Journal of
Zoological and Botanical Gardens

MDPI

Article

Comparing Space Use and Fecal Glucocorticoid Concentrations during and after the COVID-19 Closure to Investigate Visitor Effects in Multiple Species

Ashley N. Edes [1,*], Nathan C. Liu [2], Eli Baskir [1], Karen L. Bauman [1], Corinne P. Kozlowski [1], Helen L. Clawitter [1] and David M. Powell [1]

[1] Department of Reproductive and Behavioral Sciences, Saint Louis Zoo, St. Louis, MO 63110, USA; baskir@stlzoo.org (E.B.); kbauman@stlzoo.org (K.L.B.); kozlowski@stlzoo.org (C.P.K.); hclawitter@stlzoo.org (H.L.C.); dpowell@stlzoo.org (D.M.P.)

[2] College of Arts & Sciences, Washington University in St. Louis, St. Louis, MO 63130, USA; nathanliu@wustl.edu

* Correspondence: aedes@stlzoo.org

Abstract: We used the COVID-19 pandemic closure at the Saint Louis Zoo to examine visitor effects on space use and glucocorticoid levels in banteng, grizzly bears, polar bears, and western lowland gorillas. The study was divided into four six-week phases: closure in spring 2020, reopening in summer 2020, fall 2020, and spring 2021 as a seasonal comparison. Space use data were collected using video, and fecal samples were assayed for glucocorticoids. Generalized linear models were used to examine differences in zone occupancy and glucocorticoids between phases. The banteng spent more time near visitors, and glucocorticoids were only temporarily elevated in two of five animals when visitors returned. The grizzly bears spent more time in their habitat than in the den, and the polar bear spent more time near viewing areas after visitors returned. Glucocorticoids did not differ significantly between the closure and reopening for any bears. The gorillas spent less time close to visitors immediately after reopening but this effect waned by fall; glucocorticoid data were not available. Overall, based on space use and glucocorticoid levels, we suggest visitor effects on the gorillas are neutral, on the grizzly bears are neutral or positive, and are positive on the banteng and polar bear.

Keywords: physiology; cortisol; zoo research; behavior; banteng; gorillas; grizzly bears; polar bears

Citation: Edes, A.N.; Liu, N.C.; Baskir, E.; Bauman, K.L.; Kozlowski, C.P.; Clawitter, H.L.; Powell, D.M. Comparing Space Use and Fecal Glucocorticoid Concentrations during and after the COVID-19 Closure to Investigate Visitor Effects in Multiple Species. *J. Zool. Bot. Gard.* **2022**, *3*, 328–348. https://doi.org/10.3390/jzbg3030026

Academic Editor: Steven Monfort

Received: 3 May 2022
Accepted: 27 June 2022
Published: 5 July 2022

1. Introduction

The effect of visitors on zoo-housed animals is frequently characterized as positive, negative, or neutral based on the behavioral or physiological responses of various species. For example, Mexican wolves (*Canis lupus baileyi*) spent less time resting and eating and had higher fecal glucocorticoid levels on days with larger crowds [1], suggesting a negative visitor effect. In contrast, behavioral diversity in Gentoo penguins (*Pygoscelis papua*) was associated with higher visitor counts [2], indicating a positive or stimulating effect. To lesser anteaters (*Tamandua tetradactyla*), visitors may be a neutral stimulus, as they showed no differences in behavior, the timing of activity, or space use when visitors were present or absent [3]. Although visitor effects are frequently studied in zoos, substantial inter- and intra-specific variation in responses have made it difficult to draw definitive conclusions about the effect of visitors, even within a single species. For example, one study reported no significant effect of crowd size on pacing by jaguars (*Panthera onca*) [4], while another reported a positive association [5]. For a recent comprehensive review of visitor effect research in zoos, see [6].

Visitors present a wide array of stimuli, including visual, auditory, and olfactory. As it is often difficult to distinguish between these stimuli and determine the exact cause of changes in behavior and/or physiology, many studies focus instead on whether visitors are present or absent [7–9]. One challenge in these studies is that times without visitors in a zoo are infrequent. As such, zoo closures in early 2020 as a result of the COVID-19 pandemic provided a unique opportunity to study visitor effects without this limitation. Importantly, to minimize the disruption to the animals in their care, many zoos worked to maintain normal husbandry and management routines during the closures.

Several published studies have already compared behavioral and physiological data collected during COVID-19 closures with data collected prior to the closure or after reopening. As seen in other visitor effects studies, a mixture of positive, negative, and neutral effects was reported. Nile crocodiles (*Crocodylus niloticus*) spent more time in proximity to conspecifics when the zoo was open compared to closed [10], and Japanese macaques (*Macaca fuscata*) seemed to prefer completing trials in testing booths closest to the public when the zoo reopened [11]. Five primate species, including Eastern black-and-white colobus (*Colobus guereza*), Allen's swamp monkeys (*Allenopithecus nigroviridis*), DeBrazza's monkeys (*Cercopithecus neglectus*), Bolivian gray titi monkeys (*Callicebus donacophilus*), and crowned lemurs (*Eulemur coronatus*), showed a slight but significant increase in proximity to the glass when open versus closed [12]. Catalina Island rattlesnakes (*Crotalus catalinensis*) showed more social and investigative behaviors after reopening [13]. Beaded lizards (*Heloderma horridum exasperatum*) [14] and multiple bear species, including Andean (*Tremarctos ornatus*), sloth (*Melursus ursinus*), sun (*Helarctos malayanus*), grizzly (*Ursus arctos horribilis*), and black bears (*Ursus americanus*), were more visible when visitors were present [15], suggesting a positive effect of visitors. On the other hand, European glass lizards (*Pseudopus apodus*) [13], meerkats (*Suricata suricatta*) [16], African penguins (*Spheniscus demersus*) [16], and red kangaroos (*Macropus rufus*) [17] used more of their enclosure when the zoo was closed, Chilean flamingos (*Phoenicopterus chilensis*) decreased activity and feeding when the zoo reopened (although the authors indicate these changes may be more due to weather) [18], and European glass lizards [13], Chinese crocodile lizards (*Shinisaurus crocodilurus*), and tokay geckos (*Gekko gecko*) [14] were less visible under open conditions.

Overall, however, most studies conducted during COVID-19 closures reported entirely or mainly neutral effects that suggest a minimal impact from visitors. There were no significant differences in behavior between the closures and open periods reported for western lowland gorillas (*Gorilla gorilla gorilla*) [19], African penguins [16], greater flamingos (*Phoenicopterus roseus*) [18], Amur leopards (*Panthera pardus orientalis*), snow leopards (*Panthera uncia*), Rothschild giraffes (*Giraffa camelopardalis*), Chapman's zebras (*Equus quagga chapmani*), nyala (*Tragelaphus angasii*), swamp wallabies (*Wallabia bicolor*) [20], Eastern black-and-white colobus, Allen's swamp monkeys, DeBrazza's monkeys, Bolivian gray titi monkeys, or crowned lemurs [12]. Additionally, Nile crocodiles [10], greater and Chilean flamingos [18], Amur leopards, snow leopards, Rothschild giraffes, Chapman's zebras, nyala, swamp wallabies, and Chinese gorals (*Naemorhedus griseus*) [20] showed no difference in space use between open and closed periods. There also were minimal differences in levels of behavior, space use, and/or visibility in multiple species of reptiles [13,14]. Similarly, while visibility initially declined with the return of visitors for several amphibian species, that effect waned with time, and visibility returned to or increased above closure levels [21]. In Japanese macaques, there was no difference between the closure and reopening on the number of cognitive trials animals chose to participate in, the number of trials they completed per session, or their accuracy on the tasks [11]. Physiologically, neither cheetahs (*Acinonyx jubatus*) nor giraffes (*G. c. reticulata*, *G. tippelskirchi*) showed significant differences in fecal glucocorticoid levels between matched open and closed periods [22].

Many COVID-19 closure studies also showed variation in visitor effects on a single species, either within the same group or between groups at different locations. For example, although not significant, the silverback male in a gorilla troop increased his foraging time and decreased his inactivity during the closure, while the other five members of his group decreased foraging and increased inactivity [19]. Nile crocodiles showed more agonism but also more conspecific bunting, a positive social behavior, during the closure [10].

Meerkats behaved differently across three zoos, with some groups showing increases in environmental interaction, positive social interaction, or vigilance during the closure, while other groups did not [16].

Our objective was to use the COVID-19 closure to gain insight into how visitors impact space use and physiology in four species housed at the Saint Louis Zoo, including banteng (*Bos javanicus*), grizzly bears, polar bears (*U. maritimus*), and western lowland gorillas. Increased time spent in proximity to viewing areas when guests returned was interpreted to indicate a positive effect of visitors and vice versa. A similar approach has been used in other studies. Gorillas and chimpanzees (*Pan troglodytes*) did not vary their use of a zone within 1m of the viewing glass based on crowd size [23]. Eastern black-and-white colobus, Allen's swamp monkeys, DeBrazza's monkeys, Bolivian gray titi monkeys, and crowned lemurs showed an increase in proximity to viewing windows, both with larger crowd sizes and when the zoo was open than during the COVID-19 closure [12]. Other COVID-19 studies also examined proximity to guests as an indicator of visitor effects. For example, Grevy's zebra spent more time in spaces close to public viewing areas than expected by chance when the zoo was closed and less time than expected by chance when the zoo was open [20]. Additionally, Chilean flamingos used a hill zone near visitor viewing areas more frequently after reopening [18], and meerkats at one zoo spent more time in the area closest to visitors while meerkats at two other zoos spent more time in the area furthest from visitors after reopening [16].

As an indicator of hypothalamic-pituitary-adrenal (HPA) axis activity, glucocorticoid levels can provide information on levels of arousal experienced by an individual, with higher levels generally indicating increased arousal, either positive or negative [24,25]. Fecal glucocorticoids are commonly used to investigate how animals respond to environmental changes and events from a physiological perspective and, as such, have been incorporated into visitor effect research. For example, clouded leopards (*Neofelis nebulosa*) on display had higher fecal glucocorticoids than those not on display [26], black-capped capuchins (*Cebus apella*) had lower glucocorticoids on average when one-way viewing screens were in place [27], and meerkats showed a positive association between crowd size and fecal glucocorticoids [28]. On the other hand, there were no significant correlations between visitor numbers and glucocorticoids in African penguins [29] or kangaroos (*Macropus fuliginosus*) and red kangaroos [30], and fecal glucocorticoids in gorillas did not vary based on crowd size, noise levels, or frequency of camera flashes [31]. While glucocorticoids can provide insight into how animals perceive experiences, there are also limitations to their use, especially as increases are frequently interpreted as evidence of stress. Glucocorticoids play functions outside of stress responses, such as the awakening response and energy metabolism [24,32], and fluctuations can be associated with season, breeding, or a variety of factors other than stress [33–35]. Glucocorticoid responses also have been shown to vary widely between individuals and even within the same individual at different points in time [33–39]. As such, it is critical to keep in mind that an increase in glucocorticoid levels is not synonymous with a negative experience or perspective, or with "stress" [24,25,40,41].

In this study, we compared the spring 2020 COVID-19 closure with three additional periods, including immediately following reopening in summer 2020, in fall 2020 after a period of potential re-acclimation to guests, and a time-matched period in spring 2021. The comparison one year following the closure was critical to determine if any changes observed were likely due to season rather than visitor presence, as some animals can show seasonal variation in both behavior and physiology. Recent research investigating visitor effects in ring-tailed lemurs (*Lemur catta*) [42], hornbills (*Ceratogymna atrata*) [43], and flamingos [18] has shown that observed changes in behavior and/or physiology were primarily explained by variables such as time, temperature, and weather rather than guests. Because these variables are correlated with visitor number, both accounting for and disentangling them from visitor effects is critical but difficult. Some of the previously published research on COVID-19 closures controlled for seasonal variation as well, such as by comparing the closure with time-matched data collected in 2019 [10], analyzing data for multiple years across seasons [15], or studying species housed in indoor controlled-climate habitats [14,21]. Other results may be confounded by seasonal variation. For example, red kangaroos spent more time inactive and in proximity to other group members when the zoo was open compared to closed [17]. While these results suggest a negative impact of visitors, both inactivity and proximity were positively correlated with temperature as well [17], which increased between the spring closure and summer reopening. Similarly, a study on giraffes and cheetahs examined two transition periods related to their COVID-19 closure, one in winter and spring and one in summer [22]. While there were no significant differences in fecal glucocorticoids during either transition period, both species showed a significant increase from the winter/spring transition period to the summer transition period [22].

