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HOW STRESSFUL IS IT TO MOVE? WHOOPING CRANE GLUCOCORTICOID RESPONSE DURING FACILITY TRANSFER

MEGAN E. BROWN

CHRISTOPHER MARTIN

CHRISTOPHER CROWE

SPRINA LUI

NUCHARIN SONGSASEN,

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MEGAN E. BROWN,[1,2] Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630, USA

CHRISTOPHER MARTIN, Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630, USA

CHRISTOPHER CROWE, Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630, USA

SPRINA LUI, Dallas Zoo, 650 S R L Thornton Freeway, Dallas, TX 75203 USA

NUCHARIN SONGSASEN, Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA USA

Abstract: The ability to transfer animals between different facilities for genetic management is a critical component to the success of any cooperatively managed captive animal population despite the high cost and the need for a high level of coordination in logistical planning. Two issues of concern are the magnitude of potential stress responses incurred during shipment of adult cranes to a new facility and the duration of elevated glucocorticoid production during the acclimation periods. The objectives of this project were to assess adrenal responses of whooping cranes (*Grus americana*) during transfer from the Patuxent Wildlife Research Center (PWRC) to other facilities across North America and the influence of transit time on acclimation duration. We conducted 2 separate studies to assess patterns of glucocorticoid production. When comparing the 3 stages of transfer (study 1), fecal glucocorticoid metabolite (FGM) concentrations were significantly higher in samples collected from the crate compared to both before and after transfer. During the acclimation period at the new facilities (study 2), 2 patterns emerged, where some birds showed an immediate response then little change in FGM production while others displayed variable production throughout the sampling period. We believe that these different patterns may be due to differences in personality and is an avenue for future investigation. Results from this study help inform future management decisions for individuals being transferred and acclimation to new facilities.

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Key words: Facility transport, fecal glucocorticoid metabolites, *Grus americana,* whooping crane.

The ability to transfer animals between different facilities for genetic management is a critical component to the success of any cooperatively managed captive animal population despite the high cost and the need for a high level of coordination in logistical planning. A Breeding and Transfer Plan (BTP) published for an Association of Zoos and Aquariums (AZA) Species Survival Plan combines the collective population of a given species across all AZA member facilities and makes recommendations for animal transfers and pairings to fulfill both genetic and demographic goals. On average, each BTP recommends 8 animal transfers, and the inter-planning period is 1.9 years; between 1999 and 2014 there was a total of 6,511 transfers recommended among 637 BTPs evaluated (Faust et al. 2019). Stress resulting from transportation and translocation is normal and can be considered an expected outcome (Dickens et al. 2010). However, another hallmark of cooperative animal management is to maintain high levels of animal welfare. Thus, to both facilitate the need to move animals between facilities and to maintain the highest level of care, the zoological field must understand how the transfers impact individuals on a biological level.

Stress is a naturally occurring process in which the body responds to external stimuli. When a situation is perceived as stressful, through any of the 5 senses, the hypothalamus releases corticotropin releasing hormone which signals the anterior pituitary to produce adrenocorticotropin hormone (ACTH). ACTH then acts on the adrenal cortex and stimulates the release of glucocorticoids (Norris 2006). The primary glucocorticoid produced in birds is corticosterone, and this molecule has the potential to act on all tissues within the body. A variety of actions may result, including increased gluconeogenesis, metabolism, and blood calcium (Siegel 1980). These are all classic

[1] E-mail: mbrown@aza.org

[2] Present address: Association of Zoos and Aquariums, 8403 Colesville Rd., Silver Spring, MD 20910, USA.

Table 1: Summary of travel method and duration of travel for whooping cranes during transport from Patuxent Wildlife Research Center, Laurel, Maryland, to destination facility, 2018-2019.

