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**Run access, hutch size and time-of-day affect welfare-relevant behaviour and faecal corticosterone in pair-housed pet rabbits**

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## Abstract

Although there exist several studies examining the housing needs of rabbits kept in laboratories and for meat, studies of the requirements of pet rabbits are few and focus entirely on single rabbits. Pet rabbits are recommended to be kept in pairs. We therefore conducted an experimental study to investigate the effects of common hutch sizes and access to an exercise area on the welfare of pairs of pet rabbits.

Twenty ~~pre-established pairs of adult neutered rabbits (one male, one female-pairs)~~ were kept for eight weeks in standardised housing (2 weeks habituation and 6 ~~weeks~~ experimental). Ten pairs were in small wooden hutches (0.73 m<sup>2</sup>) and ten in large (1.86 m<sup>2</sup>). An exercise area measuring 3 x 1m run was attached to each hutch and access was either unlimited or restricted to ~~three hours~~ 3h in the middle of the day. Each pair experienced each ~~exercise level~~ run access for three weeks in a counterbalanced design. We sampled behaviour at dawn, dusk and midday, and took faecal samples for corticosterone analysis at the end of each ~~run~~ access treatment period. ~~. In a subsequent study, ten of the rabbit pairs were given 24h access to the run, and their behaviours recorded.~~

More overall time was spent in locomotion when run access was restricted to 3h ( $F_{1,17}=5.26$ ,  $p=0.035$ ). Regardless of size of hutch, locomotory activity including locomotor play increased significantly when the pairs with restricted access were released into the run. This indicates a ~~behavioural motivational~~ rebound ~~after behavioural restriction~~ demonstrating the rabbits' need to ~~move~~ within each 24h cycle ~~as well as currently improved welfare~~. There was a significant interaction between hutch size and run access on corticosterone levels; ~~corticosterone levels they~~ were raised in the pairs kept in small hutches with restricted run access ( $F_{1,17}= 4.58$ ,  $p=0.047$ ). ~~In a subsequent study, ten of the rabbit pairs (were given 24 h access to the run, and their behaviours recorded~~. The mid-day period was found to be their least active period. Restricting rabbits' opportunity to move ~~around~~ and to get away from each other to times of day when they would not naturally be as active is likely to have contributed to the raised stress hormone levels in the pairs in the smaller hutches. Housing guidelines thus need to highlight the need to allow pairs of pet rabbits

freedom to exercise outside the mid-day period, even if they are kept in hutches larger than common practice. Hutches of commonly reported ~~(standard)~~-sizes of around 0.75 m<sup>2</sup> floor area should not be recommended for ~~pet~~ rabbit pairs, even with access to an exercise area for three hours per day during the middle of the day. ▲

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## Keywords

Spatial restriction, pet rabbits, circadian activity, enclosure size, faecal corticosterone

### 1. Introduction

Most experimental studies on the housing needs of domestic rabbits to date have focussed on rabbits farmed for meat, both the adult breeding stock and the immature offspring ('fattening' rabbits) and laboratory rabbits, largely with a view to informing legislation. Using a range of preference tests where individuals were given choices between solitary and social housing, the studies have revealed the importance of social companionship to domestic rabbits (e.g. Huls et al., 1991, Held et al. 1995; Chu et al. 2004). A considerable body of work now exists on the housing needs of fattening and laboratory rabbits kept in pairs or groups (reviewed by Verga et al. 2007; see also Stauffacher 1992, Morton et al. 1993, Bigler & Oester 1994, Chu et al. 2004; Princz et al. 2008, Buijs et al. 2011). This work has identified as important for socially-housed laboratory and fattening rabbits (i) how much space is provided, and (ii) how that space is designed. These two factors determine whether housed rabbits can be in close physical contact for some behaviours, and avoid contact for others, as they do in the wild (Lockley, 1961).

For pet rabbits, adequate amounts of space and provision of shelter, escape routes and ledges are likely to be as important as they are to other domestic rabbits, given their shared behavioural biology. However, no legislation specifies explicit housing requirements for pet rabbits (Mullan & Main 2006), unlike for laboratory and fattening rabbits. Space provision, cage enrichment and handling practices, vary greatly among pet rabbits whilst they are relatively