2. Materials and Methods

2.1. Subjects and Space Use Data

We collected data on space used by four species housed at the Saint Louis Zoo during four six-week phases (Table 1). Phase 1 took place during the zoo's closure for the COVID-19 pandemic in spring 2020. Phase 2 occurred in summer 2020 and was the first six weeks after the zoo was reopened, with guest counts limited to one-third capacity. Phase 3 occurred in fall 2020 after a potential period of re-acclimation to visitors, with visitor capacity increased to one-half. Phase 4 occurred in spring 2021, with capacity still at one-half, and was date-matched with the zoo's closure the previous year to serve as a seasonal comparison. To compare guest counts during the study with those prior to the COVID-19 pandemic, minimum, maximum, and average weekday and weekend guest counts on space use data collection days are presented alongside guest counts from time-matched periods from 2019 in Table 2.

Table 1. Space use data were collected during four six-week phases.

Phase	Dates	Description	Average Temp. (°C)
Phase 1	20 April–30 May 2020	Zoo closed for COVID-19	17.3°
Phase 2	9 June–18 July 2020	Zoo reopened, one-third capacity	26.9°
Phase 3	14 September–24 October 2020	Half capacity, following re-acclimation in Phase 2	16.5°
Phase 4	19 April–29 May 2021 3 May–12 June 2021 [1]	Half capacity, seasonal comparison with Phase 1	17.3° 20.5°

[1] Technical issues with gorilla habitat cameras delayed recordings for Phase 4 by two weeks.

Table 2. Comparison of guest counts on weekdays and weekends on space use collection days during the study periods with time-matched periods from 2019.

		Project Phases in 2020 & 2021		Time-Matched Comparisons from 2019	
		Weekdays	Weekends	Weekdays	Weekends
Phase 1	Range	COVID-19 closure		3484–20,516	4595–24,976
	$\bar{x}$			9066.8	17,040.8
Phase 2	Range	2928–9837	4418–10,187	5417–17,044	7825–20,824
	$\bar{x}$	5644.6	7660.3	10,310.4	14,650.8
Phase 3	Range	2438–7147	3312–13,531	2742–12,827	2581–22,982
	$\bar{x}$	3318.7	8810.9	5369.9	12,552.0
Phase 4	Range	630–7892	5175–15,312	3484–20,516	4595–24,976
	$\bar{x}$	5034.3	10,176.5	9066.8	17,040.8
Phase 4 [1]	Range	2270–14,178	7238–15,312	7215–20,516	4595–24,717
	$\bar{x}$	6593.5	12,012.3	10,543.2	17,917.6

[1] Guest count data for Phase 4 dates for gorillas only, as Phase 4 was delayed by two weeks for this species.

We observed two pair-housed sibling grizzly bears (1 male and 1 female, both aged 5 years), one singly housed polar bear (male, aged 7 years), and a bachelor group of four western lowland gorillas (4 males, aged 14–23 years). We also observed the banteng herd, consisting of four females at the start of the study (aged 8–13 years); a bull (aged 5 years) was introduced prior to the start of Phase 3 in fall 2020. Cameras mounted in the outdoor habitats were used to collect space use data to eliminate observer effects. During each phase, outdoor habitats were recorded on two weekdays and two weekend days each week. Two videos, two hours in length, were recorded each day (10:00–12:00 and 13:00–15:00).

Each outdoor habitat was divided into two or three zones based on proximity to the public. For the banteng (Figure 1), grizzly bears (Figure 2), and polar bear (Figure 3), Zone 1 was the section of the habitat closest to visitor viewing areas, while Zone 2 was the back of the habitat away from viewing areas. The grizzly and polar bears also had access to their dens during the day. Due to its shape, the gorilla habitat was divided into three zones (Figure 4), with Zone 1 bordering visitor viewing areas, Zone 2 in the middle, and Zone 3 at the back of the habitat furthest from visitors. Scan sampling was used to record the number of animals in each zone every 3 min during each two-hour video. As we were unable to distinguish between individuals on video, space use data were collected at the group level. Observers were trained to distinguish between zones and determine the locations of animals within their habitats with at least 85% accuracy before collecting data using the camera feeds. Five observers collected data for this study.

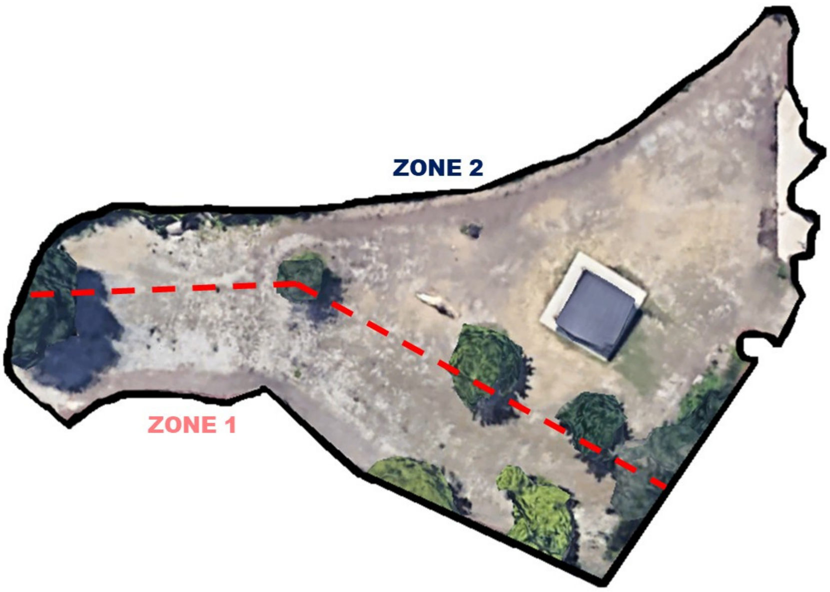

Figure 1. Map of the 1249 m^2 banteng habitat with a red line demarcating the study zones. Map of Saint Louis Zoo, Google Earth. Retrieved from earth.google.com/web/ (accessed on 20 April 2020).

Figure 2. Map of the 539 m^2 grizzly bear habitat with a red line demarcating the study zones. Map of Saint Louis Zoo, Google Earth. Retrieved from earth.google.com/web/ (accessed on 20 April 2020).

Figure 3. Map of the 762 m^2 polar bear habitat with a red line demarcating the study zones. Map of Saint Louis Zoo, Google Earth. Retrieved from earth.google.com/web/ (accessed on 20 April 2020).

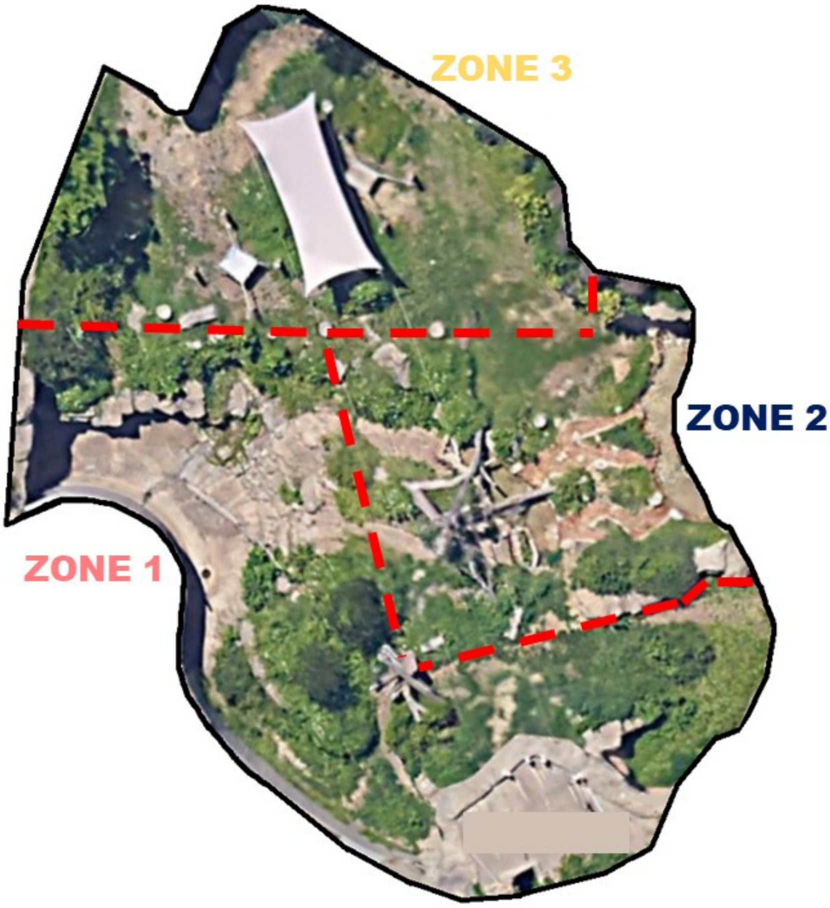

Figure 4. Map of the 1347 m^2 gorilla habitat with a red line demarcating the study zones. Map of Saint Louis Zoo, Google Earth. Retrieved from earth.google.com/web/ (accessed on 20 April 2020).

2.2. Physiological Data

During each project phase, fecal samples were collected two or three times per week for the banteng (17–18 samples per animal per phase, a total of 69–71 samples per animal) and five or more times per week for the grizzly and polar bears (31–41 samples per animal per phase, a total of 143–156 samples per animal), depending on the already established routines of the animal care teams, to compare fecal glucocorticoid levels across the phases. We were not able to collect samples for gorillas. Samples were collected in the morning (within 16 h of defecation) after animals had been housed indoors overnight. Samples were stored at −20 °C until extraction. Fecal steroids were solubilized according to [44]. Briefly, approximately 0.5 g of fecal material was shaken overnight in 5 mL modified phosphate-saline buffer containing 50% methanol. Supernatants were decanted and stored in evaporation-proof vials at −80 °C until assay following centrifugation at 4000× *g* for 60 min. Solid matter remaining in the extraction vials was weighed after drying overnight at 80 °C.

Fecal glucocorticoid concentrations were quantified using a commercially available corticosterone radioimmunoassay (DA I-125 Corticosterone RIA, ICN MP Biomedicals, Solon, OH, USA). Although cortisol is the primary circulating glucocorticoid in bears and banteng, it is excreted in feces as a mixture of glucocorticoid metabolites [45]. This assay has been previously validated for grizzly bears, polar bears [46], and banteng [47] through adrenocorticotropic hormone (ACTH) challenges. The assay's lower and upper detection limits were 0.26 ng/mL and 20 ng/mL, respectively. Assays were performed according to the manufacturer's protocols, except that standard diluent was added to the fecal extracts, and fecal extraction buffer (containing 50% methanol) was added to the kit standards. For all assays, standards, samples, and quality control pools were assayed in duplicate. Hormone concentrations were determined as ng/mL and then divided by the dry weight of the extracted feces to give the results as ng/g feces. The mean intra-assay variation of duplicate samples was 9.1%. The mean inter-assay coefficient of variation for two quality control pools was 8.7%.

2.3. Quantitative Analyses

Depending on the species and phase, total hours observed ranged from 70.5–92.6, and total observed data points for space use ranged from 1409–1851 for the whole group (Table 3). Data were collected as the number of animals per zone per phase, but for analysis, this was dichotomized as a 1–0 zone occupancy variable (1 = zone is occupied by at least one animal; 0 = zone is not occupied by any animals). We used linear mixed-effect models to test if the occupancy of each zone differed significantly across project phases for each species. To determine if space use patterns varied by day of week or time of day, weekday vs. weekend and morning vs. afternoon variables were included in the models as fixed effects. As the data are not independent, we used a split-plot design by including the date and interaction of date with time as random effects. Pairwise comparisons between all phases were then analyzed using Tukey's Honest Significant Difference (HSD) test. In case any initial differences in space use were driven by novelty immediately following the return of visitors, data were also analyzed with the first week of Phase 2 removed; there was no difference in results when the first week of Phase 2 was excluded (data not shown).

