Original Article

Investigating the Impact of Acoustic Environment and Human Presence on Okapi Faecal Glucocorticoid Metabolites

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Abstract

Background: The influence of visitor behaviour and sound pressure levels on zoo animals remains unclear. **Objective:** This study examined associations between sound pressure levels, visitor numbers and faecal glucocorticoid metabolites (FGCMs) in a male okapi (*Okapia johnstoni*) at a UK zoo. **Materials and methods:** Data were collected across two 5-day periods representing high- and low-visitor seasons, yielding 10 days of acoustic recordings and matching FGCM samples. Acoustic metrics were calculated for the open and closed periods of the zoo (L_{eqZ}, L_{10Z} and L_{90Z}). Statistical analyses included the Wilcoxon and Welch *t*-tests, as well as Pearson correlations and multiple linear regression models. **Results:** L_{eqZ} and L_{10Z} did not differ between seasons, whereas background noise (L_{90Z}) was higher in the low season ($t = –4.42$, $P = 0.005$). Sound levels were generally higher during open hours in both seasons. Correlations between visitor numbers and acoustic metrics were weak and non-significant (high season L_{eqZ} × visitors: $r = 0.005$, $P = 0.994$; low season: $r = –0.347$, $P = 0.567$). FGCM concentrations did not differ between seasons (72a: $P = 1.00$; 72T: $P = 0.37$). Regression analyses showed that L_{eqZOpen} positively predicted 72T FGCMs ($\beta = 5.30 \pm 1.85$, $P = 0.02$), while visitor numbers negatively predicted 72T ($\beta = –0.04 \pm 0.02$, $P = 0.04$). Visitor numbers did not differ between seasons. **Conclusions:** Okapi adrenocortical activity appears more responsive to acoustic conditions than to visitor numbers, suggesting noise as the primary stressor. Reducing noise through enrichment or vegetation may improve okapi welfare.

Keywords: glucocorticoids, noise, *okapia johnstoni*, stress, zoo, welfare

KEY MESSAGES

(1) Visitor presence and acoustic conditions influenced adrenocortical activity in okapis, with distinct faecal glucocorticoid metabolites (FGCM) assays revealing different sensitivities to noise components.

(2) Background noise (L_{90Z}) and visitor numbers increased FGCM levels in the 72a assay, indicating heightened physiological activation under elevated anthropogenic stimuli.

(3) High percentile noise (L_{10Z}) increased FGCM levels in the 72T assay, whereas background noise and visitor numbers showed negative or attenuated associations, suggesting assay-specific physiological pathways.

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INTRODUCTION

The relationship between zoo visitors and captive animals is complex, as interactions may act as a source of stress, enrichment or have neutral effects, with responses varying widely even within the same species.[1,2] While visitor presence effects on zoo animals are well documented, less

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is known about how specific aspects of visitor behaviour and noise influence animal welfare.[1]

Captive animal environments are influenced by multiple noise sources, including machinery, maintenance activities and urban background noise, each of which requires a specific acoustic assessment due to its distinct properties.[3-6] Adopting a soundscape approach, which considers the acoustic environment holistically, including biophonic, geophonic and anthrophonic components,[4] provides a more nuanced understanding of captive auditory conditions. Systematic soundscape monitoring in zoos and other captive settings can identify chronic noise stressors and inform targeted management strategies, ultimately mitigating stress and promoting animal welfare by aligning captive environments more closely with the species' natural acoustic ecology.

Evaluating the impact of sound on animals often combines behavioural observations with stress hormone measurements, such as glucocorticoid levels.[7-10] Behavioural habituation may occur even if physiological responses persist.[10] Non-invasive analyses of faecal glucocorticoid metabolites (FGCMs) provide insights into adrenocortical activity.[11-15] This study examines the relationship between sound pressure levels, visitor numbers and FGCM concentrations in a male adult okapi at a UK zoo during periods of low and high visitation. We hypothesised that higher sound intensity (L_{eqZ}) and greater visitor numbers would be associated with increased FGCM levels in the okapi.

METHODS

Subjects of Study – Okapi (*Okapia johnstoni*)

The okapi belongs to the same family as the giraffe (Giraffidae) but inhabits forested environments. Typically solitary, the species is classified as endangered owing to ongoing habitat loss and degradation.[16] Within the zoo, this species participates in the European Endangered Species Breeding Programme, and the institution additionally contributes to a conservation initiative in the Democratic Republic of Congo.

A single male okapi (born on 30 April 2015) was the focus of this study. The individual was housed in a paddock enclosure that allows visitors to observe through viewing windows separated by a 1-metre stand-off barrier. The animal had *ad libitum* access to an outdoor area throughout the study period. However, this area was not included in the present investigation for logistical reasons, including the large size of the outdoor space that would require extensive acoustic mapping, additional recording equipment and installation of cameras to monitor visitor interactions and animal behaviours. Consequently, all acoustic measurements and behavioural observations reported here refer solely to the indoor enclosure. It should be noted that sound exposure in the outdoor area may differ from that captured indoors, for instance, due to greater sound dissipation in open spaces, increased distance from noise sources, potential buffering by vegetation and the animal's ability to move freely away from visitors or mechanical equipment. These factors could result in lower sound pressure levels and different acoustic characteristics compared to the indoor environment.

