

How environmental conditions affect sleep? An investigation in domestic dogs (*Canis lupus familiaris*)

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Published in:
Behavioural Processes

Publication date:
2022

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This document version is the:
Peer reviewed version

The final published version is available direct from the publisher website at:
<https://doi.org/10.1016/j.beproc.2022.104662>

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Citation for published version (APA):
Schork, I. G., Manzo, I. A., De Oliveira, M. R. B., da Costa, F. V., Palme, R., Young, R. J., & de Azevedo, C. S. (2022). How environmental conditions affect sleep? An investigation in domestic dogs (*Canis lupus familiaris*). *Behavioural Processes*, 199, Article 104662. <https://doi.org/10.1016/j.beproc.2022.104662>

1 **How environmental conditions affect sleep? An investigation in domestic dogs (*Canis lupus***
2 ***familiaris*)**

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4

5 **ABSTRACT**

6

7 Studies with humans and some other animal species have shown that sleep is compromised
8 when the presence of external factors such as light, sound, and temperature surpass normal
9 levels. This study investigated the effects of these environmental conditions on 13 kennelled
10 laboratory dogs, assessing whether each variable interfered with their sleep behaviour and/or
11 increased stress responses, which could further compromise sleep quality. The behaviour of
12 dogs was video recorded for eight months. Diurnal and nocturnal behaviour were recorded,
13 along with naturally occurring levels of temperature, light and sound in the dogs' kennel
14 environment. Faecal cortisol metabolites (FCM), from samples collected every morning, were
15 used to monitor the dogs' adrenocortical activity. GLMM models and non-parametric tests
16 were conducted to evaluate the relationship between sleeping patterns, environmental
17 variables, and stress on the studied dogs. Nocturnal sleep decreased in response to increases
18 in temperature and in day light duration. No effects of sound and FCM levels on dogs' sleep
19 were observed. However, diurnal sleep was affected by sound and FCM levels, decreasing
20 when both factors increased. Additionally, noisier days increased stress responses, especially
21 in male dogs. Increased FCM levels were associated with changes in the diurnal behaviour of
22 dogs; for example, decreased activity. The decrease in daily activities and increased
23 physiological stress responses could be associated with maladaptation to the environment,
24 which could indicate poor welfare. Our study suggests that mitigating the impact of
25 environmental conditions in the kennels could improve sleep quality and the overall quality
26 of life of the dogs.

27

28 **Keywords:** environmental conditions; laboratory dogs; stress; welfare.

29

30 **1. Introduction**

31 The behavioural and stress responses of animals to environmental conditions such as
32 the levels of temperature, light and sound are the result of natural selection and directed
33 towards maintaining the individual's homeostasis (Morgan, 2004). Behavioural activity
34 patterns fluctuate across 24 hours (i.e., circadian rhythms), in a process modulated by
35 external triggers (e.g., light levels and temperature), which generate appropriate endogenous
36 responses to ensure adaptation (Adan et al., 2012; Morgan, 2004; Randler, 2014). Similar to
37 the circadian rhythm, sleep also has an adaptative function: being inactive and unconscious
38 is advantageous since sleep helps maintain homeostasis (Mader and Mader, 2016; Randler,
39 2014; Roth et al., 2010). Sleeping patterns have mostly been investigated in animals under
40 controlled laboratory conditions, without the full consideration of their ecological value and
41 without considering the importance that different ecological variables may have on sleep
42 quality (Aulsebrook et al., 2016; Tougeron and Abram, 2017). Sleep structure (duration and
43 fragmentation; that is, the time that the animal spends sleeping and the number of times it
44 wakes during sleeping) is, thus, important to be investigated to see how it influences animal
45 homeostasis.

46 The main external trigger that modulates sleep is light; the presence or absence of not
47 only luminous emittance, but of light with different wave lengths, such as blue light, is a cue
48 to promote sleep onset (Dijk and Archer, 2009; Dominoni et al., 2016). However, daylight is
49 not the only environmental trigger that affects sleep; other components such as artificial
50 lighting (Dominoni et al., 2016), sound (Muzet, 2007), temperature (Harding et al., 2019) and
51 night activity (i.e. shift in activity patterns such as remaining active during normal resting
52 hours) (Nunez et al., 2018) can influence the duration and fragmentation of sleep. For
53 instance, nocturnal artificial lights, higher temperatures, greater night activity, and noise
54 pollution have negative impacts on an array of wild species, reducing REM sleep and the total
55 time spent sleeping, increasing the latency to sleep and alertness (Chinoy et al., 2018; Harding
56 et al., 2019; Mendoza, 2021; Shannon et al., 2016; Siegel, 2011).

