



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

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## ABSTRACT

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

## 1. Introduction

The behavioural and stress responses of animals to environmental conditions such as the levels of temperature, light and sound are the result of natural selection and directed towards maintaining the individual's homeostasis (Morgan, 2004). Behavioural activity patterns fluctuate across 24 h (i.e., circadian rhythms), in a process modulated by external triggers (e.g., light levels and temperature), which generate appropriate endogenous responses to ensure adaptation (Adan et al., 2012; Morgan, 2004; Randler, 2014). Similar to the circadian rhythm, sleep also has an adaptive function: being inactive and unconscious is advantageous since sleep helps maintain homeostasis (Mader and

Mader, 2016; Randler, 2014; Roth et al., 2010). Sleeping patterns have mostly been investigated in animals under controlled laboratory conditions, without the full consideration of their ecological value and without considering the importance that different ecological variables may have on sleep quality (Aulsebrook et al., 2016; Tougeron and Abram, 2017). Sleep structure (duration and fragmentation; that is, the time that the animal spends sleeping and the number of times it wakes during sleeping) is, thus, important to be investigated to see how it influences animal homeostasis.

The main external trigger that modulates sleep is light; the presence or absence of not only luminous emittance, but of light with different wave lengths, such as blue light, is a cue to promote sleep onset (Dijk and

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Archer, 2009; Dominoni et al., 2016). However, daylight is not the only environmental trigger that affects sleep; other components such as artificial lighting (Dominoni et al., 2016), sound (Muzet, 2007), temperature (Harding et al., 2019) and night activity (i.e. shift in activity patterns such as remaining active during normal resting hours) (Nunez et al., 2018) can influence the duration and fragmentation of sleep. For instance, nocturnal artificial lights, higher temperatures, greater night activity, and noise pollution have negative impacts on an array of wild species, reducing REM sleep and the total time spent sleeping, increasing the latency to sleep and alertness (Chinoy et al., 2018; Harding et al., 2019; Mendoza, 2021; Shannon et al., 2016; Siegel, 2011).

In captive/laboratory settings artificial light also disrupts the circadian cycle (*mice Mus musculus*: Ishida et al., 2005) and affects sleep quality and quantity (rat *Rattus norvegicus*: Abou-Ismaïl et al., 2008; great tit *Parus major*: Raap, Pinxten, & Eens, 2016). It also induces a chronic stress response, which can result in brain damage and depressive states (rat: Abou-Ismaïl et al., 2008; rat *Rattus norvegicus*: Tapia-Osorio et al., 2013). Recently, a preliminary investigation of dogs associated sleep with light levels, showing variation in sleeping time and sleeping posture under different levels of illumination (Fukuzawa and Nakazato, 2015). Therefore, they are an interesting model to investigate the effects of the environment on sleep and the possible consequences of sleep disruption on their welfare.

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

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

Altogether, these environmental conditions can interfere with sleep, either by directly affecting it, or by producing stress responses that interfere with sleep quality. Stress itself is known to be the main cause of sleep disruption and insomnia in humans (Jun and Polotsky, 2016; Van Reeth et al., 2000). The secretion of cortisol (i.e., a stress hormone) is also modulated by circadian rhythms, and it has an alternating cycle with melatonin, the sleep hormone (Buckley and Schatzberg, 2005). Stressful events lead to the production of cortisol to levels that will inhibit sleep, causing delays in sleep onset and increasing waking episodes during sleep cycles. In turn, sleep problems including insomnia

and obstructive sleep apnoea can further increase – hypothalamic-pituitary–adrenal axis dysfunction (Buckley and Schatzberg, 2005; Jun and Polotsky, 2016; Sadeh et al., 2004; Van Reeth et al., 2000). Sleep disruption can also be considered a stressor by itself, since lack of sleep compromises an individual's homeostasis by overloading the adrenal function, suppressing immunity and can be responsible for depressive states (inability to cope with stressors; Orzeł-Gryglewska, 2010; Tobaldini et al., 2016). As chronic stress is also a main source of behavioural and physiological disturbances in most captive species (Brando and Buchanan-Smith, 2018; Wolfensohn et al., 2018), understanding the relationship between sleep and chronic stress could be a relevant tool to evaluate animal welfare.

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

## 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 n°12 (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;  $5.9 \pm 1.8$  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 (5.8 m x 1.6 m x 1.65 m) 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 min. 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

#### 2.3.1. Sleeping patterns and general behaviour

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

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

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

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

#### 2.3.2. Analysis of faecal cortisol metabolites

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

2011).