standardised in laboratory and fattening rabbits (e.g. Mullan & Main 2006, Schepers et al. 2009, Rooney et al. 2014). Mullan and Main (2006), for example, found that 58% of their 102 pet rabbits surveyed lived in hutches situated in gardens, 32% in hutches in sheds or other outbuildings and only 10% in hutches in the house; hutch size ranged from 0.2 m<sup>2</sup> to 1.33 m<sup>2</sup> floor area (Mullan, pers. comm). In Rooney et al.'s survey (2014) 59 % of 1254 owners reported their pet rabbits as living mainly outdoors, 28% predominantly indoors and 12% in a shed, garage or outbuilding; for 19.5% location varied with season. Median living space provided was 1.8 m<sup>2</sup> including any permanently accessible runs and multiple floors (25<sup>th</sup> percentile=0.9 m<sup>2</sup>, 75<sup>th</sup> percentile = 3.78 m<sup>2</sup>). A total of 41.0% were reported as living in pairs. For 53% of these pairs the main living space were hutches of varying types. Size of the main living accommodation ranged from 0.18 m<sup>2</sup> to 36.84 m<sup>2</sup> with the most common size 1.0 m<sup>2</sup> to 1.2 m<sup>2</sup> floor area (Rooney et al. 2012). Access to an exercise area (run or other) is similarly variable. In Rooney et al.' report (2012), for example, 43% of pet rabbits had access to an exercise area attached to their hutch; 63% had access to an exercise area away from their hutches in the summer, but only 50% in the winter. Slightly over a fifth of pet rabbits in the UK have continual access to a run to exercise throughout the year (Rooney et al. 2012; PDSA 2011). For the remaining 80%, access to an exercise area varied between seasons. In the summer, it was most common for rabbits to have access to a run every day, and for 4-8 hours at a time. In contrast, in winter, rabbits were most commonly reported to have access to a run for occasional sessions only, and for 1-2 hours at a time (Rooney et al. 2012).

Such variability in housing conditions can be reflected in differences in the health and welfare of the pet rabbits surveyed. Issues included dental disease, digestive problems and stereotypies (Mullan & Main 2006, Schepers et al. 2009, Rooney et al. 2014). Our own preliminary analyses of correlations between housing practices and welfare outcomes had suggested as key factors a) hutch size and b) access to an exercise area (including runs) (Rooney et al. 2012). When investigating the spatial requirements of singly-housed pet rabbits Dixon et al. (2010) found increased locomotion following space restriction of singly-housed pet rabbits. When housed individually in floor pens of 0.88 m<sup>2</sup>, 1.68 m<sup>2</sup> and 3.35 m<sup>2</sup>, an activity rebound was observed in the first two hours after rabbits were

moved from the smallest to the largest pen size. This finding was interpreted to indicate that 0.88 m<sup>2</sup> is insufficient for a singly-housed rabbit to adequately express active behaviours and thus compromises welfare (Dixon et al., 2010). They also investigated the effect of pen height and found that rabbits performed more rearing behaviour in pens with no ceiling or high (0.75m) ceilings compared to low ceilings (0.45m); and medium sized rabbits exhibited a rebound effect in hopping, rearing and, alert behaviours when moving from a low ceiling pen to a high, or no ceiling pen (Dixon and Cooper, 2010).

Building on these findings, we here present an experimental study of the effects of hutch size and run access on the welfare of pet rabbits kept in pairs ('Study 1'). We aimed for our sizes and run access to reflect those most commonly provided to pet rabbits in the UK. Variables selected to capture the rabbits' welfare included welfare-relevant behaviours and faecal corticosterone as a measure of stress experienced over a period of time (cf-Buijs et al. 2011a). Some of the variables are welfare-relevant as they are indicators of a rabbit's welfare state; examples would be binkying as a locomotory play behaviour ('frollicking', Held et al. 2001; Buijs et al. 2011<sup>07</sup>) with raised levels indicating current good welfare or a motivational rebound after a period of behavioural restriction (Held & Špinka, 2011; Ahloy-Dallaire et al., 2018); or lying fully stretched-out as indicating relaxation (Morton et al. 1993). Others are welfare-relevant as their performance contributes positively or negatively to a rabbit's welfare; examples here would be rearing-up and locomotion as positive for musculoskeletal development (Drescher & Loeffler, 1991; Rothfritz et al. 1992) or prolonged fighting as a negative social stressor (Bigler & Oester 1994; Girolami et al. 1996). In addition to overall differences between treatments in welfare-related behaviours and faecal corticosterone, we also investigated potential time-of-day effects (dawn, mid-day, dusk) and their interaction with hutch size and run access. Wild rabbits show crepuscular activity and nocturnal peaks (Mykytowycz & Rowley, 1958). Restricting behavioural activity in pet rabbits at those times might therefore be expected to have particularly negative welfare effects. A smaller, complementary study (Study 2) therefore investigated whether the natural circadian activity rhythm of pet rabbits would indeed mirror that of wild rabbits if given access to a run for 24h.