57
58 In captive/laboratory settings artificial light also disrupts the circadian cycle (*mice Mus*
59 *musculus*: Ishida et al., 2005) and affects sleep quality and quantity (rat *Rattus norvegicus*:
60 Abou-Ismaïl et al., 2008; great tit *Parus major*: Raap, Pinxten, & Eens, 2016). It also induces a
61 chronic stress response, which can result in brain damage and depressive states (rat: Abou-

62 Ismail et al., 2008; rat *Rattus norvegicus*: Tapia-Osorio et al., 2013). Recently, a preliminary
63 investigation of dogs associated sleep with light levels, showing variation in sleeping time and
64 sleeping posture under different levels of illumination (Fukuzawa and Nakazato, 2015).
65 Therefore, they are an interesting model to investigate the effects of the environment on
66 sleep and the possible consequences of sleep disruption on their welfare.

67 Some recent studies demonstrated that urban noise causes sleep restriction and sleep
68 fragmentation in birds, despite this the effects of noise disturbances on other non-human
69 animal groups needs further investigation (Grunst et al., 2021). Indeed, noise exposure at
70 night causes the individual to take longer to fall sleep, causes sleep fragmentation and
71 increases stress responses, which can lead to cardiovascular diseases (as reviewed for various
72 animal species e.g. Hume et al., 2012; Münzel et al., 2014; Muzet, 2007). Nonetheless, studies
73 in animal models have shown several deleterious effects of noise on animals' health, and in a
74 number of species, cortisol production increases due to noise exposure to a level that causes
75 a negative welfare response (as reviewed by Kight & Swaddle, 2011). Hence, understanding
76 how sound can affect sleep is of interest, especially as sound pollution is considered the
77 second largest environmental cause of health problems in humans (World Health
78 Organization, 2011).

79 Temperature is another external condition that can shift sleeping patterns. For
80 example, non-REM (Rapid-eye movement) sleep is associated with body cooling and warm
81 comfort during sleep (e.g., a warm bed or a nest), while sleeping in very low temperatures
82 will cause sleep debt (the cumulative effects of partial or total sleep deprivation; for example,
83 the quiescent state of hibernating mammals that are not truly sleeping but dealing with very
84 low temperatures) (Harding et al., 2019). However, while warmth may induce sleep, only the
85 warming of skin temperature can shorten sleep latency and promote non-REM sleep.
86 Research has shown that insomniac humans have elevated core temperature, signalling that
87 elevated metabolic rates producing heat in association with warmer environments could
88 cause sleep disruption (Caddick et al., 2018; Harding et al., 2019). The same phenomenon is
89 observed in other animal species. For instance, birds have elevated sleep fragmentation in
90 higher temperatures (Stuber et al., 2017, 2015). Also, mammals living in habitats with
91 extreme temperatures, such as deserts, shift their sleeping patterns seasonally, sleeping less
92 during summer (e.g., *Oryx leucoryx*, Davimes et al., 2018).

93 Altogether, these environmental conditions can interfere with sleep, either by directly
94 affecting it, or by producing stress responses that interfere with sleep quality. Stress itself is
95 known to be the main cause of sleep disruption and insomnia in humans (Jun and Polotsky,
96 2016; Van Reeth et al., 2000). The secretion of cortisol (i.e., a stress hormone) is also
97 modulated by circadian rhythms, and it has an alternating cycle with melatonin, the sleep
98 hormone (Buckley and Schatzberg, 2005). Stressful events lead to the production of cortisol
99 to levels that will inhibit sleep, causing delays in sleep onset and increasing waking episodes
100 during sleep cycles. In turn, sleep problems including insomnia and obstructive sleep apnoea
101 can further increase – hypothalamic-pituitary–adrenal axis dysfunction (Buckley and
102 Schatzberg, 2005; Jun and Polotsky, 2016; Sadeh et al., 2004; Van Reeth et al., 2000). Sleep
103 disruption can also be considered a stressor by itself, since lack of sleep compromises an
104 individual’s homeostasis by overloading the adrenal function, suppressing immunity and can
105 be responsible for depressive states (inability to cope with stressors; Orzeł-Gryglewska, 2010;
106 Tobaldini et al., 2016). As chronic stress is also a main source of behavioural and physiological
107 disturbances in most captive species (Brando and Buchanan-Smith, 2018; Wolfensohn et al.,
108 2018), understanding the relationship between sleep and chronic stress could be a relevant
109 tool to evaluate animal welfare.

110 Dogs have a diurnal circadian rhythm with polyphasic sleep, meaning the peak of
111 activity occurs during the day and bulk of sleep is concentrated at night, although sleep bouts
112 are also present during the day (Bódizs et al., 2020; Zanghi, 2010). Furthermore, sleep can be
113 associated with biological factors, such as age, sex, and level of activity (Bunford et al., 2018;
114 lotchev et al., 2019; Zanghi et al., 2013), and also with environmental factors such as feeding
115 times, social interactions, positive and negative affective experiences, and their owner’s
116 routine (Dow et al., 2009; Duranton and Gaunet, 2018; Kis et al., 2017; Zanghi et al., 2012).
117 As follows, the aim of this study was to investigate whether environmental conditions affect
118 dogs’ sleeping patterns and behaviours in controlled housing. Furthermore, we assessed
119 whether environmental variables increase stress responses, which could further compromise
120 sleep and other behaviours. As deliberately disturbing the dogs’ sleeping cycles would be
121 considered highly unethical, this study uses an observational approach to investigate the
122 associations of environmental conditions and cortisol levels with changes in the sleep
123 structure of studied dogs (see Owczarczak-Garstecka and Burman, 2016 for a similar
124 approach).

