EFFECTS OF A RECURRING LATE-NIGHT EVENT ON THE BEHAVIOR AND WELFARE OF A POPULATION OF ZOO-HOUSED GORILLAS

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ABSTRACT

The impact of visitors on the wellbeing of captive animals presents both positive and potentially negative consequences. While some amount of novelty through visitor stimulation offers the opportunity for a more complex captive environment, anecdotal evidence from primate staff observations at the Smithsonian's National Zoo and Conservation Biology Institute suggested that gorillas exhibited increased restlessness during the annual month-long late night ZooLights event than prior to it. The current study compared activity budgets, aggression (interactions involving contact between conspecifics and displays toward visitors), and abnormal behaviors in two groups of socially-housed gorillas for 1-month periods before, during, and after the 2015 ZooLights event. We also compared the fecal glucocorticoid metabolite profiles of all six gorillas across these same observation periods. Physiologically, most individuals appeared to cope appropriately with the increased visitor presence during the event. We saw little difference in contact aggression; however, abnormal behavior was observed in some gorillas during and following the event, which highlights the importance of individual analysis and data interpretation. As predicted, we found that the majority of gorillas rested less during ZooLights than during other observation periods, particularly adult females in the mixed sex troop. Preliminary results of this study aided the decision of zoo management to close initially the Great Ape House and subsequently most animal buildings during future ZooLights events to avoid the potential disruption of normal activity patterns. While the findings of this study were mixed as to the impact of the event on these gorillas, the results suggest that zoos should carefully consider the possible ramifications of keeping great ape buildings open for lengthy multi-night events.

1 **INTRODUCTION**

2 A central goal of the exhibition and management of great apes in zoos is to provide engaging visitation

3 experiences that enhance conservation awareness through proximity to representative, naturally behaving,

4 animals. Concomitant to providing close access by visitors is whether and to what degree human presence

5 itself impacts the welfare of socially grouped zoo-housed great apes. For the purposes of this study we

6 accept the AZA's definition: "Animal welfare refers to an animal's collective physical, mental, and

7 emotional states over time, and is measured on a continuum of good to poor." (Association of Zoos &

8 Aquariums, 2019).

9   Research suggests both positive and negative consequences from the presence of visitors on the daily

10 lives of zoo animals. Morris (1964) suggests that human visitation can represent a desirable component of

11 environmental variability. Other work suggests potentially negative consequences. Based on observations

12 of 15 primate species, Chamove (1988) found that increased aggressive and less affiliative behavior was

13 associated with larger visitor numbers. In another study of three groups of golden-bellied mangabeys

14 (*Cercocebus galeritus chrysogaster*), the exhibited animals showed increased aggression both within group

15 and towards zoo guests when exposed to greater numbers of visitors (Mitchell et al., 1991). Hosey (2005)

16 reviewed research concerning visitation effects on captive primates, concluding that large active groups of

17 visitors will generally be stressful to them.

18   The consequences of stress associated with zoo visitation on gorillas may be expressed through various

19 undesirable behaviors, including restlessness, increased conspecific-directed aggression, stereotypies, and

20 auto-grooming (Wells, 2005). Regurgitation and re-ingestion (R/R) is also a commonly observed

21 phenomenon in captive gorillas (Gould & Bres, 1986). Kuhar (2008) examined the impact of fluctuating

22 holiday visitor numbers on the behavior of 10 zoo-housed western lowland gorillas, finding few differences

23 between high and low crowd conditions. Stoinski et al., 2011 identified differences in the behavior of

24 individual gorillas, but were unable to conclude that larger crowds posed a significant welfare impediment

25 overall, although their study subjects remained out of sight to visitors more often during large crowd

26 conditions.

27   Our goal was to determine the potential impact, if any, of extending the evening visitation period well

28   beyond regular hours during a month-long event on a set of physiological and behavioral indicators of stress

29   in a group of zoo-housed gorillas. Our study examined the behavioral effects of ZooLights (ZL), a late-

30   night event at the Smithsonian's National Zoo (NZP), Washington, DC, held annually between

31   Thanksgiving and New Year's Day. ZL visitors have evening access to NZP grounds, illuminated with

32   holiday-themed lighting, and at the time of study to a number of animal buildings kept open past their

33   seasonal closing schedule. During the 2015-2016 ZL event, the Great Ape House (GAH) was open to

34   visitors until 2100 hr, 5 hr past routine winter closing time.