Table 3. Total number of hours observed (out of 96 possible hours) and observation points (out of 1920 possible data points) per species for each phase.

		Project Phase			
Species		**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**
Banteng	Hours	70.5	82.6	79.6	79.5
	Observation Points	1409	1651	1591	1589
Grizzly bears	Hours	73.3	87.2	92.6	87.8
	Observation Points	1466	1744	1851	1756
Polar bear	Hours	83.8	87.0	88.3	84.3
	Observation Points	1676	1739	1765	1686
Gorillas	Hours	82.4	85.3	91.5	85.7
	Observation Points	1647	1705	1829	1714

The glucocorticoid data for all individuals were right-skewed. As such, generalized linear models with a gamma distribution and log link function were used to examine differences in glucocorticoids between phases for each animal. Average daily temperature and weekday vs. weekend were included as fixed effects. Pairwise comparisons between phases were analyzed using Tukey's HSD test. To ensure the lack of visitors during Phase 1 did not mask differences in glucocorticoids between weekdays and weekend days, we also analyzed glucocorticoid data with Phase 1 removed; there was no difference in results for glucocorticoid differences between weekdays and weekend days when Phase 1 was removed (data not shown). Both space use and fecal glucocorticoid data were analyzed using the lme4 [48] and multComp [49] packages in R [50]. Results are described as significant at $p \leq 0.05$. However, as statistical significance is not necessarily equivalent to biological importance [51–53], we also discuss trends in the data.

3. Results

3.1. Banteng

Pairwise comparisons of zone occupancy by phase for the banteng are presented in Table 4 and Figure 5. There was a significant increase in Zone 1 occupancy and a significant concomitant decrease in Zone 2 occupancy during Phase 2 when guests first returned to the zoo. Zone 1 occupancy declined but not significantly while Zone 2 occupancy significantly increased during Phases 3 and 4 compared to Phase 2. Zone 1 trended toward significantly higher occupancy on the weekend (β = 0.120, SE = 0.065, p = 0.067) but was not affected by time of day (β = 0.040, SE = 0.030, p = 0.186), while Zone 2 occupancy was significantly higher in the morning (β = −0.078, SE = 0.026, p = 0.003) but was not affected by day of the week (β = −0.048, SE = 0.043, p = 0.274).

Table 4. Pairwise comparisons of zone occupancy by phase for each species.

Phase Comparison	Banteng			Grizzly Bears			Polar Bear			Gorillas		
	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
Zone 1												
Phase 2 vs. Phase 1	0.322	0.092	**0.002**	0.069	0.057	0.629	0.136	0.068	0.195	−0.076	0.041	0.243
Phase 3 vs. Phase 1	0.180	0.090	0.190	0.206	0.057	**0.002**	0.232	0.068	**0.004**	0.044	0.040	0.696
Phase 4 vs. Phase 1	0.108	0.092	0.643	0.035	0.057	0.929	0.198	0.069	**0.022**	0.003	0.041	0.999
Phase 3 vs. Phase 2	−0.142	0.091	0.401	0.137	0.056	0.071	0.096	0.067	0.476	0.120	0.041	**0.017**
Phase 4 vs. Phase 2	−0.214	0.093	0.094	−0.034	0.057	0.933	0.063	0.068	0.796	0.079	0.041	0.225
Phase 4 vs. Phase 3	−0.072	0.091	0.860	−0.171	0.056	**0.013**	−0.034	0.068	0.960	−0.041	0.041	0.754
Zone 2												
Phase 2 vs. Phase 1	−0.233	0.061	**0.001**	0.101	0.049	0.156	−0.026	0.033	0.850	−0.015	0.044	0.986
Phase 3 vs. Phase 1	0.059	0.060	0.757	−0.038	0.048	0.861	0.081	0.032	0.062	−0.224	0.044	**<0.001**
Phase 4 vs. Phase 1	−0.047	0.061	0.872	−0.046	0.048	0.775	−0.018	0.033	0.947	−0.201	0.044	**<0.001**
Phase 3 vs. Phase 2	0.293	0.061	**<0.001**	−0.139	0.047	**0.018**	0.107	0.032	**0.005**	−0.209	0.044	**<0.001**
Phase 4 vs. Phase 2	0.186	0.062	**0.013**	−0.148	0.048	**0.010**	0.008	0.033	0.994	−0.185	0.045	**<0.001**
Phase 4 vs. Phase 3	−0.106	0.061	0.302	−0.008	0.047	0.998	−0.099	0.032	**0.012**	0.024	0.045	0.952
Den/Zone 3												
Phase 2 vs. Phase 1				−0.015	0.060	0.994	−0.109	0.061	0.273	0.253	0.063	**<0.001**
Phase 3 vs. Phase 1				−0.059	0.059	0.751	−0.314	0.060	**<0.001**	0.261	0.063	**<0.001**

Table 4. *Cont.*

Phase Comparison	Banteng			Grizzly Bears			Polar Bear			Gorillas		
	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
Phase 4 vs. Phase 1				−0.047	0.059	0.857	−0.181	0.061	**0.016**	0.162	0.064	**0.054**
Phase 3 vs. Phase 2				−0.044	0.059	0.880	−0.205	0.059	**0.003**	0.007	0.064	0.999
Phase 4 vs. Phase 2				−0.032	0.059	0.949	−0.072	0.060	0.630	−0.091	0.065	0.494
Phase 4 vs. Phase 3				0.012	0.059	0.997	0.133	0.060	0.117	−0.098	0.064	0.417

Values in bold are significant at $p \leq 0.05$. Underlined values are trending toward significance at $p \leq 0.10$.

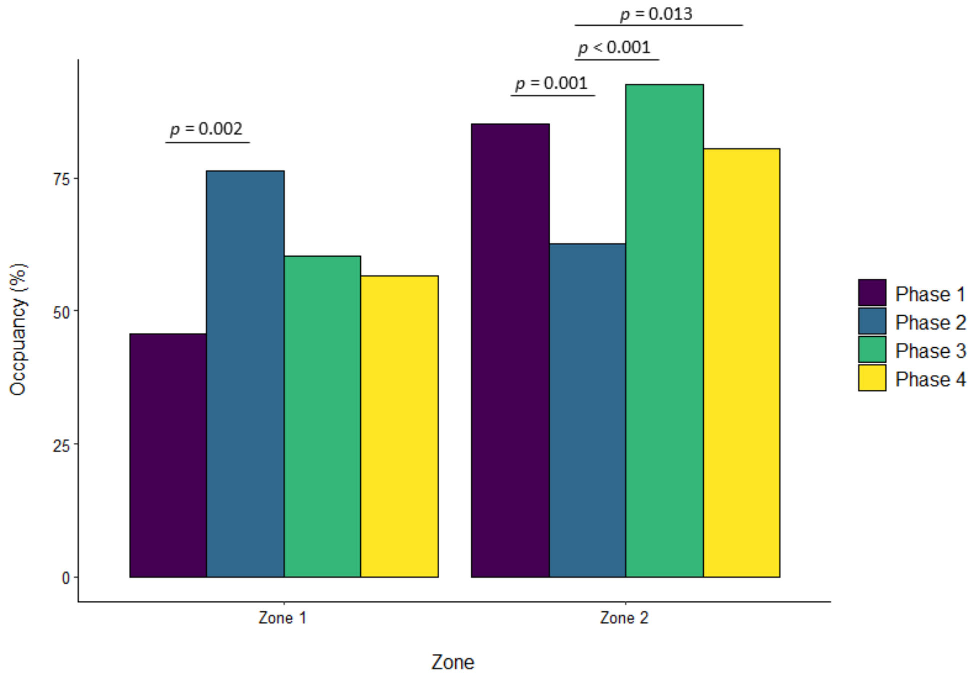

Figure 5. Percent occupancy of each zone by phase for the banteng.

Means and standard deviations for fecal glucocorticoid levels by phase for each species are presented in Table 5. Pairwise comparisons of fecal glucocorticoid levels between phases and associations with temperature and day of the week for the banteng are presented in Table 6 and Figure 6. The banteng showed considerable intra-individual variation in changes in glucocorticoids across the study periods. For banteng female 1, glucocorticoids in Phase 4 were significantly lower than in Phase 2. For female 2, glucocorticoid levels in Phase 2 were significantly higher than in Phases 1, 3, and 4; for female 4, glucocorticoid levels in Phase 2 were significantly higher than in Phases 3 and 4. Glucocorticoid levels did not vary significantly by phase in female 3. There were no other significant differences in glucocorticoid levels observed between phases for the female banteng. The banteng bull was not housed with the females or in an area that was viewable to the public in Phases 1 and 2, and glucocorticoids from Phase 3 are not presented due to an injury and temporary removal from the group. There were no significant differences in glucocorticoid levels between Phases 1, 2, and 4 for the bull. Temperature neared a significant inverse relationship with fecal glucocorticoids in female 1 but was not a significant variable in the models for any other banteng. There were no significant differences in fecal glucocorticoids between weekdays or weekend days for any banteng.

Table 5. Means and standard deviations for fecal glucocorticoids by phase for individual banteng, grizzly bears, and the polar bear.

Species	Sex		Fecal Glucocorticoid Levels (ng/g) Phase 1	Phase 2	Phase 3	Phase 4
Banteng	Male	$\bar{x} \pm$ SD	24.5 ± 2.3	25.0 ± 3.9	27.9 ± 9.8	22.4 ± 3.5
	Female 1	$\bar{x} \pm$ SD	25.8 ± 6.3	30.4 ± 6.8	31.9 ± 18.2	23.9 ± 4.6
	Female 2	$\bar{x} \pm$ SD	26.2 ± 4.8	186.1 ± 183.9	30.4 ± 11.2	37.7 ± 8.4
	Female 3	$\bar{x} \pm$ SD	75.6 ± 106.2	74.4 ± 63.7	34.8 ± 10.8	48.0 ± 13.6
	Female 4	$\bar{x} \pm$ SD	58.4 ± 97.6	125.5 ± 179.1	26.3 ± 7.2	34.7 ± 10.8
Grizzly bear	Male	$\bar{x} \pm$ SD	116.9 ± 159.4	181.0 ± 277.0	152.5 ± 197.0	187.6 ± 198.8
	Female	$\bar{x} \pm$ SD	106.6 ± 125.4	165.6 ± 249.3	85.4 ± 102.1	119.8 ± 105.6
Polar bear	Male	$\bar{x} \pm$ SD	105.4 ± 99.2	75.8 ± 82.6	52.5 ± 76.4	135.5 ± 166.9

Table 6. Pairwise comparisons of glucocorticoids by phase as well as associations with temperature and day of the week (weekday vs. weekend) for each banteng.

Phase Comparison	Male [1] β	SE	p	Female 1 β	SE	p	Female 2 β	SE	p	Female 3 β	SE	p	Female 4 β	SE	p
Phase 2 vs. Phase 1	0.043	0.069	0.808	0.326	0.143	0.100	1.924	0.234	**<0.001**	0.021	0.349	1.000	0.788	0.444	0.281
Phase 3 vs. Phase 1				0.181	0.109	0.344	0.145	0.179	0.847	−0.468	0.264	0.283	−0.696	0.338	0.163
Phase 4 vs. Phase 1	−0.090	0.048	0.142	−0.060	0.109	0.945	0.365	0.179	0.177	−0.156	0.265	0.935	−0.468	0.343	0.519
Phase 3 vs. Phase 2				−0.146	0.143	0.735	−1.779	0.234	**<0.001**	−0.489	0.342	0.477	−1.484	0.444	**0.005**
Phase 4 vs. Phase 2	−0.132	0.066	0.107	−0.387	0.140	**0.029**	−1.562	0.230	**<0.001**	−0.176	0.335	0.952	−1.257	0.434	**0.020**
Phase 4 vs. Phase 3				−0.241	0.109	0.120	0.217	0.179	0.616	0.261	0.261	0.624	0.228	0.343	0.909
Temperature	−0.001	0.003	0.674	−0.010	0.005	0.068	0.002	0.008	0.837	0.014	0.012	0.265	0.014	0.016	0.384
Day of Week	0.042	0.042	0.315	−0.102	0.083	0.224	−0.070	0.136	0.609	−0.212	0.199	0.290	−0.152	0.261	0.562

[1] Due to injury and temporary removal from the herd, glucocorticoids from Phase 3 were excluded from analyses. Values in bold are significant at $p \leq 0.05$. Underlined values are trending toward significance at $p \leq 0.10$.