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2. Methods

2.1 Ethical statement

Ethical approval was granted by the Science & Technology Research Ethics Panel of the University of Salford Manchester (STR1617-80) and by the Animal Ethics Committee of the Federal University of Ouro Preto, Minas Gerais, Brazil (Protocol 2017/04). Dogs' husbandry routines follow the National Animal experimentation Control Council, Normative Resolution nº12 (CONCEA, 2013). No changes to the dogs' routine or environment were made for the purpose of this study.

2.2 Study site and subjects

For this study, thirteen mixed-breed adult dogs (seven males and six females; 5.9 ± 1.8 years old, mean \pm SD; range: 1.5 years - 7 years old) were randomly selected from the kennel's population at the Centre for the Animal Science, in the Federal University of Ouro Preto, state of Minas Gerais, Brazil. Dogs were kept in same sex pairs and housed in outdoors kennels, which had a rectangular layout (5.8m x 1.6m x 1.65m) with bare concrete flooring and walls (the front wall had a gate, but all the other walls were bare concrete). The kennels had one-third of the space covered to provide shelter. The female section also had a small backroom, which acted as a birthing den, if the females were selected for breeding. Kennels had only natural lighting (average 12hr-12hr light cycle), ambient temperature (average 18°C throughout the year), and no isolation from external noise.

The dogs had access to water and food *ad libitum* which was replenished as necessary. Kennels were cleaned twice a day. Dogs were not walked, instead they had play/exercise same-sex group sessions every day, varying from 30 to 60 minutes. Contact with the opposite sex was not allowed to avoid unnecessary breeding. Dogs were bred and kept in the same kennels since birth at the facility (have not lived elsewhere), were clinically healthy, with no signs of behavioural problems and have never been used in any prior research that may have had an impact on their physiology and/or behaviour. Throughout the duration of our study, dogs did not participate in any other research, nor were they used for breeding.

2.3 Data collection

157 **2.3.1 Sleeping patterns and general behaviour**

158 From October 2017 to May 2018, dogs' behaviour was weekly monitored for 24
159 hours/day using CCTV cameras with night vision capability (Swann SWDVK-845504; two per
160 kennel to ensure full coverage of the area). Observations started on Monday morning and
161 ended on the morning of the subsequent Saturday, generating sleep and behavioural data for
162 five consecutive days and nights for each dog. Due to equipment restrictions (number of
163 simultaneous recordings supported by the DVR) not all dogs were assessed in the same week,
164 but pairs were always assessed together. The recording schedule ensured that every dog pair
165 had a whole week of assessment during a period of four to six weeks (i.e., in the same season)
166 to prevent bias due to acute differences in light and temperature. In any given week at least
167 four pairs of dogs were being accessed and no pair of dogs were monitored alone (i.e., without
168 other pairs being recorded simultaneously in a week).

169 In total, 130 days/nights of observations were used for data analysis. Each kennel was
170 assessed four times during the assessment period, meaning each dog had the equivalent of a
171 working month (20 days and nights) of observations. It is important to clarify that although
172 dogs may have been assessed in non-consecutive weeks, the five-day period was always
173 consecutive. If, for some reason, data collection was disrupted that week of sampling was
174 discarded, and a new assessment period began in the following Monday.

175 Data collection was separated in two time periods: Diurnal (07:00-17:59) and
176 nocturnal (18:00 – 06:59), based on the kennels normal husbandry routine. For the diurnal
177 observations, behaviour was registered using focal sampling with instantaneous recordings
178 of behaviour at a 30-second interval (Martin and Bateson, 2007) for 15 randomized minutes
179 within each hour of assessment. Behaviour was coded using an ethogram (Supplementary
180 Table S1) developed using the scientific literature (Broom and Fraser, 2015; Luescher et al.,
181 1991). In addition, every time the dogs slept, the duration and number of sleeping bouts were
182 recorded. For both nocturnal and diurnal observations, a dog was considered as being asleep
183 if eyes were closed and no movement was observed for two consecutive minutes; from that
184 point the duration of sleep was recorded. Every time the dog woke after the two minutes
185 have passed and went back to sleep (following the two-minute rule) this was considered as a
186 sleeping bout. The duration of wakefulness between bouts was only recorded during the
187 night, for the day, the behaviour was coded following the diurnal sampling rule.

188 For the nocturnal period, behaviours were registered using focal sampling with
189 continuous recordings to provide the full duration of behaviours assessed (Martin and
190 Bateson, 2007). However, due to the time-consuming nature of the method, behaviours were
191 classified in broad categories: sleeping, resting, or activity. Similarly, to the diurnal
192 observations, we also recorded the number of sleeping bouts at night. The data were coded
193 using the software Boris v.7.0.12 (Friard and Gamba, 2016).