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

#### 2.3.3. Environmental conditions

Sound pressure levels were collected manually using a digital sound level meter (Minipa MSL-1355). Sound was recorded twice per day for 15 min, once in the morning and once in the afternoon. Sampling times were decided using a Latin square design and encompassed all possible hours within the daily observation period throughout the study. Within the 15 min, one sound point was recorded per second, totalling 1800 sound pressure points per day. Due to the university campus regulations and equipment restrictions, it was not possible to visit the site at night to collect sound data. Hence, the data used to assess the influence of sound on nocturnal behaviours was an average of the sound points collected during the day; this would be a reflection of the sound profile the dogs experienced in the kennels and that could potentially contribute to cumulative stress responses leading to disruptive sleep at night (Hewison et al., 2014; Kawada, 2011).

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

#### 2.3.4. Statistical analysis

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

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

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

To test the effect of environmental conditions and FCM (explanatory variables) on dogs' sleeping patterns and behaviours (response variables) we used generalised linear models of mixed effects (GLMMs, *lmer* function for data with normal distribution and *glmer* for non-normal data, both with *lme4* package in R, Bates et al., 2015). Variables' effects were determined by model comparison and backward selection, until a minimum suitable model was reached, and significant levels between variables were then found using pair-wise comparison (Crawley, 2007). Due to the repeated measurements of kennels during studied days, sampling day was considered a random effect varying in the intercept (1 | day) (Bates et al., 2015). As some variables included in the models had different metrics (e.g. duration vs percentage), data were

scaled to avoid over dispersion of residuals (Becker et al., 1988). All models were submitted to residual analysis.

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

### 3. Results

#### 3.1. Characteristics of dogs' sleep and behaviour

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

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

#### 3.2. Faecal cortisol metabolites (FCM)

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

#### 3.3. Characteristics of dogs' environment

##### 3.3.1. Sound

The sound in the kennels ranged from 46.1 dB to 75.3 dB, with an

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

##### 3.3.2. Light

Luminous emittance in the kennels followed natural light/dark cycles. Throughout the data collection, the average day had 12h46min  $\pm$  00h36min of light, sunrise occurred at 05:18 h and sunset at 18:05 h. The difference between the longest day and the shortest day was 02:14:16 h, which meant an increase of 16.7 % of daylight time.

##### 3.3.3. Temperature

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

#### 3.4. Effects of environment on sleep and other dogs' behaviours

##### 3.4.1. Sound and faecal cortisol metabolites (FCM)

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

A noisier environment was associated with the dogs spending less time laying down or sitting inactive, while increasing time spent standing and in locomotion. It was also significantly associated with decreased expression of drinking, eating, exploring, and performing

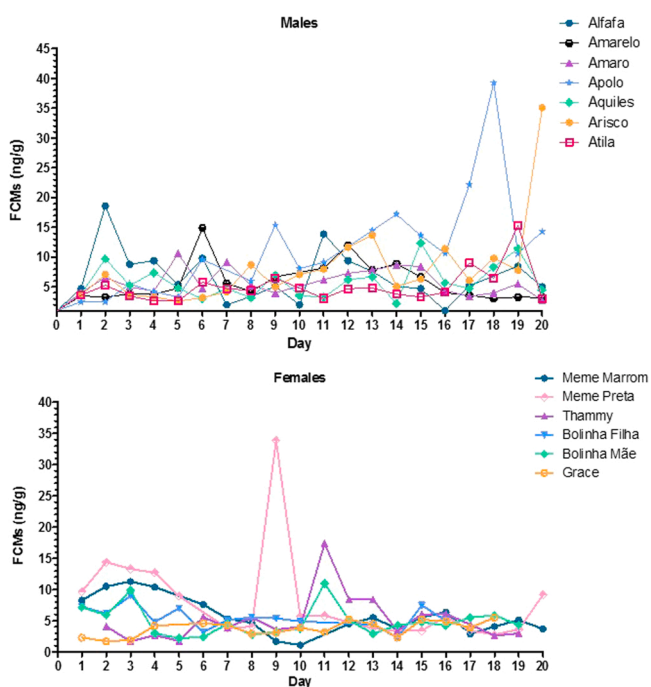


Fig. 1. Variation in individual faecal cortisol metabolites (FCMs) daily concentrations measured in males (A) and females (B) laboratory dogs participating in the study.

Table 1

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

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

\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ .

maintenance behaviours (Table 1).

