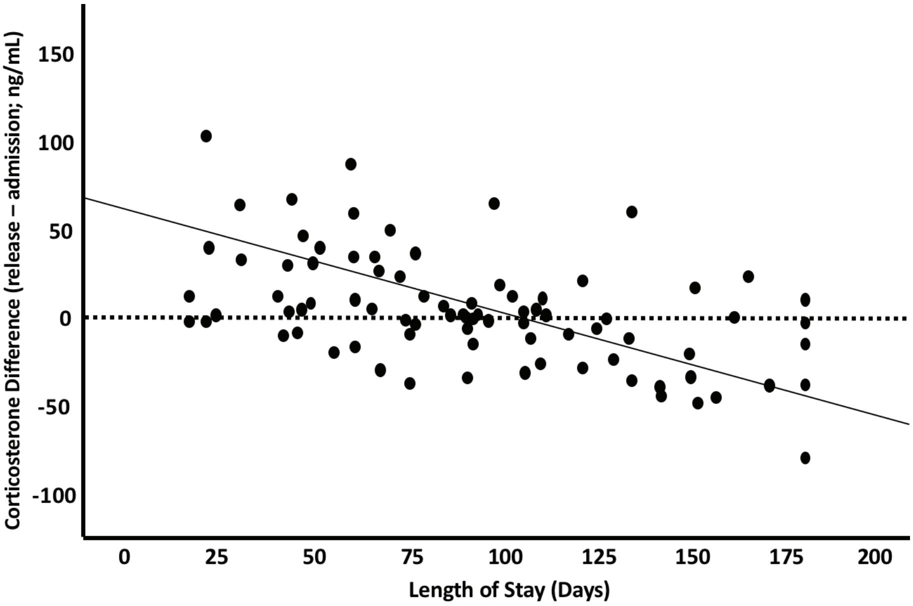

**Figure 2** Difference in corticosterone levels between admission plasma samples and release plasma samples collected from adult birds of prey in rehabilitation at the Illinois Raptor Center. *Note*: The dashed line represents "no change," with birds above that line having increased responses upon release and birds below the line having decreased responses upon release. The solid line is the line of best fit for regression.

less severe, less acute stressors over a longer period of time (Müller et al. 2011). Indeed, individual experiments with raptors (e.g., Eurasian kestrels, Müller et al. 2011) and review of glucocorticoid responses to stressors and changes in leukocyte differentials in response to stressors have shown that while there is an overlap in some areas, they are, indeed, indicative of two very different physiological responses to stress (Goessling et al. 2015). Ill or injured birds admitted for rehabilitation also have competing physiological needs for fighting infection and healing that may also influence the heterophil and lymphocyte distributions independent of the stress of capture alone.

Ultimately, when a bird is released back into the wild, the most effective mechanism to deal with competitors, prey, and predators is to mount a robust stress response when challenged. At the very least, a stress response as robust as the one with which they arrived would seem important. However, given that birds are arriving at the rehabilitation facility having faced significant stressors, their stress response may be dampened due to adrenal fatigue (Tome et al. 1985). Therefore, a better sign of preparedness for return to the wild would be a stronger CORT response to capture and holding just prior to release. This would indicate recovery from adrenal fatigue and assure that the bird is fully capable of responding to stressors in the wild. For example, in little penguins (*Eudyptula minor*) captured in the wild and immediately released after holding and sampling, birds demonstrate stronger CORT responses to a stressor upon subsequent capture when the time between captures is spent in natural, free-living conditions (Carroll et al. 2016). When examining the CORT data in our study (Figs. 2 and 3), approximately half of the birds (across all lengths of stay) were very near the "zero" line where the response upon admission and the response upon release are the same, while the other half consistently showed deviation from zero, with a clear pattern where birds released prior to 100 days in captivity tending to have stronger responses and those released after 100 days having consistently lower CORT responses, with the widest variation occurring between 45 and 70 days.

