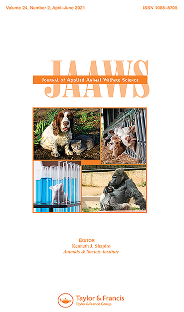

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A Behavioral Index for Assessing Bison Stress Level during Handling and Demographic Predictors of Stress Response

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ABSTRACT

There are an estimated half-a-million Plains Bison (*Bison bison*) present in North America in commercial and conservation herds. Most bison are rounded up and "worked" annually for parasite control, veterinarian attention, and processing, making it important to understand the impacts of these operations. Research indicates bison generally experience higher levels of stress than cattle during similar handling processes. However, most methods for assessing stress-level during working are invasive, increase handling time, and paradoxically increase stress levels. We designed a behavioral index to assess bison stress level during handling and used it to evaluate various predictors of stress response in a semi-wild bison herd. We examined how sex, age, herd of origin, previous experience, calf rearing, and body condition influenced bison stress response during working operations from 2015 to 2017. Our results indicate that stress level decreased with age and previous experience being worked through a particular facility. Additionally, herd of origin influenced stress level, indicating that stress response may have a genetic or epigenetic component. Our study provides an easily applicable tool for monitoring bison stress levels.

KEYWORDS

Plains Bison; *Bison bison bison*; Bison handling; low-stress handling; Bison behavior; stress response

Introduction

Plains bison (*Bison bison bison*; hereafter "bison") once numbered in the millions, but the number of bison plummeted in the mid-to-late 1800s to around a few hundred individuals as a result of overhunting and targeted eradication efforts meant to subjugate Native American tribes (Freese et al., 2007; Gates, Freese, Gorgan, & Kotzman, 2010; Lott, 2003). However, the American Bison Society, private ranchers (e.g., Charles Goodnight), and others started a movement to conserve this species and there are now an estimated half-a-million bison present in North America (Freese et al., 2007; Steenweg et al., 2016). Most bison currently exist in privately owned, generally production-oriented, herds and only about 4% remain in conservation herds on public lands (Freese et al., 2007; Metzger & Anderson, 1998; Ranglack & Du Toit, 2015). Regardless of management contexts, most will be rounded up and "worked" through a corral system for parasite control, veterinary attention, sorting, and/or processing annually (Duysen et al., 2017; Finocchiaro, 2019; Kossler, 2015). Techniques common in cattle ranching, including round up via all-terrain vehicles or horses and pushing individuals through corral systems are occasionally employed for bison (Duysen et al., 2017; Finocchiaro, 2019; Kossler, 2015; Wolfe, Shipka, & Kimball, 1999). However, given the wild or semi-wild nature of most bison, they are more likely to be injured or killed while being handled than cattle (Duysen et al., 2017; Grandin, 1999b; Lanier, Grandin, Chaffin, & Chaffin, 1999). Bison regularly

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experience high levels of stress during handling that can result in erratic behavior such as attempting to jump holding pens or goring other bison (Lanier & Grandin, 2001; Lanier et al., 1999). Even less extreme stress responses can promote sickness, limit growth, and reduce meat quality (Grandin, 2008; Grandin, Oldfield, & Boyd, 1998b; McCorkell et al., 2013; Shoemaker, 2014). Additionally, highly stressed bison pose a larger threat to the safety of bison management personnel (Duysen et al., 2017; Finocchiaro, 2019; Lanier et al., 1999). Furthermore, handling can serve as a selective pressure promoting domestication (Gates et al., 2010; Jones & Dratch, 2017). For these reasons, the bison conservation and ranching communities have promoted low-stress handling techniques as a best practice ([CBA and NFACC] Canadian Bison Association and the National Farm Animal Care Council, 2017; Church, Galbraith, McCorkell, Rioja-Lang, & Silzer, 2016; Finocchiaro, 2019; Gates et al., 2010; Gegner, 2001; Grandin et al., 1998b; Kossler, 2015; Lanier et al., 1999; Rioja-Lang, Galbraith, McCorkell, Spooner, & Church, 2019).