**Commented [NR1]:** Not in ref list and I cant find it on web of science is it wrong suzanne?

**Commented [SH2R1]:** Yes, it's 2011.

**Commented [NR3]:** In the introduction, binkying is called a welfare-relevant behavior. What does that mean in terms of run access when it was mainly shown with restricted access? Is binkying an indicator of good or poor welfare? Normally, you would call the environment with more frequent positive behavior more welfare friendly. You explain this paradox with the rebound effect which seems likely. However, shouldn't you stop calling binkying an indicator of good welfare when it indicates a rebound effect and thus the presence of a previous negative welfare state? This has to be discussed. The same might apply to allogrooming. Allogrooming is a natural behavior and strengthens the social bond. However, it could also be a sign for boredom if the frequency or duration is too long. It might have been interesting to distinguish where the behavior took place (in the hutch or in the run). If this has been recorded it would be interesting to analyze it. There are times (e.g. bad weather) when access to an outside run cannot be provided. The hutch should be sufficient during those times. Corticosterone is affected by exercise. Therefore it is not clear if it is an indicator of stress in this study because it could also be due to the increased exercise level of restricted rabbits. Please discuss.

Suzanne to add to intro and/discussion as appropriate ro

Both studies were undertaken with a view to informing housing guidelines for pet rabbits kept in pairs.

## 2. Study 1 - Hutch size and run access

### 2.1 Materials and Methods

#### 2.1.1 Animals and study design

The project was approved by the Animal Welfare & Ethical Review Board of the University of Bristol ([Ref no: UB/11/041](#)).

We tested two sizes of hutch and two levels of access to an attached run in a 2 x 2 design. Hutch size was a between-subjects factor, run access a within-subjects factor. Representative small and large hutch sizes were chosen based on 25<sup>th</sup> and 75<sup>th</sup> percentiles of sizes recorded during previous visits to pet owners' homes (Held et al. 2012). They were 0.73 m<sup>2</sup> for the small hutches and 1.86 m<sup>2</sup> for the large ones. Access to an attached run was either restricted to three hours in the middle of the day ('mid-day only') or unrestricted, that is the rabbits had 24-hour access to the run ('24 h'). We had previously found that owners reported in surveys to most commonly provide access for a few hours over the mid-day period between 10:00 and 16:00 hs (Held et al. 2012). All runs measured 1 m x 3 m and were permanently attached to the hutches such that opening of the hutch door allowed the rabbits access to the run. Further details of hutches, runs and husbandry are given below.

Twenty pre-established pairs of adult neutered rabbits (one male, one female) were recruited to the experiment from re-homing centres throughout the UK. They thus varied in breed and size. Pairs had been living together for at least a month before arrival and were reported as established and compatible, that is as not showing any prolonged agonistic behaviours towards each other. The experiment lasted six weeks and started after rabbits had settled in for two weeks ('habituation phase'). Ten pairs of rabbits were housed in large hutches throughout, the other ten pairs were kept in the small hutches. The level of run access was swapped after the first three weeks of the experiment such that one half of the pairs in large hutches had unrestricted access first (Weeks 1-3), then restricted access for the second three weeks (Weeks 4-6); the other five pairs in large hutches first

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had restricted, then unrestricted access. The same pattern was followed for the ten pairs kept in small hutches. Behaviour video recordings were taken of all pairs in experimental weeks 1, 3, 4 and 6, to cover the beginning and end of each of the two run-access treatments. For the purposes here, we report on behavioural recordings from Weeks 3 and 6, that is at the ends of the two different access treatments periods when any effects of differences in run access were expected to be maximised. Behavioural data were complemented with faecal corticosterone measures as physiological stress indicators (cf Buijs et al. 2011). Further details are given below.

### 2.1.2 Housing and husbandry

Hutches were wooden and consisted of a nesting and an open area. The nesting area was a box with a gap of 0.3 m (small hutches) or 0.5 m (large) for moving to and from the open area of the hutch. The front of the open area was covered by a mesh and could be opened as a door into the run (Fig.1). The floor of the hutches was covered with wood shavings throughout, with additional hay provided in the nesting area. All hutches were 0.75 m high based on Dixon & Cooper (2010) and on most common heights recorded in our previous owner visits (Held et al. 2012). Runs were permanently attached to hutches with hooks. The run floor was concrete and partly covered with a 0.2m x 1.0m strip of fresh turf, renewed at least once every two weeks. The run sides were wire mesh with a wooden frame (height 1.0m), and one side had an additional wooden partition to preclude visual access to the adjacent enclosure. Other enrichments provided to all pairs in the run were an upturned three-sided cardboard box in the run (to provide for shelter and a platform), a wicker ball and cardboard toilet roll inners. Lighting was by natural daylight, ventilation via airflow from windows, and the temperature ranged from 13°C- 27° over the experimental period. All rabbits were fed Burgess Excel rabbit concentrate daily at 40g per rabbit. In addition, they received one piece of fresh greens/root vegetable daily, plus their body size in hay. Water was provided *ad libitum* from a water bottle attached to the hutch. All hutches and runs were cleaned out fully once a week by University of Bristol animal care staff; dirty and wet litter was removed from hutches every other day. Every rabbit was given a health check to include body scoring and weighing upon arrival by one of the two veterinary surgeons on the



project team (SM and RS), and once a week thereafter. After Studies 1 and 2 were completed, all rabbits were rehomed in their established pairs to vetted adopters.