35   Events such as ZL present a valuable opportunity to promote conservation and animal care awareness

36   with the general public. It is important, however, to consider what, if any, impact increased visitation might

37   have on zoo animals. We conducted behavioral observations on our gorillas daily for 1 month before (pre-

38   ZL), during, and 1 month after (post-ZL) the event. Behavioral data, feeding, and abnormal, undesirable

39   behaviors such as stereotypies and R/R, were taken during daytime and evening periods by trained

40   volunteers. Additionally, adrenocortical activity (stress response) in each gorilla was examined through

41   analysis of glucocorticoid metabolites from fecal samples collected daily throughout the study.

42   Based on observations during previous ZL events, we hypothesized that the gorillas would rest

43   proportionately less in the evenings during ZL than the pre-ZL period, particularly during periods of high

44   visitation. Anticipating that lower levels of rest after normal zoo hours might result in the need for additional

45   sleep during the day, we further predicted that the gorillas would rest more during daytime ZL hours than

46   either pre- or post-ZL. We also predicted greater frequencies of abnormal behavior, contact aggression, and

47   higher visitor-directed behavior during normal zoo hours during the ZL event compared to the other two

48   periods. Finally, considering the possibility of a "carryover" effect due to reduction in rest during ZL, we

49   expected greater frequencies of abnormal behavior post- compared to pre-ZL.

50

51   **MATERIALS/METHODS**

52   **Subjects**

53   Study subjects were six western lowland gorillas, housed in two social groups with visual, auditory,

54   and olfactory access to each other at the NZP: (1) a mixed sex troop of one adult male (Baraka: 23 yrs old

55   at time of study), two adult females (Mandara: 33, Calaya: 13), and a juvenile female offspring of Mandara

56   (Kibibi: 6); and (2) a bachelor group of two adult males (Kwame: 16, Kojo: 14). Calaya had no previous

57   exposure to a ZL event, but Baraka, Mandara, Kwame and Kojo all experienced 8 and Kibibi 5 prior years

58   of NZP's ZL.

59

60   **Gorilla Exhibit**

61   The gorilla exhibit included six indoor enclosures, each with climbing structures, platforms, fabric

62   hammocks, and a 0.1ha grassy outdoor yard with climbing structures, dry moat, and vegetation. Viewing

63   areas included public space within the GAH, which also housed six orangutans, and a walking path around

64   the outside yard. The indoor public viewing area was separated from four gorilla enclosures by multi-paned

65   glass. Two remaining enclosures were not viewable from the public area, affording some privacy from

66   visitors. We chose not to include orangutans in this study because we judged based on pre-study anecdotal

67   observations that their nighttime routines were substantially different from those of gorillas, building more

68   elaborate nests that often include a full-body covering and typically positioning them farther away from

69   exhibit glass, often off exhibit. We were further limited in project scope by the availability of volunteer

70   observers and their ability to cover multiple areas in the GAH.

71

72   **Behavioral Data Collection**

73   Data collection spanned three, approximately 1-month periods: (1) Pre-ZL (October 27, 2015 –

74   November 27, 2015); (2) ZL (November 28, 2015 – January 2, 2016); and (3) Post-ZL (January 3, 2016 –

75   January 31, 2016). 23 behavior watchers (trained by M.L. Bastian) conducted between one and three 2-hr

76   data collection sessions per day, balanced over mornings (0700-0900, 0900-1100), afternoons (1100-1300,

77   1300-1500), and evenings (1700-1900, 1900-2100). A total of 134 data collection sessions were recorded:

78   43 pre-ZL, 56 ZL, and 35 post-ZL. For purposes of analysis, collection sessions were collapsed and

79 categorized as either "Day" (0700 - 1700) or "Evening" (1700 - 2100) to distinguish between time periods

80 outside the ZL event when the animals were most typically subject to visitation (Day) and not (Evening).

81 During each 2-hr session, observers recorded behavioral data using one-zero and 2 min instantaneous

82 sampling (Altmann, 1974) in 30 min blocks, alternating between the mixed-sex and bachelor groups. Data

83 collection was balanced across all six gorillas to ensure equal coverage. Gorilla management was identical

84 across pre-ZL, ZL, and post-ZL periods, although differed between day and during the evening ZL event.

85 Specifically, the gorilla groups time-shared the outdoor yard as temperature and weather restrictions

86 allowed during normal zoo hours but remained inside overnight.