Despite including a relatively universal set of core techniques (low noise, habituation to working facilities, etc.), low-stress handling practices often differ across taxa depending on a particular species' natural history (Grandin, 1989, 1998a, 1999a, 1999b, 1999c, 2008; Grandin et al., 1998b). For instance, it is important to consider bison social relationships to effectively implement low-stress handling practices (Gegner, 2001; Lanier & Grandin, 2001; Rioja-Lang et al., 2019; Shaw, 2012). Bison prefer to be in groups, and when they are alone, they are more likely to respond to threats with comparatively dangerous fight-or-flight behaviors (Gegner, 2001; Lanier & Grandin, 2001; Rioja-Lang et al., 2019). Conversely, keeping bison at lower densities within the corral system helps prevent a stressed individual from injuring others (Grandin, 1998a; Lammers, 2011; Lanier & Grandin, 2001; Shaw, 2012). Experts generally recommend moving bison through the corral system in small groups (e.g., 2–5 individuals depending on facilities) and keeping mothers close to their calves (Kossler, 2015; Lammers, 2011; Lanier & Grandin, 2001). Nonetheless, in very tight spaces, such as the alleyway just before the compression chute, it is better to move bison individually (Gegner, 2001; Lanier & Grandin, 2001). Similarly, low-stress handling involves the calm habituation of animals to working facilities through repeat exposure and often employs positive reinforcement (Gegner, 2001; Grandin, 1998a, 1999b; Lanier et al., 1999). For example, baiting can be used to move bison through corral systems as opposed to pushing them via off-road vehicles (Lammers, 2011). The structural features of working facilities are also essential to practicing safe and low-stress handling techniques (Duysen et al., 2017; Grandin, 1998a, 1999c, 2008; Lammers, 2011; Rioja-Lang et al., 2019). Fences and chutes should be 7 feet tall (2.1 m), curves within the alley way system should be rounded, and persons should not be visible through panels (Grandin, 1998a, 1999c, 2008; Lammers, 2011; Rioja-Lang et al., 2019). The elimination of distractions such as puddles, shadows, and loud noises may also help bison move through the corral systems efficiently (Grandin, 1998a, 1999b; Rioja-Lang et al., 2019).

Though substantial effort has been dedicated to establishing best practices for low-stress handing, it remains as much of an art as a science (Kossler, 2015). Moreover, nearly all working facilities are unique, with most needing iterative adjustment to effectively facilitate low-stress handling (Grandin, 1998a; Kossler, 2015; Lammers, 2011). Therefore, it is important to have a tool to monitor the effectiveness of working practices for maintaining a low-stress environment (Lanier & Grandin, 2001). Several physiological indicators of stress exist such as blood cortisol level, plasma epinephrine, heart rate, respiration rate, and rectal temperature (Hawley & Peden, 1982; McCorkell et al., 2013; Shoemaker, 2014). However, these methods often require veterinary expertise, sample processing can be expensive, and are often invasive, increase handling time, and therefore paradoxically increasing stress levels (Hawley & Peden, 1982; Kossler, 2015; McCorkell et al., 2013; Shoemaker, 2014). As Lanier and Grandin (2001) note, bison behavior can be used as an indicator of an individual's stress level. For instance, initial signs of stress include more subtle behaviors such as circular movements, blinking, and licking, but as stress level increases it can lead to heavy breathing, foaming at the mouth, attacking, and trying to escape the pen (Lanier & Grandin, 2001). We used existing literature to construct a behaviorally based bison stress index, with the goal of creating an objective yet observational tool for monitoring bison stress levels during handling that is accessible to a wide range of personnel (Lanier & Grandin, 2001; Lanier et al., 1999; Lee, 1990). We applied the behavioral stress index during bison working efforts from 2015 to 2017 on a semi-wild herd on land owned and managed by a private conservation organization. We examined variation in the stress index in relation to demographic and physiological covariates as a test of the index's sensitivity and to provide insight into those animals typically experiencing the highest levels of stress during handling.

Methods

Study site and Bison Herd

The Crane Trust is a nonprofit conservation organization dedicated to protecting the ecological integrity of the Central Platte River Valley for the benefit of Whooping Cranes, Sandhill Cranes, other migratory birds, and species of concern (VanDerwalker, 1982; www.cranetrust.org). The Crane Trust protects over 9,000 acres of lowland tallgrass prairie, wet meadow, riparian woodlands, and associated habitats (See Currier, 1982) through land ownership and conservation easements, and grazes bison on over 1,000 acres of that land (40.78° N, −98.47° W, 600 m elevation). The organization takes a holistic approach to habitat conservation and reintroduced bison on a significant scale in January of 2015, following a smaller pilot program from 2013 to 2014, in the hope of improving the ecological function of its wet meadows and prairies by reestablishing this historic grazer (Steuter & Hidinger, 1999; Truett, Phillips, Kunkel, & Miller, 2001). The Crane Trust herd included 41 bison upon reintroduction in 2015 and totaled 148 individuals, including calves, as of September 2020. The first bison brought to the Crane Trust were from the privately owned Rim Rock (RR) herd, in Crawford, Nebraska, which included two recently added bulls from Turner Ranches in Nebraska. In 2016, bison from the Rocky Mountain Arsenal Wildlife Refuge (AWR) in Colorado and the north unit of Theodore Roosevelt National Park (TNP) in North Dakota were also reintroduced to the Crane Trust. We considered bison born at the Crane Trust, as a result of this herd integration, to be Crane Trust (CT) bison.

