



<https://helda.helsinki.fi>

Helda

Exploration of potential triggers for self-directed behaviours and regurgitation and reingestion in zoo-housed chimpanzees

Wallace, Emma K.

Elsevier B.V.

2019-12

Wallace, E K, Herrelko, E S, Koski, S, Vick, S-J, Buchanan-Smith, H M & Slocombe, K E 2019, 'Exploration of potential triggers for self-directed behaviours and regurgitation and reingestion in zoo-housed chimpanzees', Applied Animal Behaviour Science, vol. 221, 104878. <https://doi.org/10.1016/j.applanim.2019.104878>

<http://hdl.handle.net/10138/319586>

10.1016/j.applanim.2019.104878

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

1 **Exploration of potential triggers for self-directed behaviours and regurgitation and reingestion in zoo-**
2 **housed chimpanzees**

3 Emma K. Wallace ^a, Elizabeth S. Herrelko ^{bcd}, Sonja E. Koski ^e, Sarah-Jane Vick ^b, Hannah M. Buchanan-Smith ^b
4 and Katie E. Slocombe ^a

5 a) Department of Psychology, University of York, Heslington, York, YO10 5DD

6 b) Psychology, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA

7 c) Edinburgh Zoo, Royal Zoological Society of Scotland, Edinburgh EH12 6TS

8 d) Animal Care Sciences, Smithsonian's National Zoo, 3001 Connecticut Ave NW, Washington DC 20008

9 e) During Data Collection: Department of Biological Anthropology, University of Cambridge, Pembroke Street,
10 Cambridge, CB2 3QG. Current Affiliation: University of Helsinki, Department of Social Sciences, Unioninkatu
11 35, 00014

12 **Corresponding Author:**

13 **Katie Slocombe**

14 **Ks553@york.ac.uk**

15 **01904 322905**

16

17

18 **Exploration of potential triggers for self-directed behaviours and regurgitation and reingestion in zoo-**
19 **housed chimpanzees**

20 **Abstract**

21 The unique challenges faced by animals living in zoos can lead to the production of anxiety-related
22 behaviours. In this study we aimed to understand what specific factors may cause chimpanzees to display
23 these behaviours. In non-human primates, displacement behaviours, such as self-scratching and yawning,
24 are considered markers of anxiety and stress, and Regurgitation and Reingestion (R/R) is considered an
25 abnormal behaviour with negative consequences for physical health. We examined the possible triggers of
26 R/R, scratching, and yawning in a group of zoo-housed chimpanzees and followed this up with an analysis of
27 long-term data to examine further aspects of R/R behaviour. In the first study we conducted focal
28 observations on 18 adult chimpanzees at Edinburgh Zoo, UK, in addition to all occurrence sampling of visitors
29 using flash photography, screaming and banging on the glass in the exhibit. 158 hours of data were analysed
30 and Generalised Linear Mixed Models revealed that yawning was significantly more likely if there was a long
31 period of time since the last feed and when there were moderate numbers of visitors in the zoo. There were
32 trends that yawning was more likely to occur if children screamed and that scratching was more likely to
33 occur if visitors used flash photography. R/R occurred most often within 40 minutes of a feed, but was not
34 affected by the inter-feed interval preceding that feed, positive or negative social interactions, or visitor
35 numbers or behaviour. As there was no obvious daily trigger for R/R, an analysis of long-term data (2009 to
36 2015) was conducted to investigate if social or dietary factors affected rates R/R over a larger timescale. It
37 was found that R/R rates in the months before a significant diet change were not different from R/R rates in
38 the months after, but it was found that R/R rates decreased over the five-year period. Lastly, we found no
39 evidence that the introduction of individuals engaging in R/R lead to resident chimpanzees habitually
40 adopting the behaviour, despite considerable opportunities to observe it. These findings have implications
41 for welfare interventions aimed to reduce R/R and/or anxiety behaviours in captive populations and for the
42 translocation of individuals that are known to engage in R/R between groups.

43
44 **Keywords**

45 Regurgitation and Reingestion; Chimpanzees; Animal Welfare; Scratching; Yawning; Anxiety-related
46 behaviour

47

48 **1. Introduction**

49 Zoo environments present a unique set of challenges, with animals regularly exposed to high numbers of
50 unfamiliar visitors, restricted space, and unnatural social group compositions (Hosey, 2005). Animal welfare
51 is conceived as a balance of positive and negative welfare states, and our goal is to minimise negative and
52 maximise positive welfare (e.g. Mellor and Beusoleil, 2015). It is not only vital for animal welfare, but also
53 valid research findings and the education of visitors, that potential stressors in the zoo environment are
54 identified and, when deemed to be damaging to wellbeing, minimised. For any given species, it is thus
55 important to identify and monitor anxiety-related and abnormal behaviours that may indicate low welfare
56 states and the factors that may trigger their performance.

57 Within primates, two self-directed behaviours (SDBs), self-scratching and yawning are commonly interpreted
58 as indicators of anxiety (Troisi, 2002; Maestriperi et al. 1992). SDBs are suggested to be coping mechanisms,
59 as wild female olive baboons (*Papio hamadryas anubis*) that display SDBs have lower cortisol levels than
60 those who do not (Higham et al., 2009). The evidence linking self-scratching to anxiety comes from both
61 experimental and observational work: anxiolytic drugs induce scratching in long-tailed macaques (*Macaca*
62 *fascicularis*; Schino et al., 1991) and natural rates of scratching increase after aggression in Japanese
63 macaques (*Macaca fuscata*; Schino et al., 1998) and when captive chimpanzees (*Pan troglodytes*) perceive
64 the threat of intra-group aggression (Baker and Aureli, 1997). Yawning has also been related to anxiety and
65 viewed as an SDB in primates (Maestriperi et al., 1992), as rates of yawning increase in captive chimpanzees
66 during periods of social tension (Baker and Aureli, 1997) and in wild chimpanzees when in close proximity to
67 humans (Nishida, 1970).

68 Previous research has indicated that a range of factors can increase anxiety levels in zoo-housed primates,
69 which is manifested in elevated rates of self-scratching and yawning. When not given enrichment, high visitor
70 numbers were associated with high rates of scratching in two groups of captive gorillas (*Gorilla gorilla gorilla*)
71 (Carder and Semple, 2008). In an Indian zoo, where the lion-tailed macaques were often 'taunted' by visitors,
72 yawning rates were higher when animals were 'on-exhibit' compared to when they were 'off-exhibit'

73 (Mallapur et al., 2005).

74 It is not just high levels of SDBs that can occur in response to captive environments; abnormal behaviours
75 can arise, which are defined as a set of behaviours that are performed either solely in captivity or at a much
76 higher level than in the wild and are thought to be indicators of poor welfare (Birkett and Newton-Fisher,
77 2011; Mason, 1991; Bloomsmith et al., 2019). A recent study found that 64% of sampled chimpanzees
78 within the United States had been seen to engage in at least one type of abnormal behaviour in the past
79 two years (Jacobson, 2016), which shows that these behaviours are prevalent within captivity. One
80 abnormal behaviour that has been observed across a range of captive primates is regurgitation and
81 reingestion (R/R). It has been observed in chimpanzees (Baker and Easley, 1996), bonobos (*Pan paniscus*)
82 (Miller and Tobey, 2012), gorillas (Akers and Schildkraut, 1985; Hill, 2009), and lion-tailed macaques
83 (Mallapur et al., 2005). The behaviour is defined as the voluntary movement of food from the stomach or
84 the oesophagus into the hand, the mouth or on to a substrate followed by the consumption of the
85 regurgitant (Gould and Bres, 1986). It is similar to rumination, a human abnormal behaviour that can lead
86 to serious health issues, such as oesophageal strictures, ulcers, reflux, oesophagitis, intestinal obstruction,
87 oesophageal motor disorders and pulmonary aspiration (Wyngaarden et al., 1992; Hill, 2009). To date no
88 single trigger for R/R has been identified; rather multiple factors have been suggested. Life history and
89 demographic factors have been shown to influence the likelihood of individuals engaging in R/R. A recent
90 survey of chimpanzees living in research facilities in the United States, conducted by Bloomsmith et al.
91 (2019), found that adults over the age of 40 were more likely to engage in R/R than adults 12- 39 years old,
92 possibly because older adult animals may have lived through a time when the welfare levels within
93 research centres were not as high as today. In addition, non-mother reared/non-wild born individuals living
94 in pairs were more likely to engage in R/R than mother reared or wild born chimpanzees, possibly due to
95 the lack of mother rearing. It has been shown that being deprived of mother rearing can cause emotional
96 trauma and lead to the development of abnormal behaviours (Kalcher et al., 2008).