87   Average daily indoor animal area temperature was recorded during all three observation periods,

88 but we did not measure additional environmental differences or relative noise levels (except *ad libitum*, as

89 noted below), as have some other studies (e.g. Morgan & Tromborg, 2007; Kuhar, 2008; Stoinski et al.,

90 2011; Quadros et al., 2014). Instead, we considered the total impact of the late-night event as a singular,

91 albeit extended, "disruption" to the gorillas' normal after-hours experience. A Kruskal-Wallis test indicated

92 that temperature was closely associated with observation period, with post-hoc tests indicating that daily

93 temperatures post-ZL were significantly lower than during either the pre-ZL ($p = 0.0238$) or ZL ($p = 0.0010$)

94 periods. A Spearman Rank correlation revealed that the relationship between daily temperature means and

95 proportion of scans gorillas spent resting post-ZL was not significant ($p = 0.3231$), so temperature was not

96 included as a variable in subsequent analyses.

97   Activity budgets included social, movement, feeding, and rest state behaviors (mutually exclusive

98 in that order of priority) and, in the case of the juvenile female, whether those activities occurred while in

99 maternal contact (see Table 1). Social behavior was categorized as either "affiliative" or "aggressive"

100 (contact or non-contact aggression). "Feeding" included foraging and drinking water. Behavior watchers

101 recorded all observed instances of "self-directed" behavior (while not observed during this study, any

102 abnormal or self-injurious behaviors would have been considered as "abnormal" behavior), "abnormal"

103 behavior (R/R, consuming feces, drinking urine, and stereotypies), "human-directed" behavior (including

104 touch/bang a barrier where there was a human presence, distinguishing between staff and visitors), and

105 "aggressive-contact" (agonistic contact with a conspecific). Unusual environmental conditions (e.g. high

106 noise levels) were recorded *ad libitum*. One-zero scoring was used to code the proportion of 2 min time

107 samples that included at least one occurrence of abnormal, human-directed behavior towards visitors, and/or

108 contact aggression with a conspecific. We also consulted keeper reports when we believed they might

109 provide insight. Crowd size (≤5, 6-15, 16-30 and >30) in the GAH was recorded at the start of each 30 min

110 observation session and as categories changed within each session. Specifically, as crowd size changed,

111 observers indicated the new category in the two-minute observation interval during which it changed. Zoo-

112 wide ZL visitor numbers were determined from official entrance records.

113

114 Insert Table 1

115

116 **Observer Reliability**

117 As the primary aim of this study was to assess the degree to which the ZL event affected the overall

118 proportion of scans each gorilla spent resting, observer reliability was considered most critical for all

119 mutually exclusive primary and secondary behaviors and positional behaviors when gorillas were

120 resting/inactive, as defined in Table 1.

121 Because each behavior watcher required different amounts of focused training on the variety of

122 behaviors before being approved to collect data, each was assessed separately against a set of master pre-

123 scored video keys rather than against one another.

124 Cohen's kappa, a robust statistic that controls for chance agreement between raters (Cohen, 1960;

125 McHugh, 2012), was calculated for each observer and against a pre-scored video key to ensure an

126 acceptable level of reliability across all mutually exclusive primary and secondary behaviors, and across

127 all-occurrence behaviors (Table 1). Reliability for aggressive contact behavior was assessed along with

128 other secondary behaviors. To participate, observers achieved a Cohen's kappa score of no lower than 0.70.

129 Observers with scores lower than 0.70 for any category of data were given additional training and re-tested

130 using a different set of pre-recorded video clips prior to beginning data collection.

131

132 **Fecal Sample Collection**

133   Fresh fecal samples were collected daily from all subjects during the period October 27, 2015 -

134 January 31, 2016. Sample collection occurred during morning enclosure maintenance, approximately 0730-

135 1030 hr. To facilitate fecal sample identification by animal, subjects in each of the two groups were orally

136 administered, via applesauce or similar food item, either 7ml green dye, 1.5 tbsp millet, a combination of

137 these, or none (see Table 2). Following collection, samples were bagged and immediately frozen at -20°C

138 before being transported weekly to the Smithsonian Conservation Biology Institute for glucocorticoid

139 metabolite analysis (Brown et al. 1994).