The Crane Trust handles bison once annually in the fall to meet several management objectives, including: genetic monitoring of newly introduced bison and recently born calves (blood and hair collection), treatment of parasites (topical moxidectin application), fecal parasite monitoring (droppings from chute or manually extracted from rectum), vaccinations (Vision-8, Merck Animal Health, Madison, NJ), necessary sorting of animals for management purposes, and application of identification equipment (ear tag and electronic identification tag) (See Douglas et al., 2011; Woodbury & Lewis, 2011). The bison are moved through a working system designed to follow recommendations for low-stress handling, which includes multiple corrals and an alleyway that is partitioned with sliding doors for sorting that eventually narrows as it approaches a compression chute (Grandin, 1999c; Kossler, 2015; Lammers, 2011; Rioja-Lang et al., 2019). Each bison is secured in the compression chute before biological samples are collected and veterinary medicine treatments are applied. The alleyway is > 2 m tall and constructed with solid metal to prevent bison from seeing through to the workers, directional turns within the system are gradual curves without obvious sharp angles, and the crash gate is wire mesh which allows the bison to partially view the open corral past the compression chute (Grandin, 1999c; Kossler, 2015; Lammers, 2011; Rioja-Lang et al., 2019). Crane Trust staff attempt to practice low-stress handling techniques by minimizing noise, limiting the time individuals are within the working system, particularly the compression chute, limiting the number of bison held together in holding pens within the alleyway (< 5 individuals), keeping mothers with their calves, using non-electric prods (rattle paddles), restricting the number personnel to those essential for operations, and maintaining a calm attitude among assisting personnel (Grandin, 1999c; Kossler, 2015; Lammers, 2011; Rioja-Lang et al., 2019).

Data collection

We worked bison on four occasions in October or November from 2015 to 2017 (2015–11-18, 2016–10-11, 2016–11-12, and 2017–10-17). We assessed bison stress levels using a scalar index we developed based on bison behaviors, indicative of various levels of stress from the existing literature as well as our own observations (Lee, 1990; Lanier et al., 1999; Lanier & Grandin, 2001; King, Caven, Leung, Ranglack, & Arcilla, 2019; Table 1). The stress index ranged from 0 to 7, with 0 being no detectable stress and 7 being severe stress (Table 1a). The index includes a range of stress-related behaviors from those regularly observed (lower stress) to those only displayed in highly restrictive unnatural situations or under threat of mortality (higher stress; Table 1a). The index is paired with a descriptive catalog of behaviors reflective of various levels of stress, which is divided into three broader lists, including those behaviors generally associated with “Low to Moderate (1–3)”, “Moderate to High (4–5)”, or “High to Severe (6–7)” stress (Table 1b). Each behavior within this catalog is also paired with a range of stress scores (Table 1b). For instance, “bucking” behavior, depending on how severe, could be indicative of a stress-score from 5 to 6 (Table 1b). The overall score for each individual represented an average of all stress-related behaviors observed. Each animal was assessed by at least two personnel, with one observer providing an assessment from near the end of the alley way, and the other observer estimating the stress-level at the compression chute. Observers were consistent across the study period. The final stress score for each bison was the median value between both observers rounded to the nearest 0.5. A body condition score (BCS) was also assigned to each bison. This ranged from 1 to 5 with 1 being the lowest body condition (very thin) and 5 being the best body condition (very fat; Alberta Agriculture and Forestry, 2010; Norman, 2010; Ranglack & Du Toit, 2015; Zielke, Wrage-Mönnig, & Müller, 2018). The score was determined by the visual appearance of the ribs, spine, hip bones, rump, tail head, and hump as the animal passed through the end of the alley way and the compression chute (Alberta Agriculture and Forestry, 2010; Norman, 2010; Ranglack & Du Toit, 2015; Zielke et al., 2018). The bison BCS was also scored by at least two observers with the final BCS being the median value between observers rounded to the nearest 0.5. Herd of origin (RR, CT, AWR, TNP), sex, age (Fuller 1959), whether the bison had been worked at the Crane Trust previously, whether female bison had a calf, and ear tag number were also recorded for all bison.

Table 1. Bison stress indicator scale for assessing low stress handling techniques and quantifying individual responses during working (a) with a description of common “low to moderate”, “moderate to high”, and “high to severe” stress indicating behaviors (b).

a. Stress behavior indicator scale from 0 (non-detectable) to 7 (severe).

Stress Score	0	1	2	3	4	5	6	7
Level	Non-Detectable	Very Low	Low	Moderately Low	Moderately High	High	Very High	Severe

b. Observable behaviors associated with various levels of stress.