97 Indeed, rumination in humans has been linked to anxiety (Landis and Lambroza, 2001), which suggests that
98 this could also be a more immediate trigger for the behaviour in animals. Previous research has suggested
99 other immediate triggers for R/R may include boredom (Baker, 1997, 2004), diet (Morgan et al., 1993; Lukas

100 et al., 2014) and visitor presence and behaviour (Mallapur et al., 2005; Wells, 2005). Taken together, it seems
101 a range of factors may influence engagement in this behaviour, with some suggesting a link to current or
102 previous stress, but a lack of consistency across studies and populations highlights a need for further
103 research. A better understanding of the causes of R/R may enable effective interventions to be implemented,
104 which would be valuable as R/R is likely to be negatively perceived by zoo visitors (Ackers and Schildkraut,
105 1985) and could affect the educational potential of the exhibit by giving false impressions of the species
106 (Carlstead, 1998; Ironmonger et al., 1992; Ackers and Schildkraut, 1985).

107 In order to reduce anxiety-related and abnormal behaviours in zoo-living animals, it is first important to
108 understand the aspects of this captive environment that may increase stress or abnormal behaviour. Zoo
109 visitors are a potential source of anxiety, in terms of their numbers and behaviour. For example, mandrills
110 (*Mandrillus sphinx*) exhibit higher levels of leg/hair pulling, stereotyped locomotion and masturbation in the
111 presence of high visitor numbers (Chamove et al., 1988). In terms of visitor behaviour, in a multi-species
112 study at Sacramento zoo it was found that active groups of visitors (where at least one individual attempted
113 to attract an animal's attention), regardless of size, induced more locomotion and audience-directed
114 behaviours than passive groups, although the authors do not state if they believed that was a sign of a
115 positive or negative welfare change (Mitchell et al., 1992). Previous studies have found, however, that the
116 activity or noise level of visitors negatively affects the behaviour of captive primates (Chamove et al., 1988;
117 Birke, 2002). If the presence or behaviour of visitors in zoos has a negative effect on animal welfare then
118 more research is needed into exactly which behaviours and numbers of visitors causes these effects and how
119 they can be minimised.

120 The type and availability of food in captive environments are very different from that found in most species'
121 natural environments (Oftedal and Allen, 1996) and, therefore, feeding regimes could be a potential source
122 of stress and/or triggers for R/R. Fruit and starchy vegetables increased R/R rates in laboratory chimpanzees
123 and zoo-housed gorillas (Morgan et al., 1993; Lukas et al., 2014). In addition, increasing the amount of time
124 since eating has also been linked to higher R/R rates in chimpanzees (Baker and Easley, 1996). This may be
125 due to longer periods between feeds violating anticipated feeding times, as delays to expected feeds
126 increases abnormal behaviours in macaques (Waite & Buchanan-Smith, 2001).

127 Agonistic interactions with conspecifics can be a source of anxiety, leading to higher rates of SDBs (Castles et
128 al., 1999), but affiliative interactions can be a protective factor against abnormal behaviours and anxiety-
129 related behaviours. For instance, in captive bonobos, R/R was positively correlated with aggression, but
130 negatively correlated with social grooming (Miller and Tobey, 2012). It is unclear whether these social
131 behaviours had a direct casual impact on R/R behaviour or whether the relationship was mediated through
132 changes in stress levels, however it highlights the importance of considering social factors in the occurrence
133 or rate of abnormal behaviour such as R/R.

134 Given the importance of minimising factors that cause anxiety or facilitate the development of abnormal
135 behaviours in captive animals, we investigated the possible triggers of SDBs and R/R in a large group of zoo-
136 housed chimpanzees at Edinburgh Zoo over two studies. We chose to investigate R/R within this group as it
137 was regularly observed in a number of individuals, whereas other abnormal behaviours were too infrequent
138 or displayed by too few individuals to generate sufficient data in the time-period available. In the first study,
139 to test whether environmental factors affected the production of the behaviours, we examined whether the
140 length of time between feeds, the type of food the animals received, grooming within the group, visitor
141 behaviour, and the number of visitors in the zoo effected the occurrence of self-scratching, yawning, or
142 engaging in R/R. In the second, we investigated longer-term influences on R/R prevalence in the group. In
143 study 1, we predicted that high visitor numbers would cause an increase in rates of R/R and SDBs, in line with
144 previous findings (Carder and Semple, 2008; Mallapur et al., 2005). Previous work has shown that the
145 behaviour of zoo visitors can have negative effects on the behaviour of zoo animals (Hosey, 2000). We
146 predicted that zoo visitors displaying specific potentially negative behaviours that have previously been
147 observed at this facility (screaming, banging on windows, flash photography) would increase rates of SDBs
148 and R/R. In terms of social interactions with group members, we predicted that being involved in social
149 grooming would decrease an individual's rates of R/R and SDBs (Miller and Tobey, 2012). We predicted that
150 that SDB and R/R rates would increase when the duration between feeds was long (Baker and Easley, 1996)
151 and finally that consumption of fruit and starchy vegetables would increase rates of R/R (Morgan et al., 1993;
152 Lukas et al., 2014).

153 **2. Study One: Investigation into potential triggers of scratching, yawning and R/R**

154 **2.1 Methods**

155 *2.1.1 Study Site*

156 The study was undertaken at Budongo Trail Chimpanzee enclosure, Edinburgh Zoo, Scotland. The enclosure
157 comprises of three large indoor areas or 'pods' that include wooden climbing structures, a bedding area,
158 smaller 'pods' used for conducting cognitive research and an outdoor enclosure with further climbing
159 structures. These areas are all connected by tunnels and the whole enclosure spans over 1500m² (see
160 Herrelko et al., 2015 for more details) This layout allows the animals to split into sub-groups that vary in
161 composition of individuals, allowing their natural fission-fusion social system to be expressed. Budongo Trail
162 exhibit receives approximately 800,000 visitors each year (Whitehouse et al., 2014).

163 *2.1.2 Subjects*

164 The group of chimpanzees at Edinburgh Zoo comprised of two recently integrated groups (Schel et al., 2013)
165 that originated from Edinburgh (EZ) and Beekse-Bergen Safari Park (BB), The Netherlands (see Table S1 for
166 individual demographic details). The BB chimpanzees were introduced in 2010 and prior to living at the
167 Beekse-Bergen Safari Park in 2007, these individuals were housed in a medical testing facility and their history
168 was largely unknown.