All research reported here was approved by the animal ethics committees of the authors' institution (University of Salford, UK) and the zoo (ethics application ST1617-46).

Data Collection

Data were collected across two periods: the high-visitor season (September 2017) and the low-visitor season (December 2017), each spanning 5 consecutive days and including weekends. The monitoring periods were determined by the zoo staff in coordination with animal care routines and logistical constraints, ensuring that both visitor seasons were adequately represented. During these periods, the zoo operated from 10:00 to 17:00 in the high season and from 10:00 to 15:30 in the low season. Visitor numbers were recorded at five consistent time points each day (early morning, late morning, lunchtime, early afternoon and late afternoon), yielding a total of 25 observation time points per season. During each period, all visitors who approached the viewing windows were counted using a handheld mechanical counter, providing the total number of visitors for that interval. The same procedure was applied consistently across all sampling days to ensure comparability between observations.

Acoustic data were obtained using a sound level meter (SLM; Svantek SVAN 957, Class 1, IEC 61672-1 compliant, Svantek Sp. z o.o., Warsaw, Poland) and a passive acoustic monitoring device (Wildlife Acoustics Song Meter SM3, Wildlife Acoustics, Inc., Massachusetts, USA). The SLM was calibrated before and after each recording session using the manufacturer's calibrator (SVAN 957 kit), and no significant drift was observed. Both devices were installed inside the okapi enclosure, with microphones positioned approximately 2.5 m above the floor, oriented towards the public passageway and the animal's activity area, to capture sounds entering the space. They were equipped with a windshield to reduce environmental interference. This height was selected in consultation with zoo staff to approximate the animal's ear level (approximately 2 m when standing), while ensuring both animal and visitor safety and preventing any physical contact with the equipment. The precise monitoring points were chosen based on accessibility for equipment maintenance and proximity to a suitable power source required for the sound level meter. Sound levels were sampled at 30-second intervals, which may slightly smooth out transient peak events; however, this integration time was applied consistently across all recordings.

This meter model enables the simultaneous recording of multiple acoustic metrics, including L_{eq}, L_{10} and L_{90}, across 1/3-octave frequency bands, with Z-weighting (10 Hz–20 kHz, reported in dBZ) to preserve low-frequency components. Spectral analyses confirmed that low-frequency contributions from the ventilation and heating systems dominated background noise during closed hours. This study focused on three Z-weighted sound measures: L_{eqZ}, $L_{10\text{Z}}$ and $L_{90\text{Z}}$.[17] L_{eq} estimates the overall energy from the acoustic environment. L_{10}, the sound level exceeded 10% of the time, serves as a stable metric for peak noise and captures sudden loud events, such as a vehicle backfire, that may elicit startle or surprise responses in animals. L_{90} defines the ambient background noise (level exceeded 90% of the time) by excluding extremes. Z-weighting was chosen as it applies no frequency filtering, reflecting actual sound pressure levels (10 Hz to 20 kHz).[18] This preserves low-frequency components, providing a precise representation of the absolute sound pressure levels for background noise analysis. Additionally, the acoustic recorder was employed to allow identification of noise sources for individual decibel measurements.

Extraction of FGCMs

Faecal samples were collected each morning by the okapi keeper team, approximately 20 hours after the end of the previous day's acoustic recordings. This sampling schedule ensured that the FGCM concentrations reflected the hormonal output corresponding to the period of acoustic exposure recorded on the preceding day. Accordingly, the dataset comprised 5 consecutive days of acoustic recordings and five corresponding faecal samples in each visitor season, with each faecal sample aligned to the sound data collected on the previous day (e.g., sound data from Day 1 paired with the faecal sample collected on Day 2). This temporal alignment accurately captured the expected physiological lag between glucocorticoid secretion and faecal metabolite excretion.[9] Each faecal sample was placed in a labelled, airtight plastic bag and immediately frozen at −20°C for subsequent FGCM extraction.[9] Establishing a true "noise-free" physiological baseline for FGCM measurements was not feasible. Even when the zoo is closed to the public, the enclosure remains continuously exposed to low-frequency mechanical noise generated by essential life-support systems (ventilation and climate control), which operate 24/7 for welfare reasons and cannot be switched off. Therefore, no sampling period represented a condition without anthropogenic noise. To address this limitation, we incorporated two relative baseline comparisons within the study design. First, we contrasted the low visitor season versus the high visitor season, reflecting the lowest and highest naturally occurring levels of visitor presence and acoustic disturbance. Second, within each season, we compared periods when the zoo was open versus closed to the public, capturing the additional influence of direct visitor activity. Together, these two complementary contrasts allowed us to evaluate FGCM responses across the full range of realistic acoustic conditions present in the enclosure, despite the impossibility of defining an accurate noise-free baseline.