140

141 Insert Table 2

142 *Fecal Extraction*

143   Fecal samples were lyophilized, pulverized into fine powder, and sifted to remove non-fecal matter.

144 Fecal glucocorticoid metabolites (fGCM) were then extracted from fecal material using a shaking-

145 extraction method similar to that described by Bernstein et al. (2009). In brief, 0.2g (±0.01g) of dried fecal

146 material was added to 5ml of 90% ethanol, vortexed, and placed on a multi-tube mixer for 30 min. Samples

147 were centrifuged at 1200g for 20 min before the supernatant was decanted into clean tubes. The remaining

148 fecal pellet was resuspended in 5ml of 90% ethanol, re-vortexed for 30 sec and re-centrifuged at 1200g for

149 a further 15 min. Supernatants were combined, evaporated to dryness under air, and resuspended in 1ml

150 phosphate buffer (0.2M $NaH_2\text{-}PO_4$, 0.2M Na_2HPO_4, 0.15M NaCl; pH 7.0). Steroid extraction efficiency

151 averaged 72% as determined by the recovery of ^3H corticosterone added to feces before extraction. Fecal

152 extracts were stored at -20°C until analysis.

153

154 **Glucocorticoid Metabolite Analysis**

155   fGCMs were measured using an ImmuChem™ Double Antibody Corticosterone ^{125}I

156 radioimmunoassay kit (MP Biomedicals, Orangeburg, NY), with some modifications. In brief, 50µL of

157   each calibrator (12.5-1000ng/ml), control (high and low) and sample (diluted 1:10 in phosphate buffer

158   [0.039M NaH_2-PO_4, 0.061M Na_2HPO_4, 0.15M NaCl; pH 7.0]) were added to glass tubes in duplicate,

159   followed by 100µL of ^{125}I-labelled corticosterone and 100µL of anti-corticosterone antibody solution.

160   Tubes were mixed briefly and incubated for 2 hr at room temperature. Following incubation, 250µL of

161   precipitant solution was added, mixed and centrifuged for 15 min at 1800g. Tubes were decanted before

162   being counted in a gamma counter (Iso data 20/20 series).

163   Cross reactivities for the corticosterone RIA are as follows: corticosterone 100.00%,

164   desoxycorticosterone 0.34%, testosterone 0.10%, cortisol 0.05%, aldosterone 0.03%, progesterone 0.02%

165   androstenedione 0.01% and 5α-dihydrotestosterone 0.01%. This radioimmunoassay was biochemically

166   validated for measuring glucocorticoids in lowland gorilla fecal extract through parallelism and matrix

167   interference assessment. Serial dilutions of fecal extract yielded a displacement curve parallel to the

168   standard curve (males: y = 0.902x + 0.607, R^2 = 0.985, $F_{1,5}$ = 332.049, P < 0.001; females: y = 0.728x +

169   10.717, R^2 = 0.966, $F_{1,5}$ = 141.424, P < 0.001). There was no evidence of matrix interference, as addition

170   of diluted fecal extract to assay standards did not alter the amount observed (males: y = 1.180x − 15.266,

171   R^2 = 0.996, $F_{1,5}$ = 1164.902, P < 0.001; females: y = 0.933x + 10.185, R^2 = 0.997, $F_{1,5}$ = 1452.028, P <

172   0.001). For each sample, calibrator and control run in duplicate, coefficient of variation (CVs) were all

173   below 10%.

174

175   **High Performance Liquid Chromatography**

176   The suitability of the corticosterone RIA for measuring glucocorticoid metabolites in lowland gorilla

177   feces was assessed by reverse-phase high-performance liquid chromatography (HPLC). Male and female

178   fecal extracts were prepared as described (Edwards et al. 2013), with some modifications. High fGCM

179   concentration samples from the study period (10 from males, 10 from females) were extracted using 90%

180   ethanol as described above, pooled by sex, reconstituted in 1ml 20% methanol, and loaded onto a pre-

181   conditioned Hypersep™ C8 cartridge (Thermo Fisher Scientific, Waltham, MA). Cartridges were washed

182   with 5ml distilled water, before steroids were eluted with 5ml 100% methanol. These filtered fecal extracts

183   were each spiked with ^{3}H tracers (cortisol, corticosterone, testosterone and 5α-dihydrotestosterone, Perkin

184   Elmer, Waltham, MA), evaporated to dryness, and reconstituted in 300μl 100% methanol. A 50μl aliquot

185   of each pool was separated using Microsorb-MV 100-5 C18 250 X 4.6mm columns (Agilent, Santa Clara,

186   CA), and a combination stepwise and isocratic gradient of acetonitrile (A) in water over 90 min (min 0-10

187   20%A, min 11-16 25%A, min 17-22 30%A, min 23-28 35%A, min 29-34 40%A, min 35-90 45%A;

188   1ml/min flow rate, fractions collected every 20 sec). A separate tracer run with the addition of an unlabeled

189   desoxycorticosterone standard (Steraloids, Newport, RI) was also fractionated as described above to

190   determine the relative elution position of this standard that was unavailable as a radiolabeled version.