Furthermore, noisier days were associated with increased FCM levels on the following morning ( $N = 5$ ,  $r_s = 0.366$ ,  $p = 0.026$ , Fig. 2). Females and males were affected differently by sound levels. For females, their FCM levels remained constant during the week ( $N = 70$ ,  $r_s = 0.0038$ ,  $p = 0.775$ ); whereas for males, noisier days preceded an increase in FCM ( $N = 74$ ,  $r_s = 0.366$ ,  $p = 0.026$ ).

### 3.4.2. Light

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

### 3.4.3. Temperature

The only effect of temperature found was for the duration of sleep, which decreased when the average daily temperature increased, as shown by a linear regression ( $r^2 = 0.13$ ,  $p < 0.05$ , Fig. 3).

## 4. Discussion

We found that environmental factors influenced sleep in dogs in many ways. An increase in environmental noise decreased sleeping during day, but not during the night. An increase in faecal cortisol metabolites (FCM) was associated with less sleep during the day. Furthermore, longer daylengths increased sleeping bouts at night, and an increase in temperature decreased sleeping duration at night.

The sleeping cycle of the observed dogs was very different from what has been found in the literature. The studied dogs slept less during the night than shelter dogs (44 % less; Owczarczak-Garstecka and Burman, 2016) and laboratory dogs (50 % less; Zanghi et al., 2012). Additionally, patterns of sleep fragmentation were unusual, with an average of 10 bouts of sleep per night compared to the 57–66 bouts (Zanghi et al., 2013) and 33 bouts (Takeuchi and Harada, 2002) found in other studies. Sleep during the day was rarely observed in the present study, despite

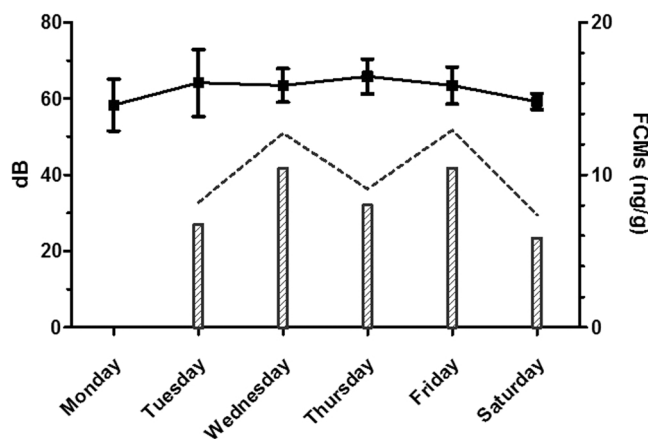


Fig. 2. Variation of sound levels (dB) and faecal cortisol metabolites (FCMs) between different days of the week. Bars indicate mean levels of FCMs, and dashed line shows standard error of the mean. Tick line represents decibels median, whiskers show maximum and minimum values, box represents interquartile ranges.

Table 2

GLMM results for the optimal models describing the effects of environmental conditions on sleep related behaviours and physiological components in kennelled 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	1.96e-02 **
Day length (Poisson)	Sleep bouts	1.0414 ± 0.2319	4.491	7.39e-06 ***
	FCM	-2.8143 ± 0.4165	-6.756	2.19e-06 ***

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

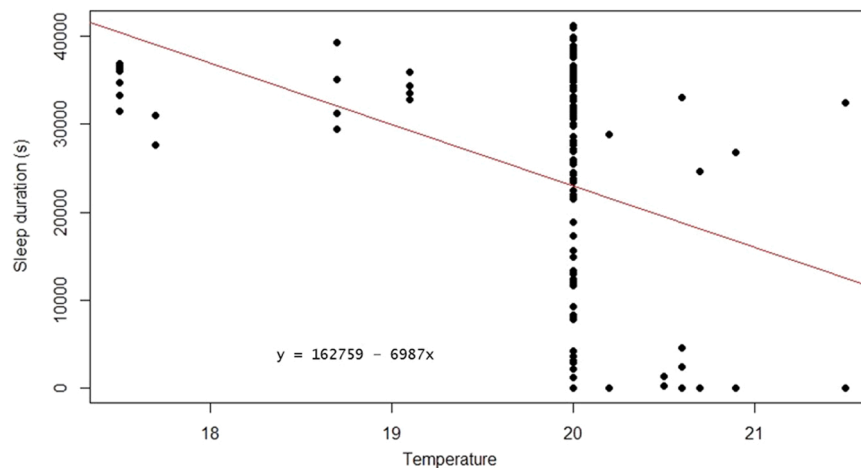
the fact that daytime sleep is considered a normal behaviour for kennelled dogs, accounting to up to 40 % of their daily activities (Takeuchi and Harada, 2002). These differences in sleep structure were responsible for modifications in the dogs' activities patterns, and were also affected by factors such as sex and age; results that have been broadly discussed in a previous study (see Schork et al., 2022).