Faecal samples (0.5 g each, thoroughly homogenised) were extracted following a methanol-based protocol.[19] To each sample, 5 mL of 80% methanol was added, and the mixture was mixed on a multivortex for approximately 90 seconds. The suspension was then centrifuged for 15 minutes at $2500 \times g$ at room temperature (≈20–22 °C). After centrifugation, the supernatant was carefully decanted and aliquoted (0.5 mL, in duplicate), while the sediment was discarded. Each aliquot was dried in a heat block at 60 °C until the solvent had evaporated entirely.[19] Dried extracts were stable at room temperature and subsequently shipped by post to the University of Veterinary Medicine, Vienna (Austria) for FGCM quantification.

Quantification of FGCMs was performed using two 11-oxoaeticholanolone enzyme immunoassays (EIAs): the 72T assay (targeting metabolites with a 5β-3α-ol-11-one structure) and the 72a assay (targeting 11,17-dioxoandrostanes, representing a broader range of cortisol/corticosterone metabolites).[20-22] The rationale for dual assays is that faecal steroid metabolite profiles vary by species, sex, diet, gut passage time and biotransformation pathways, using both assays provides complementary sensitivity to detect Hypothalamic-Pituitary-Adrenal axis (herein HPA axis) axis activation under different conditions.[20-22]

Statistical Analysis

Sound pressure level (SPL) data were analysed using two complementary approaches. First, differences in SPL metrics were examined between periods when the zoo was open (low season: 10:00–15:30; high season: 10:00–17:00) and closed (low season: 15:30–09:00; high season: 17:00–09:00), as well as between visitor seasons. To account for the unequal durations of open hours between seasons and to avoid pseudoreplication, all 30-second SPL measurements within each period were first converted into 30-second L_{eq} values, and these L_{eq} values were then averaged per day. In other words, each data point represents the mean of multiple L_{eq} (30 seconds) measurements rather than a simple average of raw decibel readings, ensuring a standardised representation of acoustic exposure despite differences in sampling effort. These daily averages were used in all subsequent statistical analyses, ensuring that each value represented an independent sampling unit rather than individual 30-second recordings. This approach reflects the average acoustic environment experienced by the animal rather than cumulative exposure over time.

Before hypothesis testing, normality of each variable within season groups was assessed using Shapiro-Wilk tests. Variables meeting parametric assumptions (L_{eqZ}, $L_{10\text{Z}}$, $L_{90\text{Z}}$, L_{eqZOpen}, $L_{10\text{ZOpen}}$, $L_{90\text{ZOpen}}$, 72T and visitor numbers) were analysed with Welch *t*-tests for comparisons between seasons. Variables violating normality assumptions (e.g., 72a) were analysed using the Wilcoxon rank-sum test.

To investigate the influence of acoustic exposure on FGCM levels, multiple linear regression models (LMs) were fitted, with FGCM concentrations (72T or 72a) as the response variables. The first set of models included $L_{eqZOpen}$ and the total number of visitors as predictors. $L_{eqZOpen}$ was selected as the primary acoustic predictor because it represents the Z-weighted equivalent continuous sound level calculated exclusively during zoo opening hours, thereby capturing noise exposure specifically associated with visitor presence while excluding periods without public activity. This allowed us to test the effect of visitor-related acoustic disturbance more directly. Predictors were used in their original units without centring or scaling. Variance inflation factors (VIFs) were calculated using the vif() function from the car package and indicated no multicollinearity issues (VIF = 1.007 for both L_{eqZ} and visitors). L_{10Z} and L_{90Z} were analysed in separate exploratory models to assess the potential effects of percentile sound metrics.

A second set of linear models was fitted to examine whether percentile-based noise metrics explained additional variation in FGCM levels. These models included L_{10Z}, L_{90Z} and total visitor numbers as simultaneous predictors (FGCM ~ $L_{10Z} + L_{90Z}$ + visitors) for both 72T and 72a. This allowed assessment of whether different components of the acoustic environment (higher-intensity peaks vs. background levels) were independently associated with FGCM concentrations, regardless of visitor numbers. Predictors were kept at their original scales, and VIF values indicated low multicollinearity across all models (VIFs: $L_{10Z} = 1.08$, $L_{90Z} = 1.31$, visitors = 1.33).

Model diagnostics included examining residuals versus fitted values, QQ plots, Shapiro-Wilk tests for the normality of residuals and influence plots to identify points with high leverage. Leave-one-out (LOO) sensitivity analyses were performed to assess the robustness of the regression coefficients, given the small sample size. Scatterplots with fitted regression lines and 95% confidence intervals were generated to inspect bivariate relationships visually.