194

195 **2.3.2 Analysis of faecal cortisol metabolites**

196 Adrenocortical activity was assessed non-invasively by measuring faecal cortisol
197 metabolites (FCM; Palme, 2019) . Fresh faecal samples were collected each morning in every
198 week of behavioural data collection (five samples/week, totalling 20 samples per dog for the
199 entire period). Morning collection ensured that quantity of FCM present would reflect
200 adrenocortical activity of the previous day (Palme, 2005, Schatz and Palme, 2001). To identify
201 the faeces of paired dogs, a non-toxic blue food colouring (Mix Alimentos) was added to wet-
202 food and fed to one of the dogs of the pair in the afternoon prior to the collection (Fuller et
203 al., 2011).

204 The extraction of FCM followed the protocol proposed by Palme et al. (2013). From
205 the original samples, 0.5 g of defrosted faecal matter were added to 5 ml of a premixed
206 solution of 80% methanol, then shaken using a hand-vortex for two minutes and centrifuged
207 (15 min; 2500 g). After this procedure, 1 ml of the supernatant was transferred to a 1.5ml
208 Eppendorf tube, lyophilized and then sent to the Unit of Physiology, Pathophysiology and
209 Experimental Endocrinology of the University of Veterinary Medicine, in Vienna, Austria for
210 further analysis. FCM were measured with a cortisol enzyme immunoassay, previously
211 validated for dogs (Schatz and Palme, 2001). For details of the assay, including cross-reactions
212 see Palme and Mostl (1997).

213

214 **2.3.3 Environmental conditions**

215 Sound pressure levels were collected manually using a digital sound level meter
216 (Minipa MSL- 1355). Sound was recorded twice per day for 15 minutes, once in the morning
217 and once in the afternoon. Sampling times were decided using a Latin square design and
218 encompassed all possible hours within the daily observation period throughout the study.
219 Within the 15 minutes, one sound point was recorded per second, totalling 1800 sound

220 pressure points per day. Due to the university campus regulations and equipment restrictions,
221 it was not possible to visit the site at night to collect sound data. Hence, the data used to
222 assess the influence of sound on nocturnal behaviours was an average of the sound points
223 collected during the day; this would be a reflection of the sound profile the dogs experienced
224 in the kennels and that could potentially contribute to cumulative stress responses leading to
225 disruptive sleep at night (Hewison et al., 2014; Kawada, 2011).

226 As the kennels did not have any artificial lighting, the effect of luminosity in the sleep
227 behaviour was assessed using data of sunset and sunrise timing (the exact hour of sunrise and
228 sunset converted to seconds), and day length (total duration of day light between sunrise and
229 sunset in seconds) retrieved from the Time and Date AS database (© Time and Date AS 1995-
230 2019, Norway). Similarly, the average temperature for the studied days and nights was
231 retrieved from the National Institute of Spatial Research of Brazil website (CPTEC/INPE, Brazil;
232 meteorological station located 30 km from the kennels).

233

234 **2.3.5 Statistical analysis**

235 All data were tested for normality using the Anderson-Darling test. Descriptive metrics
236 were calculated for behaviours, environmental conditions and FCM levels and results were
237 reported as either counts or percentages with standard deviation.

238 To determine inter-observer reliability during behavioural sampling, the Kendall's
239 coefficient of concordance (W) was used. The coefficient value can range from 0 to 1. The
240 stronger the agreement, the higher Kendall's value (Kendall and Smith, 1939). For this study,
241 a similarity of at least 95% was used as the standard observers had to achieve.

242 Comparisons of sleep between day and night and variation on FCM levels among
243 sampled days, individuals and age were analysed using Kruskal-Wallis (H) with Dunn's *post-*
244 *hoc* tests. Variation of sound level throughout the day was analysed using a Mann-Whitney
245 test (U). Furthermore, the relationship between specific days and environmental metrics was
246 explored using the Spearman's rank correlation test (r^2).

247 To test the effect of environmental conditions and FCM (explanatory variables) on
248 dogs' sleeping patterns and behaviours (response variables) we used generalized linear
249 models of mixed effects (GLMMs, *lmer* function for data with normal distribution and *glmer*
250 for non-normal data, both with *lme4* package in R, Bates et al., 2015). Variables' effects were
251 determined by model comparison and backward selection, until a minimum suitable model

252 was reached, and significant levels between variables were then found using pair-wise
253 comparison (Crawley, 2007). Due to the repeated measurements of kennels during studied
254 days, sampling day was considered a random effect varying in the intercept (1 | day) (Bates et
255 al., 2015). As some variables included in the models had different metrics (e.g. duration vs
256 percentage), data were scaled to avoid over dispersion of residuals (Becker et al., 1988). All
257 models were submitted to residual analysis.

258 Data analysis was performed in RStudio (Team, 2019). Data generated and analysed
259 in this study are available at Mendeley Data website (doi: 10.17632/7nnwc3f3kv.1).

260

261 **3. RESULTS**

262 **3.1 Characteristics of dogs' sleep and behaviour**

263 Overall, sleep was the most frequent behaviour expressed at night (72%, $H = 177.5$,
264 $p < 0.001$, $df = 2$) and dogs slept an average of 6.1 ± 3.9 hours (mean \pm SD), with 10.8 ± 7.01
265 bouts per night.