\*Figure 1 here\*

### 2.1.3 Data collection

On arrival, rabbits were allocated to their hutches such that larger pairs (>2kg kg per rabbit) and smaller-sized pairs (< 2kg) were approximately evenly distributed between large and small hutches. They were then allowed to settle in, undisturbed, for two weeks before behavioural recording started ('habituation phase'). During habituation, all pairs were allowed to access the attached run for six hours a day from 09:00 - 15:00h.

During the experimental period, 'restricted access' meant the door of the hutch was secured open to allow access to the attached run for only three hours daily ('midday only'; 12:00 -15:00 for Weeks 1-3; 11:00 – 14:00 for Weeks 4-6; the one hour adjustment was for seasonal change). 'Unrestricted' rabbits had 24h continuous access to their run.

#### 2.1.3.1 Behavioural recording

Ten Sanyo VCC-6695P colour CCD cameras were mounted on the far ends of ten runs, filming the main areas of the accommodation, i.e. the hutch and attached run. After each 24-hour filming period, the cameras were moved onto a neighbouring pen. Each pen was filmed for two 24 h periods in each of Weeks 1, 3, 4 and 6 starting from either 10am, 11am, 1pm or 2pm. Out of the two 24-hour periods recorded per pair and week, the one with the better visual quality was then chosen for behaviour analysis using the Observer 10.5 (Noldus). We used continuous sampling for three 30-minute periods of each 24-hour period: one at dawn, when natural light levels were sufficient to observe the rabbits' behaviour; one at dusk, 30 minutes before natural light faded; and one 30-minute period at midday, starting five minutes after the time at which rabbits with restricted access were allowed into the run.

Note that the same observation times were used for filming rabbits in the restricted and unrestricted conditions. Frequencies and durations of behaviours were recorded. The behaviour of the two rabbits in a pair was scored from the video for the same 30-min period and averaged (states) or totalled (events) to give one value per pair.

Table 1 summarises the ethogram used for behavioural analysis. After video analysis, the duration, frequencies and percentage time spent were calculated. One pair of rabbits was excluded from the behaviour analysis due to ill health.

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Commented [NR4]: Line 202: To which group did the excluded group belong? - sorry Suzanne I don't think I have the data

\* Table 1 here \*

#### 2.1.3.2 Faecal Sampling

Fresh faecal deposits were collected from the hutch and attached run on the second week of the habituation phase followed by Weeks 1, 3, 4 and 6. Data reported here are from Weeks 3 and 6 as for the behavioural variables. Faecal deposits were collected on Fridays at 1500h and placed in a plastic 20ml sterile screw cap bottle and frozen at -80°C for analysis. Faecal corticosterone was extracted and quantified using enzyme-linked immunosorbent assay kits (DetectX®, Arbor Assays), at the immunology lab of Bristol Vet School.

#### 2.1.4 Data analysis

We report here our analyses of behavioural and faecal data collected on Weeks 3 and 6, that is at the end of each run-access treatment. Unless otherwise reported, state-type behaviours are expressed as the average percentage for each pair. This was quantified as the total time spent by each rabbit in the pair in the 90 min of the three observation periods, expressed as % of observation time, then averaged for the pair. Event-type behaviours are quantified as the total number of times a

pair showed the behaviour in the 90 minutes observed per 24h recording unless specified otherwise. Error bars show 95% confidence intervals.

Unless stated otherwise, data were analysed in SPSS 19.0 using general linear models (GLMs) for the state-type behaviours after Kolmogorov-Smirnov tests for normality; event-type behaviours were analysed using non-parametric statistics. In the GLMs we analysed hutch size and run access as the main effects plus their interaction. Run access was a within-subjects factor with two levels ('24h' and 'mid-day only'), hutch size a between-subjects factor with two levels (0.73m<sup>2</sup> and 1.86 m<sup>2</sup>). Analysis of their interaction allowed us to determine whether any effects of run access depended on the size of the pairs' hutches. Where we were interested in the effects of time of day on the measured behaviour, we included it and its interactions with hutch size and run access in the GLM models. This was a within-subjects factor with three levels (dawn, mid-day or dusk). Only significant ( $p < 0.05$ ) or marginal ( $0.05 < p < 0.1$ ) results are reported.