169 *2.1.3 Data collection*

170 Data collection occurred over two study periods; 13th March 2014 to 8th July 2014 and 6th January 2015 to 2nd
171 March 2015. Ethical approval was obtained from the Animal Welfare Ethical Review Board of the Dept. of
172 Biology, University of York. Twenty minute focal samples (Altmann, 1974) were carried out on all 18 adult
173 individuals within the group (mean = 27.8 samples/individual; range = 20-57). No more than three
174 consecutive focal samples, lasting an hour in total, were collected within each 'pod' within the enclosure. No
175 animal was observed more than once each day and individuals with the least focal minutes were
176 preferentially chosen as focal animals from those available in the pod. Only complete focal samples where
177 the individual was observed for the full 20 minutes were included in the analysis, making a total of 474 focal
178 samples (158 hours). During the focal period, we used one/zero sampling to record if visitors used flash
179 photography (Y/N), percussed (banging, tapping etc.) on the windows of the enclosure (Y/N) and if children
180 screamed or vocalised loudly (Y/N). All occurrence data were collected on whether the focal individual was

181 involved in dyadic grooming (including roles in these interactions; self-grooming was not recorded) whilst
182 the frequency of yawning, scratching and engaging in R/R were recorded. To be counted as separate events,
183 an inter-event period of at least 2 seconds was required for all behaviours (e.g. two yawns 1 second apart
184 would be counted as 1 yawn; two scratches 5 seconds apart would be counted as 2 events). Due to the large
185 number of samples where zero events were recorded, the frequency data we collected was extremely
186 skewed and transformation was ineffective. Therefore, we converted these behavioural measures into
187 categorical variables where the behaviour was either present or absent within a focal sample period.

188 Examination of the visitor number data through Q-Q plots and the acquisition of significant Shapiro-Wilk tests
189 of normality indicated that this variable was also not normally distributed, even after transformation.
190 Therefore, this variable was also converted into a categorical variable. Total visitor numbers within the zoo
191 (data based on gate numbers provided by Edinburgh Zoo) were categorised into low (0-1000), medium (1001-
192 4000) and high (4000+) visitor numbers. The category boundaries for this variable was chosen as they gave a
193 roughly equal distribution of data in each category.

194 The keepers provided detailed records of the time, type and quantities of food given to the chimpanzees on
195 a daily basis. Food data were categorised based on methods used by Plowman (2013) into starchy vegetables,
196 fruit or other. If feeds were given that contained multiple food types, each type of food was categorised
197 separately so there were multiple data points for that feed. To account for taking multiple samples from
198 some feeding events, feeding event was entered as a random factor into our statistical models. Before data
199 analysis was undertaken, it was noted that only six of the 18 chimpanzees were regularly seen to engage in
200 R/R and the majority of these events (16 out of a total of 27 observed during study period; 59.3%) happened
201 within 40 minutes of the most recent feed. For these reasons, the data for all analysis of R/R came from just
202 those six chimpanzees and focal samples that occurred within 40 minutes of a keeper feeding event.

203 **2.2 Data Analysis**

204 *2.2.1 Statistical Analysis*

205 General Linear Mixed Models (GLMMs) with a binomial error structure and a logit link were used to
206 investigate the influence of categorical and continuous explanatory variables on whether or not the
207 chimpanzees displayed the behaviours in question. Individual identity was included as a random factor to

208 address the issue of pseudoreplication due to each individual contributing multiple data points to the
209 analyses. Likelihood ratio tests were run for full models and to determine the contribution of each variable
210 in the model. If a factor that explained significant variation in a dependent variable contained three
211 categories, post-hoc GLMMs were run, each containing two of the three categories within the factor. All tests
212 were run using SPSS v.21 with an alpha value of .05, but with Bonferroni corrected alpha levels of .017 applied
213 to post hoc tests.

214 Table 1 shows each of the research questions and the breakdown of the variables included in each of the
215 GLMMs that were run in order to answer each of those questions. For question (iv) that related to R/R, as
216 the majority of R/R events were observed occurring when food was available during or shortly before the
217 focal observation period, we looked at if the duration between the most recent feed (within the last 40
218 minutes from the focal period) and the previous feed affected the occurrence of R/R. The sample was limited
219 to sessions where the previous feeding opportunity was known (i.e. sessions where the previous feed was
220 the day before were omitted to control for opportunistic overnight eating).

221 -----Table 1 ----

222 **2.3 Results**

223 *2.3.1 Descriptive Results*

224 The raw frequencies of scratching, yawning and R/R events can be seen in Table S2. The proportion of focal
225 samples (N = 474) where the focal individual was observed (i) scratching was 0.62, (ii) yawning was 0.20 and
226 (iii) engaging in R/R was 0.04. For R/R, if we just examined data from the six individuals who had been known
227 to regularly engage in R/R prior to the study period, they were observed to engage in R/R in 0.15 of their
228 total 183 focal samples or 0.28 of the 68 focal samples within 40 minutes of a feed. Although the likelihood
229 of yawning and scratching occurring was higher in the six individuals who regularly engaged in R/R compared
230 to the 12 individuals who did not regularly engage in R/R, this pattern was not significant (Median proportion
231 of focal samples where yawning occurred for R/R individuals = 0.23 (IQR = 0.20) and for Non R/R individuals
232 = 0.16 (IQR = 0.06); Mann Whitney U test U = 24.50, p = .279; Median proportion of focal samples where
233 scratching occurred for R/R individuals = 0.63 (IQR = 0.12) and for Non R/R individuals = 0.56 (IQR = 0.30);
234 Mann Whitney U test U = 27.50, p = .425)

235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261

2.3.2 Does the number of visitors affect SDBs and R/R in chimpanzees?

Visitor numbers in the zoo did not explain a significant amount of variation in whether R/R or scratching behaviour occurred (Table 2) but did explain a significant amount of variation in whether the chimpanzees yawned (Table 2). Post-hoc GLMMs revealed that a significantly higher proportion of focal samples contained yawning when there were a medium number of visitors in the zoo compared with a low number of visitors ($F = 8.13 (1, 402), p = 0.005$; Figure 1). The likelihood of the focal chimpanzee yawning was not different for any other pairwise comparisons in the post-hoc GLMMs (see table S3).

-----Table 2 -----

-----Figure 1 -----

2.3.3 Does visitor behaviour affect SDBs and R/R in chimpanzees?

None of the different types of potentially disruptive visitor behaviours explained a significant amount of variation in whether or not the chimpanzees engaged in R/R (Table 2). Overall visitor behaviour did not explain a significant amount of variation in whether or not chimpanzees scratched (Table 2), however, when individual factors within the model were examined, there was a trend for a higher proportion of focal samples to contain scratching when flash photography was used (0.70) than when it was absent (0.59; Table 2). Again, overall visitor behaviour did not explain a significant amount of variation in whether or not chimpanzees yawned but there was a trend for a higher proportion of focal samples to contain yawning when children screamed (0.26) than when they did not (0.18; Table 2).

2.3.4 Does involvement in grooming affect SDBs and R/R in chimpanzees?

Receiving or giving grooming at any time during the focal period did not affect whether or not the chimpanzees engaged in R/R, scratched or yawned (Table 2).

2.3.5 Does length of time since being fed affect SDBs and R/R in chimpanzees?

The amount of time from the most recent feeding event influenced the likelihood of yawning, which increased as interval between feeding increased (Figure 2), but not the likelihood of scratching or R/R (Table 2).

----Figure 2-----

262 *2.3.6 Does the type of food consumed affect the likelihood of Regurgitation and Reingestion?*

263 The type of food given did not affect R/R ($F(2,88) = 1.05$ $p = 0.354$).

264 **2.4 Discussion**

265 The main finding of this study is that, contrary to the predictions, few of the potential environmental triggers
266 we examined significantly affected the occurrence of SDBs or R/R within this group of chimpanzees. One
267 factor of the captive environment we did find to contribute to increases in SDBs was the duration between
268 feeding events. In the wild, chimpanzees spend 6.68 hours per day foraging or eating (Leonard and
269 Robertson, 1994) whilst in captivity this is greatly reduced (Chamove et al., 1982) and can mean that there
270 are long periods of time between feeding events. Our results show that when the chimpanzees have to wait
271 longer to eat they are more likely to yawn. Apart from one visitor talk feed which occurs at a standard time
272 each day, the keepers aimed to feed at irregular intervals to prevent anticipatory behaviours, and it is unlikely
273 that yawning is an anticipatory response in this group. Automatic feeders that release food at specific times
274 or random intervals could help negate this issue and reduce potential stress in captive chimpanzees.