266 During the day, inactivity ("Sitting Inactive", "Standing inactive" and "Laying down
267 inactive") with an average of 17370 records per individual ($57.8\% \pm 7.9\%$ of total
268 observations), was the most expressed diurnal behavioural category when compared to the
269 other behaviours ($H = 1844$, $p < 0.0001$, $df = 2$). The second most expressed diurnal behaviour
270 was locomotion (12.2%), followed by sleep (6%) and exploring (5.2%). All other behaviours
271 accounted for less than 5% of the total records. Sleeping time ($U = 5.65$, $df = 1$, $p < 0.001$), and
272 the number of sleeping bouts ($U = 31.39$, $df = 1$, $p < 0.001$) were also significantly different
273 between day and night, with sleep being most expressed at night. Mean sleeping duration
274 during the day was 4 ± 7 minutes, with less than one bout per day (0.14 ± 0.43).

275

276 **3.2 Faecal cortisol metabolites (FCM)**

277 FCM levels varied between males and females (Figure 1), with males having higher
278 concentrations than females (GLMM; $t = 0.682$, $p = 0.049$, Family = Gaussian); age did not
279 affect FCM levels ($p > 0.05$). No significant differences among individuals were found ($p > 0.05$).

280 _____ Insert Figure 1 here _____

281

282

283 **3.3 Characteristics of dogs' environment**

284 **3.3.1 Sound**

285 The sound in the kennels ranged from 46.1 dB to 75.3 dB, with an average of $63.9 \pm$
286 7.0 decibels and with mornings being louder than afternoons ($U = 160200$, $p < 0.0001$) (Figure
287 2). A Kruskal-Wallis test indicated the average levels of sound were significantly different
288 among the days of the week ($p < 0.05$). Some days (i.e., Tuesdays and Wednesdays) presented
289 many outlier values in their residuals and were removed from the analysis. Significant results
290 were still found after removing outliers ($H = 2.95$, $df = 3$, $p < 0.05$) and the post-hoc test
291 showed Thursdays to be the loudest days at the kennels with a mean value of 64.8 ± 5.9
292 decibels.

293 _____-Insert Figure 2 here_____

294

295 **3.3.2 Light**

296 Luminous emittance in the kennels followed natural light/dark cycles. Throughout the
297 data collection, the average day had $12\text{h}46\text{min} \pm 00\text{h}36\text{min}$ of light, sunrise occurred at
298 $05:18\text{h}$ and sunset at $18:05\text{h}$. The difference between the longest day and the shortest day
299 was $02:14:16$ hours, which meant an increase of 16.7% of daylight time.

300

301 **3.3.3 Temperature**

302 Temperature varied between 14.8°C and 25°C during the experimental period. A
303 mean variation of six degrees occurred between the analysed months, but temperature
304 changes were observed mostly at the beginning and at the end of the day. Average
305 temperature at midday was $19.9^\circ\text{C} \pm 1.12^\circ\text{C}$.

306

307 **3.4 Effects of environment on sleep and other dogs' behaviours**

308 **3.4.1 Sound and faecal cortisol metabolites (FCM)**

309 The variation in sound levels and FCM did not influence the duration of sleep at night
310 and the number of sleeping bouts (GLMM results; $p > 0.05$). However, both variables
311 influenced sleep and other behaviours during the day. An increase in stress responses was
312 associated with the dogs sleeping less during the day and by becoming more alert. It was also
313 associated with decreased time spent eating, drinking, playing, performing maintenance and
314 overall locomotion; glucocorticoid levels were positively correlated to dogs' vocalizations
315 (Table 1).

316 A noisier environment was associated with the dogs spending less time laying down
317 or sitting inactive, while increasing time spent standing and in locomotion. It was also
318 significantly associated with decreased expression of drinking, eating, exploring, and
319 performing maintenance behaviours (Table 1).

320 _____-Insert Table 1 here_____

321 Furthermore, noisier days were associated with increased FCM levels on the following
322 morning (N= 5, $r_s = 0.366$, $p = 0.026$, Figure 3). Females and males were affected differently
323 by sound levels. For females, their FCM levels remained constant during the week (N=70 $r_s =$
324 0.038 , $p = 0.775$); whereas for males, noisier days preceded an increase in FCM (N = 74, $r_s =$
325 0.366 , $p = 0.026$).

326 _____-Insert Figure 3 here_____

327 **3.4.2 Light**

328 Variation of light had a direct effect on the dogs' sleeping patterns. Increased day
329 length was associated with increasing number of sleeping bouts in the dogs at night, while
330 later sunsets diminished the number of bouts at night (our data showed that later sunsets
331 were not related to increased day length; days with the latest sunsets had also latest sunrises,
332 see Table 2). No effect was found for sunrise. Latency to first sleep did not change with any
333 of the measured variables, but the awakening time of the dogs did vary with sunrise and
334 sunset. Dogs woke up closer to the kennel staff starting time with later sunsets and later
335 sunrises (Table 2). Similarly, FCM levels of the dogs varied according to day length: a longer
336 day was associated with lower FCM concentrations (Table 2).