Commented [NR5]: Line 225: Was the within pair factor a random effect in the analyses? -suzanne

Commented [SH6R5]: No, we used the GLM repeated measures procedure with different within-subjects repeated measures (factors): one was run access (2 levels), another was time of day (3 levels)

## 2.2 Results

### 2.2.1 Behaviour

More overall time was spent in locomotion when run access was restricted to 3h (GLM, main effect of run access:  $F_{1,17}=5.26$ ,  $p=0.035$ ); 'locomotion' was all the time spent hopping, walking and jumping added together (Table 1). This effect was independent of hutch size. Fig. 2 shows that it can be explained by significantly increased locomotor activity during the 30 min mid-day observations in pairs which were only let out at that time (GLM, interaction of run access x time of day:  $F_{2,24}=20.56$ ,  $p < 0.001$ ).

\*Figure 2 here\*

Pairs of rabbits with mid-day only access also tended to spend more time allogrooming (GLM, main effect of run access:  $F_{1,17}=3.25$ ,  $p=0.064$ ). Again, this effect was independent of hutch size.

Analysis of the event-type welfare-related behaviours revealed an effect on binkying only. The frequency of binkying was independent of hutch size but affected by run access with more

total binkying being recorded for pairs with restricted run access (Wilcoxon Signed Rank Test:  $W_{\text{standardized}}=2.95$ ,  $N=19$ ,  $p=0.03$ ). This was due to much increased binkying during the 30 min mid-day recordings in restricted pairs which was significantly higher in restricted than in unrestricted rabbits during that observation period (Fig.3; Wilcoxon Signed Rank Test:  $W_{\text{standardized}}=3.19$ ,  $N=19$ ,  $p=0.001$ ).

\*Figure 3 here\*

### 2.2.2 Faecal corticosterone

Neither hutch size nor run access, alone, affected corticosterone levels in Weeks 3 and 6. Their interaction, however, did such that corticosterone levels were raised in pairs which had been kept in small hutches and had had access to the run only for three hours midday (GLM, interaction of hutch size x run access:  $F_{1,17}=4.58$ ,  $p=0.047$ ; Fig. 4). The difference in corticosterone levels between unrestricted and restricted run access was significantly larger for pairs in the small hutches (Fig. 5; Independent Samples T-test:  $T_{1,17}=8.09$ ,  $p=0.046$ ).

\*Figure 4 here \*

\*Figure 5 here\*

### 3. Study 2 – Space use and 24h activity levels when run access is unrestricted (24h)

This study investigated space use and time-of-day changes in behavioural activity in pairs of pet rabbits given continuous access to a hutch and attached run. Its main aims were to determine where rabbits would spend most of their time and whether they would show peaks in activity during crepuscular periods as do their wild counterparts. Study 2 started after the end of Study 1 and used five Study 1 pairs in small hutches and five in large hutches. All pairs had unrestricted access to the attached run throughout. Through continuous behavioural sampling of one rabbit from each pair, 24-hour activity profiles and hour-by-hour behavioural time budgets were established. In addition, we

established where rabbits spent their time over the 24h: the open hutch area, nesting hutch area or the run; and how close to each other the two individuals in a pair were ('proximity').

### 3.1. Materials and Methods

#### 3.1.1. Animals

Only one rabbit from each of the pairs was used for the behavioural observations, five males and five females in total. These included five smaller and five larger individuals.

Commented [NR7]: How did we select them?

Commented [SH8R7]: We selected such that we had a sex and size balance. Beyond that I am not there was much choice.

#### 3.1.2. Housing and Husbandry

Rabbit pairs were housed as in Study 1 except that all pairs had unrestricted access to the run. Husbandry protocols were as for Study 1.

#### 3.1.3 Data collection

Cameras were fixed to runs as before to maximise the view of the rabbits' location and behaviour in the hutch and run. Each rabbit pair was recorded for one 24-hour period over a period of three days, with location and behaviour data collected for one rabbit in each pair.

Continuous behavioural sampling of each 24-hour video was performed using Observer 10.1 (Noldus Information Technology; 2010). Behavioural variables recorded were as in Study 1 (see Tab. 1). The durations of all behaviours are reported as percentages of time spent expressing the behaviour for each hour of the day.

We used instantaneous scan sampling at two-minute intervals on the same 24-hour videos (total of 720 scans per pair; using Observer 10.1; Noldus Information Technology) to record proximity between the two individuals in a pair as either: 'touching', 'one body length apart', 'two to five body lengths apart', 'more than five body lengths apart', or 'unknown' (rabbits out of sight).

## 2.5. Data analysis

All data were evaluated for normality by visual inspection and Kolmogorov–Smirnov tests with distributions all found to be normal. Thus, behaviour and space data were analysed using a Repeated Measures General Linear Model with a within-subjects factor of time of day (each hour of the day), and between-subject factors of sex (male, female), hutch size (small, large), and rabbit size (small, medium). Potential sex effects were included as they might inform housing guidelines specific to males or females. Proximity data were similarly analysed using a repeated measures general linear model with a within-subjects factor of time (each hour of the day), but just one between-subject factor of hutch size (small, large).