All FGCM analyses were temporally adjusted to account for the approximately 20-hour physiological lag between glucocorticoid secretion and faecal metabolite excretion, aligning each faecal sample with the corresponding acoustic data from the previous day. Holm–Bonferroni corrections were applied across all regression coefficients for both assays and all time windows to adjust for multiple comparisons, and effect sizes with 95% confidence intervals were reported. All statistical analyses were conducted in RStudio (R version 4.5.1; RStudio, PBC, Boston, MA, USA).[23] All statistical tests were two-tailed, with a significance level of $\alpha = 0.05$.

RESULTS

Acoustic Patterns in the Okapi Enclosure between Seasons (High × Low)

Within each visitor season, the okapi enclosure exhibited temporal variation in sound levels. Overall noise levels (L_{eqZ}) and peak noise levels (L_{10Z}) were slightly higher during the high season compared to the low season; however, the differences were not statistically significant (L_{eqZ}: high 67.72–77.56 dB, low 69.22–73.77 dB; L_{10Z}: high 70.56–81.55 dB, low 70.28–76.55 dB; Table 1). In contrast, background noise (L_{90Z}) was significantly higher during the low season compared to the high season (high 59.89–65.94 dB, low 66.83–69.54 dB; Figure 1). Additionally, effect sizes indicated that the seasonal difference in background noise (L_{90Z}) was large (Cohen $d = -2.79$), whereas differences in L_{eqZ} and L_{10Z} were small to moderate ($d = 0.37$ and 0.73, respectively), consistent with the non-significant statistical outcomes for these metrics. When comparing operational hours within each season, noise levels were generally higher during open hours. For the high season, L_{eqZ} and L_{10Z} during open hours were slightly higher than during closed hours ($L_{eqZOpen}$ 69.62–81.67 dB vs. $L_{eqZClose}$ 65.08–73.67 dB; $L_{10ZOpen}$ 72.43–85.73 dB vs. $L_{10ZClose}$ 67.93–77.52 dB; Table 1), while background noise decreased significantly during open hours ($L_{90ZOpen}$ 58.90–67.55 dB vs. $L_{90ZClose}$ 60.19–65.28 dB; Table 1). For the low season, the differences between L_{eqZ} and $L_{10ZOpen}$ versus $L_{10ZClose}$ were minimal and non-significant ($L_{eqZOpen}$ 70.95–74.12 dB vs. $L_{eqZClose}$ 67.92–74.02 dB; $L_{10ZOpen}$

Table 1: Acoustic metrics during high and low visitor seasons

Metric	High season Mean ± SE	Low season Mean ± SE	*t*-test	*P*-value	Effect size
L_{eqZ}	72.64 ± 1.77	71.5 ± 0.82	0.59	0.581	0.37
L_{10Z}	76.05 ± 1.98	73.41 ± 1.13	1.16	0.288	0.73
L_{90Z}	62.92 ± 1.09	68.19 ± 0.49	−4.42	**0.005**	−2.79
$L_{eqZOpen}$	75.65 ± 2.17	72.53 ± 0.57	1.39	0.230	0.88
$L_{eqZClose}$	69.38 ± 1.55	70.97 ± 1.1	−0.84	0.426	−0.53
$L_{10ZOpen}$	79.08 ± 2.4	74.49 ± 0.73	1.83	0.130	1.16
$L_{10ZClose}$	72.72 ± 1.73	72.85 ± 1.48	−0.06	0.957	−0.04
$L_{90ZOpen}$	63.23 ± 1.56	68.09 ± 0.67	−2.87	**0.032**	−1.81
$L_{90ZClose}$	62.74 ± 0.92	68.24 ± 0.53	−5.20	**0.002**	−3.29

Note: Summary of acoustic metrics (L_{eqZ}, L_{10Z}, L_{90Z}) during high- and low-visitor seasons, including sample size (*N*), mean, standard error (SE), *t*-test results, *P*-values and effect sizes (Cohen *d*). Values of *P* in bold indicate statistically significant differences between high and low seasons.