While it is unknown if this pattern persists after they are, in fact, facing challenges in the wild, the possibility that this is their new, long-term physiological norm is troublesome and likely decreases their risk of post-rehabilitation success. This could be particularly problematic for young birds whose physiological systems have not been patterned, or trained, from years of experience in the wild, and who may be more susceptible to habituation (Drummond & Ancona 2015). Among the birds in this study, 54.95% were hatch year (juvenile) birds and 45.05% were adults. The strength of the relationship between length of stay and difference in admission to release stress response was much greater in juvenile birds than in adults, despite the pattern being the same for adults and juveniles. With regard to age, other studies have demonstrated changes in the CORT response to

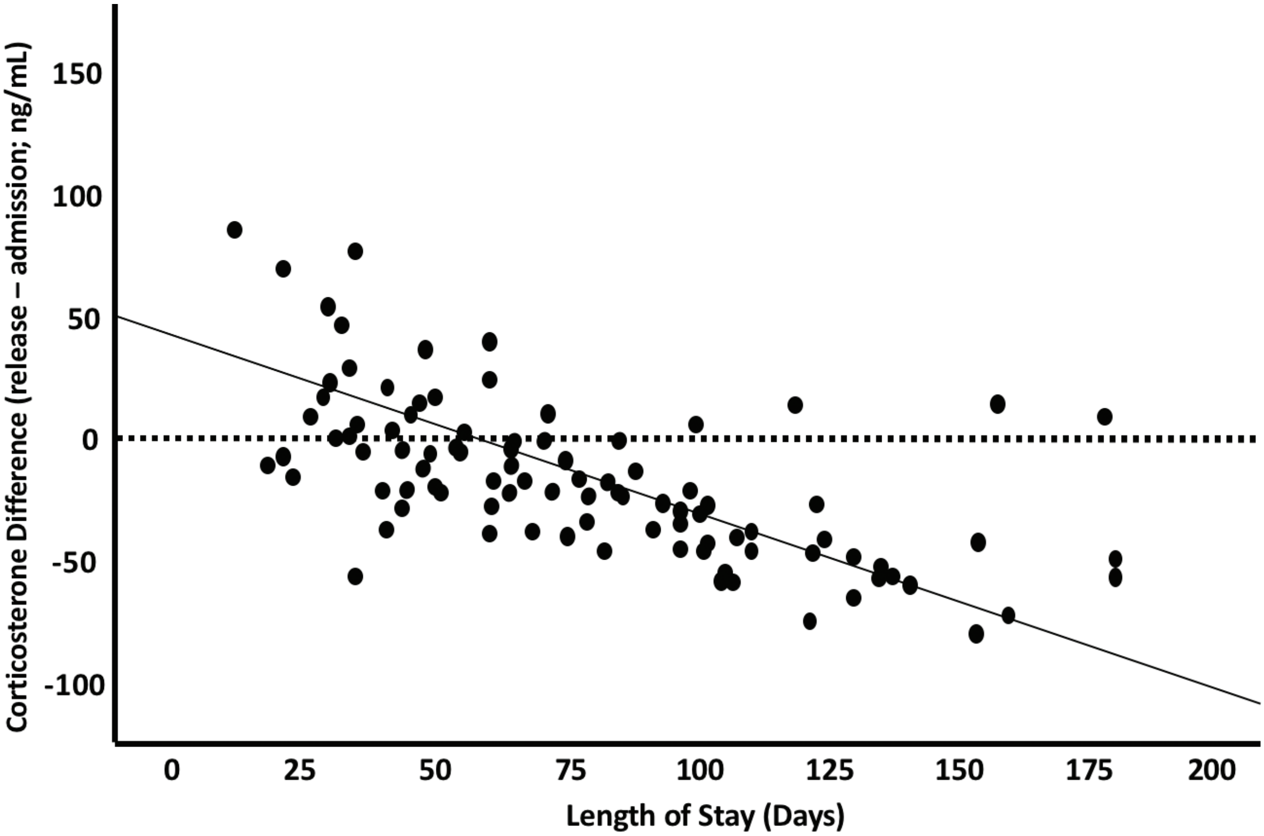

**Figure 3** Difference in corticosterone levels between admission plasma samples and release plasma samples collected from juvenile birds of prey in rehabilitation at the Illinois Raptor Center. *Note*: The dashed line represents "no change," with birds above that line having increased responses upon release and birds below the line having decreased responses upon release. The solid line is the line of best fit for regression.

handling stress in some species (Heidinger et al. 2008; Wilcoxen et al. 2011), but those studies come from a single sample and do not allow for individual changes between handling events in a paired-sample design as was used here.

Related studies have found similar results, such as Black et al. (2011) who analysed white blood cell counts, including H:L ratios, of many of the same raptor species sampled in this study and compared free-living and captive raptors. However, their study focussed on birds in captivity in general , not birds in rehabilitation facilities. Healthy birds may be brought into captivity for research purposes and planning such experiments should carefully consider the potential impacts of that time in captivity on future free-living success. In this study, healthy birds were not admitted for experimental purposes; instead, illness or injury had struck these birds, and they needed to be in captivity in order to receive rehabilitative care. It is important for the rehabilitation community to understand these effects of captivity not only on healthy birds but on sick birds as well in order to create a plan of care that minimises the effects of captivity on these birds while still maximising their ability to be rehabilitated.