191   Radioactivity in 100μl aliquots of each fraction was determined using a scintillation counter (LS6500,

192   Beckman Coulter). Remaining fractions were evaporated to dryness, reconstituted in 300μl phosphate

193   buffer [0.039M NaH_2-PO_4, 0.061M Na_2HPO_4, 0.15M NaCl; pH 7.0] and an aliquot (50μl) analyzed for

194   immunoreactivity on the corticosterone RIA.

195

196   Insert Figure 1

197

198   **Statistical Analysis**

199   Behavioral data were converted into the proportion of total observation scans performed during a

200   given day or evening where gorillas engaged in each behavior. For purposes of analysis, total observation

201   scans excluded periods gorillas were out of sight of the observer, or when the primary activity was recorded

202   as "unknown". Thirty-minute data sessions were excluded from analysis if they included less than 15 min

203   of useable data, allowing observers to switch focals after 15min if their focal was out of sight and ensuring

204   that only data sessions in which the observer was aware of at least half of the actual activity of the focal

205   were analyzed.

206   Data were analyzed using generalized linear mixed models (GLMM) in MLwiN version 2.02

207 (Rashbash et al., 2005). Random effects were incorporated to control for repeated behavioral observations

208 and fecal samples collected from individual gorillas over the study period. As we were interested in

209 investigating individual-level differences in response to the ZL event, separate models were created for

210 each gorilla, and are reported in addition to the overall (individuals combined) models. Observation day

211 was included as a random effect in all individual models, and both individual and observation day in the

212 overall models. Binomial models were used to compare each behavior variable (proportion of scans spent

213 resting, exhibiting abnormal behavior, contact aggression and visitor-directed behavior) across time-periods

214 (pre-ZL, ZL and post-ZL), with pre-ZL as the reference category throughout. To analyze fGCM

215 concentrations between time-periods, data were $\log_{10}$ transformed to normalize the distribution ($\log_{10}$

216 fGCM), and a normal error structure was used. The proportion of scans spent resting during the evening

217 observation session was also compared relative to the estimated GAH crowd size and zoo-wide visitor

218 numbers. Binomial models were used to compare the proportion of scans spent resting in the evenings

219 between the pre-ZL period (when crowd size was always 0), and ZL period (for which modal crowd size

220 was collapsed into categories ≤15 and >15 for purposes of analysis), and with zoo-wide crowd size as a

221 continuous fixed effect. For all models, the significance of each fixed effect was determined using the Wald

222 statistic and chi-squared (χ^2) distribution, with alpha set to 0.05. Data are presented as the mean prediction

223 ± standard error (SE) to control for non-independence of data.

224

225 **RESULTS**

226 **High Performance Liquid Chromatography**

227   Metabolites measured by the corticosterone RIA in male and female pooled fecal extracts displayed

228 polarity similar to that of native corticosterone and that of another unidentified metabolite. In the male

229 extract, peak immunoreactivity occurred at fraction 96, just after the ^{3}H corticosterone tracer eluted at

230 fraction 94 (Fig. 1a), with a second smaller immunoreactive peak at fraction 110. In the female extract,

231 immunoreactivity occurred at fraction 92, just before elution of the ^{3}H corticosterone tracer at fraction 93

232 (Fig. 1b), with a second immunoreactive peak at fraction 110.

233

234 **Evening Behavior Analysis**

235   Table 3 reports results of behavioral analyses performed on data collected after normal zoo hours

236 during the three observation periods, pre-ZL, ZL and post-ZL. As predicted, gorillas spent significantly less

237 time resting during ZL than pre-ZL, with a tendency to rest less during ZL than post-ZL (Fig. 2).

238   Both adult females, Calaya and Mandara, rested less during ZL than either pre- or post-ZL (Table

239 3a), irrespective of their different historical exposure to late night events. The silverback, Baraka, also rested

240 significantly less during ZL than post-ZL, with a tendency to rest less during ZL than pre-ZL. By contrast,

241 no significant differences were detected across observation periods in proportion of scans spent resting for

242 the juvenile female, Kibibi. For the teenage males, neither Kwame nor Kojo had sufficient data to conduct

243 an analysis of time spent resting.

244   Overall, increased evening crowd size, both in regard to zoo-wide crowd estimates (GLMM

245 coefficient = -0.004, SE = 0.001, χ^2 = 39.553, df = 1, P < 0.001) and modal GAH (Table 3b), was associated

246 with decreased resting during ZL as predicted. Contrary to prediction, the gorillas rested more during ZL

247 when GAH crowd sizes exceeded 15 compared to crowd sizes of ≤15. As expected, they rested less with

248 both ZL crowd size categories than with no crowds pre-ZL (Fig. 3).