Destination facility	Number of birds (male,female)	Transport method	Total time in crate (hr)	Included in study	
				1	2
Dallas (DZ)	8 (5,3)	Airplane then van	12	X	X
White Oak Conservation (WOC)	6 (3,3)	Van	12	X	X
Calgary Zoo (CZ)	8 (4,4)	Airplane then van	24	X	X
Smithsonian Conservation Biology Institute (SCBI)	10 (5,5)	Van	4		X

actions seen in the fight or flight response, which help an individual cope and survive in short term stressful situations (Siegel 1980, Norris 2006). The reaction of an individual to stressors depends on the length of time during which the stressor is perceived, an individual's previous experience and physiological status, and the environment in which the stressor is experienced (Fazio and Ferlazzo 2003). Although the secretion of corticosterone helps individuals cope with stressful situations, persistent elevation of this hormone (chronic stress) compromises other bodily functions such as reproduction and immune response (Angelier et al. 2009).

While numerous studies have documented the physiological (Franceschini et al. 2008, Fazio et al. 2020) and behavioral responses (Dembiec et al. 2004, Ross et al. 2011) of mammals to handling and transport, there have been very few studies conducted in avian species, especially whooping cranes (*Grus americana*). This project utilized an already occurring event, animal transfer, to assess individuals' adrenal responses to potentially stressful stimuli (i.e., being moved from a facility to another). It is the first attempt to assess stress response in a large number of individuals during the same event over a similar time period.

We did not know the magnitude of potential stress responses incurred during shipment of adult cranes to a new facility or the duration during which glucocorticoid production will remain elevated during initial adjustment periods. For the first, we collected samples comparing the 3 stages of transfer: during the pre-shipment period, from the crate, and within 48 hours of transfer. The second study assessed glucocorticoid production during the acclimation period at a new facility; fecal samples were collected prior to transfer and continued opportunistically once per week for 6 weeks at the birds' respective new facilities. Our objectives were to determine the response 1) during and immediately following the physical transfer, and 2) during acclimation to the new facility. We hypothesized that fecal glucocorticoid metabolite (FGM) levels elevate during shipment and initial acclimation to new facilities and then decline as birds acclimate to the new environment.

METHODS

Thirty-two birds were moved from Patuxent Wildlife Research Center (PWRC) to 4 new facilities across North America during 2018 and 2019, including Calgary Zoo (CZ), Dallas Zoo (DZ), Smithsonian Conservation Biology Institute (SCBI), and White Oak Conservation (WOC). The number of birds, transportation type, transportation duration, and study inclusion for each destination facility are summarized in Table 1. This study was approved by the Smithsonian National Zoological Park Animal Care and Use Committee (#17-19), Dallas Zoo's Coordinator of Research Projects, and Calgary Zoo's Welfare, Research, and Ethics Committee.

Study Design

Study 1: Fecal glucocorticoid metabolite response of whooping cranes during physical transfer to new facility. Three fecal samples were collected from each individual (n = 22): 1) day of shipment prior to being crated, 2) all fecal material from the crate that was produced during the shipment period (range: 12-24 hr), and 3) all fecal material produced after the first 48 hours following arrival at the new facility. Birds transported to WOC, DZ, and CZ were used in this analysis, while birds transferred to SCBI were excluded as they had not been crated long enough (~4 hr) to defecate in the crate.

Study 2: Fecal glucocorticoid metabolite response of whooping cranes during acclimation period at