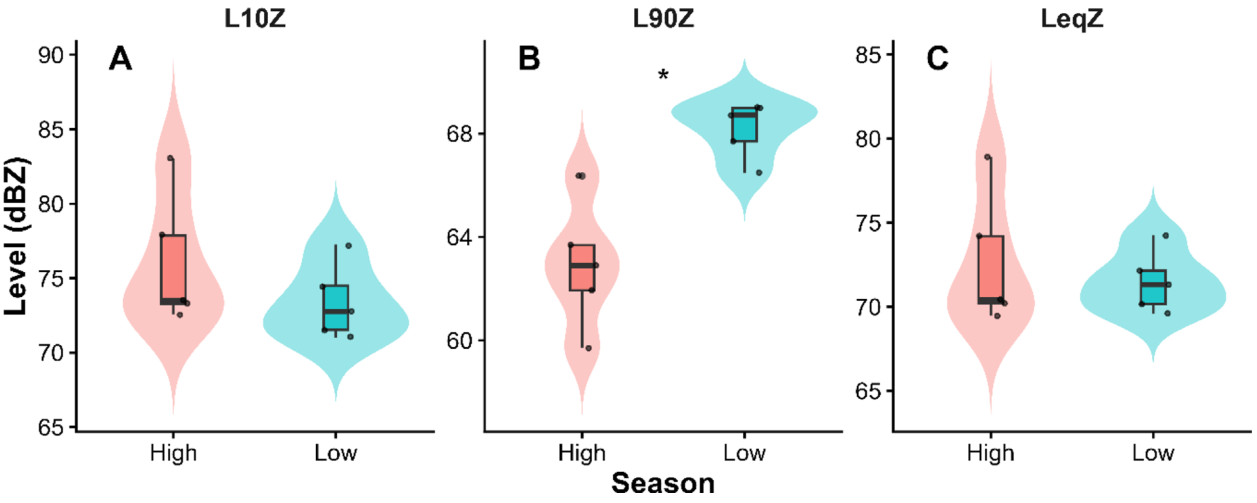

Figure 1: Z-weighted sound levels (A) L_{10Z}, (B) L_{90Z}, and (C) L_{eqZ} (dBZ) recorded in the okapi (*Okapia johnstoni*) enclosure at a UK zoo during high and low visitor seasons.

Note: Data represent 24-hour values, including open and closed hours. Boxplots (overlaid on violin distributions) show median, interquartile range and data density for each acoustic metric and season. L_{eqz} represents the equivalent continuous sound level, L_{10Z} represents the maximum sound level for 10% of the time (peak levels) and L_{90Z} represents the sound level occurring 90% of the time (background noise). Statistical significance is indicated between the low and high seasons for L_{90Z} (*$P < 0.01$).

72.46–76.51 dB vs. $L_{10ZClose}$ 68.73–76.97 dB; Table 1), whereas background noise remained higher overall during both open and closed hours ($L_{90ZOpen}$ 66.22–69.96 dB vs. $L_{90ZClose}$ 66.77–69.72 dB; Table 1).

Correlations between visitor numbers and sound levels were generally weak and non-significant [Table 2].

Faecal Glucocorticoid Metabolites (FGCMs)

The non-parametric Wilcoxon test indicated no difference between seasons for FGCMs in the 72a assay [Table 3]. The 72T assay showed a similar pattern, with no seasonal difference by *t*-test [Table 3]. Pearson correlations between FGCMs and visitor numbers were weak and non-significant (72a: $r = 0.46$, $P = 0.17$; 72T: $r = –0.38$, $P = 0.27$).

Linear Regression Analysis

Multiple linear regression analysis revealed that zoo opening-time equivalent sound levels ($L_{eqZ,openzoo}$) and the total number of visitors significantly predicted FGCM levels measured with the 72T EIA [Table 4]. The effect of L_{eqZ} was positive and significant, indicating that higher broadband sound levels were associated with increased FGCM concentrations [Table 4]. In contrast, the number of visitors showed a significant negative association with FGCM levels [Table 4]. Both predictors remained significant after Holm–Bonferroni correction. The final model equation was $\mathrm{FGCM} = –320.94 + 5.30 \times L_{eqZ} – 0.04 \times \mathrm{visitors}$. In comparison, the multiple LM for FGCM levels measured with the 72a assay did not reach statistical significance, whereas the model for 72T was significant [Table 4]. Shapiro-Wilk tests indicated that residuals were approximately normally distributed for both assays (72T: $W = 0.93$, $P = 0.49$; 72a: $W = 0.94$, $P = 0.56$). Leave-one-out sensitivity analyses showed that coefficients remained generally consistent across models, with ranges for 72T ($L_{eqZOpen}$: –4.49 to 6.06; visitors: –0.05 to –0.00) and for 72a ($L_{eqZOpen}$: 1.56–7.05; visitors: –0.00 to 0.02), indicating that single observations (i.e., outliers) did not significantly influence the results.

Table 2: Correlations between visitor numbers and acoustic metrics

Season	Metric	Correlation (*r*)	*P*-value
High	L_{eqZ} × visitors	0.005	0.994
High	L_{10Z} × visitors	0.004	0.994
High	L_{90Z} × visitors	0.087	0.889
Low	L_{eqZ} × visitors	–0.347	0.567
Low	L_{10Z} × visitors	–0.411	0.492
Low	L_{90Z} × visitors	0.254	0.680

Note: Pearson correlations between daily visitor numbers and Z-weighted acoustic metrics (L_{eqZ}, L_{10Z}, L_{90Z}) during high and low visitor seasons in the okapi enclosure. Correlation coefficients (*r*) and associated *P*-values are reported for each season.