275 Several studies have shown that high visitor numbers can negatively affect behaviour (Birke, 2002) leading
276 us to predict that having a high number of visitors would lead to an increase in SDBs. We found no evidence
277 of visitor numbers affecting scratching or R/R, but, in line with our prediction, we did find that there was a
278 higher proportion of focal samples where the focal animal yawned when there were medium zoo gate
279 numbers (1001 to 4000 people) compared to low number of visitors (0-1000). However, contrary to the
280 prediction, yawning was not more likely when high rather than medium or low numbers of visitors were in
281 the zoo. This result is unexpected and shows that further research into other associated factors, such as
282 duration of visitor stay at enclosure windows and visitor noise levels, are required to establish what is driving
283 this effect. Although visitor behaviour did not explain a significant amount of variation in whether SDBs or
284 R/R occurred, there were trends for yawning being more likely when children were screaming and scratching
285 being more likely when flash photography was used. This highlights these visitor behaviours as potentially
286 problematic, and future research with more groups and individuals is needed to investigate these factors
287 further.

288 Another unexpected result was that grooming did not appear to influence the likelihood of SDBs or R/R. This

289 contrasts with work on long-tailed macaques (Schino et al., 1988), crested black macaques (Aureli and Yates,
290 2010), and bonobos (Miller and Tobey, 2012), but supports previous findings in barbary macaques (*Macaca*
291 *sylvanus*) (Semple et al., 2013). Semple et al. suggest that when the macaques terminated a grooming event
292 it may have led to an increase in anxiety, which counter-acted the positive, anxiety reducing effect of
293 grooming that would have been expected to lead to a reduction in scratching.

294 Previous research has suggested that the type of food given to the chimpanzees (Morgan, 1993) and
295 increased time between feeds (Baker and Easley, 1996) can affect R/R behaviour, however, this was not
296 found to be the case with this group of animals. Although we found no evidence that R/R was linked to
297 potentially stressful concurrent events, we had an excellent opportunity to track whether large scale events
298 affected the frequency of this behaviour.

299 **3. Study Two: Longer-term influences on R/R prevalence**

300 **3.1 Aims and Research Questions**

301 Given the lack of immediate factors influencing R/R in this group, we wanted to investigate longer term
302 influences on this behaviour. More specifically we aimed to examine if the translocation and integration into
303 a new social group and major diet changes affected the rates of R/R. We also examined the stability of R/R
304 rates from 2009 to 2015. Analyses were focussed on the nine of the 11 BB chimpanzees who were integrated
305 into the Edinburgh group in 2010, were still alive in 2015 and who had relatively high levels of R/R behaviour
306 at their previous facility. These nine individuals included all six individuals who were observed to engage in
307 R/R in study one. In addition, given that anecdotal reports from keepers and researchers indicated that the
308 original EZ individuals did not engage in R/R prior to the arrival of the BB group, we wanted to test whether
309 this behaviour spread through social learning. Many chimpanzees in captivity are moved between facilities
310 for breeding programmes so it is important to understand if this is a socially learnt negative behaviour, as
311 coprophagy has been suggested to be (Hopper et al., 2016).

312 More specifically, in our second study we aimed to address the following questions:

313 1) Did the introduction of the BB individuals to Edinburgh Zoo cause their R/R rates to increase? It was
314 predicted that R/R rates would increase during the introduction between the two groups of individuals as
315 this was believed to be a stressful time for the animals.

316 2) Are any changes in R/R rates related to changes in the diet given to the chimpanzees? Changes in the
317 chimpanzees' diet, as recommended by zoo veterinarians and nutritionists in order to improve the digestion
318 of the BB individuals, may have led to changes in R/R rates. Mulder et al. (2016) found that changes to the
319 diet of the chimpanzees at Amersfoort Zoo by increasing fibre did lead to a significant reduction in R/R rates
320 but descriptive data showed that the rates of R/R were lowest immediately after the change in diet and began
321 to increase afterwards.

322 3) Did R/R rates in BB individuals change over time? Being integrated into a large and socially complex group
323 living in an enclosure designed to encourage natural behaviours, such as 'fission-fusion' dynamics (Aureli et
324 al., 2008), in addition to several small diet changes over this period could have led to reductions in the BB
325 individuals' rates of R/R.

326 4) Did observing R/R in the BB individuals lead to the adoption of the behaviour by EZ individuals? We
327 predicted that R/R might be socially learnt, which would mean that observing the BB individuals engaging in
328 R/R could lead to the performance and adoption of the behaviour by the EZ individuals.

329 **3.2 Methods**

330 The data analysed in this study was collected during three distinct time periods, which are described in detail
331 below. Each data collection used slightly different methodologies but they were similar enough to allow this
332 very important long-term analysis to be undertaken.

333 1) May to September 2009 at Beekse-Bergen Safari Park. Data were collected by SK and students using 10-
334 minute long focal samples (Altmann, 1974) to record the duration of time spent engaging in R/R by the focal
335 individual. Prior to data collection, inter-observer reliability test between SK and each student showed >90%
336 identical data coding. 836 focal samples were collected (139.3 hours). 836 focal samples were collected
337 (139.3 hours).

338 2) March to October 2010 at Edinburgh Zoo (pre-, during and immediately post integration of BB and EZ
339 groups) 10-minute focal samples were conducted and the frequency of R/R within each sample period
340 recorded. Data were collected by ESH and 2 research assistants, with interobserver reliability tested at 2 time
341 points and agreement on presence or absence of R/R completely reliable ($R = 1.0$). 1133 focal samples were
342 collected (188.8 hours).

343 3) July 2010 to February 2015 at Edinburgh Zoo. Much of the data from this time period came from University
344 of York researchers working on independent research projects in 2010-11, as well as long-term observational
345 data collection that was introduced in 2012 by KS. All long-term researchers conducting independent
346 research projects at Budongo Trail contributed to the data set, once they had passed an identification test to
347 ensure they could reliably identify all individuals. Detailed instructions were given to researchers and they
348 submitted data regularly for checking by KS or EW, so they received feedback on their data collection, but no
349 formal measures of interobserver reliability were taken. 10-minute focal samples were conducted where all
350 instances of the focal engaging in R/R were recorded, along with details of which other chimpanzees were in
351 the same pod as the focal animal and which of those were within 3 meters of the focal individual at the time
352 of each R/R event. Data on changes to diets and the dates of the integration process were obtained from
353 the keepers. 3612 focal samples were collected (602 hours).

354 In order to make all three sets of data comparable, each focal observation was scored for whether or not at
355 least one R/R event occurred in the 10-minute time period and only complete focal periods were considered.

356 **3.3 Data Analysis**

357 *3.3.1 Are the changes to R/R related to the integration process?*

358 Individual proportions of focal observations where R/R was observed per month for the BB individuals were
359 calculated. These monthly proportions were averaged for three time periods: pre-integration of the BB
360 individuals (May to September 2009), during the integration (May to July 2010) and immediately post
361 integration (August to December 2010). A Friedman test (N= 9) was used to compare the average rates of
362 R/R for the three time periods.