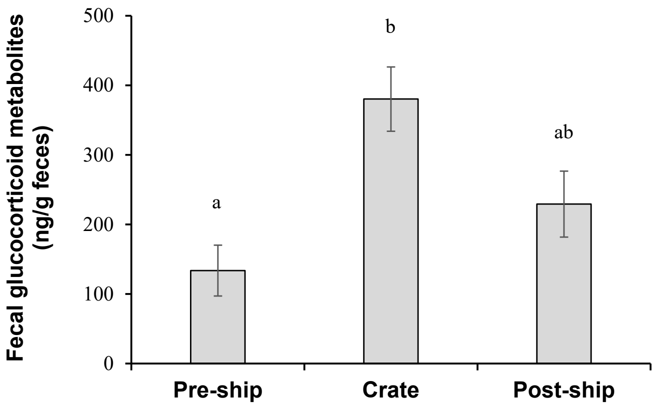

Figure 1. Fecal glucocorticoid metabolite production during stages of transfer in adult whooping cranes (± SE). Fecal samples were collected from adult whooping cranes (*n* = 22) during the 3 stages of transfer: 1) Pre-ship: day of transfer prior to being crated, 2) Crate: from the crate itself, and 3) Post-ship: within 48 hours of arrival at new facility. Birds transported to Dallas Zoo, Calgary Zoo, and White Oak Conservation were utilized in this analysis, while birds transferred to Smithsonian Conservation Biology Institute were excluded as they had not been crated long enough to defecate in the crate. As indicated by letters above the bars, fecal glucocorticoid metabolite concentrations were higher in samples collected from the crate (P < 0.001) compared to pre-ship values but not post-ship.

a new facility. Fecal samples were collected from each individual (n = 32) on the following schedule as possible: 1) 1 sample 2 weeks prior to shipment to establish an individual baseline value, 2) within 24-48 hours (2 days) of arriving at new facility, and 3) 1 sample weekly for next 5 weeks during acclimation at the new facility. Samples were not collected if entering the pen was deemed too stressful based on keeper assessment of how the bird was behaving on that collection day. A total of 5-8 samples was obtained from each individual crane.

Hormone Extraction and Analysis

Fecal samples were stored at –20°C following collection until shipment to SCBI for processing. Hormone extraction was performed using previously described methods (Brown et al. 2016, Brown et al. 2020). Briefly, fecal samples were freeze-dried to remove moisture content differences (Millspaugh and Washburn 2004), 0.2 ± 0.08 g of homogenized sample was measured into a 16×125-mm glass test tube, chemically extracted using 5 mL of 70% ethanol (EtOH; v:v deionized water, vortexed for 30 sec, and then vigorously shaken for 30 min. The samples were then centrifuged (500 g for 20 min), and the supernatant poured off into a clean glass tube. A second 5 mL of 70% EtOH was added to the fecal pellet, the sample was re-vortexed and re-centrifuged. The supernatants from the same sample were then combined for a total of 10 mL. This mixture was dried under forced air stream, and the dried extract was resuspended in 1 mL of phosphate assay buffer (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, 0.15 M NaCl; pH 7.0) and stored at –20°C until hormone analysis.

All samples were evaluated for adrenal (glucocorticoid) hormone metabolites measured at SCBI's Wildlife Endocrinology Laboratory using an in-house corticosterone enzyme immunoassay system and the R0006 Corticosterone antibody obtained from C. Munro (University of California, Davis, CA, USA). Samples were diluted with buffer (1:10) and processed in duplicate according to assay specifications. For all assays, intra-assay coefficients of variation were ≤10% and inter-assay coefficients of variation were ≤15%. Data are expressed as mass units of hormone per gram of feces.

Statistical Methods

Prior to analysis, normality was assessed through frequency distribution and Q-Q plots. The data were normally distributed, and no further transformation was required. Individual was included as a random effect to account for repeated sampling from the same individual throughout the study. For each research objective we performed a separate linear regression using a mixed model framework in the R language (R Core Team 2014) and the lme4 package (Bates et al. 2012). We then used the phia package (De Rosario-Martinez et al. 2015) to calculate estimated marginal means from the model and perform post-hoc analysis comparing day and facility. Marginal means and SEMs are reported below.