337

338 _____-Insert Table 2 here_____

339 **3.4.3 Temperature**

340 The only effect of temperature found was for the duration of sleep, which decreased
341 when the average daily temperature increased, as shown by a linear regression ($r^2 = 0.13$, $p <$
342 0.05 , Figure 4).

343 _____-Insert Figure 4 here_____

344

345 **4. Discussion**

346 We found that environmental factors influenced sleep in dogs in many ways. An
347 increase in environmental noise decreased sleeping during day, but not during the night. An

348 increase in faecal cortisol metabolites (FCM) was associated with less sleep during the day.
349 Furthermore, longer daylengths increased sleeping bouts at night, and an increase in
350 temperature decreased sleeping duration at night.

351 The sleeping cycle of the observed dogs was very different from what has been found
352 in the literature. The studied dogs slept less during the night than shelter dogs (44% less;
353 Owczarczak-Garstecka and Burman, 2016) and laboratory dogs (50% less; Zanghi et al., 2012).
354 Additionally, patterns of sleep fragmentation were unusual, with an average of 10 bouts of
355 sleep per night compared to the 57-66 bouts (Zanghi et al., 2013) and 33 bouts (Takeuchi and
356 Harada, 2002) found in other studies. Sleep during the day was rarely observed in the present
357 study, despite the fact that daytime sleep is considered a normal behaviour for kennelled
358 dogs, accounting to up to 40% of their daily activities (Takeuchi and Harada, 2002). These
359 differences in sleep structure were responsible for modifications in the dogs' activities
360 patterns, and were also affected by factors such as sex and age; results that have been broadly
361 discussed in a previous study (see Schork et al., 2022).

362 An altered sleep structure indicates a failure to maintain a regular circadian rhythm,
363 which can further generate stress responses. Previous studies have shown that disruption of
364 the sleeping cycles caused by external factors such as light, impairs organisms' circadian
365 rhythms (Tapia-Osorio et al., 2013; Touitou et al., 2017). Moreover, stress responses also
366 disrupt the normal cycles and further compromise sleep quality and quantity (Abou-Ismael et
367 al., 2008; Tahara et al., 2015). A cumulative effect of disrupted sleeping nights associated with
368 the lack of daily sleep and the associated increase in FCM secretion could cause a decrease in
369 our dogs' welfare. This is further corroborated by the presence of high inactivity during the
370 day, as this behaviour is used as a common indication of maladaptation to the environment
371 and indicates poor welfare (Fureix & Meagher, 2015; Meagher et al., 2013). Also, the higher
372 inactivity during the day could be related to an environment poor in stimuli (Sampaio et al.,
373 2019).

374 Noise was the environmental component that most affected the dogs' behaviour and
375 probably wellbeing in our study. Noise sensitivity and noise phobia are recurrent behavioural
376 problems in dogs and as previously reported, dogs produced stress responses to acute loud
377 noise exposure (Ballamwar et al., 2008; Beerda et al., 1997; Sherman and Mills, 2008;
378 Storengen and Lingaas, 2015). Similarly, noise exposure causes several deleterious effects for
379 dogs in kennels (Polgár et al., 2019), and increases cortisol secretion triggering abnormal

380 behaviours, aggression, reduces the immune response, and induces apathy (Protopopova,
381 2016).

382 The sound in our kennels ranged from 46.1 dB to 75.3 dB and the increase of sound
383 above 70 decibels were usually observed when the dogs were barking in situations of high
384 emotional arousal, such as when the staff arrived to clean the kennels and feed the dogs
385 (characterizing events of acute and loud noise). Barking sometimes can reach to up 100 dB
386 (Sales et al., 1997), and indeed, shelters/kennels are considered one of the loudest
387 environments for dogs to be housed (Coppola et al., 2006). In these locations, noise produced
388 by visitors, husbandry procedures (cleaning and renovations nearby the studied kennels), and
389 barking are responsible for increasing the levels of cortisol, the expression of abnormal
390 behaviours, compromising the immune function and the auditory perception in individuals
391 (Kogan et al., 2012; Protopopova, 2016; Taylor & Mills, 2007).

392 At the studied kennels, the environmental noise produced by daily routine and people
393 was characterised as being constant and loud and our results corroborate the deleterious
394 effects of noise: dogs became more restless, reduced eating and drinking, slept less during
395 the day, and decreased their maintenance behaviour. These behavioural changes in
396 association with the observed cortisol levels could be indicating that dogs were experiencing
397 chronic stress (Beerda et al., 1997; Part et al., 2014).

398 The presence of constant barking and other noises during the day were probably
399 interfering with the dogs' resting. This result was associated with an increased accumulated
400 stress response, as FCM peaks were observed on Fridays following the noisiest day of the
401 week. This cause-effect relationship should be considered with caution because dogs could
402 be awake and stressed because of the noise or they could be barking because they were
403 stressed, increasing noise. However, the idea of noise causing sleep disruption at day and a
404 consequent cascade effect of increased stress leading to sleep disruption at night, seems very
405 plausible with the overall results of the present study.