Low to Moderate (1–3)

- Blinking (1)
- Licking (1–2)
- Huddling (2)
- Tail slightly elevated or arched with/without defecation (2–3)
- Head shaking normally (2–3)
- Vocalization (i.e. low grunt, sharp grunt, snort, alarm) (2–3)
- Circular movement (3–4)
- Backing up (3–4)
- Balking (3–4)
- Stomping (3–4)
- Quickened pace (3–4)

Moderate to High (4–5)

- Bulging eyes (4–5)
- Tail vertically raised (4–5)
- Head shaking vigorously with/without vocalization (4–5)
- Labored breathing (deep, increased) (4–5)
- Frothing at mouth (medium) (4–5)
- Ramming corral (occasionally, not full speed) (4–5)
- Pushing with entire body weight (5)
- Trotting-galloping (5)
- Vocalization (i.e. growl, rapid grunt, hiss and spit) (5–6)
- Bucking (5–6)
- Attacking others in view (5–6)

High to Severe (6–7)

- Ramming corral (vigorously, full speed) (6–7)
- Head shaking suddenly and heavily with/without vocalization (6)
- Running in circles/laps (6)
- Pushing/overexerting body weight (6)
- Sitting inappropriately (6)
- Labored breathing (shallow, quick) (6–7)
- Frothing at mouth (heavy) (6–7)
- Attacking sharply and intensely (6–7)
- Immobility (7)

Data analysis

We summarized variation in our sample using mean, median, interquartile range, minimum, and maximum value summary statistics for scalar and numeric variables. We additionally examined variation in the behavioral stress index across values and categories of independent variables. We then fit multiple generalized linear models (Nelder & Baker, 1972) to examine how stress levels during working operations were influenced by age, sex, experience, herd of origin, calf rearing, and body condition using the "glm" function in the "stats" package for R version 3.3.2 (R Core Team, 2020). Given that the relationship between stress and our covariates may be nonlinear, we evaluated multiple functional forms (linear, quadratic, pseudothreshold) for age and body condition. To examine direct comparison of the importance for each covariate in our model, the age and body condition were standardized by subtracting the mean and dividing by two times the standard deviation (Gelman, 2008; Lele, 2009).

The top model from all the factors was found by using a multi-tiered approach to model selection (Franklin, Anderson, Gutierrez, & Burnham, 2000). The multi-tiered approach lowered the number of competing models (Burnham & Anderson, 2002). Tier one was an exploratory analysis of the selected functional forms (linear, quadratic, and/or pseudothreshold) for age and body condition in relation to stress level. All models were ranked for each covariate using Akaike's Information Criterion (AIC), and those of which the functional forms were within 2 AIC delta were advanced to the next tier (Burnham, Anderson, & Huyvaert, 2011; Ranglack et al., 2017). In tier two, we combined the top functional forms for age and body condition with every possible combination of the categorical covariates to determine the top supported model of bison stress during working operations. All covariates were screened for multi-collinearity using Person's correlation/association coefficients. Any collinear covariates were not included in the same model using |0.6| as a basis of determining correlation (Dormann et al., 2013). Following recommendations from Arnold (2010), we culled uninformative parameters from our reported models to improve their interpretability. Lastly, the resulting models were ranked using AIC to determine the best-supported model predicting bison stress during working operations.

Results

There were 50, 72, and 65 individual bison worked at Crane Trust facilities in 2015, 2016 (2 events), and 2017, respectively. A total of 100 individuals were worked from 1 (n = 34) to 3 (n = 21) times, with most individuals being worked twice (n = 45; $\bar{x} \pm$ = 1.9 ± 0.7) across the three-year study period for a total sample of 187 cases. No bison were worked four times. Forty individuals were from the founding RR herd, 37 were from the CT herd, 13 were from the AWR herd, and 10 were from the TNP herd. Fifty-eight percent of the observations included female bison and 42% were male. The mean age of bison worked from 2015 to 2017 was 3.3 years of age, with a median value of 2.5 years (range = 0.5 to 9.5 yrs.). Body condition score averaged 3.4 on a 5-point scale indicating that our bison were generally between "moderate" and "moderately fat" body condition (range = 2 to 4).