RESULTS

Study 1: Fecal glucocorticoid metabolite response of whooping cranes during physical transfer to new facility. We compared samples collected prior to shipment, from the transfer crate, and within 48 hours of arrival at the new facility. FGM concentrations were higher in samples collected from the crate compared to pre-ship values ($P < 0.001$; Fig. 1) but not compared to

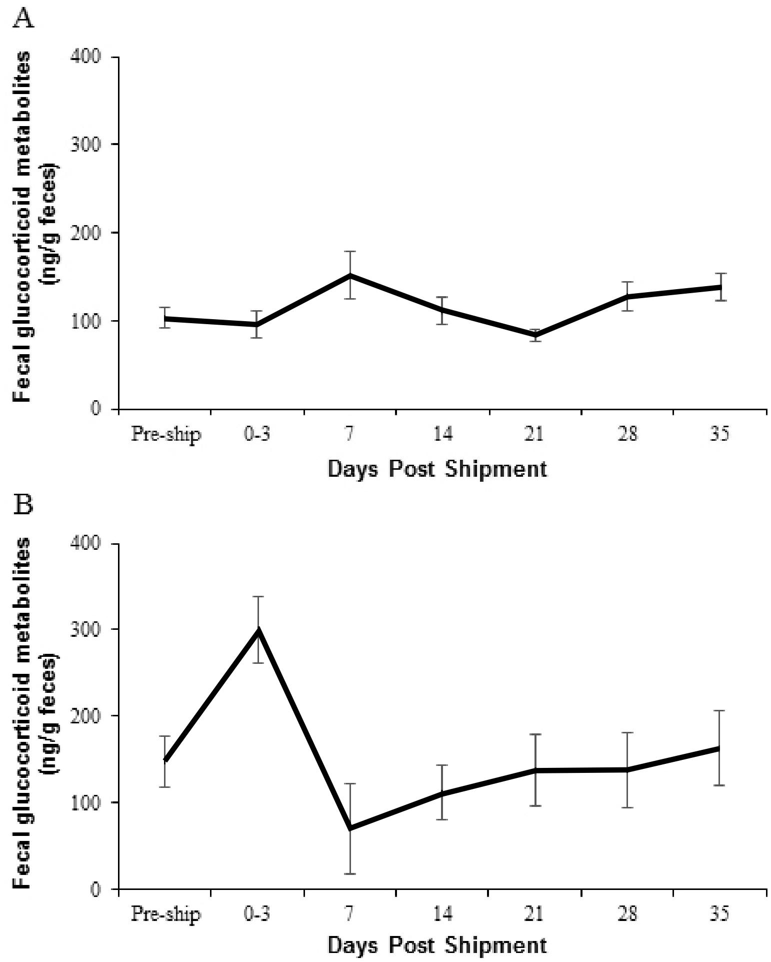

Figure 2. Whooping crane fecal glucocorticoid metabolite ($\bar{x}$ ± SE) production following facility transfer to A) Smithsonian Conservation Biology Institute (*n* = 10) and B) Dallas Zoo, Calgary Zoo, and White Oak Conservation (*n* = 22). Concentrations from samples collected within the first 48 hours (2 days) following transfer were not significantly different for birds transferred to SCBI but were significantly higher for birds moving to the other facilities (*P* = 0.04). These concentrations returned to and remained at similar levels within 1 week of acclimation and throughout the rest of the study.

immediate post-ship values.

Study 2: Fecal glucocorticoid metabolite response of whooping cranes during acclimation period at a new facility. We observed significant differences between facilities ($P < 0.01$), specifically as birds transferring to SCBI produced overall lower FGM concentrations compared to birds transferred to other facilities, so we elected to separate the data into separate analyses for short (SCBI) and long transits (DZ, CZ, and WOC). There were no other differences based on facility or date between the long-travel facilities. For the birds transferred to SCBI (Fig. 2A) there was no significant difference following shipment. The birds transferred to the other 3 facilities (Fig. 2B) displayed a 2-fold increase in FGM concentrations for samples collected within the first 48 hours of arrival at the new facility (P = 0.047). FGM concentrations returned to levels comparable to the pre-shipment values after 1 week of acclimation and remained at similar levels for the next 5 weeks.