406 We also found different effects for males and female dogs, with male dogs having
407 higher FCM levels. Research is still needed to conclude if female and male dogs do express a
408 different stress response. Though some studies have not found an association between
409 cortisol and sex (e.g. Part et al., 2014; Stephen and Ledger, 2006), others point to an increased
410 susceptibility for females when spatial restriction and social isolation are present (Beerda et
411 al., 1999). The females in our study had larger kennel than the males and had a back room,

412 which provided full isolation of the external area and both characteristics could be
413 contributing to lower FCM levels when compared to the males. Quality of the environment is
414 considered a main criterion for the welfare of dogs housed in kennel environments (Beerda
415 et al., 2000; Hubrecht et al., 1992) and space restriction is a known cause of chronic stress
416 (Beerda et al., 1999). Furthermore, dogs in our study have restricted social contact and
417 physical activity, which also are factors that decrease dogs' welfare and increase stress
418 responses (Dalla Villa et al., 2013; Normando et al., 2014).

419 Both light and temperature were also factors that promoted changes in the stress
420 responses of the dogs in this study and their sleeping cycles. The mammal circadian clock
421 mediates behaviour and physiology in response to variation in light levels, which also
422 regulates cycles of sleep and activity (Adan et al., 2012; Randler, 2014). It is predicted that
423 environments that are not affected by artificial lights at night would be more suitable for
424 animals, as their sleeping patterns would follow the natural light cycle. However, as sleep is
425 also regulated homeostatically, sleep timing and duration are also dependent on prior
426 sleeping cycles (Friedman et al., 1978; Vyazovskiy and Tobler, 2012). When individuals have
427 normal sleeping patterns disturbed in response to variation in light level, sleep deprivation
428 motivates the body to seek rest in a different time of the day, and when this is not possible,
429 as it is the case for the studied dogs, it will disrupt their circadian rhythms (Boivin & Boudreau,
430 2014; Nunez et al., 2018).

431 Although the studied kennels did not have any source of artificial light, we still found
432 an effect of duration of the day and sunset times in the dogs. The extended light hours in
433 most of the months caused the dogs to sleep less for most of the time studied. Similarly, the
434 same was observed for days where the sunset occurred later (days with later sunsets were
435 not days with the highest lengths). This response could indicate a physiological adjustment
436 since dogs were exposed only to a natural light cycle. Thus far, most studies verifying the
437 effect of light on sleeping patterns are based on the presence of artificial light at night. A
438 recent study found that a nocturnal species of rodent, when exposed to a variation of lights
439 in normal light/dark cycles, also changed their sleeping patterns according to light colour, due
440 to their perception of some wave lengths as day light (de Oliveira et al., 2019). In the present
441 study, no variation in the light/dark cycle was applied. Hence, it is possible that natural light
442 can affect behaviour in ways that are not predictable with normal activity cycles and the
443 matter needs to be further explored.

444 An impact was also found for temperature, meaning that dogs slept less when
445 temperatures were higher. In endotherms (including humans), sleeping patterns can be
446 disturbed when individuals are exposed to temperatures exceeding an individual's
447 thermoneutral zone. A negative influence of the high temperatures on sleep was also
448 observed in humans (Okamoto-Mizuno & Mizuno, 2012; Zheng et al., 2019), in mice (Jhaveri
449 et al., 2007), and in the Javan slow loris (*Nycticebus javanicus*) (Reinhardt et al., 2019). The
450 combination of shifts in seasonal temperature and light levels has been shown to cause major
451 behavioural changes in these species, disrupting especially NREM sleep (Harding et al., 2019).

452 The layout of the outdoor kennels allowed the animals to have exposure to weather
453 conditions. This meant that sunlight reached most of the space, increasing light exposure and
454 temperature, but it also meant that on colder days, or when during rainy days, the
455 temperature dropped significantly. Although our analysis did not indicate that males and
456 females were affected differently by temperature, it is possible that the increased stress
457 responses in males may also be related to the difference in the kennel layout, as females were
458 provided with better shelter conditions (i.e., denning room at the rear).

459 The environmental variables present at the kennels could be contributing to producing
460 a situation of chronic stress. Loud noises in the kennel, associated with longer and hotter
461 days, disrupted dogs' sleeping cycles, decreasing the quantity and quality of sleeping during
462 the day and night, reflecting on the levels of FCM, which increased through the studied weeks.
463 In this way, the cumulative effects of this disrupted sleeping cycles, that do not permit
464 recovery of body functions, possibly resulted in chronic stress.