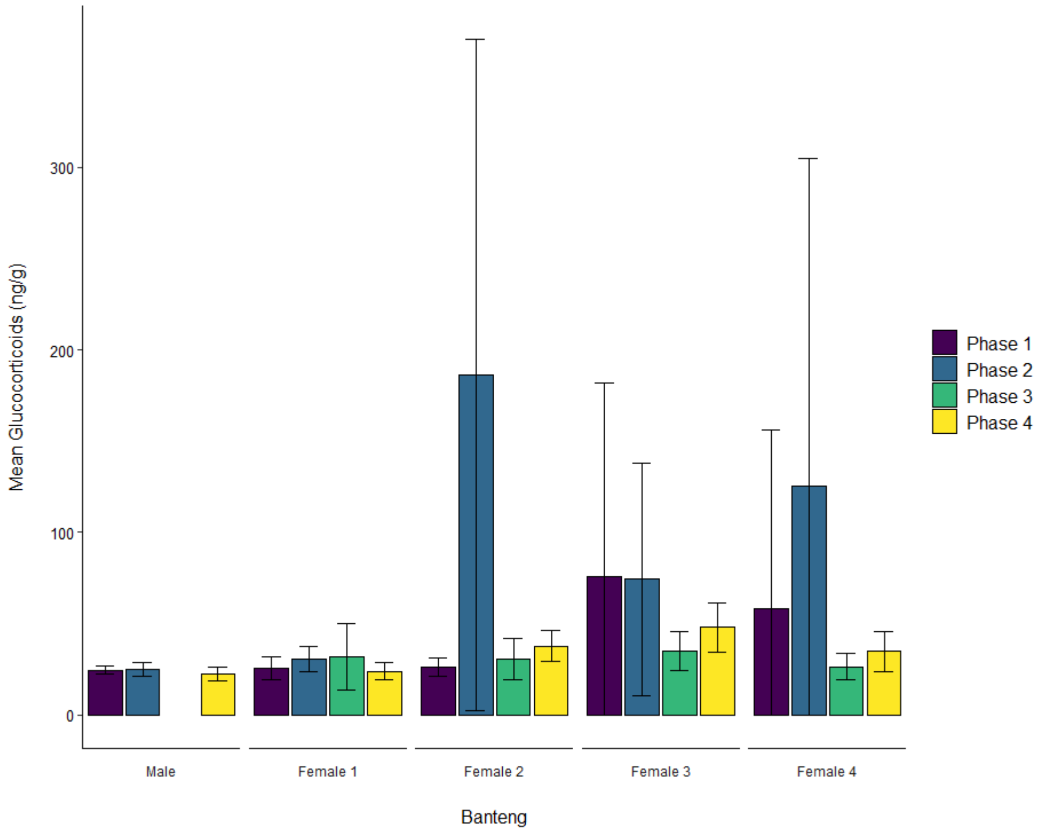

Figure 6. Means and standard deviations for fecal glucocorticoids by phase for individual banteng.

3.2. Grizzly Bears

Pairwise comparisons of zone occupancy by phase for the grizzly bears are presented in Table 4 and Figure 7. In Phase 2 when guests returned, there was a non-significant increase in occupancy for both Zone 1 and Zone 2, and a non-significant decrease in Den occupancy. Zone 1 occupancy was significantly higher in Phase 3 compared to Phase 1 and neared significantly higher than in Phase 2. Zone 1 occupancy then significantly decreased in Phase 4, which was higher than but not significantly different from Phase 1. Zone 2 occupancy was significantly lower during Phases 3 and 4 than in Phase 2. There were no significant differences in Den occupancy by phase. Neither day of the week ($\beta = -0.050$, SE = 0.040, $p = 0.212$) nor time of day ($\beta = -0.002$, SE = 0.030, $p = 0.955$) significantly impacted Zone 1 occupancy, Zone 2 occupancy was significantly higher in the morning ($\beta = -0.129$, SE = 0.027, $p < 0.001$) but was not affected by day of the week ($\beta = -0.051$, SE = 0.034, $p = 0.134$), and Den occupancy was significantly higher on weekend days ($\beta = 0.096$, SE = 0.042, $p = 0.024$) and afternoons ($\beta = 0.088$, SE = 0.033, $p = 0.009$).

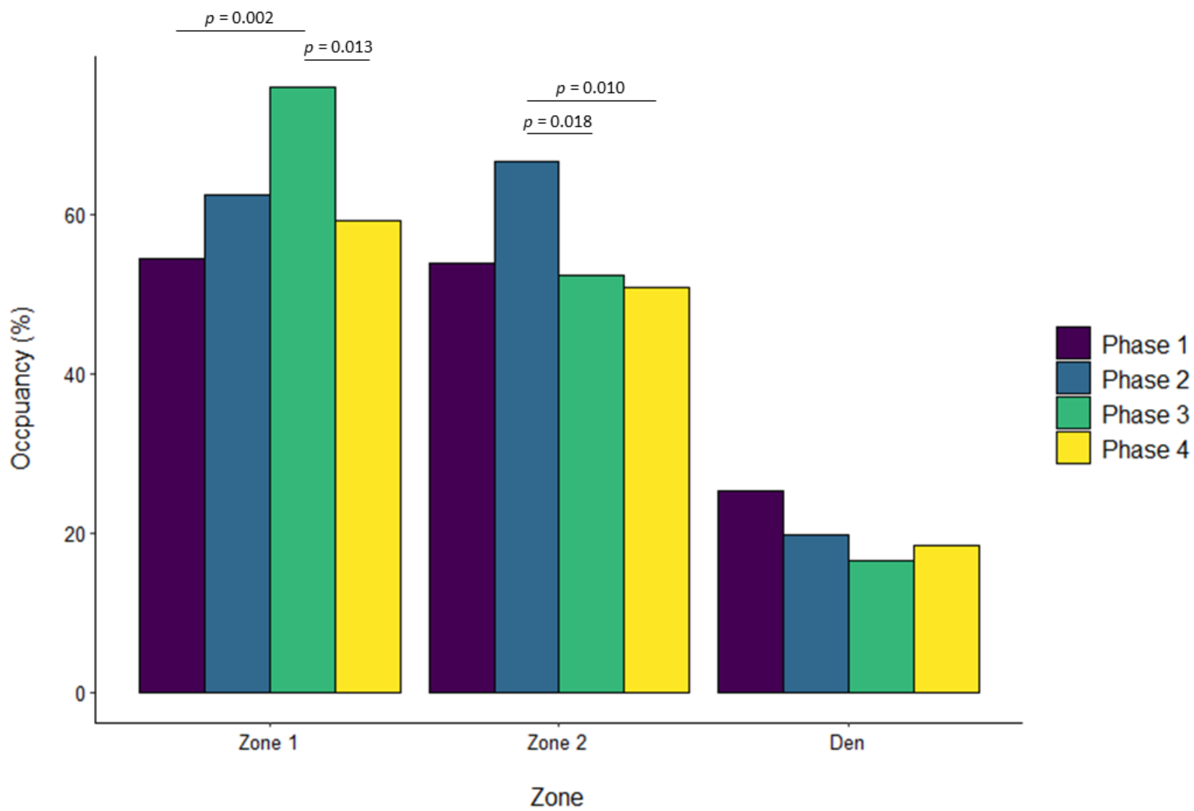

Figure 7. Percent occupancy of each zone by phase for the grizzly bears.

Means and standard deviations for fecal glucocorticoid levels by phase for each species are presented in Table 5. Pairwise comparisons of fecal glucocorticoid levels between phases and associations with temperature and day of the week for the grizzly bears are presented in Table 7 and Figure 8. There were no significant differences in glucocorticoid levels between phases for either grizzly bear. Temperature was significantly associated with fecal glucocorticoid levels in the female grizzly bear but not the male. Fecal glucocorticoids did not significantly vary between weekdays and weekends for either grizzly bear.

Table 7. Pairwise comparisons of glucocorticoids by phase as well as associations with temperature and day of the week (weekday vs. weekend) for each grizzly bear.

	Male			Female		
Phase Comparison	**β**	**SE**	***p***	**β**	**SE**	***p***
Phase 2 vs. Phase 1	0.358	0.378	0.777	0.079	0.341	0.996
Phase 3 vs. Phase 1	0.263	0.301	0.817	−0.223	0.273	0.844
Phase 4 vs. Phase 1	0.471	0.295	0.379	0.218	0.269	0.848
Phase 3 vs. Phase 2	−0.096	0.382	0.994	−0.302	0.338	0.806
Phase 4 vs. Phase 2	0.112	0.375	0.991	0.139	0.344	0.978
Phase 4 vs. Phase 3	0.208	0.299	0.898	0.441	0.273	0.367
Temperature	0.005	0.013	0.701	0.024	0.012	**0.051**
Day of Week	0.057	0.217	0.794	−0.157	0.198	0.428

Values in bold are significant at $p \leq 0.05$.

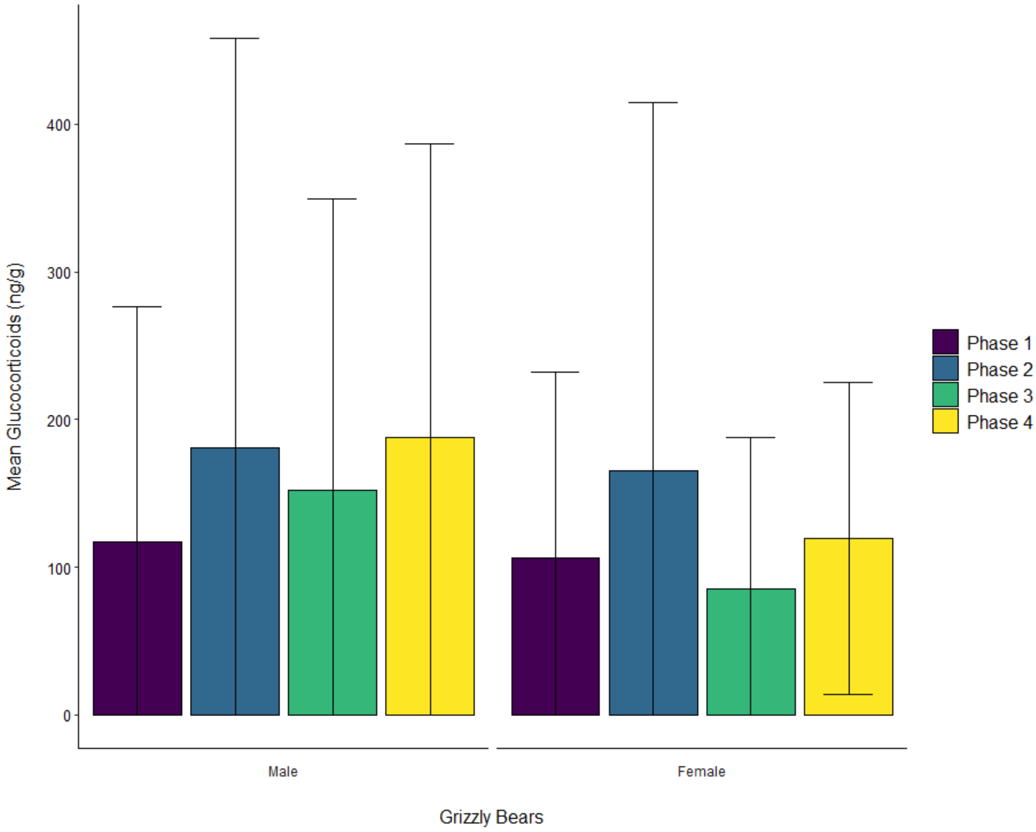

Figure 8. Means and standard deviations for fecal glucocorticoids by phase for individual grizzly bears.