363 *3.3.2 Are the changes to R/R related to diet changes?*

364 The monthly proportion of focal observations where R/R was observed for the BB individuals, were used to
365 compare the average R/R rates of the three months before and after a major diet change. On 20th October
366 2010 an additional 50kg of grapes, pears and mangos, 15kg of apples and four loaves of white bread were
367 included in the weekly diet of the chimpanzees. Individuals were observed for a minimum of 18 focal samples
368 (three hours) during each three- month period. A Wilcoxon test (N= 9) was used to compare the mean of the
369 proportions of observations where R/R occurred in each three-month period before and after the diet

370 change.

371 *3.3.3 Have the proportion of focals where R/R was observed changed over time?*

372 For each year, from 2009 to 2015, a yearly proportion of focals where R/R was observed for each individual
373 was calculated by averaging the available monthly proportions in each year. These values for the 9 BB
374 individuals were then averaged to create a group annual mean. The relationship between time (year) and
375 R/R proportions was examined using a Kendall's-tau correlation, due to the small sample size.

376 *3.3.4 Have the EZ individuals socially learnt the behaviour from the BB individuals?*

377 We wanted to establish if observing the BB chimpanzees engaging in R/R lead to the EZ individuals adopting
378 the behaviour. We calculated the total number of R/R events that the BB individuals were recorded as
379 engaging in. We also determined the number of these events where at least one EZ chimpanzee was present
380 within 3 metres of a BB individual, from where they could have observed the behaviour closely. The data
381 used for this came from August 2010 – 2015, once the BB had been fully integrated into the group. We then
382 calculated the total number of times each of the EZ individuals were recorded to have engaged in R/R to see
383 if the behaviour was adopted by those animals.

384 *3.3.5 Statistical analysis*

385 All tests run were two-tailed with alpha level set at 0.05 and Bonferroni corrected to $p = 0.017$ for post hoc
386 tests. Wilcoxon Signed Ranks, Friedmans and Kendall's-tau tests were run using SPSS v.21. Effect sizes (*d* and
387 *r*) were calculated using an online tool (<http://www.uccs.edu/~lbecker/>). When using Cohen's *d* as an effect
388 size, .80 is considered a large effect, .50 a medium sized effect, and 0.20 a small effect (Cohen, 1992). *r* was
389 used as an effect size for non-parametric Wilcoxon Signed Rank tests, in which 0.50 or above is a large effect,
390 above 0.30 a medium effect and 0.10 a small effect (Pallant, 2007).

391 **3.4 Results**

392 *3.4.1 Are the changes in R/R related to the integration process?*

393 There were significant differences between the proportions of observations where R/R occurred pre-, during
394 and post integration (Friedman $X^2(2) = 9.60$ $N = 9$, $p = 0.008$; Figure 3). Using Bonferroni corrected alpha levels,
395 post-hoc Wilcoxon signed ranks tests show that the proportions of R/R were significantly higher in the pre-
396 integration than during the post-integration period ($Z = -2.38$, $p = 0.017$; $r = 0.24$). There were trends for the

397 proportions of R/R being higher during pre-integration than in the integration period ($Z = -2.24$, $p = 0.025$;
398 $r = 0.61$) and in integration than post-integration ($Z = -2.20$, $p = 0.028$; $r = 0.50$). Figure 3 illustrates that all
399 individuals observed to engage in R/R showed a decrease over the integration process and that this pattern
400 was not driven by a single individual.

401 ----Figure 3 ----

402 3.4.2 Are the changes in R/R related to diet changes?

403 R/R proportions were not significantly higher after the diet change (median = 0.073; IQR = 0.13) than before
404 (median = 0.071; IQR = 0.16; Wilcoxon $Z = -0.41$ $N = 10$ $p = 0.686$; $r = 0.16$).

405 3.4.3 Has the proportion of focals where R/R was observed changed over time?

406 There was a trend for the proportion of focals where R/R was observed decreasing over the period from 2009
407 to 2015 ($\tau_b = -0.62$, $n = 9$, $p = 0.051$). Figure S4 shows how proportions of R/R change over time for nine BB
408 individuals and indicates that all individuals contribute to the overall group decrease rather than one
409 individual driving it.

410 3.4.4 Have the EZ individuals socially learnt the behaviour from the BB individuals?

411 We found that the EZ chimpanzees were within 3 metres of 89 R/R events and the BB chimpanzees were
412 recorded to engage in R/R a total of 160 times from August 2010-2015, which means that the EZ chimpanzees
413 were in proximity to 55.6% of all observed R/R events. Despite this, no EZ individual was seen to engage in
414 R/R themselves more than four times between August 2010 and July 2013 (see Table S5). The number of R/R
415 events by the EZ individuals was low and sporadic and no events were recorded after July 2013.

416 **3.5 Discussion**

417 It was predicted that observations of R/R would increase during the introduction between the two groups of
418 individuals as this was believed to be a stressful time for the animals. However, R/R proportions were
419 significantly higher before the introduction. This suggests that either the integration process did not involve
420 as much negative stress as assumed or more likely, given the convergent results of study 1, R/R is not a
421 response to current stress levels. It is possible that R/R is related to boredom (Baker, 1997; 2004) and that
422 the cognitive challenge presented to the BB chimpanzees by the introduction to a new physical and social
423 environment may have reduced their boredom and, therefore, their proportions of focal samples where R/R

424 was observed. Previous studies have found that the provision of foraging related enrichment (Baker, 1997)
425 and increased human caretaker interaction (Baker, 2004) has led to reductions in R/R rates. We therefore
426 recommended that further research investigating the link between boredom and R/R should be undertaken.
427 In October 2010, the diet of the chimpanzees changed and the amount of fruit given weekly increased. Based
428 on the results of Morgan et al. (1993) it was predicted that this would lead to an increase in R/R. However,
429 comparing the proportions of R/R for three months on either side of this diet change showed there was no
430 significant difference, however, this could have been because rates were already low before the change (only
431 8/743 samples prior to the diet change contained an R/R event). There were other small changes to the diet
432 of these chimpanzees from April 2010 onwards but we lacked sufficient data to examine three months prior
433 to and after each of these changes. It is possible, therefore, that each of these small changes may have
434 contributed to the overall reduction in R/R within the BB individuals.

435 By looking longitudinally at the R/R performed by the chimpanzees at Edinburgh Zoo we have been able to
436 identify that the proportion of the BB individuals' focal periods where R/R was observed has a trend for
437 reducing over time, which suggests an improvement in their welfare. Some BB individuals (Pearl, Edith, Eva
438 and Heleen) were no longer observed engaging in R/R by 2015. The design of Budongo Trail and being part
439 of the large, socially complex group of chimpanzees is the most probable cause of the reduction of R/R rates
440 since 2009 in the BB individuals. The fact that six of the animals still occasionally engage in R/R is likely due
441 to the persistent nature of the behaviour and is possibly a form of coping strategy, similar to stereotypical
442 behaviours (Higham et al., 2009). In humans, the same behaviour, known as rumination (Nakanishi and
443 Anderson, 1982), has been linked to periods of distress in individuals of average intelligence but the
444 behaviour is very difficult to eradicate (Nakanishi and Anderson, 1982). Mulder et al. (2016) found that
445 feeding a higher fibre diet did reduce rates of R/R in the chimpanzees at Amersfoort Zoo but the behaviour
446 did not disappear, suggesting the behaviour had become a habit. Although we do not know the full history
447 of the BB animals during their time in the medical testing facility, it is likely that the experience may have
448 been stressful and this is where they first performed R/R. Once established as a behaviour pattern, it may be
449 difficult to eliminate, hence why the BB individuals still perform the behaviour, albeit at much lower levels.