465 However, we did not observe the expression of abnormal behaviours, instead, the
466 dogs remained mainly inactive, which could be evidence of learned helplessness, which has
467 been associated with poor affective states (Fureix and Meagher, 2015). Past studies of
468 kennelled dogs found that inactivity is connected to increased cortisol levels (Hiby et al.,
469 2006). Conversely, increased cortisol levels do not always change behavioural patterns in dogs
470 (Beerda et al., 2000; Hennessy et al., 2001; Rooney et al., 2007), hence the importance of
471 using different investigative techniques to understand the effects that kennelling has on dogs.
472 Although we have not been able to link the fluctuation of FCM levels with the expressions of
473 abnormal behaviours - a clear indication of failure to adapt to conditions present - there is an
474 agreement among animal welfare researchers that dogs which remain a long time in

475 kennelled environments are more prone to develop chronic stress and reduced welfare (as
476 reviewed in Polgár et al., 2019).

477 Overall, the sleep and the behaviour of the dogs appeared to be affected by the
478 different environmental components analysed. Sound remains one of the most important
479 factors that can negatively impact dogs' welfare in kennels, but light and temperature also
480 play an important part. Aligning their effects with defective kennel structures (i.e., lack of
481 shelter or poor insulation) influenced dogs' behaviour and generated stress responses, which
482 further compromised dogs' sleep. Further studies are still necessary to better comprehend
483 the full effect that long-term kennelling/chronic stress may have on dogs' sleep. Nonetheless,
484 our investigation presents evidence that sleep is an important measure to be evaluated when
485 fully assessing the welfare of dogs and that should be taken into consideration when devising
486 management guidelines for dogs in kennels.

487

488 **5. Conclusion**

489 In conclusion, our study shows that surrounding environmental conditions affected
490 sleeping behaviour, stress responses and general welfare of the studied dogs. These findings
491 suggest that improvements in kennels are needed to try to mitigate such issues. The
492 construction of barriers to block street noise and kennels with comfortable light and
493 temperature levels are suggested. These environmental modifications could contribute to
494 improve the sleep quality of the dogs and, consequently, their welfare.

495

496 **6. Funding**

497 PhD Scholarship and research funding provided to IGS by CNPq (Conselho Nacional de
498 Desenvolvimento Científico e Tecnológico) process nº 202351/2015-7.

499

500 **7. Acknowledgements**

501 Authors express their gratitude to Hugo Costa, DVM; Chief Veterinarian of the CCA Kennels
502 and his staff for the constant support during data collection. Authors would like to thank
503 Roberta Santos and Lucas France Sol for their aid on the early stages of data
504 collection. Authors express their gratitude to Edith Klobetz-Rassam for performing the FCM
505 analysis. FVC thanks CAPES for research scholarship funding.

506

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772 **Table 1. GLMM results for the optimal models describing the effects of environmental**
 773 **conditions on different behaviours in kennelled laboratory dogs. Error distribution of each**
 774 **response variable is indicated inside parenthesis. FCM – faecal cortisol metabolites**

Behaviour	Response	Estimate ± SE	Coefficient value	p
Alert (Poisson)	FCM	-0.0263 ± 0.0126	-2.081	0.037*
	Decibels	0.0049 ± 0.0079	0.630	ns
Drinking (Poisson)	FCM	-0.1853 ± 0.0641	-2.890	0.003**
	Decibels	-0.0595 ± 0.0236	-2.522	0.011*
Eating (Poisson)	FCM	-0.0333 ± 0.0095	-3.500	4.65e-03***
	Decibels	-0.0337 ± 0.0099	-3.382	7.20e-03***
Laydown (Poisson)	Decibels	-0.0372 ± 0.0023	-15.543	2.16e-16***
Locomotion (Poisson)	FCM	-0.0226 ± 0.0045	-5.017	5.24e-07***
	Decibels	0.0161 ± 0.0036	4.469	7.85e-06***
Maintenance (Poisson)	FCM	-0.0356 ± 0.0105	-3.386	7.09e-03***
	Decibels	-0.0367 ± 0.0072	-5.100	3.39e-07***
Play (Poisson)	FCM	-0.0807 ± 0.0427	-1.886	0.059*
Sleeping (day) (Poisson)	FCM	-0.0226 ± 0.0070	-3.192	0.001**
Standing (Poisson)	FCM	-0.0089 ± 0.0039	-2.276	0.022*
	Decibels	0.0205 ± 0.0032	6.265	3.37e-10***
Vocalizing (Poisson)	FCM	0.0261 ± 0.0122	2.627	0.008**

775 *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

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780 **Table 1. GLMM results for the optimal models describing the effects of environmental**
 781 **conditions on sleep related behaviours and physiological components in kennelled**
 782 **laboratory dogs. FCM – faecal cortisol metabolites**

Condition	Independent variable	Estimate ± SE	Coefficient value	p
Sunrise (Poisson)	Sleep bouts	-0.0529 ± 0.0099	-5.333	9.68e-08***
	Last bout	-0.5644 ± 0.1225	-4.606	4.10e-06***
Sunset (Poisson)	Sleep bouts	-1.2503 ± 0.2311	-5.410	6.29e-08***
	Last bout	-0.2728 ± 0.1168	-2.333	1.96-02**
Day length (Poisson)	Sleep bouts	1.0414 ± 0.2319	4.491	7.39e-06***
	FCM	-2.8143 ± 0.4165	-6.756	2.19 e-06***

783 *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

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