Mean stress level during working was 4.3 on our 7-point behavioral index, which would equate to "moderately high" stress levels (e.g., bulging eyes, labored breathing, etc.; Table 1, Figure 1). However, scores for individual observations ranged from 1.5 to 7 on our behavioral stress index, with about 12% of bison scoring a 6.0 or higher and about 6% exhibiting a 2.0 or lower (Figure 1). The lower interquartile range value of the bison behavioral stress index was 3.5, the median 4.5, and the upper interquartile range 5.0 indicating that 25% of bison in this study experienced "high" stress behavior (e.g., bucking, hiss and spit vocalization, sitting inappropriately, etc.; Figure 1). Bison two years of age or younger averaged 4.7 on the stress index, while bison older than 2 years of age averaged 4.0. Bison with a BCS of 3.5 or higher averaged 4.2 on the stress index while bison with a BCS of 2.5 or lower averaged 4.7. Finally, bison that had been worked previously at Crane Trust facilities averaged 3.8 on the stress

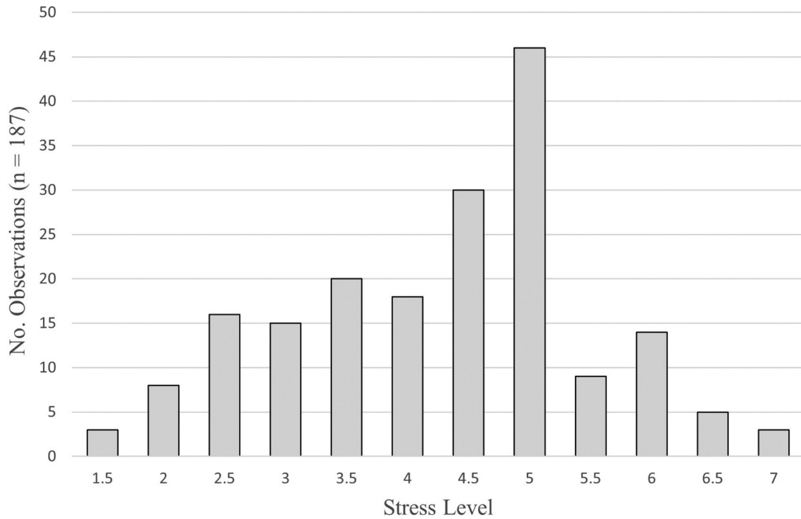

Figure 1. Histogram of bison stress levels across 187 observations of bison worked through Crane Trust handling facilities from November 2015 to October 2017.

index compared to 4.7 for those that had not. No bison mortalities or life-threatening injuries were recorded during handling efforts associated with this study. We incidentally noted contusions to the nostril area with localized bleeding and broken horn caps as a result of the high-speed ramming of corral walls by bison experiencing higher levels of stress (≥5).

Quadratic and pseudothreshold were the most supported functional forms for the relationship between age and stress level from the first tier of our analysis. Linear, quadratic, and pseudothreshold forms were all supported for the relationship between BCS and stress level from the first tier of our analysis. These functional forms were moved forward into the tier two analysis. Sex, if females had a calf, herd of origin, and if they had been worked at the Crane Trust previously were all included in tier two analyses as categorical variables. We removed BCS and if an individual had a calf from reported models as they were uninformative covariates. The resulting top model from tier two contained age as a pseudothreshold, herd of origin, sex, and if bison had been worked previously at the Crane Trust (Table 2).

The most important factor influencing estimated stress levels was herd of origin, with an estimated effect of 0.93 (SE = 0.26, p < 0.001, Table 3 and Figure 2) for the Rim Rock herd, which was the only herd that was significantly different than Arsenal, which was used as a dummy variable. The second most important factor was age as a pseudothreshold, with an estimated effect on stress levels of −0.81 (SE = 0.27, p = 0.001, Table 3 and Figure 3). Having been worked previously was the third most important factor influencing stress levels, with an estimated effect of −0.73 (SE = 0.19, p < 0.001, Table 3 and Figure 4). The estimated effect of sex on stress levels was −0.32 (SE = 0.17, p = 0.07, Table 3 and Figure 5) for males relative to females.

Discussion

The behavioral stress index demonstrated variability and sensitivity across a range of contexts as well as a relatively normal statistical distribution (Figure 1, Table 2). Despite efforts to construct low-stress working facilities and employ associated handling techniques, our data suggests that most bison processed at the Crane Trust are still experiencing "moderately high" to "high" levels of stress during handling (Table 1; Lanier & Grandin, 2001). High stress experiences can result in both acute

Table 2. Model selection table for tier-2 analysis including only informative covariates and the top functional forms of linear variables with models ranked by Akaike Information Criterion corrected for small sample sizes (AIC_c) including model covariates (Model), degrees of freedom (df), log Likelihood, AIC_c score, AIC_c delta score, and AIC_c weight for each individual model. Model variables include the behavioral stress index score (Stress – Dependent Variable), pseudothreshold form of age (Age_{ps} – Independent Variable (IV)), herd of origin (Origin – IV), sex as male relative to female (Sex – IV), and whether a bison had been previously worked through Crane Trust facilities (Previously Worked – IV).