3.3. Polar Bear

Pairwise comparisons of zone occupancy by phase for the polar bear are presented in Table 4 and Figure 9. Occupancy of Zone 1 increased across Phases 2–4, with the increases reaching significance in Phases 3 and 4. Zone 2 occupancy was significantly higher in Phase 3 than in Phases 1, 2, or 4. Den occupancy was significantly lower in Phase 3 than in Phases 1 and 2, and in Phase 4 than in Phase 1. Zone 1 occupancy was higher during morning hours (β = −0.224, SE = 0.037, $p < 0.001$) and Den occupancy was higher in the afternoon (β = 0.190, SE = 0.037, $p < 0.001$), but Zone 2 occupancy was not affected by time of day (β = 0.030, SE = 0.022, $p = 0.175$). Day of the week did not significantly impact occupancy of Zone 1 (β = −0.054, SE = 0.048, $p = 0.268$), Zone 2 (β = −0.008, SE = 0.023, $p = 0.710$), or the Den (β = 0.063, SE = 0.043, $p = 0.146$).

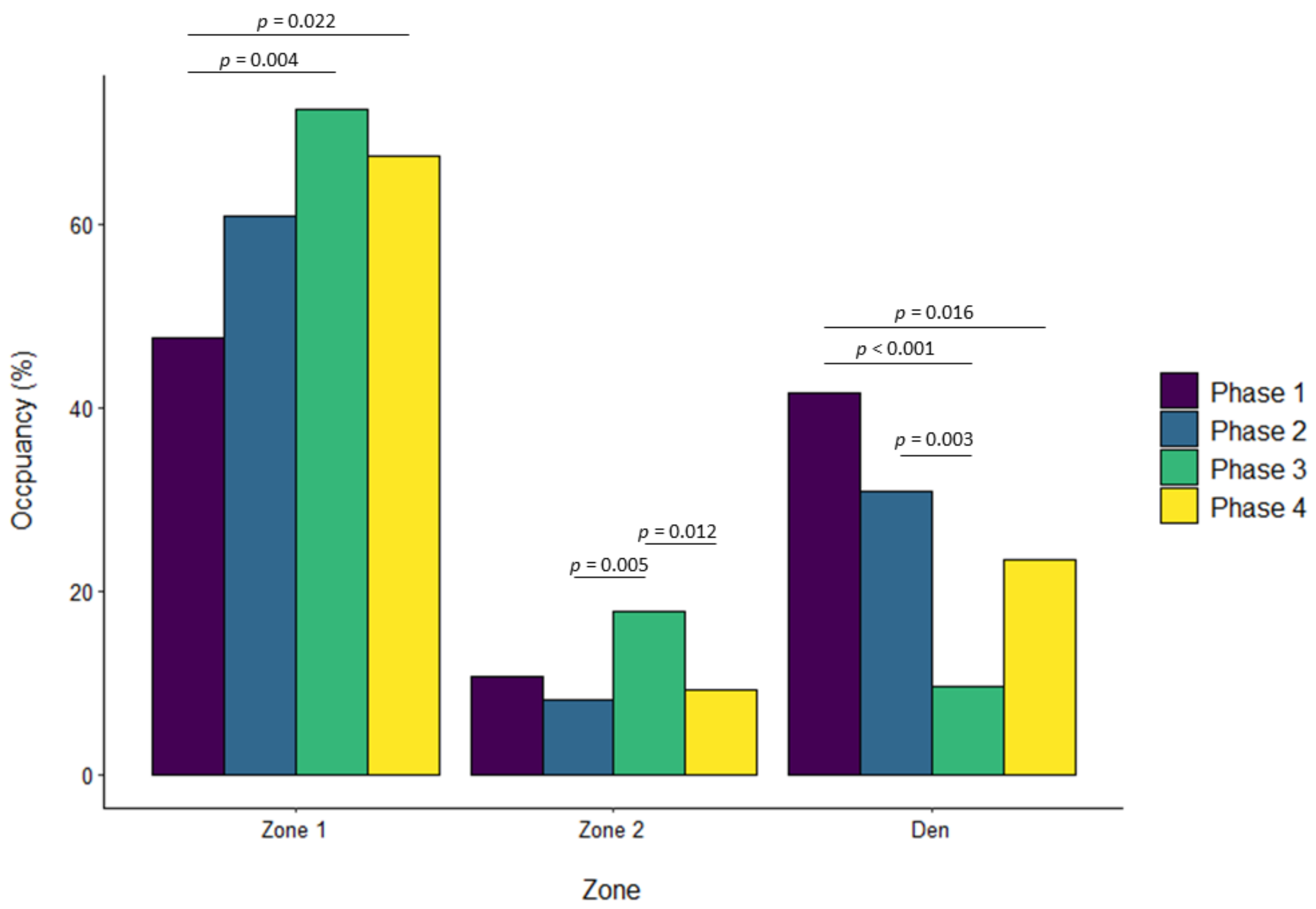

Figure 9. Percent occupancy of each zone by phase for the polar bear.

Means and standard deviations for fecal glucocorticoid levels by phase for each species are presented in Table 5. Pairwise comparisons of fecal glucocorticoid levels between phases and associations with temperature and day of the week for the polar bear are presented in Table 8 and Figure 10. Glucocorticoid levels in Phase 3 neared being significantly lower than those observed in Phase 1 and were significantly lower than those observed in Phase 4. Glucocorticoid levels were not significantly different for any other phase comparisons. Fecal glucocorticoid levels also did not vary significantly by temperature or weekday versus weekend day.

Table 8. Pairwise comparisons of glucocorticoids by phase as well as associations with temperature and day of the week (weekday vs. weekend) for the polar bear.

Phase Comparison	β	SE	*p*
Phase 2 vs. Phase 1	−0.433	0.362	0.627
Phase 3 vs. Phase 1	−0.676	0.290	<u>0.090</u>
Phase 4 vs. Phase 1	0.285	0.299	0.775
Phase 3 vs. Phase 2	−0.244	0.358	0.903
Phase 4 vs. Phase 2	0.717	0.359	0.187
Phase 4 vs. Phase 3	0.961	0.281	**0.003**
Temperature	0.008	0.013	0.548
Day of Week	−0.037	0.207	0.860

Values in bold are significant at $p \leq 0.05$. Underlined values are trending toward significance at $p \leq 0.10$.

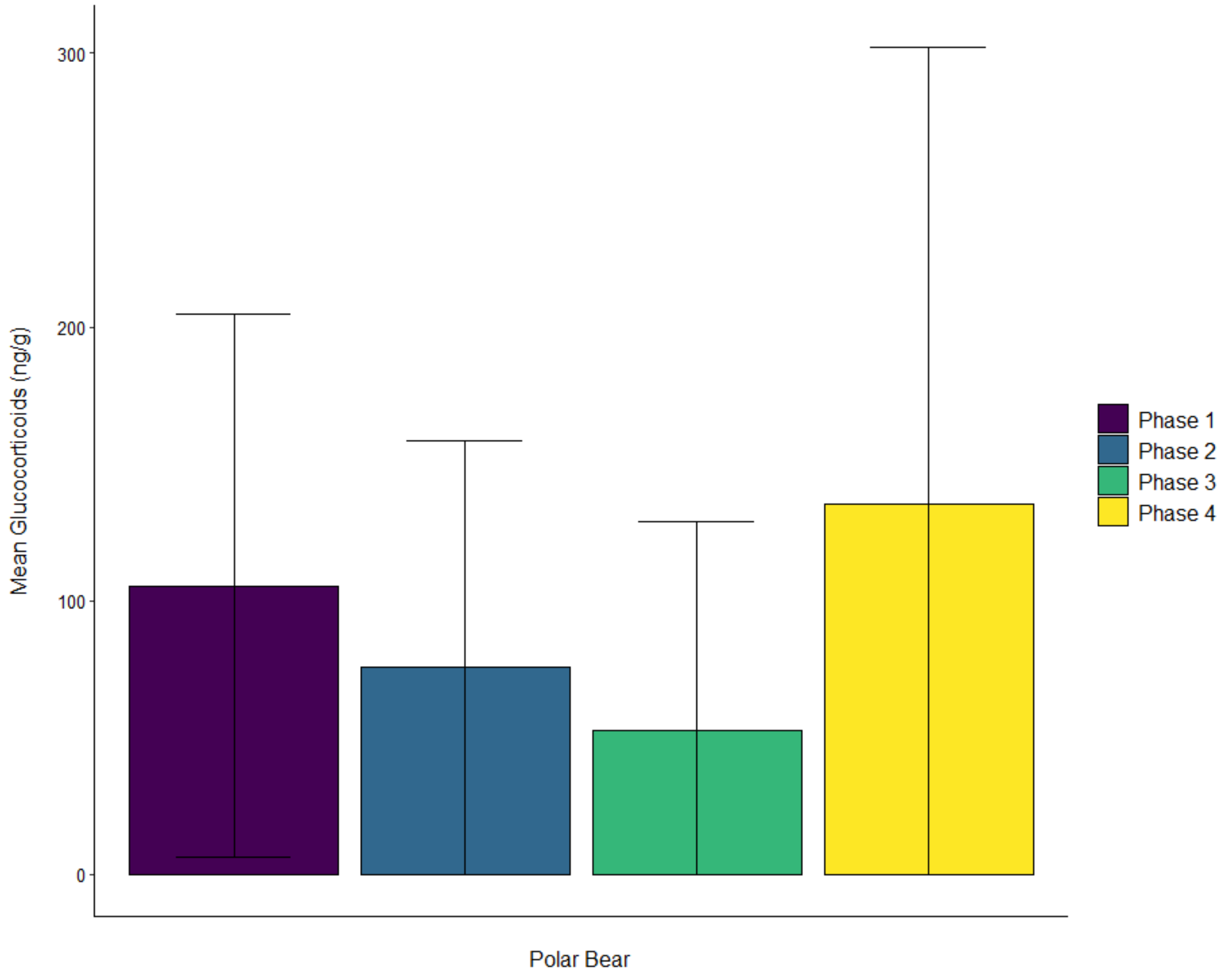

Figure 10. Means and standard deviations for fecal glucocorticoids by phase for the polar bear.

3.4. Western Lowland Gorillas

Pairwise comparisons of zone occupancy by phase for the gorillas are presented in Table 4 and Figure 11. There was a small decrease in Zone 1 occupancy in Phase 2, when guests returned, compared to Phase 1. However, in Phase 3, Zone 1 occupancy was significantly higher than in Phase 2. Zone 2 occupancy decreased significantly in Phases 3 and 4 compared to Phases 1 and 2. Occupancy in Zone 3 was significantly higher in Phases 2–4 relative to Phase 1. Time of day did not impact the occupancy of Zones 1 ($\beta = 0.009$, SE = 0.023, $p = 0.688$) or 2 ($\beta = -0.053$, SE = 0.031, $p = 0.095$) but the occupancy of Zone 3 was significantly higher in the morning ($\beta = -0.176$, SE = 0.029, $p < 0.001$). There were no significant associations between day of the week and occupancy for Zones 1 ($\beta = -0.012$, SE = 0.029, $p = 0.684$), 2 ($\beta = 0.028$, SE = 0.031, $p = 0.374$), or 3 ($\beta < 0.001$, SE = 0.045, $p = 0.994$).