Given our sample sizes of 182 and 133, respectively, distributed among seven species and the lack of available genetic tools to determine the sex of each bird, we were not able to add sex of the raptors as an independent variable. The reason for rehabilitation was also not included as an independent variable. We did not have full replication of all ailments or injuries with any statistical power that would allow a thorough analysis. While we have no specific reason to believe that there would be differences among sexes or causes of ailment or injury that would bias our results, particularly because a paired design was used with each bird, it would be worthwhile to see if a difference is present among these groups should a large enough sample size to do so be accumulated. Still, it is worth considering that Dufty, Jr. and Belthoff (1997) found no significant differences in the acute CORT response to handling stress between male and female Western screech owls (*Megascops kennicottii*). In terms of the reason for rehabilitation, it would be worthwhile to analyse the difference between a raptor being admitted for a minor illness compared to a major injury. However, in this study specifically we analysed the difference upon admission and release and therefore looked at their value from an absolute value standpoint, controlling for much of that variation in our paired-sample design.

The Five Domains Model for animal welfare assessment (Mellor & Beausoleil 2015; Mellor et al. 2020) provides valuable insight into how rehabilitators may use enrichment activities to create positive states in birds undergoing rehabilitation, including simulations of experiences the animals may face upon release, potentially reducing the risk of habituation of the physiological stress response. In studies of stress in captivity that incorporate such interventions, positive outcomes have been reported for Japanese quail (*Coturnix japonica*) (Laurence et al. 2015) and owls (Potts 2016), among others. For example, flying models at the raptors to mimic predation could provide meaningful activation of the stress response and high-tech muscle-building activities such as time in flight tunnels may as well (Granati et al. 2021). Likewise, simulating foraging by having the raptor search for food, as opposed to being served, is a good option to maintain the sense of urgency needed to seek live prey. There is also the possibility that the capture, restraint, and sampling protocol associated with examination of birds upon admission and release are not akin to the most severe stressful stimuli birds may face and do not initiate their greatest stress response. For example, Pakkala et al. (2013) studied pigeons exposed to a simulated predatory attack and capture and restraint by humans, and found CORT levels to measure more than twice as high in response to threat of predation as the CORT levels measured during the classic capture-restraint method. Our study utilised the capture-restraint method on the admission exam and the release exam as it is excessively utilised in many studies, and is representative of a high-level stress situation for the bird. However, simulating stressors in the wild like predation or competition as a part of the plan of care in rehabilitation would allow the raptors to mount a CORT response on a routine basis. It is important to highlight that this study only simulated the capture and examination stressor on the admission and release examinations and therefore, the birds in this study may have only been exposed to a high-level stressor twice over the course of six months, in the most extreme cases.

It is also important to consider that the CORT-mediated stress response is just one tool to assess stress responsiveness in animals. While the effects of glucocorticoids on multiple physiological systems are far-reaching, there are other methods to assess stress responsiveness throughout rehabilitation, and when evaluating an animal for release, which are also important to consider. The Minimum Standards for Wildlife Rehabilitation (Miller 2012) includes “demonstrate the fight or flight response” as one of the minimum requirements for release; the physiological data provided here represent the internal fight or flight response, but there are also behavioural signs that the response is appropriate. Those behavioural signs may include avoidance behaviour or defensive behaviour, with regard to the rehabilitators when retrieving the bird from its holding facility, vocalisation and bill snapping toward rehabilitators, and active approach to prey inside the holding facility. These are all useful signs of a bird’s preparation for release, although they are directed toward a more predictable and familiar stimulus than what is likely to be experienced after release.

Mason (2010) reviewed studies of multiple vertebrate taxa and many different forms of captivity including zoos, farms, and captive breeding programmes, documenting lasting effects on behaviour associated with time in captivity, specifically noting patterns of habituation. In rehabilitation settings, the cost of lengthened captivity may be greater than the benefits of extended time with high-quality food and a competition-free and predator-free environment once the animal is well enough for return to the wild. Given the findings of this study and others that have addressed behavioural and physiological changes associated with time in captivity, the authors recommend that rehabilitators closely follow the minimum standards for release of wildlife following rehabilitation (Miller 2012; Miller & Schlieps 2021). Rehabilitators should pay close attention to when animals are ready for release, and release them as immediately as possible once ready to reduce the likelihood of any unintended negative effects of extended periods of stay.

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## Disclosure statement

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