Model	df	log Likelihood	AIC_c	Δ AIC_c	AIC_c wt.
Stress ~ Age_{ps} + Origin + Sex + Previously Worked	8	−268.00	552.81	0.00	0.54
Stress ~ Age_{ps} + Origin + Previously Worked	7	−269.78	554.19	1.37	0.27
Stress ~ Origin + Previously Worked	6	−271.75	555.97	3.16	0.11
Stress ~ Origin + Sex + Previously Worked	7	−271.15	556.93	4.11	0.07
Stress ~ Age_{ps} + Origin + Sex	7	−275.40	565.43	12.61	0.00
Stress ~ Age_{ps} + Origin	6	−277.60	567.67	14.86	0.00
Stress ~ Age_{ps} + Sex + Previously Worked	5	−279.41	569.15	16.34	0.00
Stress ~ Age_{ps} + Previously Worked	4	−281.64	571.51	18.70	0.00
Stress ~ Previously Worked	3	−283.69	573.50	20.69	0.00
Stress ~ Sex + Previously Worked	4	−282.79	573.79	20.98	0.00
Stress ~ Age_{ps} + Sex	4	−288.67	585.57	32.75	0.00
Stress ~ Age_{ps}	3	−290.56	587.26	34.45	0.00
Stress ~ Origin	5	−289.25	588.84	36.03	0.00
Stress ~ Origin + Sex	6	−289.24	590.94	38.13	0.00
Stress ~ Null	2	−299.64	603.35	50.54	0.00
Stress ~ Sex	3	−299.62	605.37	52.56	0.00

Table 3. Covariate parameter estimates (*B*) with associated standard errors (*SE*) and p-values (*p*) for significant variables from the top performing model predicting stress levels of bison processed through Crane Trust working facilities.

Covariate	*B*	*SE*	*p*	
Intercept	4.19	0.27	< 0.001	***
Age (*pseudothreshold*)	−0.81	0.33	0.001	**
Previously Worked-Yes	−0.73	0.19	< 0.001	***
Origin-Crane Trust	0.42	0.37	0.264	
Origin-Rim Rock	0.93	0.26	< 0.001	***
Origin-Teddy Roosevelt	−0.15	0.36	0.677	
Sex-Male	−0.32	0.17	0.065	^

and long-term negative health consequences including capture myopathy, increased in utero mortality (i.e., abortion), and increased disease risk, (Grandin, 2008; Rioja-Lang et al., 2019; Shaw, 2012; Shoemaker, 2014). The undomesticated character of bison presents a unique and challenging management situation, making low-stress working methods essential (Lanier & Grandin, 2001). Understanding the demographic predictors of stress can help us determine which animals are most at risk during processing and adapt our working procedures accordingly.

Our results indicate that stress level declined significantly with age, particularly across the first two years of life (Figure 3). Stress level in bison less than or equal to one year of age at the time of handling averaged 5.2, which is associated with potentially dangerous behaviors such as "attacking others in view" or "labored breathing" (Table 1; Lee, 1990, Lanier et al., 1999, Lanier & Grandin, 2001). Calves are generally the most vulnerable individuals in a herd to predation and depend on their mothers and the herd for protection (Carbyn & Trottier, 1987). Calves reared in a wild or semi-wild setting, such as at the Crane Trust, are not artificially weaned from their mothers and annual working operations likely represent the first occasion that calves are completely separated from them (Green, 1986; Lott, 2003). Furthermore, calves were being worked through a facility for the first time and novel artificial environments tend to be more stressful for ungulates than familiar settings (Grandin, 1999b; Lanier & Grandin, 2001; Lanier et al., 1999). Concurrently, bison that had been previously worked through Crane Trust facilities had lower stress levels than those that had not (Figure 4). Bison that had been worked previously

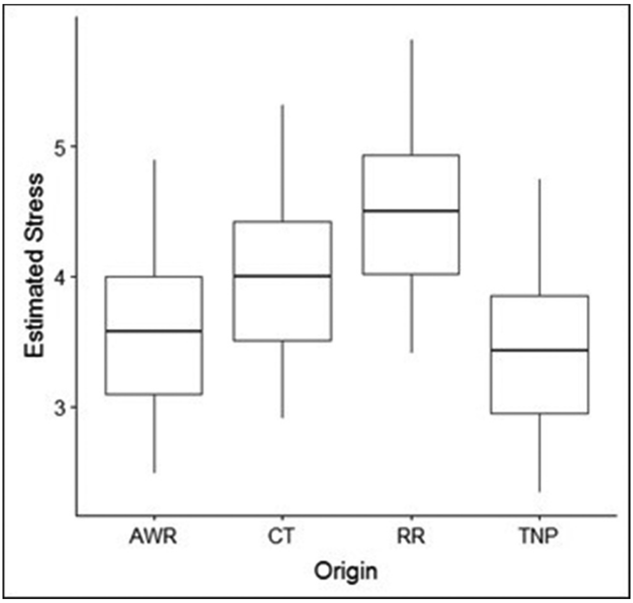

Figure 2. The estimated effect of herd of origin on stress during working operations for the years 2015–2017 when holding all other covariates constant.