249

250 Insert Figure 2

251

252 Insert Table 3

253

254 Insert Figure 3

255

256 **Daytime Behavior Analysis**

257   Tables 4A-D show results of analyses performed on data collected during normal zoo hours during

258   the three observation periods, pre-ZL, ZL and post-ZL. Overall, gorillas spent a significantly lower

259   proportion of scans resting in the daytime during pre-ZL than either ZL or post-ZL, a result that, according

260   to individual analyses, appears to be driven by Mandara and Kojo, subordinate members of their respective

261   groups. Baraka spent more time resting daytime during post-ZL than ZL (Table 4A). No significant

262   differences across pre-ZL, ZL and post-ZL periods were detected in daytime crowd sizes during normal

263   zoo hours (H = 4.663, P = 0.0971).

264   Overall, gorillas engaged in abnormal behaviors in the daytime more often during ZL and post-ZL

265   than pre-ZL, with the highest frequency of abnormal behaviors observed post-ZL (Table 4B). Individual

266   analyses revealed that abnormal behaviors (primarily R/R) were significantly less common during pre-ZL

267   than either ZL or post-ZL for both adult females, Calaya and Mandara, with Mandara exhibiting more

268   abnormal behavior during ZL.

269   Silverback male Baraka and young female Kibibi engaged in significantly more abnormal

270   behaviors (primarily R/R for Baraka, ear cupping for Kibibi) post-ZL than ZL (Table 4B). The proportion

271   of scans spent engaged in these behaviors was also significantly lower for Baraka and trended lower for

272   Kibibi during ZL than pre-ZL. Like Calaya and Mandara, the proportion of scans spent engaged in abnormal

273   behavior for Baraka post-ZL was greater than pre-ZL.

274   The proportion of scans spent in contact aggression among the gorillas overall was highest during

275   the ZL period, a result that appears to be driven by Kojo's data (Table 4C). Although no overall differences

276   were found in visitor-directed behavior across observation periods, individual analyses indicate significant

277   differences across observation periods for teenage gorillas Kwame, who exhibited more post-ZL than pre-

278   ZL, and Kojo, who exhibited an overall tendency for differences across observation periods, exhibiting

279   lower occurrences of visitor-directed behavior post-ZL than either the pre-ZL or ZL periods (Table 4D).

280

281   Insert Tables 4(A-D)

282

283 **Hormonal analysis**

284   Profiles of fGCM concentrations for each of the six gorillas across the study period are provided in Figure

285 4. There were no differences in fGCM concentrations across observation periods, either when combining

286 all individuals (χ^2 = 0.915, df=2, P=0.633), or applying individual models (P > 0.076). Although in some

287 individuals (e.g. Baraka, Fig 1a) the variability in fGCM appeared to be reduced during the ZL period

288 compared to both pre- and post-ZL, this was not significant when comparing either the standard deviation

289 (χ^2 = 0.884, df=2, P=0.643) or coefficient of variation (χ^2 = 0.808, df=2, P=0.668) across observation

290 periods.

291

292 Insert Figure 4

293

294 **DISCUSSION**

295   Historically, there has been concern for the potential ill-effects that human visitation, specifically large

296 crowd visitation, poses to the well-being of non-human primates in a zoo setting (Davis et al., 2005; Hosey,

297 2000; Mitchell, et al., 1992). In this study, we measured behavioral and physiological responses to a month-

298 long late-night event that introduced a large number of visitors to NZP-housed gorillas during evening hours

299 when the animals were unaccustomed to human presence. Behaviorally, we observed impacts on rest and

300 vigilance during the extended evening hours in most study subjects, except the single juvenile female.

301 Additionally, higher rates of abnormal behavior occurred during and after the event than before. We did

302 not observe any significant changes in fGCM concentrations across the three periods, indicating that visitor

303 presence and increased noise and lighting after normal zoo hours had a minimal effect on adrenal activity,

304 perhaps meaning that behavioral changes mitigated physiological responses.