All statistical analyses were conducted using SPSS Version 19. Mauchly's test indicated that the assumption of sphericity had been violated for all data. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (Field 2009). Time-of-day plots do not display standard error bars as they are not appropriate for a repeated measures design (Belia et al. 2005).

## 3.2 Results

### 3.2.1 Space use and proximity over 24h

Space use was significantly affected by hutch size (Fig. 6). Rabbits in large hutches used the main hutch area ( $F_{1,8} = 12.19$ ,  $p = 0.008$ ) and nest (bedroom) area ( $F_{1,8} = 10.13$ ,  $p = 0.013$ ) significantly more than rabbits in small hutches (Fig. 6). In contrast, rabbits in small hutches used the run significantly more throughout the day than rabbits in large hutches ( $F_{1,8} = 13.073$ ,  $p = 0.007$ ). Figure 6 shows that rabbits in small hutches spent on average over 80% of 24h in the run, whereas the rabbits in the large hutches split their 24h roughly 50:50 between run and hutch.

No significant within-subject effects of time-of-day were found for any of the proximity categories; nor did hutch size significantly affect proximity between individuals ( $P > 0.050$  for all categories). Proximity data for individuals in each pair are summarised in Figure 7, totalled over 24

hours. They show that all pairs spent ~~the majority~~ a large amount of their time 2-5 body lengths apart.

**Commented [NR9]:** Line 326: There seems to be a large variation in proximity among pairs. Two pairs were touching during 30 or 35% of the time. If you add these values with the time when they were 1 body length apart, I don't think that your statement 'all pairs spent the majority of their time 2-5 body lengths apart' is correct.

\*Figure 6 here \*

\*Figure 7 here\*

### 3.2.2 Daily behavioural activity cycles

The analyses revealed no interactions between time-of-day, sex, rabbit size or hutch size for any of the behavioural variables. However, time-of-day as a single factor significantly affected the proportion of time spent ~~feeding~~ eating, drinking, inactive, self-grooming, alert and hopping (Tab. 2).

\*Table 2 here \*

Figure 8 shows representative peaks in the 24h profiles for two of the six behaviours that varied significantly with time-of-day. All show a similar pattern of peaks around dawn and dusk except of 'inactive' which showed the opposite pattern.

\*Figure 8 here\*

**Commented [NR10]:** Reviewer says Reviewer notes Figure 8: The terms feeding and binkyng do not correspond to the terms in the graphs

I cant see any reference to binkyng

Male rabbits spent significantly more time 'inactive' (any sitting and lying) than females ( $F_{1,8} = 15.22$ ,  $p = 0.005$ ). On average, males spent 40% of their daily time budget inactive, whilst females were inactive just 25% of the time. However, sex did not have a significant effect on the proportion of time spent on any other behaviours. The proportion of time the rabbits spent in different locations of their accommodation over a 24-hour period was not affected by sex.

## 4. Discussion

The aim of the present studies was to investigate the effects of hutch size and access to an exercise area on the welfare of pet rabbits kept in pairs. The exercise area was a run attached to the hutches, and rabbits had either unlimited access to it, or access was restricted to three hours at mid-day.

The main study (Study 1) found that pet rabbit pairs with run access of only 3h showed more locomotion (hopping plus walking plus jumping), binkying and allogrooming compared to pairs with unlimited run access. This was independent of hutch size. For locomotion and binkying this difference was due to particularly high levels during the 30 min mid-day observation sessions which fell into the period of the day when restricted rabbits had access to the run. This increase above levels of unrestricted pairs indicates a behavioural rebound. An 'activity-motivational rebound', 'behavioural rebound' or 'post-inhibitory rebound' is understood as an increase in performance of a specific behaviour after a period of confinement during which performance was restricted (Vestergaard, 1982; Nicol 1987). It is thus a response to release from restriction and interpreted as an indicator of preceding behavioural deprivation and compromised welfare (see Freire et al. 2009). A key feature of behavioural rebound is that locomotory activity levels rise above those recorded under control conditions that is under unrestricted conditions. Laying hens, for example, respond to release from wire cages into larger pens with marked increases in stretching, locomotion and wing flapping which they had been restricted in expressing in cages (Nicol 1987); calves buck and gallop more in open-field tests when previously kept in spatially restricted pens than when kept in larger control pens (e.g. Dellmeier et al., 1985; Jensen *et al.*, 1999); and horses show intense locomotor activity including trotting, rolling, cantering and bucking following release from confinement in stables (Freire et al. 2009).