In both statistical models performed for study 2, we noted high levels of residual variance attributed to the random effect of individual. Because our sample size was too small to further assess differences between individuals, FGM patterns were then visually assessed. Two obvious patterns emerged: some birds had a large initial response with 1 high peak (Fig. 3A), while others showed a more variable response which fluctuated around baseline for the length of the sampling period (Fig. 3B).

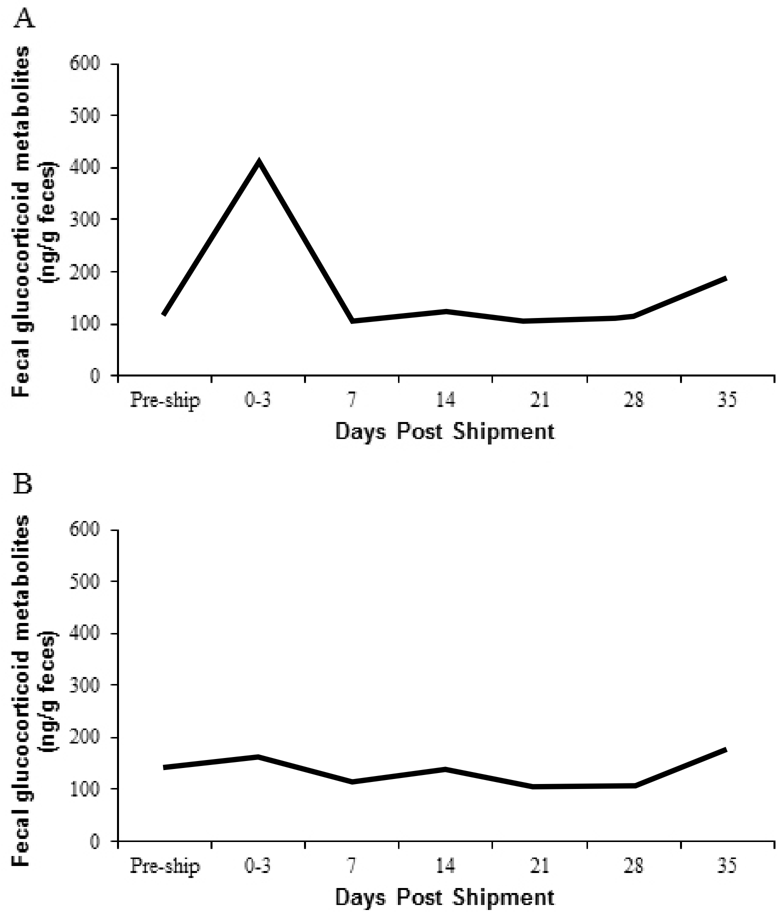

Figure 3. Representative graphs of the 2 different fecal glucocorticoid metabolite excretion patterns (± SE) observed in individuals transferred to a new facility, either A) a robust response immediately following transfer or B) a less pronounced and more variable response in the weeks following transfer. These representative individuals were both moved to Dallas Zoo, but all individuals included in the study exhibited 1 of these same patterns.

DISCUSSION

Through the utilization of non-invasive endocrine monitoring, we describe different patterns in FGM concentrations from samples collected during and immediately following transport in a large number of whooping cranes. In our first study, FGM concentrations were significantly higher in samples collected from the crate compared to samples both before and after the transfer, indicating the acute response experience during the actual event. In our second study, we observed differences in FGM production based on the length of travel, as short-term transfers appeared to produce a more limited response in the weeks following transfer compared to prolonged transfers. Individuals in transit for longer periods producing higher FGM immediately post shipment, but in most cases FGM levels returning to baseline within 1 week. However, even within these patterns there was high variability between individuals, where the magnitude of response and the fluctuation of FGM, and possibly the speed at which an individual acclimated to a new facility, differed possibly due to differences in individual personalities.