LMs incorporating acoustic predictors revealed additional patterns. In the 72a assay, FGCM levels were positively

Table 3: Seasonal comparison of FGCM concentrations

Assay	Test	Statistic	*P*-value
72a	Wilcoxon	$W = 13.00$	1.00
72T	Welch *t*-test	$t = 0.99$	0.37

Note: Seasonal comparison of faecal glucocorticoid metabolite (FGCM) concentrations for the two immunoassays (72a and 72T). The 72a assay was analysed using a Wilcoxon rank-sum test due to non-normal data distribution, while the 72T assay was analysed using Welch *t*-test. Test statistics and *P*-values are reported for comparisons between high and low visitor seasons.

Table 4: Linear regression models predicting FGCM levels

Assay	R^2adj	*F* (df)	*P* (model)	Predictor	*β*	SE	*t*	*P*	95% CI
72T	0.50	5.40 (2.70)	<**0.05**	$L_{eqZOpen}$	5.30	1.85	2.87	**0.02**	[0.93, 9.68]
				Visitors	–0.04	0.02	–2.54	**0.04**	[–0.08, –0.00]
	0.62	5.97 (6)	**0.03**	L_{10Z}	5.30	1.58	3.35	**0.02**	[1.43, 9.18]
				L_{90Z}	–4.64	1.94	–2.40	**0.05**	[–9.38, 0.01]
				Visitors	–0.05	0.02	–3.18	**0.02**	[–0.08, –0.01]
72a	0.17	1.91(2.70)	0.22	$L_{eqZOpen}$	2.27	1.88	1.21	0.27	[–2.15, 6.70]
				Visitors	0.02	0.02	0.99	0.36	[–0.02, 0.05]
	0.62	5.81 (6)	**0.03**	L_{10Z}	1.21	1.24	0.97	0.37	[1.43, 9.18]
				L_{90Z}	4.77	1.52	3.14	**0.02**	[–9.38, 0.10]
				Visitors	0.04	0.01	3.21	**0.02**	[–0.08, –0.01]

Note: Multiple linear regression results predicting FGCM levels (*β*, SE, *t*, *P*, 95% CI) for two assays (72T and 72a). The table lists the regression coefficients (*β*) ± standard error (SE), *t*-values, *P*-values, and 95% confidence intervals (CI) for each predictor. Significant *P*-values ($P < 0.05$) are indicated in bold.

associated with L_{90Z} ($\beta = 4.77$, SE = 1.52, $t = 3.14$, $P = 0.02$) and with visitor numbers ($\beta = 0.04$, SE = 0.01, $t = 3.21$, $P = 0.02$), whereas L_{10Z} was not significant ($\beta = 1.21$, SE = 1.24, $t = 0.97$, $P = 0.37$). In contrast, in the 72T assay, L_{10Z} showed a positive association with FGCM ($\beta = 5.30$, SE = 1.58, $t = 3.35$, $P = 0.02$), L_{90Z} showed a negative association that approached significance ($\beta = –4.64$, SE = 1.94, $t = –2.40$, $P = 0.05$) and visitor numbers were negatively associated with FGCM ($\beta = –0.05$, SE = 0.02, $t = –3.18$, $P = 0.02$) [Table 4].

Visitor numbers tended to be higher during the high season, although this difference was not statistically significant (mean ± SE: high season: 1049 ± 222.49; low season: 568 ± 79.69; $t = 2.035$, df = 5.01, $P = 0.097$) [Figure 2].

DISCUSSION

Our findings revealed that acoustic conditions, rather than visitor numbers, were more strongly associated with okapi stress physiological responses, highlighting noise as the primary driver of hypothalamic–pituitary–adrenal axis (HPA-axis) activation in this species. Unexpectedly, acoustic analysis of the okapi enclosure revealed higher sound levels during the low-visitor season and during closed hours. This was primarily due to the ventilation/heating system, which operated at approximately 50 dB(A) during the UK's colder winter months, necessary for the okapi's welfare, given its native tropical habitat.[24] Internal spectral analyses confirmed that low-frequency contributions, particularly from the ventilation/heating system, dominated background noise during closed hours, supporting our interpretation that mechanical sources were the main contributors to enclosure sound levels.[25] In contrast, during the high-visitor season, despite anticipated visitor-generated noise, the ventilation system generated lower baseline sound levels (approximately 30 dB L_{eq}) and ran for shorter durations. These findings suggest that reduced visitor numbers or non-visiting hours do not guarantee a quieter environment, emphasising the persistent impact of mechanical infrastructure on zoo soundscapes, a factor also highlighted by Quadros *et al.*[3]