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

Noise was the environmental component that most affected the dogs' behaviour and probably wellbeing in our study. Noise sensitivity and noise phobia are recurrent behavioural problems in dogs and as previously reported, dogs produced stress responses to acute loud noise exposure (Ballamwar et al., 2008; Beerda et al., 1997; Sherman and Mills, 2008; Storengen and Langaas, 2015). Similarly, noise exposure causes several deleterious effects for dogs in kennels (Polgár et al., 2019), and increases cortisol secretion triggering abnormal behaviours, aggression, reduces the immune response, and induces apathy (Protopopova, 2016).

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

At the studied kennels, the environmental noise produced by daily routine and people was characterised as being constant and loud and our results corroborate the deleterious effects of noise: dogs became more



**Fig. 3.** Sleeping duration in response to variation of temperature. Trend line based on linear regression result: Sleep duration = 162759-6987(temperature),  $r^2 = 0.13$ .

restless, reduced eating and drinking, slept less during the day, and decreased their maintenance behaviour. These behavioural changes in association with the observed cortisol levels could be indicating that dogs were experiencing chronic stress (Beerda et al., 1997; Part et al., 2014).

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

We also found different effects for males and female dogs, with male dogs having higher FCM levels. Research is still needed to conclude if female and male dogs do express a different stress response. Though some studies have not found an association between cortisol and sex (e.g. Part et al., 2014; Stephen and Ledger, 2006), others point to an increased susceptibility for females when spatial restriction and social isolation are present (Beerda et al., 1999). The females in our study had larger kennel than the males and had a back room, which provided full isolation of the external area and both characteristics could be contributing to lower FCM levels when compared to the males. Quality of the environment is considered a main criterion for the welfare of dogs housed in kennel environments (Beerda et al., 2000; Hubrecht et al., 1992) and space restriction is a known cause of chronic stress (Beerda et al., 1999). Furthermore, dogs in our study have restricted social contact and physical activity, which also are factors that decrease dogs' welfare and increase stress responses (Dalla Villa et al., 2013; Normando et al., 2014).

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

studied dogs, it will disrupt their circadian rhythms (Boivin and Boudreau, 2014; Nunez et al., 2018).

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

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

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

The environmental variables present at the kennels could be contributing to producing a situation of chronic stress. Loud noises in the kennel, associated with longer and hotter days, disrupted dogs' sleeping cycles, decreasing the quantity and quality of sleeping during the day and night, reflecting on the levels of FCM, which increased through the studied weeks. In this way, the cumulative effects of this disrupted

sleeping cycles, that do not permit recovery of body functions, possibly resulted in chronic stress.

However, we did not observe the expression of abnormal behaviours, instead, the dogs remained mainly inactive, which could be evidence of learned helplessness, which has been associated with poor affective states (Fureix and Meagher, 2015). Past studies of kennelled dogs found that inactivity is connected to increased cortisol levels (Hiby et al., 2006). Conversely, increased cortisol levels do not always change behavioural patterns in dogs (Beerda et al., 2000; Hennessy et al., 2001; Rooney et al., 2007), hence the importance of using different investigative techniques to understand the effects that kennelling has on dogs. Although we have not been able to link the fluctuation of FCM levels with the expressions of abnormal behaviours - a clear indication of failure to adapt to conditions present - there is an agreement among animal welfare researchers that dogs which remain a long time in kennelled environments are more prone to develop chronic stress and reduced welfare (as reviewed in Polgár et al., 2019).

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

## 5. Conclusion

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

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## CRediT authorship contribution statement

**Ivana Gabriela Schork:** Conceptualization, Methodology, Data analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Isabele Aparecida Manzo:** Methodology, Writing – review & editing. **Marcos Roberto Beiral De Oliveira:** Methodology, Writing – review & editing. **Fernanda Vieira Costa:** Data analysis, Writing – review & editing. **Rupert Palme:** Methodology, Writing – review & editing. **Robert John Young:** Conceptualization, Writing – original draft, Writing – review & editing. **Cristiano Schetini de Azevedo:** Conceptualization, Methodology, Data analysis, Writing – original draft, Writing – review & editing.

## Competing interests

The authors declare no competing interests.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2022.104662.

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