For domestic rabbits, Dixon et al. (2010) had found increased locomotion after space restriction in singly-housed pets. Rabbits were housed individually in floor pens of 0.88 m<sup>2</sup>, 1.68 m<sup>2</sup> and 3.35 m<sup>2</sup>. The activity rebound was observed in the first two hours after they were moved from the smallest to the largest pen size. This finding was interpreted as indicating 0.88 m<sup>2</sup> is insufficient for a singly-housed rabbit to adequately express active behaviours and thus compromises welfare (Dixon et al. 2010). In our study, pairs were housed in hutches of 0.73 m<sup>2</sup> or 1.86 m<sup>2</sup> and had

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permanent or restricted access to an additional 3 m<sup>2</sup> in the shape of a long run (1m x 3m). For pairs with permanent run access, the actual living spaces thus measured 3.73 m<sup>2</sup> or 4.86 m<sup>2</sup> (small or large hutches plus runs), both of which are relatively large. This most likely explains why hutch size alone did not affect locomotory activity including binkying in our study even though our smaller hutches were smaller than Dixon et al.'s (2010) smallest pens for individual rabbits.

Binkying as a form of locomotor play also dramatically increased in the mid-day observation session in the 'restricted' pairs, that is in the pairs that were only let out that time. This increase in binkying, as the other locomotory activities, was again unaffected by hutch size and indicates a behavioural rebound after restricted [opportunity to move](#). Furthermore, [if we take binkying is a form of locomotory play in rabbits. Increased levels of locomotory play can indicate current good welfare as well as a sign of good welfare previous earlier deprivation.](#) (Held & Špinka 2011; Ahloy-Dallaire *et al.* 2018), [its increase](#) Our finding thus suggests that access to additional space for exercise is rewarding to pet rabbits regardless of whether they are otherwise living in a small or large hutch. [This](#) It thus supports the need to incorporate the opportunity to exercise for more than 3h mid-day into any future housing designs or guidelines for pairs of pet rabbits even where the main accommodation measures 1.86 m<sup>2</sup>.

Pairs of rabbits with only 3h access to the run also tended to spend more time allogrooming. Again, this effect was independent of hutch size. Allogrooming is known to stabilise and express the strength of social bonds, for example in cattle (e.g. Sato et al. 1993), and to have a function in maintaining social dominance relationships, such as in female primates (Schino, 2001). An interesting question for further investigation is whether in the present study the increase in allogrooming in space-restricted rabbits was a time budget effect (not much else to do), the sign of stronger bonds formed, or indicative of a greater need to maintain affiliative relationships due to higher stress levels experienced in close physical proximity than unrestricted pairs.

Faecal corticosterone measurements showed higher levels in pairs in small hutches with restricted access to the run for three hours midday only. Faecal glucocorticoid levels have been validated as indicators of longer-term stress in a variety of species, with evidence for an increase in

levels with space restriction in captivity in some species (e.g. female margay, Moreira et al. 2007 cited in Buijs et al 2011), but not in others (e.g. mink and chicken (Hansen et al. 2007, Nicol et al. 2006; cited in Buijs et al. 2011). Monclus et al. (2006) validated the use of faecal corticosterone metabolites as a non-invasive method to assess the physiological stress response of wild rabbits, in response to predator odour. In Buijs et al. (2011)'s study of domestic meat rabbits, faecal glucocorticoid metabolite (GCM) levels were used to assess the effects of transport and enrichment. Levels were raised after 30 minutes of transport in a van, and in unenriched as opposed to enriched wire cages before and after transport. Enrichment was a wooden, U-shaped structure used for shelter, oral manipulation and resting on a solid surface instead of on the wire mesh floor. Their results suggest faecal GCM concentrations may reflect differences in acute (transport) as well as chronic (lack of enrichment) stress (Buijs et al. 2011). Our results show interacting effects of hutch size and run access on faecal corticosterone in pairs of pet rabbits. Corticosterone levels were highest in pairs kept in small hutches (0.73 m<sup>2</sup>) with only 3h of run access a day. In the rabbit pairs living in large hutches (1.86 m<sup>2</sup>), the difference in corticosterone levels between having or not having permanent access to a run was negligible. [Glucocorticoids can increase in response to both rewarding \(fitness-enhancing\) and aversive \(fitness-threatening\) situations \(Buwalda et al., 2012\).](#) [Furthermore, raised levels can reflect aversive psychological stressors such as unexpected noise, unhabituated transport or lack of enrichment 'above and beyond' physiological stressors such as temperature changes or increased physical activity \(Jinemo et al., 2018\).](#) [Our corticosterone findings in rabbits -in small hutches with only limited access to additional space, may thus reflect both the motivational rebound in locomotor activity during access to the extra space and psychological stress caused by spatial restriction during most of the day.](#)