450 It was predicted that R/R might be socially learnt but whilst nine of the 11 EZ individuals were recorded as

451 engaging in R/R, only 18 instances were observed from integration with the BB group (July 2010) to July
452 2013. The first recorded instances of R/R by EZ individuals were during October 2010 and R/R then occurred
453 rarely until 2013, after which time the behaviour seemingly disappeared. Prior to July 2010 and the start of
454 the integration, R/R was not systematically monitored for the EZ group because it was very rarely observed
455 by keepers and therefore was not considered a welfare issue. Despite having ample opportunity to observe
456 the behaviour being displayed by the BB chimpanzees, the behaviour was only ever performed at negligible
457 rates by the EZ individuals. This study demonstrates that the integration of individuals that engage in R/R
458 into an established group that does not regularly display the behaviour does not seem to lead to the spread
459 of the behaviour.

460 **4. Conclusions**

461 Our two studies together show that surprisingly few environmental events were associated with increases in
462 SBDs or R/R in this group of zoo-housed chimpanzees. Yawning was significantly more likely to occur when
463 the period between feeds was greater and when there were a medium rather than low number of visitors in
464 the zoo, but visitor behaviour and grooming within the group did not significantly influence SBDs. We also
465 found no links between R/R and environmental stressors as neither the presence nor behaviour of visitors
466 affected the production of the behaviour and the number of observed R/R events actually decreased during
467 and after the social integration of the two groups. No obvious trigger for R/R was identified for this group,
468 suggesting it may be a behaviour that has persisted from previous periods of potentially suboptimal
469 conditions. However, R/R decreased in frequency following integration into a complex physical and social
470 environment. We also demonstrated that the movement of individuals known to engage in this behaviour
471 into groups where R/R is absent is unlikely to lead to the spread of this behaviour.

472 **Acknowledgements**

473 We are grateful to RZSS and the keepers of Budongo Trail for their permission and support in conducting this
474 research. Thanks to Jeffrey Ridder, Annette van de Kraats, Thomas Bionda, Lauren Marshall, Joana Gričiute
475 and long-term researchers at Budongo Trail who assisted with data collection. Funding for ESH was provided
476 by University of Stirling, Burning Gold Productions (with the BBC and Animal Planet), the Royal Zoological
477 Society of Scotland, and the Scottish Funding Council.

478 **References**

- 479 Akers, J. S., & Schildkraut, D. S. (1985). Regurgitation/reingestion and coprophagy in captive gorillas. *Zoo*
480 *Biology*, 4(2), 99-109.
- 481 Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, 49(3), 227-266.
- 482 Aureli, Filippo, Colleen M. Schaffner, Christophe Boesch, Simon K. Bearder, Josep Call, Colin A. Chapman,
483 Richard Connor et al. (2008) "Fission-fusion dynamics: new research frameworks." *Current Anthropology*,
484 49(4), 627-654.
- 485 Aureli, F., & Yates, K. (2010). Distress prevention by grooming others in crested black macaques. *Biology*
486 *letters*, 6(1), 27-29.
- 487 Baker, K. C. (1997). Straw and forage material ameliorate abnormal behaviors in adult chimpanzees. *Zoo*
488 *Biology*, 16(3), 225-236.
- 489 Baker, K. C. (2004). Benefits of positive human interaction for socially-housed chimpanzees. *Animal Welfare*,
490 13(2), 239.
- 491 Baker, K. C., & Aureli, F. (1997). Behavioural indicators of anxiety: an empirical test in chimpanzees.
492 *Behaviour*, 134(13), 1031-1050.
- 493 Baker, K. C., & Easley, S. P. (1996). An analysis of regurgitation and reingestion in captive chimpanzees.
494 *Applied Animal Behaviour Science*, 49(4), 403-415.
- 495 Birke, L. (2002). Effects of browse, human visitors and noise on the behaviour of captive orangutans. *Animal*
496 *Welfare*, 11(2), 189-202.
- 497 Birkett, L. P., & Newton-Fisher, N. E. (2011). How abnormal is the behaviour of captive, zoo-living
498 chimpanzees?. *PloS one*, 6(6), e20101.
- 499 Bloomsmith, M. A., Clay, A. W., Lambeth, S. P., Lutz, C. K., Breaux, S. D., Lammey, M. L., ... & Mareno, M. C.
500 (2019). Survey of Behavioral Indices of Welfare in Research Chimpanzees (*Pan troglodytes*) in the United
501 States. *Journal of the American Association for Laboratory Animal Science*, 58(2), 160-177.
- 502 Carder, G., & Semple, S. (2008). Visitor effects on anxiety in two captive groups of western lowland gorillas.
503 *Applied Animal Behaviour Science*, 115(3), 211-220.
- 504 Carlstead, K. (1998). Determining the causes of stereotypic behaviors in zoo carnivores: toward appropriate

505 enrichment strategies. *Second nature: Environmental enrichment for captive animals*, 172-183.

506 Castles, D. L., Whiten, A., & Aureli, F. (1999). Social anxiety, relationships and self-directed behaviour among
507 wild female olive baboons. *Animal Behaviour*, 58(6), 1207-1215.

508 Chamove, A. S., Hosey, G. R., & Schaetzel, P. (1988). Visitors excite primates in zoos. *Zoo Biology*, 7(4), 359-
509 369.

510 Chamove, A. S., Anderson, J. R., Morgan-Jones, S. C., & Jones, S. P. (1982). Deep woodchip litter: hygiene,
511 feeding, and behavioral enhancement in eight primate species.

512 Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112(1), 155.

513 Gould, E., & Bres, M. (1986). Regurgitation and reingestion in captive gorillas: description and intervention.
514 *Zoo Biology*, 5(3), 241-250.

515 Herrelko, E.S., Buchanan-Smith, H.M., & Vick, S-J. (2015). Perception of Available Space During Chimpanzee
516 Introductions: Number of Accessible Areas Is More Important Than Enclosure Size. *Zoo Biology*, 34, 397-405.

517 Higham, J. P., MacLarnon, A. M., Heistermann, M., Ross, C., & Semple, S. (2009). Rates of self-directed
518 behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (*Papio hamadryas*
519 *anubis*). *Stress*, 12(6), 526-532.

520 Hill, S. P. (2009). Do gorillas regurgitate potentially-injurious stomach acid during 'regurgitation and
521 reingestion?'. *Animal Welfare*, 18, 123-127.

522 Hopper, L. M., Freeman, H. D., & Ross, S. R. (2016). Reconsidering coprophagy as an indicator of negative
523 welfare for captive chimpanzees. *Applied Animal Behaviour Science*, 176, 112-119.

524 Hosey, G. R. (2000). Zoo animals and their human audiences: what is the visitor effect? *Animal Welfare*, 9(4),
525 343-358.

526 Hosey, G. R. (2005). How does the zoo environment affect the behaviour of captive primates?. *Applied Animal*
527 *Behaviour Science*, 90(2), 107-129.

528 Ironmonger, J., Ironmonger, S., & Heaton, R. (1992). *The Good Zoo Guide*. HarperCollins.

529 Jacobson, S. L., Ross, S. R., & Bloomsmith, M. A. (2016). Characterizing abnormal behavior in a large
530 population of zoo-housed chimpanzees: prevalence and potential influencing factors. *PeerJ*, 4, e2225.

531 Kalcher, E., Franz, C., Crailsheim, K., & Preuschoft, S. (2008). Differential onset of infantile deprivation
532 produces distinctive long-term effects in adult ex-laboratory chimpanzees (*Pan troglodytes*). *Developmental*
533 *Psychobiology: The Journal of the International Society for Developmental Psychobiology*, 50(8), 777-788.

534 Landis, B., & Lambroza, A. (2001, March). A respiratory biofeedback solution for the rumination syndrome.
535 In *Biological Psychology* (Vol. 56, No. 1, pp. 79-80), NETHERLANDS: ELSEVIER SCIENCE BV.