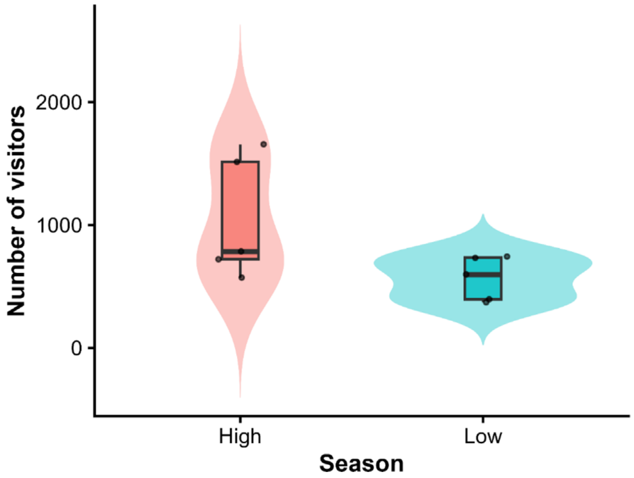

Figure 2: Daily visitor numbers at the okapi (*Okapia johnstoni*) enclosure in a UK zoo during periods of high and low visitation. ***Note:*** Violin and boxplots illustrate the distribution, median and interquartile range of visitor counts for each season.

Although FGCM levels showed no significant seasonal differences or direct correlation with visitor numbers, they were influenced by acoustic conditions. Broadband Z-weighted noise during open hours was positively associated with FGCMs, indicating a stress response to increased environmental stimulation, whereas percentile-based noise metrics (L_{10Z} and L_{90Z}) showed negative associations. This divergence suggests that distinct acoustic properties, such as transient peaks versus persistent low-frequency background, may differentially modulate adrenocortical activity. Such modulation could reflect HPA-axis habituation or altered sensitivity rather than straightforward seasonal or visitor effects.[6,26-28] Complementary behavioural observations from the same okapi indicate that sound levels influenced activity, feeding, alertness and time spent outside,[25] supporting the notion that acoustic conditions are biologically relevant. For instance, during the high season, higher sound pressure levels decreased active feeding and alert behaviours but increased time spent outside, while visitor presence had weaker and opposite effects.[25] During the low season, increased sound levels were associated with more active and alert behaviours and reduced feeding.[25] These results suggest that the observed FGCM responses may be linked to behavioural adjustments to noise, rather than representing purely speculative HPA dysregulation.

Our results indicate that visitor presence and the acoustic environment are biologically relevant correlates of adrenocortical activity in okapis. For the 72a metric, FGCM levels increased with higher L_{90Z} values and with greater visitor numbers, suggesting that both elevated broadband noise and direct human presence contribute to heightened adrenocortical activation. In contrast, the 72T metric exhibited a more complex pattern: L_{10Z} was positively associated with FGCM levels, whereas L_{90Z} and visitor numbers were negatively associated. This divergence between assays suggests that distinct components of the acoustic landscape, namely background intensity, high-percentile noise and anthropogenic activity, may influence glucocorticoid metabolite profiles through different physiological pathways or temporal dynamics. Although some associations approached statistical significance, the overall patterns were consistent and biologically interpretable, despite the limited sample size.

The observed negative relationship between FGCM levels and percentile-based noise metrics (L_{90Z} for background noise and L_{10Z} for peak noise) is counterintuitive. One possible explanation is modulation of the HPA axis or habituation to frequent, loud, yet transient noises and persistent background noise, which could lead to a blunted acute stress response or lower baseline FGCM levels. Alternative explanations include hypocortisolism resulting from chronic stress, acclimation or facilitation.[29,30] Similar patterns have been noted in poultry,[31] horses[32] and bovines,[33] underscoring the need for comprehensive studies with larger sample sizes and controlled environmental factors to fully understand HPA axis dysregulation in okapis. Comparable HPA-axis modulation, often reflecting adaptive physiological adjustments or dysregulation of glucocorticoid responses to prolonged or repetitive stressors, has been observed across taxa. For instance, studies in non-human primates, such as rhesus macaques (*Macaca mulatta*), demonstrate how social capital can buffer physiological stress and influence glucocorticoid levels.[34] In marmosets (*Callithrix jacchus*), social dynamics, particularly dominance, influence diurnal cortisol patterns.[35] In contrast, Sulawesi crested macaques (*Macaca nigra*) exhibit adaptive acute glucocorticoid increases in response to tourist presence, which subsequently return to baseline, indicating the absence of chronic stress.[36] Similarly, among ungulates like red deer (*Cervus elaphus*), seasonal glucocorticoid peaks are noted as an adaptation to environmental challenges,[37] with broader reviews highlighting the complex and varied nature of glucocorticoid responses to both acute and chronic stressors in Cervidae and Bovidae, not always directly correlating with distress.[38]