The elevation of FGM concentrations during animal transport observed in the present study was consistent with that of our previous observation where relocation of whooping cranes to a novel enclosure within the same facility resulted in elevation of FGM concentrations that lasted for roughly 10 days before the hormone levels returned to that of pre-move concentrations (M. Brown, unpublished data). For birds transferred to SCBI, the transfer itself involved a 2-hour car ride (crated ~4 hours). Often birds were crated for a similar amount of time during normal maintenance practices at PWRC such as mowing or tree trimming (B. A. Clauss, PWRC, personal communication). It is possible that birds were already acclimated to being crated for this length of time and therefore this short transit/crate time did not produce a detectible glucocorticoid response. Interestingly, SCBI birds showed a modest increase in FGM during the first week post shipment, which is likely attributed to the acclimation to the new environment, keepers, and routine. Nevertheless, even with this slight increase, the measured concentrations of FGM did not indicate that these individuals experienced long-term or "chronic" stress.

Birds that were transferred to other facilities were subjected to a 10-hour drive, a multi-hour flight, or some combination (crated between 12 to 24 hr). On average, we observed a 2-fold increase in FGM following these extended transit periods, regardless of facility. Nevertheless, FGM levels returned to pre-shipment levels in less than 1 week after the arrival to the new facilities, suggestive that the transportation did not have a long-lasting effect at least at the adrenal level.

In the present study, we also show the evidence of individual variations in physiological responses to translocation. Due to the small sample sizes, we were not able to perform statistical analysis to assess variations among individuals. Nevertheless, the visual observation of hormone profiles revealed 2 distinct patterns among birds that were transferred to the same facility: modest versus significant rise in FGM within the first 48 hours at the new facility. Anecdotal assessments (based on keepers' observation) indicate that the association between high glucocorticoid excretion and birds' temperament could be the cause of this discrepancy. We know in many other species that physiologic reactions, especially endocrine responses, can be modulated by different life history experiences and individual personalities as well as an animal's age and sex (Crino and Breuner 2015, Edwards et al. 2019, Prado et al. 2020). In order to study temperament influence in the future, an unbiased personality survey for avian species needs to be developed and implemented. Understanding of this relationship between temperament and stressful life history events would allow for improved welfare of captive populations and could influence the success of both captive breeding and reintroduction programs by informing rearing and release strategies.

Fecal samples have also been collected from whooping crane juveniles being transferred to their reintroduction site (M. E. Brown, SCBI; and B. Hartup, International Crane Foundation; unpublished data). We are unable to make direct comparisons between the juvenile and adult transfer data sets because the data sets in question used different antibodies to measure FGM, making direct comparison of the values invalid. However, similar patterns both during shipment and during acclimation were observed during the juvenile transfer. In that study, significant elevations in FGM were observed in samples collected from the crate compared to pre-shipment and post-shipment levels, and from limited opportunistic fecal sampling in the soft-release holding pens at the release site. Even without direct comparison we can appreciate the similarities in FGM production patterns associated with translocation across multiple whooping crane age classes.

Transport of wildlife and acclimation to a new facility can be very hazardous and may have a long-lasting impact on animal health and welfare (Millspaugh and Washburn 2004). Better understanding of how individuals respond to stressful stimuli based on their personalities could allow for a more specialized and personalized approach to mediating potential effects of novel environments and potentially stressful stimuli. It could also benefit as an early screening tool to determine an individual's suitability to reintroduction and provide feedback for effectiveness of reintroduction strategies. These findings help to demonstrate the significant physiological changes occurring during the transfer process and increases our knowledge of stress physiology in whooping cranes. These results can help inform future management decisions based on individual reaction during facility transfers and during acclimation to new facilities. Overall, while there were differences on the individual level, on the whole it appears that the whooping cranes included in this study acclimated quickly and did not experience long-term stress in terms of FGM production.

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