536 Leonard, W. R., & Robertson, M. L. (1994). Evolutionary perspectives on human nutrition: the influence of
537 brain and body size on diet and metabolism. *American Journal of Human Biology*, 6(1), 77-88.

538 Lukas, K. E., Bergl, R., Ball, R., Kuhar, C. W., Lavin, S. R., Raghanti, M. A., ... & Dennis, P. M. (2014).
539 Implementing a low-starch biscuit-free diet in zoo gorillas: The impact on health. *Zoo Biology*, 33(1), 74-80.

540 Maestriperi, D., Schino, G., Aureli, F., & Troisi, A. (1992). A modest proposal: displacement activities as an
541 indicator of emotions in primates. *Animal Behaviour*, 44(5), 967-979.

542 Mallapur, A., Sinha, A., & Waran, N. (2005). Influence of visitor presence on the behaviour of captive lion-
543 tailed macaques (*Macaca silenus*) housed in Indian zoos. *Applied Animal Behaviour Science*, 94(3), 341-352.

544 Mellor, D. J., & Beausoleil, N. J. (2015). Extending the 'Five Domains' model for animal welfare assessment
545 to incorporate positive welfare states. *Animal Welfare*, 24(3), 241-253.

546 Mason, G. J. (1991). Stereotypies: a critical review. *Animal Behaviour*, 41(6), 1015-1037.

547 Miller, L. J., & Tobey, J. R. (2012). Regurgitation and reingestion in bonobos (*Pan paniscus*): Relationships
548 between abnormal and social behavior. *Applied Animal Behaviour Science*, 141(1), 65-70.

549 Mitchell, G., Tromborg, C. T., Kaufman, J., Bargabus, S., Simoni, R., & Geissler, V. (1992). More on the
550 'influence' of zoo visitors on the behaviour of captive primates. *Applied Animal Behaviour Science*, 35(2), 189-
551 198.

552 Morgan, L., Howell, S. M., & Fritz, J. (1993). Regurgitation and reingestion in a captive chimpanzee (*Pan*
553 *troglodytes*). *Lab Animal*, 22:42-45.

554 Mulder, I., van der Meer, R., de Vries, H., & Sterck, E. H. M. (2016). The relationship between diet change and
555 regurgitation and reingestion in captive chimpanzees. *Journal of Zoo and Aquarium Research*, 4(4), 196.

556 Nakanishi, D. A., & Anderson, D. R. (1982). Behavioral Treatment of Psychogenic Vomiting Among Children-
557 A Review and Case Example. *Journal Of Psychosocial Nursing And Mental Health Services*, 20(11), 17-20.

558 Nishida, T. (1970). Social behavior and relationship among wild chimpanzees of the Mahali Mountains.
559 *Primates*, 11(1), 47-87.

560 Oftedal, O. T., & Allen, M. E. (1996). Nutrition and dietary evaluation in zoos. In *Wild mammals in Captivity*.
561 109–116. Kleiman, D. G., Allen, M. E., Thompson, K. V., & Lumpkin, S. (Eds). Chicago, IL: The University of
562 Chicago Press.

563 Pallant, J. (2007). *SPSS Survival Manual*. 3rd edition. McGrath Hill.

564 Plowman, A. (2013). Diet review and change for monkeys at Paignton Zoo Environmental Park. *Journal of*
565 *Zoo and Aquarium Research*, 1(2), 73-77.

566 Schel, A., Rawlings, B., Claidiere, N., Wilke, C., Wathan, J., Richardson, J., ... & Slocombe, K. (2013). Network
567 analysis of social changes in a captive chimpanzee community following the successful integration of two
568 adult groups. *American Journal of Primatology*, 75(3), 254-266.

569 Schino, G., Rosati, L., & Aureli, F. (1998). Intragroup variation in conciliatory tendencies in captive Japanese
570 macaques. *Behaviour*, 135(7), 897-912.

571 Schino, G., Troisi, A., Perretta, G., & Monaco, V. (1991). Measuring anxiety in nonhuman primates: effect of
572 lorazepam on macaque scratching. *Pharmacology Biochemistry and Behavior*, 38(4), 889-891.

573 Semple, S., Harrison, C., & Lehmann, J. (2013). Grooming and anxiety in Barbary macaques. *Ethology*, 119(9),
574 779-785.

575 Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human
576 subjects. *Stress*, 5(1), 47-54.

577 Waitt, C., & Buchanan-Smith, H. M. (2001). What time is feeding?: How delays and anticipation of feeding
578 schedules affect stump-tailed macaque behavior. *Applied Animal Behaviour Science*, 75(1), 75-85.

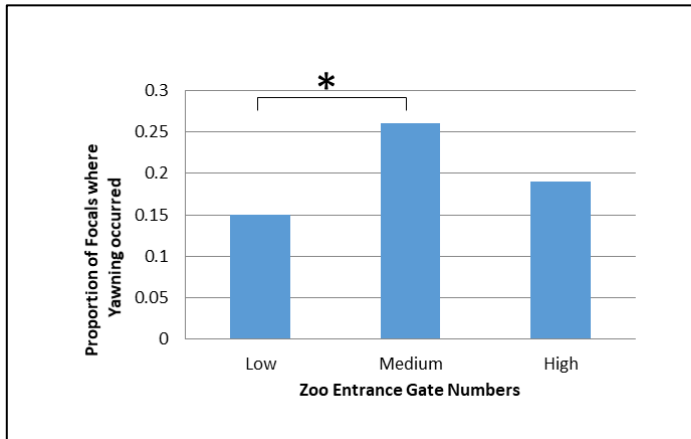
579 Wells, D. L. (2005). A note on the influence of visitors on the behaviour and welfare of zoo-housed gorillas.
580 *Applied Animal Behaviour Science*, 93(1), 13-17.

581 Whitehouse, J., Waller, B. M., Chanvin, M., Wallace, E. K., Schel, A. M., Peirce, K., ... & Slocombe, K. (2014).
582 Evaluation of public engagement activities to promote science in a zoo environment. *PloS one*, 9(11),
583 e113395.

584 Wyngaarden, J. B. (1992). Smith L. *Cecil textbook of medicine*, 19.

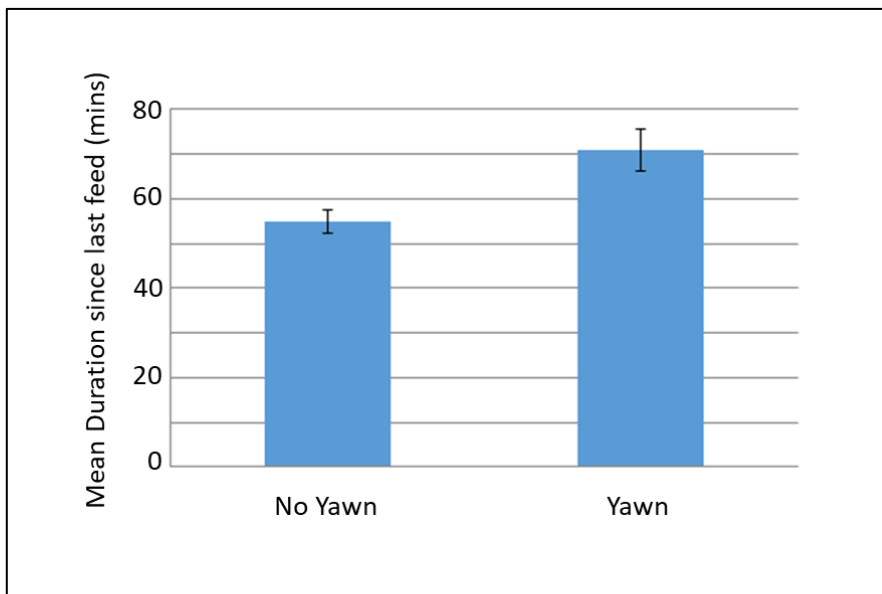
585

586 Figure 1 – The proportion of focal samples where the focal individual was seen to yawn when the Zoo
587 entrance numbers were low (0-1000), medium (1001-4000) and high (4001+). * denotes post-hoc GLMM
588 showed a significant difference (p=0.005).