305

306 **Evening**

307   As predicted, during the evenings, the mixed-sex group (excepting juvenile Kibibi) showed lower

308 periods of resting during ZL than pre-ZL. Given that the ZL period was marked by ~5 hrs of extended

309 lighting and the potentially disrupting presence of visitors, this was not surprising. Kibibi's relative

310 uniformity of time spent resting across observation periods suggests that unlike the increase in vigilance

311 behavior with the onset of the ZL event by the group's silverback and adult females, such state of alertness

312 may not be required by immature individuals within a social group. Due to difficulties observers had

313 distinguishing the bachelor pair during the pre-ZL period when their enclosures were dark and one of the

314 two typically slept off exhibit, there was insufficient data to analyze the proportion of scans spent resting

315 after normal zoo hours for them.

316   Comparing rest in the context of evening crowd sizes, using both zoo-wide and GAH crowd

317 calculations, as predicted, increased crowd size was associated with decreased rest in both circumstances.

318 Contrary to our prediction, the mixed-sex group rested more at the higher GAH-specific crowd level (>15

319 visitors) then when fewer visitors were present. While this is not easily explained by observer *ad libitum*

320 notes, we suspect that because fewer observations were made when GAH crowd sizes were <15, more

321 boisterous visitor behavior during those periods could have had a greater impact on the statistical outcome.

322 Further, observers were not asked to note the relative distribution of visitors within the GAH, so although

323 total visitor numbers were higher at times, their distribution across gorilla and orangutan enclosures may

324 have muted the effect of human presence in the immediate vicinity of the mixed-sex gorilla group.

325

326 **Daytime**

327   We predicted that during daytime hours, the gorillas would rest proportionately less pre-ZL than

328 either during or after the event, which the data supported. Individual analyses revealed this result was driven

329 by Mandara and Kojo, both subordinate members of their respective groups. Dominant silverback Baraka

330 rested less during the day in the ZL period than either pre- or post-ZL, a finding consistent with heightened

331 vigilance behavior throughout the late-night event. The individual findings indicate the importance of

332 focusing on each gorilla, as there can be significant variation in behavioral responses, especially across

333 age/sex classes. When we analyzed the potential impact of daytime crowd size on proportion of scans

334 gorillas spent resting, whether zoo-wide or the GAH, we found no significant relationships across

335 observation periods.

336   Whether a change in rest patterns should trigger a welfare concern is not clear. It may be a natural

337 coping strategy for zoo-housed gorillas to rest less during prolonged late-night events, or it may signal a

338 potential for concern given increased awareness for the benefits of sleep in human and non-human primates

339 (Nunn, et al., 2016; Fruth, et al., 2017). We were unable to assess whether cumulative rest across the 24-

340 hour period was affected by the ZL event due to limitations in our ability to collect data over night.

341 However, it is unlikely that the average 9% decrease in evening rest during ZL compared to the preceding

342 month was fully compensated for by a 3.3% increase in daytime inactivity, when rest may be interrupted

343 by other factors associated with daytime animal care and basic husbandry routines.

344   As predicted, the gorillas spent a greater proportion of scans exhibiting abnormal behaviors during

345 ZL than before the event. Calaya and Mandara showed significant increases in R/R activity during and post-

346 ZL than pre-ZL. These results suggest the potential for compounded effects of chronic events to disrupt

347 routine rest schedules of zoo-housed animals, particularly events that introduce large crowds and associated

348 lighting during otherwise off-hour timeframes.

349   Although the data indicates an increase in overall daytime conspecific aggression during ZL

350 compared to pre- or post-ZL, this derives primarily from a single individual, Kojo, who tended to be the

351 instigator of aggressive encounters with Kwame. The mixed-sex group, on the other hand, appears to have

352 maintained daytime intra-group relationships consistently across periods, perhaps due to the increase in

353 daytime resting. Our prediction of increased visitor-directed behavior by gorillas during ZL compared with

354 pre-ZL, was not supported by the data. However, post-hoc analysis did indicate that Kwame, who exhibited

355 more visitor-directed behavior post-ZL than pre-ZL, may have experienced escalated frustration over the

356 course of the study.

357   The fact that fGCM were not significantly altered during the ZL period suggests that although this

358 event was sufficient to affect the gorillas' behavior, it did not appear to affect adrenal activity. Previous

359 studies investigating the impact of zoo visitors on the stress response have reported varying results. In spider

360   monkeys, increased visitor numbers were associated with higher urinary glucocorticoids (Davis et al.,

361   2005), Mexican wolves had higher fGCM concentrations following higher visitor days (Pifarré et al., 2012),

362   and in koalas, increased visitor encounters led to variable adrenal responses between individuals (Webster

363   et al., 2017). Similar to that reported here, however, a 9-month study of lowland gorillas at the London Zoo

364   found no differences in fGCM concentrations in response to environmental variables including crowd size

365   and noise level (Clark et al., 2011), although that study reported an increase in vigilance and a decrease in

366   food-related behaviors with increasing visitor noise and numbers, respectively. The impact of visitors on

367   stress physiology of zoo-housed wildlife may depend on context or the availability of other coping

368   mechanisms. In giant pandas, brief periods of loud noise resulted in behavioral signs of distress

369   (locomotion, vocalization and scratching at the door to the off-exhibit area), but not in adrenal activation.