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Study 2 showed the existence of daily rhythms in behavioural activity. Many of the recorded behavioural variables showed significant within-subject effects of time of day. Males spent significantly more time inactive (lying and sitting). However, both sexes showed a synchronised daily rhythm of locomotor activity, with major activity peaks just after dawn and before dusk, activity minima during the middle of the day and between 03:00hrs to 04:00hrs. The circadian feeding

activity rhythm of the study rabbits is illustrated in Figure 7. The feeding pattern of the wild rabbit is characterized by two major periods of eating, at dawn and dusk (Southern 1940; Van Hof et al. 1963; Fraser 1992). A bimodal crepuscular activity pattern is assumed to be the result of predation risk (Lima & Dill 1990; in rabbits see e.g. Bakker et al. 2005). However, despite the absence of predation risk in a captive environment, Horton et al. (1974) found two main peaks of feeding also in laboratory rabbits, one at the beginning and one at the end of the light period. Our results similarly indicate that pet rabbits maintained under natural lighting and fed a mix of concentrate, hay and vegetables maintain a feeding pattern like that of wild rabbits. The study rabbits' circadian rhythm of hopping was also characterised by two major peaks, one at dawn and one at dusk. This corresponds to locomotor activity patterns common in wild rabbits (e.g. Mykytowycz & Rowley 1958; Van Hof et al. 1963; Villafuerte et al. 1993) and diurnal patterns in other domestic, non-pet rabbits. Gunn and Morton (1995), for example, investigated behaviour patterns of laboratory rabbits kept individually in standard laboratory cages of 0.3 m<sup>2</sup> floor space and a height of 0.48 m under an artificial lighting of 10.5h dark:1h dim:21.5h light. While normal locomotion was impossible in these cages, rabbits still showed increases in adapted locomotory behaviours during the dark periods (Gunn & Morton 1995). Buijs et al (2011) and Trocino et al. (2013) similarly report raised levels of activity and movement at night, dawn and dusk in fattening (meat) rabbits, with lowest levels at midday, under natural light conditions or artificial lighting including simulated two-hour dawn and dusk periods.

The final significant finding was that rabbits in small hutches used their runs significantly more throughout the day than rabbits in large hutches when they all had constant access to the runs in Study 2. If rabbits were using the space randomly we may have expected more run use in rabbits with small hutches since the run represents a greater proportion of the total available area. However, they used the run for a slightly larger proportion of the time than the area would predict (85% of time compared to 80% of area). In near-natural conditions, that is with near unlimited space available, domestic rabbits spend up to 90% of their resting periods during the day in body contact with one or more other rabbits when given the opportunity (Stauffacher, 1986). However, during

**Commented [NR12]:** One of the significant findings was that space use was affected by hutch size (lines 447ff). The explanation given is that the small hutch was too small for rabbits to keep the preferred interindividual distance. However, a more parsimonious explanation should be mentioned. For small hutches, the run was about 80% of the total area, whereas for large hutches, the run was only 62% of the total area. Likewise, in the treatment with the small hutches, the hutch presented only 20% of the total area. In the treatment with the large hutches, the hutch presented 38% of the total area. If rabbits were using the space randomly, we would expect rabbits with large hutches to spend more time in the hutch than rabbits with smaller hutches. Random movement to some extent could explain Fig. 6. This should be discussed.

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active periods their distances to each other increase, and they are less likely to be observed in close proximity (distances between individuals greater than or equal to one body length; Lockley, 1961). Correspondingly, it has been shown in preference tests that domestic rabbits choose solitary vis-a-vis group-housing for the expression of different behaviours (Held et al. 1995). Our present data show that rabbits spent the greatest amount of time 2-5 body length apart and that small hutches of 0.73 m<sup>2</sup> alone are too small for pair members to spend time apart as they did when able to use the run. This may have contributed to the raised levels of corticosterone found in the Study 1 in pairs in small hutches with restricted access to additional space in the form of a run.

## 5. Summary and Conclusion

Regardless of whether hutches measured 0.73 m<sup>2</sup> or 1.86 m<sup>2</sup>, locomotory activity including locomotor play increased significantly when the pairs with access only for 3h mid-day were allowed into the run which increased the space available by 3 m<sup>2</sup>. This is in comparison to pairs allowed access all the time. A peak in locomotion in the restricted rabbits at this time of day thus indicates a behavioural rebound demonstrating the rabbits' need to move within each 24h cycle. Results of the circadian activity rhythm study showed that the mid-day period is the least active period for pet rabbits when they have full freedom to move. Restricting to unnatural activity periods their opportunity to move around and to get away from each for periods of time is likely to have contributed to the raised stress hormone levels in the pairs in the small hutches. Housing guidelines thus need to highlight the need to allow pairs of pet rabbits freedom to exercise outside the mid-day period even if they are kept in hutches larger than common practice. Hutches of commonly reported (standard) sizes of around 0.75 m<sup>2</sup> floor area should not be recommended for pet rabbit pairs even with access to an exercise area for three hours per day during the middle of the day.

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