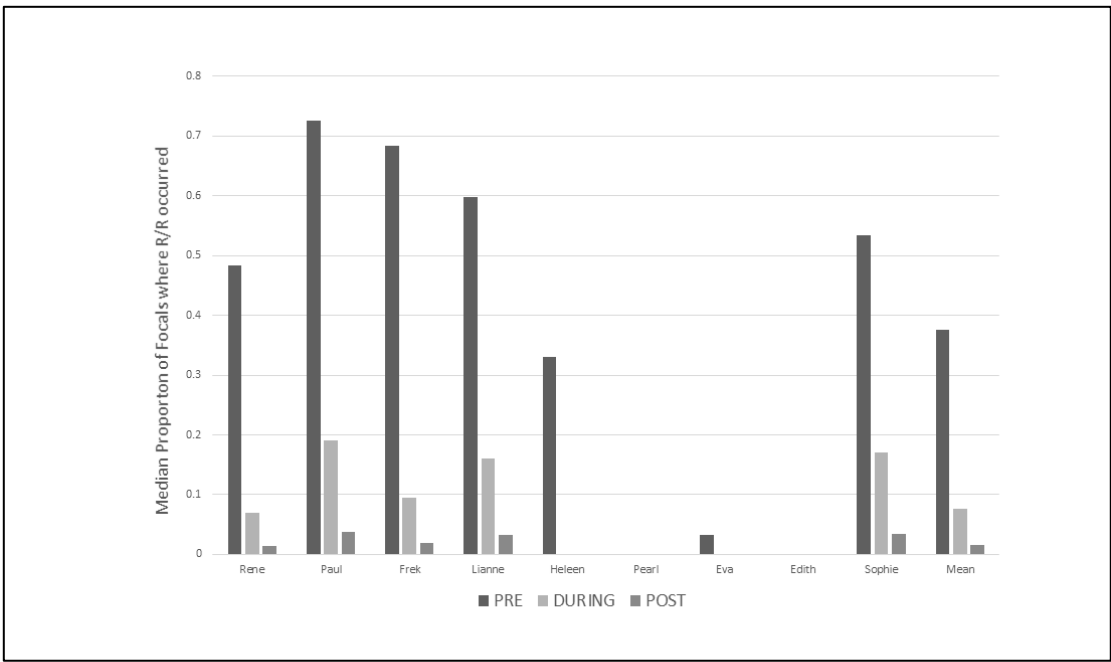
589

590 Figure 2- The mean duration in minutes from the last feeding event to the start of the focal samples where
591 the focal individual was seen to yawn and not yawn. Error bars show ± 1 SEM.



592

593 Figure 3- The median proportions of focal samples where R/R was observed for each of the nine BB individuals
594 throughout the integration process



595

596

Tables

599 Table 1. Variables included in GLMMs to answer each research question.

| Research Questions: Are the abnormal or anxiety behaviours of chimpanzees affected by: | Dependent Variables (1 variable/ model) | N data points entered into model (N = individuals) | Independent Variable 1 | Independent Variable 2 | Independent Variable 3 | Random Effect(s) |
|---|--|---|--|--|-------------------------------|-------------------------|
| (i) number of visitors | Focal engaged in R/R? (Y/N) | 68 (N = 6 individuals known to engage in R/R) | The level of visitor numbers present in the zoo on that day (low, medium and high) | N/A | N/A | Chimp Identity |
| | Focal scratched? (Y/N) | 474 (N = 18) | | | | |
| | Focal yawned? (Y/N) | 474 (N = 18) | | | | |
| (ii) visitor behaviour | Focal engaged in R/R? (Y/N) | 68 (N= 6 individuals known to engage in R/R) | If a visitor used flash photography (Y/N) | If a visitor banged on the window of the focal pod (Y/N) | If a child screamed (Y/N) | Chimp Identity |
| | Focal scratched? (Y/N) | 474 (N = 18) | | | | |
| | Focal yawned? (Y/N) | 474 (N = 18) | | | | |
| (iii) involvement in grooming events | Focal engaged in R/R? (Y/N) | 68 (N= 6 individuals known to engage in R/R) | Whether the focal animal received or gave grooming at any time | N/A | N/A | Chimp Identity |

| | | | | | | |
|--|-----------------------------|--|---|-----|-----|--------------------------------|
| | Focal scratched? (Y/N) | 474 (N = 18) | during the focal sample (Y/N) | | | |
| | Focal yawned? (Y/N) | 474 (N = 18) | | | | |
| (iv) duration since being fed - scratching and yawning | Focal scratched? (Y/N) | 358 (N = 18) | The interval between previous feeding time and start of focal period | N/A | N/A | Chimp Identity |
| | Focal yawned? (Y/N) | 358 (N = 18) | | | | |
| (iv) duration since being fed - R/R | Focal engaged in R/R? (Y/N) | 51 (from the 6 individuals known to engage in R/R) | Interval between the most recent and previous feeding event and the start of the focal period | N/A | N/A | Chimp Identity |
| (v) the type of food consumed | Focal engaged in R/R? (Y/N) | 91 (from the 6 individuals known to engage in R/R) | The type of food recently provided (starchy vegetable, fruit or neither) | N/A | N/A | Chimp Identity ; Feeding event |

600

601

602 Table 2. Results of the 15 GLMMs run to address each of the four research questions for each behaviour of interest
 603 (R/R, scratching and yawning). F, df and p values derived from likelihood ratio tests that compared the full model with
 604 a null model (intercept and random factors only), or the full model with a reduced model, designed to assess the
 605 contribution of a specific variable to explaining variation in the DV.

| Are the abnormal or anxiety behaviours of chimpanzees affected by: | Independent Variables | R/R | | | Scratching | | | Yawning | | |
|--|---------------------------------------|------|--------|-------|------------|--------|-------|--------------|---------------|--------------|
| | | F | df | p | F | df | p | F | df | p |
| (i) Visitor numbers | Total numbers of visitors in the zoo | 2.07 | 2, 180 | 0.129 | 1.53 | 2, 471 | 0.217 | 4.84, | 2, 471 | 0.016 |
| (ii) visitor behaviour | Full Model | 0.72 | 3, 64 | 0.546 | 1.56 | 3, 470 | 0.198 | 0.10 | 3, 470 | 0.395 |
| | Children Screaming | 1.25 | 1, 64 | 0.268 | 0.003 | 1, 470 | 0.957 | 2.98 | 1, 470 | 0.085 |
| | Banging on Windows | 0.04 | 1, 64 | 0.841 | 0.46 | 1, 470 | 0.496 | 1.67 | 1, 470 | 0.198 |
| | Camera Flashes | 0.97 | 1, 64 | 0.328 | 3.59 | 1, 470 | 0.059 | 0.003 | 1, 470 | 0.956 |
| (iii) involvement in grooming events | Grooming | 2.51 | 1, 181 | 0.115 | 1.04 | 1, 181 | 0.309 | 0.49 | 1, 181 | 0.486 |
| (iv) length of time since being fed | Length of Time Between Feeding events | 1.63 | 1, 49 | 0.208 | 0.08 | 1, 355 | 0.783 | 5.30 | 1, 355 | 0.022 |

606
607