370   By contrast, longer-term noise was associated with higher urinary glucocorticoid concentrations (Owen et

371   al., 2004). The availability of alternative coping mechanisms, such as social support (Cheney and Seyfarth,

372   2009) and displacement behaviors (Troisi, 2002) could be a way by which individuals respond to potential

373   stressors, as opposed to an adrenal response.

374   Notwithstanding the potential benefit of the novelty of human visitation on the zoo-housed

375   population we studied (Morris, 1964), the increase in frequency of R/R is troublesome from a welfare

376   perspective. This finding, along with the observed pattern of increased restlessness after normal zoo hours

377   during ZL, supported the concerns leading us to conduct this study. While the physiological profiles of the

378   gorillas did not indicate a clear stress response, the increased prevalence of abnormal behavior and lowered

379   proportion of scans spent resting during ZL indicated a concerning pattern. Following the presentation of

380   preliminary data collected during this study, it was decided that the GAH would remain closed during future

381   ZL events and other after-hours events lasting longer than 1-2 consecutive evenings.

382

383   **CONCLUSIONS**

384   1. ZooLights, a month-long event that exposed NZP gorillas to increased periods of artificial light and

385   crowds, reduced evening rest during the event.

2. Overall, gorillas engaged in abnormal behaviors in the daytime more often during ZL and post-ZL than pre-ZL.

3. No significant relationship was found between the ZL event and the gorillas' adrenal function based on fGCM analyses.

4. Assessing both behavioral and physiological responses to potentially stressful events is critical in evaluating the welfare of zoo-housed gorillas, as evidenced here where the two measures yielded different information. Care also should be taken when planning events that disrupt normal routines of zoo-housed gorillas.

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TABLE LEGENDS

Table 1. Ethogram of Behaviors used during the Study. Table adapted from Kramer, 2016.
Table 2. Fecal markers used for each individual gorilla to distinguish feces.
Table 3. Summary of Models Investigating the Effects of A. Observation Period and B. GAH
Crowd Size on Evening Resting Behavior of Gorillas.
Table 4A: Summary of Model Investigating the Effects of Observation Period on Daytime
Resting of Gorillas.
Table 4B: Summary of Model Investigating the Effects of Observation Period on Daytime
Abnormal Behavior of Gorillas.
Table 4C: Summary of Model Investigating the Effects of Observation Period on Daytime
Contact Aggression of Gorillas.
Table 4D: Summary of Model Investigating the Effects of Observation Period on Daytime
Visitor-Directed Behavior of Gorillas.

FIGURE LEGENDS

Figure 1: Elution profile of immunoreactive glucocorticoid metabolites in male (top) and female (bottom)
lowland gorilla fecal extracts on the corticosterone RIA, separated by reverse-phase HPLC. Numbered
markers indicate the elution positions of synthetic standards 1) cortisol, 2) corticosterone, 3) testosterone,
4) desoxycorticosterone, and 5) 5α-dihydrotestosterone.

Figure 2: The proportion of scans spent resting during the a) daytime and b) evening in six zoo-housed
western lowland gorillas in the month before (Pre-), during (ZooLights) and after (Post-) a ZooLights event,
where the great ape house was open to the public for 5 hours beyond typical closing time for the season.
Bars represent the mean prediction from the GLMM, taking into account non-independence of data; error
bars represent 95% confidence intervals; letters denote significant differences between the proportion of
scans spent resting.

Figure 3: Proportion of scans spent resting with large (n=73 sessions) or small (n=28 sessions) crowd sizes
during the Zoolights event, compared to pre-ZL when crowd size was zero (n=58 sessions). Bars represent

the prediction from the GLMM, taking into account non-independence of data; error bars represent 95% confidence intervals; letters denote significant differences between the proportion of scans spent resting.

Figure 4: Fecal glucocorticoid metabolite profiles for six lowland gorillas (a: Baraka; b: Mandara; c: Calaya; d: Kibibi; e: Kwame; f: Kojo) prior to, during (grey shaded), and following the public ZooLights event during which time the Great Ape House was open to the public for 5 hours beyond typical closing time for the season.