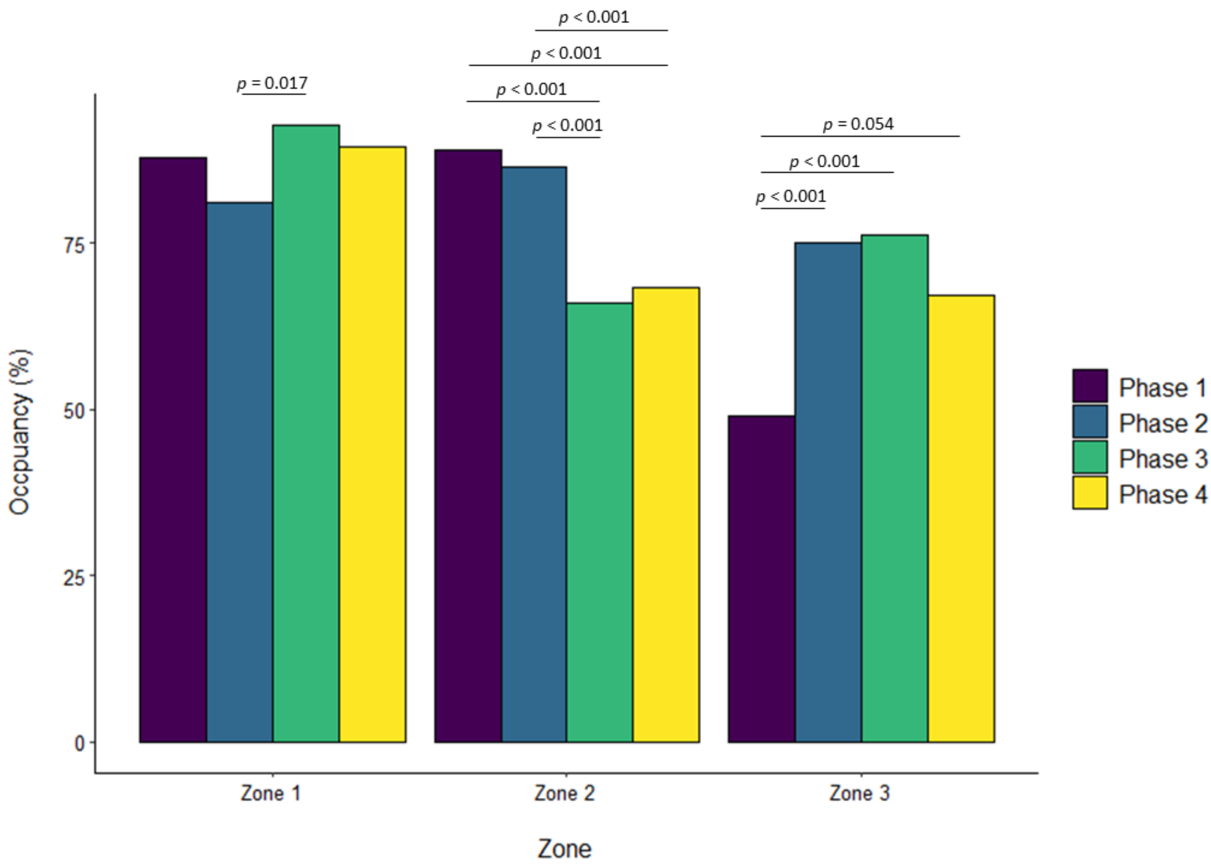

Figure 11. Percent occupancy of each zone by phase for the western lowland gorillas.

4. Discussion

This study examined space use and fecal glucocorticoids during the COVID-19 closure in comparison with multiple periods after reopening to investigate the effects of visitors on four species housed at the Saint Louis Zoo. For the banteng, in Phase 2, when guests first returned to the zoo, there was a more than 20% increase in Zone 1 occupancy near visitor viewing areas. While zone occupancy is not mutually exclusive (i.e., both zones in the banteng habitat could be occupied simultaneously), the increase in Zone 1 occupancy coincided with a nearly 23% decrease in Zone 2 occupancy, suggesting a change in space use by the herd toward visitor viewing areas. This is especially notable as Phase 2 occurred during summer, which had the highest average temperatures of any phase, and the primary shade structures for the banteng are in Zone 2. During Phases 3 and 4, space use shifted back toward patterns observed while the zoo was closed, although occupancy of Zone 1 remained higher than Phase 1 levels. Additionally, Zone 1 occupancy neared being significantly higher on weekends, which has higher visitation rates. Behaviorally, these data suggest the return of guests may have stimulated the banteng to use areas of their habitat closer to visitors. The bull was introduced to the herd at the start of Phase 3, and changing social dynamics may also have impacted space used by the herd.

One cow showed significantly higher fecal glucocorticoids in Phase 2, when the zoo re-opened to guests, compared to any other phase. Another cow had a non-significant increase in fecal glucocorticoids in Phase 2 compared to Phase 1, and these levels were significantly higher than those in Phases 3 and 4. These data suggest that, at least for these individuals, the return of guests may have presented an aversive stimulus. However, glucocorticoids can elevate in response to non-negative stimuli. For example, our previous measures of fecal glucocorticoids in the banteng herd have found distinct seasonal differences, with the highest concentrations overall during the summer months [47]. These months may correspond with the breeding season for most banteng in human care, although this species can breed year-round in North America [54]. In a number of species, glucocorticoid production is known to increase during the breeding season [55], likely reflecting the increase in energy expenditure during this time. Unfortunately, space use data were not collected at the individual level and so it is not possible to determine where these two females spent their time, which may have provided insight into how they perceived visitors. Ultimately, if visitors returning was initially stressful for these females, the impact was short-term as their glucocorticoids returned to lower levels in Phases 3 and 4. While some other comparisons in the cows showed significant differences in fecal glucocorticoids between phases, the absolute differences are minimal and within the normal range of variation based on our routine hormone monitoring (data not shown), suggesting the differences are unlikely to be biologically meaningful. The fecal glucocorticoid data also suggest the bull did not find visitors aversive; his fecal glucocorticoid levels were similar when he was not exposed to visitors (Phases 1 and 2) and when he was (Phase 4). There also was no difference in fecal glucocorticoids between weekdays and weekends for any of the banteng despite differences in visitation levels.

To our knowledge, this is the first study to examine visitor effects on zoo-housed banteng. Our results are inconsistent with previous research in other bovid ungulates such as Indian blackbuck (*Antelope cervicapra*) [56] and Indian gaur (*Bos gaurus gaurus*) [8], which showed increased activity and aggression and decreased resting in response to visitors. Alternatively, at two zoos, bison (*Bison bonasus*) showed no difference in fecal glucocorticoids between weekdays and weekends, as we observed in this banteng herd, and bison at another zoo had lower glucocorticoid levels on weekends than on weekdays [57], suggesting a neutral or even positive impact of visitors, depending on the herd. Although little has been published on banteng in zoos, they have been described as resilient and not timid [58–60], suggesting banteng may thrive in the zoo environment, especially if they find visitors positively stimulating.

The grizzly bears showed an 8% increase in Zone 1 occupancy during Phase 2 and another 12% increase in Phase 3. Additionally, Zone 2 occupancy increased 13%, while Den occupancy declined 5.5% in Phase 2 and stayed low throughout the remaining phases. With less time in the Den and similar amounts of time in Zones 1 and 2 during Phase 2, these data indicate the grizzly bears were more visible in their habitat when guests returned, suggesting a neutral or potentially positive effect of visitors on these two individuals. This suggestion is further supported by the physiological evidence, as neither grizzly bear showed significant differences in fecal glucocorticoid levels by phase. Our routine hormonal monitoring of the grizzly bears has shown high levels of variation in fecal glucocorticoid levels in the past (data not shown), as was also observed during this study. Fecal glucocorticoids in the female were positively associated with temperature, but there was no significant variation between weekdays and weekend days for either grizzly bear.

Zone 1 occupancy by the polar bear was 13–24% higher after visitors returned than during the COVID-19 closure, Zone 2 occupancy was minimal throughout the study, and Den occupancy declined following reopening. This result suggests a positive impact of visitors on the polar bear, as he chose to spend substantial proportions of his time near visitor viewing areas. While the polar bear's pool is located in Zone 1, occupancy of this zone was highest during the fall and seasonal comparison the following spring, suggesting the initial increase in Zone 1 occupancy when guests returned was not simply due to pool use in summer. The polar bear's fecal glucocorticoids also declined in Phases 2 and 3, although, like the grizzly bears, this individual shows substantial variation in fecal glucocorticoid levels during routine monitoring (data not shown). Neither temperature nor day of the week was significantly associated with the polar bear's fecal glucocorticoid levels. The lack of a significant association with temperature is interesting given a recent study showing significant increases in fecal glucocorticoids in adult polar bears when temperatures were above 20 °C [61]. In our study, approximately half of the fecal samples (73 of 143) were collected on or immediately after days with temperatures over this threshold.

Few studies have investigated visitor effects on bears of any species. However, contrary to our results, previous research has suggested that visitors may have a negative impact on both grizzly [62] and polar bears [63]. For example, grizzly bears spent more time in areas away from visitor viewing, regardless of visitor presence, and engaged in high levels of stereotypic behavior, inactivity, activity, and vigilance when visitors were present [62]. Our results do, however, support the findings of a study conducted partially during a COVID-19 closure on multiple bear species, including grizzly bears, that found no significant behavioral changes when visitors were absent, including in stereotypical or social behavior. Additionally, the results indicated that bears were more visible after visitors returned [15].

The gorillas showed a slight decrease in occupancy of Zones 1 and 2 and a more than 25% increase in occupancy of Zone 3 in Phase 2 when guests first returned to the zoo. These results indicate that, unlike the banteng, grizzly bears, and polar bears, the return of guests may have initially been aversive to the gorilla troop. However, it should be noted that some of the shade structures in the gorilla habitat are located in Zone 3. As Phase 2 occurred during the summer months with the highest average temperature, it is possible that the space used by the gorillas in Phase 2 had less to do with visitors and more to do with the season. Additionally, while there was initially a decrease in time spent in Zone 1 in Phase 2, occupancy of the zone nearest visitor viewing areas returned to levels higher than observed during the COVID-19 closure during Phases 3 and 4. Due to a camera malfunction, the seasonal comparison in Phase 4 for the gorillas happened two weeks later than Phase 4 for all the other animals in the study, pushing the timeline for that phase toward summer months with higher temperatures. This demonstrates that even as temperatures started increasing toward those observed in Phase 2, the gorillas still spent as much time in Zone 1 in Phase 4 as they did in Phase 1. Together, these results suggest that if the return of visitors initially had a negative impact on the gorillas (rather than changes in space use being due to shade availability or other factors), the negative impacts were short-lived, supporting an overall neutral effect of visitors.

Although well-studied relative to banteng and bears, visitor effect research on gorillas has been inconclusive thus far. When monitored over the long term, our results are consistent with those that indicate a potentially neutral impact of visitors on gorillas. For example, studies have shown no impact of visitors on the use of habitat space [23,64,65], aggression [23], wounding rates [66], timing of parturition [67], or fecal glucocorticoid levels [31]. Our results also support a study of western lowland gorillas during a COVID-19 zoo closure, which similarly demonstrated a neutral effect of visitors on behavior [19]. However, there are studies that suggest visitors may have a negative impact, at least on some behaviors, such as a reduction in time spent foraging [31,68–70] or engaged in active behaviors [68,70] as well as an increase in time spent out of sight [70,71] or engaged in anxiety- or vigilance-related behaviors [70,72]. Alternatively, one recent study documented locomotion was significantly more likely with increasing crowd size [70], and another that gorillas spent less time inactive and more time foraging with larger crowd sizes [69], suggesting visitors may positively impact some behaviors in some troops. Although much of the research conducted on gorillas has indicated that visitors may have a negative impact, multiple researchers have recently stated that the effects are likely not as negative as typically hypothesized in the early literature [31,66,67].

The inability to identify animals on camera required collecting space use data at the group level and prohibited us from understanding how visitors impact space use in individuals. Research on visitor effects in a variety of species has shown there can be variation in responses between individuals [19,72]. However, this limitation was partially overcome with our individual data on glucocorticoid levels across the phases for the banteng, grizzly bears, and polar bears. This study would have been further improved by the ability to document certain types of behaviors for a better understanding of how these species may behave when visitors have a positive or neutral effect, as research has typically emphasized signs of negative welfare instead [73]. Instead of specific behaviors, we used zone occupancy as a proxy for visitor effects, but it may not always be the case that space use is directly impacted by proximity to guests. Especially for those taxa where visitors are a neutral stimulus, group dynamics may be a more important regulator of space use. For example, gorillas manage potential conflict through increased social distance [74,75] and thus may occupy different habitat zones to maintain group dynamics rather than be closer to or further from guests. All four gorillas were located in a single zone in 12% or fewer scans, depending on the phase, and even during the closure, the gorillas spent more than 90% of their time spread across multiple zones. However, if guests were a strongly aversive stimulus, we believe it would be evident in the space use data. Additionally, the social housing of these species may have influenced how they responded to visitors overall. Male gorillas could be more responsive to visitors when housed in breeding groups than in bachelor groups due to their role in protecting females and offspring. Similarly, changing social dynamics among the banteng with the introduction of the bull into a previously all-female herd could have affected how individuals chose to occupy their habitat space.