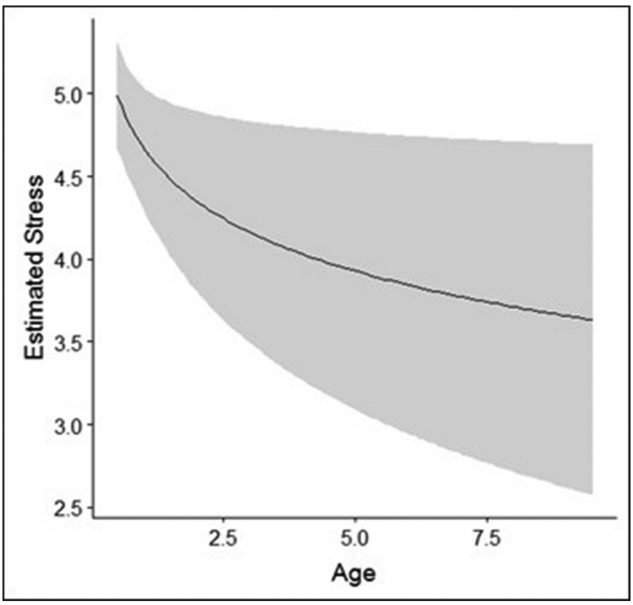

Figure 3. The estimated effect of age on stress during working operations at the Crane Trust for the years 2015–2017 when holding all other covariates constant.

at this facility likely recognized that they were not in serious danger during this process (Grandin et al., 1998b; Lanier et al., 1999). However, as Grandin et al. (1998b), Grandin (1999a), 1999b, 1999c) notes, experience does not always equate to reduced stress levels as extremely negative experiences in particular contexts can increase stress response in those same environments in the future. Research indicates that stress responses can be reduced by habituating bison to working

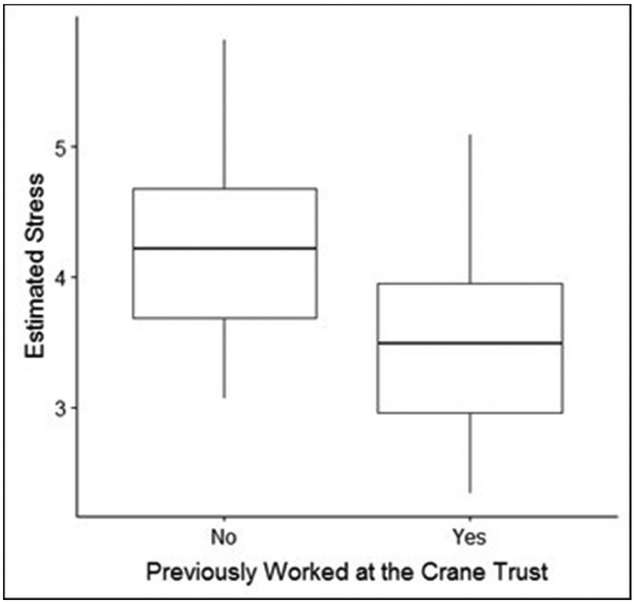

Figure 4. The estimated effect of having been previously worked at the Crane Trust on stress during working operations for the years 2015–2017 when holding all other covariates constant.

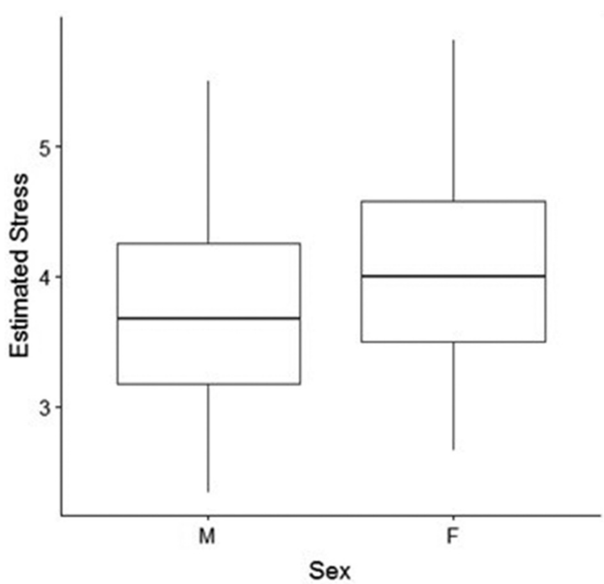

Figure 5. The estimated effect of sex on stress during working operations at the Crane Trust for the years 2015–2017 when holding all other covariates constant.

facilities, particularly by employing positive reinforcement (operant condition) at challenging areas within the handling facilities (e.g., squeeze chute; Grandin, 1999b; Lanier & Grandin, 2001; Lanier et al., 1999). Our data suggests that we should increase our efforts to habituate our bison to processing facilities, particularly regarding calves and newly acquired adults.