While the 72T assay showed that FGCM levels negatively correlated with visitor numbers, and the 72a assay levels exhibited significant positive associations with higher visitor numbers (particularly during closed periods), the direction and magnitude of these effects differed between the two assays. These opposite trends likely reflect differences in antibody sensitivity and cross-reactivity between the 72T and 72a assays. The 72T assay is more specific to tetrahydrocortisol/cortisone metabolites, whereas the 72a assay has a broader affinity to androstanes, metabolites that have lost the side chain of the cortisol molecule. While in general levels of different EIAs for measuring FGCMs are correlated [11], measures of both EIAs may show differences in capturing acute or chronic activation of adrenocortical activity in individuals.[20] Thus, apparent differences between assays likely reflect analytical specificity rather than contradictory biological responses. This suggests that the impact of human presence is more complex than a simple count, being potentially more closely linked to specific visitor-generated acoustic characteristics and behaviours than to total visitor numbers.[2,3] Future research should investigate the behavioural and acoustic components of visitor effects to better elucidate their physiological impact on zoo animals.Although FGCM analysis is non-invasive, it reflects cumulative responses from roughly 20 hours earlier and cannot capture short-term environmental changes.[9,30] Nevertheless, the observed link between FGCM levels and louder periods has practical implications for zoo management. Environmental enrichment, as shown in leopard cats (*Felis bengalensis*), can reduce FGCM by promoting a broader range of behaviours.[39] For okapis, enhancing enclosure complexity and incorporating dense, layered vegetation (such as their native rainforest in the Democratic Republic of Congo) could attenuate noise, particularly high-frequency sounds, creating a quieter, more naturalistic environment and potentially lowering stress levels.[1,3,5,6]

Our findings highlight the importance of proactively managing acoustic conditions in zoo enclosures to safeguard animal welfare. To reduce chronic stress caused by environmental noise, zoos could consider upgrading their ventilation and heating systems to quieter alternatives and incorporating sound-insulating structures within indoor enclosures. Vegetative barriers or dense planting can help attenuate both visitor-generated and mechanical noise. At the same time, enclosure design should allow animals to access outdoor areas freely, enabling them to avoid stressful soundscapes when desired. Continuous acoustic monitoring, paired with behavioural and hormonal assessments, can provide early detection of potential stressors and guide management decisions. Operational strategies may include limiting the number of visitors per session, installing educational signage and promoting visitor awareness to prevent loud or disruptive behaviours. Together, these measures can create a more controlled acoustic environment, support natural behavioural expression and reduce HPA-axis activation in sensitive species such as okapis.

This study is based on a single male okapi, which limits the generalisability of the results to other individuals, sexes or populations. We did not have an accurate baseline without visitors, making it impossible to fully disentangle the effects of visitors from other environmental factors. Acoustic and FGCM measurements were collected over consecutive days, which introduces pseudoreplication and reduces independence among observations. Seasonal differences in enclosure management, weather and mechanical noise may confound the results. Additionally, visitor numbers were recorded at only five discrete time points per day, which may not capture peak crowding or dwell time near the enclosure. The animal had *ad libitum* access to an outdoor area, which was not monitored in this study; sound exposure and visitor interactions in this space may differ from those measured indoors. Consequently, our findings should be interpreted cautiously, avoiding causal claims. Future research should include multiple individuals of both sexes, extended long-term monitoring of acoustic and physiological variables and comparisons across different zoos and enclosures to better understand the generality of noise effects on okapi welfare. Nevertheless, the study offers valuable insights for zoo management regarding the potential impact of acoustic conditions on okapi welfare.

CONCLUSION

Noise was identified as the primary stressor for this male okapi, with elevated broadband (Z-weighted) sound levels being more strongly associated with adrenocortical activity than visitor numbers. Zoo management should consider noise mitigation strategies, such as environmental enrichment, denser vegetation and quieter enclosure infrastructure, to enhance the welfare of okapis. Future research should involve multiple individuals, controlled conditions and cross-species comparisons to validate and generalise these findings.

Availability of Data and Materials

All data are available at: Queiroz, Marina; Azevedo, Cristiano; Schork, Ivana; Figueiredo Passos, Luiza; Davies, Willian; Young, Robert (2025), “Effect of noise and zoo visitors on zoo mammals’ behaviour and enclosures’ soundscapes”, Mendeley Data, V1, doi: 10.17632/2nf4xd8ync.1.

Author Contributions

Marina Bonde de Queiroz: conceptualization, methodology, data collection, visualization, data analysis, writing – original draft, reviewing and editing.

Cristiano Schetini de Azevedo: writing – original draft, reviewing, and editing.

Ivana Gabriela Schork: writing – original draft, reviewing, and editing.

Luiza Figueiredo Passos: data analysis, writing – original draft, reviewing, and editing.

Rupert Palme: data analysis, writing – original draft, reviewing, and editing.

William J. Davies: conceptualization, methodology, supervision, writing – original draft, reviewing, and editing.

Robert Joh Young: supervision, conceptualization, methodology, supervision, data collection, visualization, data analysis, writing – original draft, reviewing, and editing.

Ethics Approval and Consent to Participate

All the research reported here was approved by the Zoo and by the University of Salford Science and Technology Ethics Panel (ethics application ST1617-46). Consent to Participate is not applicable since no interviews or citizens were participating.

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Conflicts of Interest

The authors declare no conflict of interest.

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