One common constraint that visitor effect studies face is the inability to have extended periods with no visitors without other confounding variables such as construction or maintenance. This study overcame that limitation by taking advantage of the COVID-19 closure to compare the closure with periods after reopening to determine if there was a difference in space use and fecal glucocorticoid levels for multiple species. We demonstrated that visitors have a potentially positive effect on the banteng and polar bear, and a potentially neutral or positive effect on the grizzly bears and gorillas. Our conclusion of neutral and positive effects is further supported by a lack of differences in fecal glucocorticoids between weekdays and weekend days, the latter of which have higher guest counts. Like many of the other visitor effect studies that occurred during COVID-19 closures [18–20,22] and as has been suggested elsewhere [31,66,67,76–78], this study contributes to the recent evidence across multiple taxa indicating that the effects of visitors are not as negative as typically predicted based on some of the early research in this area. Identifying the impacts of visitors as well as the factors that contribute to these impacts is important for ensuring that animals in human care can thrive.

Author Contributions: Conceptualization, A.N.E., E.B., K.L.B., C.P.K. and D.M.P.; Methodology, A.N.E., E.B., K.L.B., C.P.K. and H.L.C.; Software, A.N.E.; Validation, A.N.E.; Formal Analysis, A.N.E.; Investigation, A.N.E., N.C.L. and H.L.C.; Resources, C.P.K. and D.M.P.; Data Curation, A.N.E.; Writing—Original Draft Preparation, A.N.E., N.C.L. and C.P.K.; Writing—Review & Editing, A.N.E., N.C.L., E.B., K.L.B., C.P.K., H.L.C. and D.M.P.; Visualization, A.N.E. and E.B.; Supervision, A.N.E.; Project Administration, A.N.E.; Funding Acquisition, A.N.E. and D.M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Saint Louis Zoo's Research Review Committee (16 April 2020).

Data Availability Statement: Data are available upon reasonable request from the corresponding author.

Acknowledgments: We would like to thank the animal care staff at the Saint Louis Zoo, who worked throughout 2020 and beyond to maintain normal care for their animals during a period that was anything but normal. We also extend our gratitude to our institution's technology department, who worked quickly to allow us to access, view, and download video footage remotely during the closure. We also thank our team of interns who collected zone occupancy data from the videos, including Julia Monk, Andrea Montecinos-Padilla, Michelle Pollowitz, Lauren Puleo, and Sadie Scott. Finally, we appreciate the feedback of two anonymous reviewers, whose comments helped improve this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pifarré, M.; Valdez, R.; González-Rebeles, C.; Vázquez, C.; Romano, M.; Galindo, F. The Effect of Zoo Visitors on the Behaviour and Faecal Cortisol of the Mexican Wolf (*Canis lupus baileyi*). *Appl. Anim. Behav. Sci.* **2012**, *136*, 57–62. [CrossRef]
2. Collins, C.; Quirke, T.; Overy, L.; Flannery, K.; O'Riordan, R. The Effect of the Zoo Setting on the Behavioural Diversity of Captive Gentoo Penguins and the Implications for Their Educational Potential. *J. Zoo Aquar. Res.* **2016**, *4*, 85–90.
3. Chiapero, F.; Ferrari, H.R.; Prieto, M.V.; García Capocasa, M.C.; Busso, J.M. Multivariate Analyses of the Activity Pattern and Behavior of the Lesser Anteater on Open and Closed Days at Córdoba Zoo, Argentina. *J. Appl. Anim. Welf. Sci.* **2021**, *24*, 83–97. [CrossRef]
4. Sellinger, R.L.; Ha, J.C. The Effects of Visitor Density and Intensity on the Behavior of Two Captive Jaguars (*Panthera onca*). *J. Appl. Anim. Welf. Sci.* **2005**, *8*, 233–244. [CrossRef]
5. Vidal, L.S.; Guilherme, F.R.; Silva, V.F.; Faccio, M.C.S.R.; Martins, M.M.; Briani, D.C. The Effect of Visitor Number and Spice Provisioning in Pacing Expression by Jaguars Evaluated through a Case Study. *Braz. J. Biol.* **2016**, *76*, 506–510. [CrossRef]
6. Sherwen, S.L.; Hemsworth, P.H. The Visitor Effect on Zoo Animals: Implications and Opportunities for Zoo Animal Welfare. *Animals* **2019**, *9*, 366. [CrossRef]
7. Quadros, S.; Goulart, V.D.L.; Passos, L.; Vecci, M.A.M.; Young, R.J. Zoo Visitor Effect on Mammal Behaviour: Does Noise Matter? *Appl. Anim. Behav. Sci.* **2014**, *156*, 78–84. [CrossRef]
8. Sekar, M.; Rajagopal, T.; Archunan, G. Influence of Zoo Visitor Presence on the Behavior of Captive Indian Gaur (*Bos gaurus gaurus*) in a Zoological Park. *J. Appl. Anim. Welf. Sci.* **2008**, *11*, 352–357. [CrossRef]
9. Learmonth, M.J.; Sherwen, S.; Hemsworth, P.H. The Effects of Zoo Visitors on Quokka (*Setonix brachyurus*) Avoidance Behavior in a Walk-through Exhibit. *Zoo Biol.* **2018**, *37*, 223–228. [CrossRef]
10. Riley, A.; Terry, M.; Freeman, H.; Alba, A.C.; Soltis, J.; Leeds, A. Evaluating the Effect of Visitor Presence on Nile Crocodile (*Crocodylus niloticus*) Behavior. *J. Zool. Bot. Gard.* **2021**, *2*, 115–129. [CrossRef]
11. Huskisson, S.M.; Doelling, C.R.; Ross, S.R.; Hopper, L.M. Assessing the Potential Impact of Zoo Visitors on the Welfare and Cognitive Performance of Japanese Macaques. *Appl. Anim. Behav. Sci.* **2021**, *243*, 105453. [CrossRef]
12. Cairo-Evans, A.; Wierzal, N.K.; Wark, J.D.; Cronin, K.A. Do Zoo-Housed Primates Retreat from Crowds? A Simple Study of Five Primate Species. *Am. J. Primatol.* **2022**, e23386. [CrossRef]
13. Hamilton, J.; Gartland, K.N.; Jones, M.; Fuller, G. Behavioral Assessment of Six Reptile Species during a Temporary Zoo Closure and Reopening. *Animals* **2022**, *12*, 1034. [CrossRef]
14. Carter, K.C.; Keane, I.A.T.; Clifforde, L.M.; Rowden, L.J.; Fieschi-Méric, L.; Michaels, C.J. The Effect of Visitors on Zoo Reptile Behaviour during the COVID-19 Pandemic. *J. Zool. Bot. Gard.* **2021**, *2*, 664–676. [CrossRef]
15. Bernstein-Kurtycz, L.M.; Koester, D.C.; Snyder, R.J.; Vonk, J.; Willis, M.A.; Lukas, K.E. "Bearly" Changing with the Seasons: Bears of Five Species Show Few Behavioral Changes across Seasons and at Varying Visitor Densities. *Anim. Behav. Cogn.* **2021**, *8*, 538–557. [CrossRef]
16. Williams, E.; Carter, A.; Rendle, J.; Ward, S.J. Understanding Impacts of Zoo Visitors: Quantifying Behavioural Changes of Two Popular Zoo Species during COVID-19 Closures. *Appl. Anim. Behav. Sci.* **2021**, *236*, 105253. [CrossRef]
17. Jones, M.; Gartland, K.N.; Fuller, G. Effects of Visitor Presence and Crowd Size on Zoo-Housed Red Kangaroos (*Macropus rufus*) during and after a COVID-19 Closure. *Anim. Behav. Cogn.* **2021**, *8*, 521–537. [CrossRef]
18. Kidd, P.; Ford, S.; Rose, P.E. Exploring the Effect of the COVID-19 Zoo Closure Period on Flamingo Behaviour and Enclosure Use at Two Institutions. *Birds N. Am.* **2022**, *3*, 117–137. [CrossRef]
19. Miller, M.E.; Robinson, C.M.; Margulis, S.W. Behavioral Implications of the Complete Absence of Guests on a Zoo-Housed Gorilla Troop. *Animals* **2021**, *11*, 1346. [CrossRef]
20. Williams, E.; Carter, A.; Rendle, J.; Ward, S.J. Impacts of COVID-19 on Animals in Zoos: A Longitudinal Multi-Species Analysis. *J. Zool. Bot. Gard.* **2021**, *2*, 130–145. [CrossRef]
21. Boultwood, J.; O'Brien, M.; Rose, P. Bold Frogs or Shy Toads? How Did the COVID-19 Closure of Zoological Organisations Affect Amphibian Activity? *Animals* **2021**, *11*, 1982. [CrossRef]
22. Fink, L.B.; Scarlata, C.D.; VanBeek, B.; Bodner, T.E.; Wielebnowski, N.C. Applying Behavioral and Physiological Measures to Assess the Relative Impact of the Prolonged COVID-19 Pandemic Closure on Two Mammal Species at the Oregon Zoo: Cheetah (*A. jubatus*) and Giraffe (*G. c. reticulata* and *G. c. tippelskirchii*). *Animals* **2021**, *11*, 3526. [CrossRef]
23. Bonnie, K.E.; Ang, M.Y.L.; Ross, S.R. Effects of Crowd Size on Exhibit Use by and Behavior of Chimpanzees (*Pan Troglodytes*) and Western Lowland Gorillas (*Gorilla gorilla*) at a Zoo. *Appl. Anim. Behav. Sci.* **2016**, *178*, 102–110. [CrossRef]
24. Pollard, T.M. Use of Cortisol as a Stress Marker: Practical and Theoretical Problems. *Am. J. Hum. Biol.* **1995**, *7*, 265–274. [CrossRef]
25. McEwen, B.S. What is the Confusion with Cortisol? *Chronic Stress* **2019**, *3*, 2470547019833647. [CrossRef]
26. Wielebnowski, N.C.; Fletchall, N.; Carlstead, K.; Busso, J.M.; Brown, J.L. Noninvasive Assessment of Adrenal Activity Associated with Husbandry and Behavioral Factors in the North American Clouded Leopard Population. *Zoo Biol.* **2002**, *21*, 77–98. [CrossRef]
27. Sherwen, S.L.; Harvey, T.J.; Magrath, M.J.L.; Butler, K.L.; Fanson, K.V.; Hemsworth, P.H. Effects of Visual Contact with Zoo Visitors on Black-Capped Capuchin Welfare. *Appl. Anim. Behav. Sci.* **2015**, *167*, 65–73. [CrossRef]
28. Scott, K.; Heistermann, M.; Cant, M.A.; Vitikainen, E.I.K. Group Size and Visitor Numbers Predict Faecal Glucocorticoid Concentrations in Zoo Meerkats. *R. Soc. Open Sci.* **2017**, *4*, 161017. [CrossRef]