The fact that herd of origin was highly predictive of bison stress level was somewhat surprising and may potentially be explained by a couple of different factors. First, it is possible that behavioral differences can largely be explained by genetic influences, both those derived from natural and artificial selection (Grandin & Deesing, 2014; Isvaran, 2005). Animal behavior is phenotypic, in other words it, it represents an observable trait resulting from genetic expression in distinctive environmental contexts (Jensen & Wright, 2014; Wolf & Weissing, 2012). Behavior varies not only across individuals, but also across populations, and it is an important and observable factor in evolution (Wolf & Weissing, 2012). For instance, Found and St Clair (2019) demonstrate how "boldness" in elk (*Cervus canadensis*) is promoting residency over migratory behavior within National Parks in the Canadian Rocky Mountains. The "genetic" explanation is supported by the finding that CT origin bison, which represent the genetic integration of all parent herds (RR, AWR, TNP), demonstrated a stress response mirroring the weighted average of the source herds (Figure 2).

An alternative explanation is that the different herds in our study have experienced varying levels of domestication, promoting contrasting levels of docility over successive generations before arriving at the Crane Trust (Jensen & Wright, 2014). On the other hand, it is possible that the collective experiences of individual herds before their arrivals at the Crane Trust were influencing stress responses to their contemporary working facilities (Grandin, 1999a, 1999c; Merkle, Fortin, Morales, & Grether, 2014). Bison have impressive memories that guide their spatial habitat selection and inform responses to landscape and habitat features (Bailey et al., 1996; Merkle et al., 2014). Processing within the Crane Trust working facilities may be relatively stressful compared to their previous experiences, or alternatively, their preceding experiences could have been highly stressful, resulting in particular herds having a stronger aversion to all similar working processes (Grandin, 1999a, 1999c). In any case, it is possible that previous collective experiences could be driving differences in herd stress responses to handling at the Crane Trust. Epigenetics provides a third explanation that bridges the gap between genetic and behavioral explanations (Darnaudéry & Maccari, 2008; David, Canario, Combes, & Demars, 2019; Veenema, 2009). Significant and/or recurrent environmental stressors alter neuroendocrine activity patterns, which in turn influences gene expression and behavioral patterns without altering underlying genetic material (Darnaudéry & Maccari, 2008; David et al., 2019; Veenema, 2009). Furthermore, these phenotypical expressions can be transmitted across successive generations (David et al., 2019; Lind & Spagopoulou, 2018).

It is notable that female bison appear marginally more stressed during working than male bison, but that does not seem to be related to having a calf, which was not a significant predictor variable in our model. Adult male bison generally experience a reduced predation risk in the wild because of their significantly larger size, which may result in them being less likely to identify external stimuli as a serious danger (Meagher, 1986). It is possible that males were less likely to identify the working process as a threat in our study.

Conclusions

Our behavioral stress index displayed variation between individuals and across demographic groups suggesting that the measure is sensitive to differences in expressed behavior indicative of stress. Further work should validate this index using biological and physiological indicators of stress (blood or fecal cortisol, etc.). This behavioral index provides a widely accessible and applicable tool for monitoring bison stress levels during handling processes and in other non-natural environments. For instance, to move genetic material between herds and to move bison to markets, it is necessary both for conservation and production purposes to transport bison (Hedrick, 2009, Woodford & Rossiter, 1994). This behavioral stress index can easily be used to track the stress levels of bison during translocations and similar intensively human-managed and unnatural processes. Even in production-oriented situations, which is a growing business, limiting stress levels generally equates to better meat quality (Metzger & Anderson, 1998, McCorkell et al. 2013). Despite our efforts to follow recommendations for low-stress handling practices (facilities, worker behavior, etc.) our data suggests that bison at the Crane Trust are regularly experiencing high levels of stress, particularly regarding calves. Increased habituation of young and newly acquired bison to our working facilities prior to processing efforts may reduce stress levels during handling (Grandin, 1999b; Lanier & Grandin, 2001; Lanier et al., 1999).

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