29. Ozella, L.; Anfossi, L.; Di Nardo, F.; Pessani, D. Effect of Weather Conditions and Presence of Visitors on Adrenocortical Activity in Captive African Penguins (*Spheniscus demersus*). *Gen. Comp. Endocrinol.* **2017**, *242*, 49–58. [CrossRef]
30. Sherwen, S.L.; Hemsworth, P.H.; Butler, K.L.; Fanson, K.V.; Magrath, M.J.L. Impacts of Visitor Number on Kangaroos Housed in Free-Range Exhibits. *Zoo Biol.* **2015**, *34*, 287–295. [CrossRef]
31. Clark, F.E.; Fitzpatrick, M.; Hartley, A.; King, A.J.; Lee, T.; Routh, A.; Walker, S.L.; George, K. Relationship Between Behavior, Adrenal Activity, and Environment in Zoo-Housed Western Lowland Gorillas (*Gorilla gorilla gorilla*). *Zoo Biol.* **2012**, *31*, 306–321. [CrossRef]
32. Clow, A.; Hucklebridge, F.; Thorn, L. The Cortisol Awakening Response in Context. In *International Review of Neurobiology*; Clow, A., Thorn, L., Eds.; Academic Press: Cambridge, MA, USA, 2010; Volume 93, pp. 153–175.
33. Bonier, F.; Moore, I.T.; Martin, P.R.; Robertson, R.J. The Relationship between Fitness and Baseline Glucocorticoids in a Passerine Bird. *Gen. Comp. Endocrinol.* **2009**, *163*, 208–213. [CrossRef]
34. Busch, D.S.; Hayward, L.S. Stress in a Conservation Context: A Discussion of Glucocorticoid Actions and How Levels Change with Conservation-Relevant Variables. *Biol. Conserv.* **2009**, *142*, 2844–2853. [CrossRef]
35. Madliger, C.L.; Love, O.P. The Need for a Predictive, Context-Dependent Approach to the Application of Stress Hormones in Conservation. *Conserv. Biol.* **2013**, *28*, 283–287. [CrossRef]
36. Cockrem, J.F. Individual Variation in Glucocorticoid Stress Responses in Animals. *Gen. Comp. Endocrinol.* **2013**, *181*, 45–58. [CrossRef]
37. Dantzer, B.; Fletcher, Q.E.; Boonstra, R.; Sheriff, M.J. Measures of Physiological Stress: A Transparent or Opaque Window into the Status, Management and Conservation of Species? *Conserv. Physiol.* **2014**, *2*, cou023. [CrossRef] [PubMed]
38. Crespi, E.J.; Williams, T.D.; Jessop, T.S.; Delehanty, B. Life History and the Ecology of Stress: How Do Glucocorticoid Hormones Influence Life-History Variation in Animals? *Funct. Ecol.* **2013**, *27*, 93–106. [CrossRef]
39. Möstl, E.; Palme, R. Hormones as Indicators of Stress. *Domest. Anim. Endocrinol.* **2002**, *23*, 67–74. [CrossRef]
40. Broom, D. Cortisol: Often Not the Best Indicator of Stress and Poor Welfare. *Physiol. News* **2017**, *107*, 30–32. [CrossRef]
41. MacDougall-Shackleton, S.A.; Bonier, F.; Romero, L.M.; Moore, I.T. Glucocorticoids and "Stress" Are Not Synonymous. *Integr. Org. Biol.* **2019**, *1*, obz017. [CrossRef]
42. Goodenough, A.E.; McDonald, K.; Moody, K.; Wheeler, C. Are "Visitor Effects" Overestimated? Behaviour in Captive Lemurs Is Mainly Driven by Co-Variation with Time and Weather. *J. Zoo Aquar. Res.* **2019**, *7*, 59–66.
43. Rose, P.E.; Scales, J.S.; Brereton, J.E. Why the "Visitor Effect" Is Complicated. Unraveling Individual Animal, Visitor Number, and Climatic Influences on Behavior, Space Use and Interactions with Keepers-A Case Study on Captive Hornbills. *Front. Vet. Sci.* **2020**, *7*, 236. [CrossRef]
44. Kozlowski, C.P.; Clawitter, H.L.; Thier, T.; Fischer, M.T.; Asa, C.S. Characterization of Estrous Cycles and Pregnancy in Somali Wild Asses (*Equus africanus somaliensis*) through Fecal Hormone Analyses. *Zoo Biol.* **2018**, *37*, 35–39. [CrossRef]
45. Palme, R. Non-Invasive Measurement of Glucocorticoids: Advances and Problems. *Physiol. Behav.* **2019**, *199*, 229–243. [CrossRef]
46. White, B.C.; Kozlowski, C.; Taylor, S.R.; Franklin, J.A.; Burns, R. Faecal Glucocorticoid Metabolite Concentrations during ACTH Challenge Tests in Captive Grizzly Bears (*Ursus arctos horribilus*) and Polar Bears (*Ursus maritimus*). *J. Zoo Aquar. Res.* **2015**, *3*, 59–62.
47. Kozlowski, C.P.; Bauman, K.L.; Clawitter, H.L.; Thier, T.; Fischer, M.; Powell, D.M. Noninvasive Monitoring of Steroid Hormone Production and Activity of Zoo-Housed Banteng (*Bos javanicus*). *In review*.
48. Bates, D.; Maechler, M.; Bolker, B.; Walker, S.; Christensen, R.H.B.; Singmann, H.; Dai, B.; Scheipl, F.; Grothendieck, G.; Green, P.; et al. *Lme4: Linear Mixed-Effects Models Using "Eigen" and S4*; The R Project for Statistical Computing: Vienna, Austria, 2015.
49. Hothorn, T.; Bretz, F.; Westfall, P.; Heiberger, R.M.; Schuetzenmeister, A.; Scheibe, S. *Multcomp: Simultaneous Inference in General Parametric Models*; The R Project for Statistical Computing: Vienna, Austria, 2022.
50. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
51. Greenland, S.; Senn, S.J.; Rothman, K.J.; Carlin, J.B.; Poole, C.; Goodman, S.N.; Altman, D.G. Statistical Tests, P Values, Confidence Intervals, and Power: A Guide to Misinterpretations. *Eur. J. Epidemiol.* **2016**, *31*, 337–350. [CrossRef]
52. McShane, B.B.; Gal, D.; Gelman, A.; Robert, C.; Tackett, J.L. Abandon Statistical Significance. *Am. Stat.* **2019**, *73*, 235–245. [CrossRef]
53. Nakagawa, S.; Cuthill, I.C. Effect Size, Confidence Interval and Statistical Significance: A Practical Guide for Biologists. *Biol. Rev. Camb. Philos. Soc.* **2007**, *82*, 591–605. [CrossRef]
54. Coleman, C. *AZA Regional Studbook Banteng (Bos javanicus)*; Association of Zoos and Aquariums: Silver Spring, MD, USA, 2020.
55. Kenagy, G.J.; Place, N.J. Seasonal Changes in Plasma Glucocorticosteroids of Free-Living Female Yellow-Pine Chipmunks: Effects of Reproduction and Capture and Handling. *Gen. Comp. Endocrinol.* **2000**, *117*, 189–199. [CrossRef]
56. Rajagopal, T.; Archunan, G.; Sekar, M. Impact of Zoo Visitors on the Fecal Cortisol Levels and Behavior of an Endangered Species: Indian Blackbuck (*Antelope cervicapra* L.). *J. Appl. Anim. Welf. Sci.* **2011**, *14*, 18–32. [CrossRef]
57. Klich, D.; Łopucki, R.; Gałązka, M.; Ścibior, A.; Gołębiowska, D.; Brzezińska, R.; Kruszewski, B.; Kaleta, T.; Olech, W. Stress Hormone Level and the Welfare of Captive European Bison (*Bison bonasus*): The Effects of Visitor Pressure and the Social Structure of Herds. *Acta Vet. Scand.* **2021**, *63*, 24. [CrossRef]
58. Leake, J. The Livestock Industry. *Bull. Indones. Econ. Stud.* **1980**, *16*, 65–74. [CrossRef]

59. Martojo, H. Indigenous Bali Cattle Is Most Suitable for Sustainable Small Farming in Indonesia. *Reprod. Domest. Anim.* **2012**, *47* (Suppl. S1), 10–14. [CrossRef]
60. Copland, R.S. Observations on Banteng Cattle in Sabah. *Trop. Anim. Health Prod.* **1974**, *6*, 89–94. [CrossRef]
61. Leishman, E.M.; Franke, M.; Marvin, J.; McCart, D.; Bradford, C.; Gyimesi, Z.S.; Nichols, A.; Lessard, M.-P.; Page, D.; Breiter, C.-J.; et al. The Adrenal Cortisol Response to Increasing Ambient Temperature in Polar Bears (*Ursus maritimus*). *Animals* **2022**, *12*, 672. [CrossRef]
62. Soriano, A.I.; Vinyoles, D.; Maté, C. The Influence of Visitors on Behaviour and on the Use of Space in Two Species of Ursids: A Management Question? *Int. Zoo News* **2013**, *60*, 341–356.
63. Kelly, K.R.; Harrison, M.L.; Size, D.D.; MacDonald, S.E. Individual Effects of Seasonal Changes, Visitor Density, and Concurrent Bear Behavior on Stereotypical Behaviors in Captive Polar Bears (*Ursus maritimus*). *J. Appl. Anim. Welf. Sci.* **2015**, *18*, 17–31. [CrossRef]
64. Wells, D.L. A Note on the Influence of Visitors on the Behaviour and Welfare of Zoo-Housed Gorillas. *Appl. Anim. Behav. Sci.* **2005**, *93*, 13–17. [CrossRef]
65. Ross, S.R.; Lukas, K.E. Use of Space in a Non-Naturalistic Environment by Chimpanzees (*Pan troglodytes*) and Lowland Gorillas (*Gorilla gorilla gorilla*). *Appl. Anim. Behav. Sci.* **2006**, *96*, 143–152. [CrossRef]
66. Stoinski, T.S.; Jaicks, H.F.; Drayton, L.A. Visitor Effects on the Behavior of Captive Western Lowland Gorillas: The Importance of Individual Differences in Examining Welfare. *Zoo Biol.* **2012**, *31*, 586–599. [CrossRef]
67. Kurtycz, L.M.B.; Ross, S.R. Western Lowland Gorilla (*Gorilla gorilla gorilla*) Birth Patterns and Human Presence in Zoological Settings. *Zoo Biol.* **2015**, *34*, 518–521. [CrossRef]
68. Collins, C.K.; Marples, N.M. The Effects of Zoo Visitors on a Group of Western Lowland Gorillas *Gorilla gorilla gorilla* before and after the Birth of an Infant at Dublin Zoo. *Int. Zoo Yearb.* **2016**, *50*, 183–192. [CrossRef]
69. Hashmi, A.; Sullivan, M. The Visitor Effect in Zoo-Housed Apes: The Variable Effect on Behaviour of Visitor Number and Noise. *J. Zoo Aquar. Res.* **2020**, *8*, 268–282.
70. Lewis, R.N.; Chang, Y.-M.; Ferguson, A.; Lee, T.; Clifforde, L.; Abeyesinghe, S.M. The Effect of Visitors on the Behaviour of Zoo-Housed Western Lowland Gorillas (*Gorilla gorilla gorilla*). *Zoo Biol.* **2020**, *39*, 283–296. [CrossRef]
71. Kuhar, C.W. Group Differences in Captive Gorillas' Reaction to Large Crowds. *Appl. Anim. Behav. Sci.* **2008**, *110*, 377–385. [CrossRef]
72. Carder, G.; Semple, S. Visitor Effects on Anxiety in Two Captive Groups of Western Lowland Gorillas. *Appl. Anim. Behav. Sci.* **2008**, *115*, 211–220. [CrossRef]
73. Hill, S.P.; Broom, D.M. Measuring Zoo Animal Welfare: Theory and Practice. *Zoo Biol.* **2009**, *28*, 531–544. [CrossRef]
74. Ross, S.R.; Wagner, K.E.; Schapiro, S.J.; Hau, J. Ape Behavior in Two Alternating Environments: Comparing Exhibit and Short-Term Holding Areas. *Am. J. Primatol.* **2010**, *72*, 951–959. [CrossRef]
75. Kurtycz, L.M.; Wagner, K.E.; Ross, S.R. The Choice to Access Outdoor Areas Affects the Behavior of Great Apes. *J. Appl. Anim. Welf. Sci.* **2014**, *17*, 185–197. [CrossRef]
76. Choo, Y.; Todd, P.A.; Li, D. Visitor Effects on Zoo Orangutans in Two Novel, Naturalistic Enclosures. *Appl. Anim. Behav. Sci.* **2011**, *133*, 78–86. [CrossRef]
77. Hosey, G.R.; Melfi, V.; Formella, I.; Ward, S.J.; Tokarski, M.; Brunger, D.; Brice, S.; Hill, S.P. Is Wounding Aggression in Zoo-Housed Chimpanzees and Ring-Tailed Lemurs Related to Zoo Visitor Numbers? *Zoo Biol.* **2016**, *35*, 205–209. [CrossRef]
78. Margulis, S.W.; Hoyos, C.; Anderson, M. Effect of Felid Activity on Zoo Visitor Interest. *Zoo Biol.* **2003**, *22*, 587–599. [CrossRef]