Applied Animal Behaviour Science 262 (2023) 105919

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

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

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ARTICLE INFO

Keywords:
Spatial restriction
Pet rabbits
Circadian activity
Enclosure size
Faecal corticosterone

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) were kept for eight weeks in standardised housing, Ten pairs were in small wooden hutches (0.73 m^2) and ten in large (1.86 m^2). An exercise area measuring 3×1 m was attached to each hutch and access was either unlimited or restricted to 3 h in the middle of the day. Each pair experienced each 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 access treatment period. In a subsequent study, ten of the rabbit pairs were given 24 h access to the run, and their behaviour recorded. More overall time was spent in locomotion when run access was restricted to 3 h ($F_{1,17}$ =5.26, p = 0.035). Regardless of size of hutch, locomotory activity including play increased significantly when the pairs with restricted access were released into the run. This indicates a motivational rebound after behavioural restriction demonstrating the rabbits' need to move within each 24 h cycle, as well as improved welfare There was a significant interaction between hutch size and run access on corticosterone levels; they were raised in the pairs kept in small hutches with restricted run access ($F_{1,17}$ = 4.58, p = 0.047). The mid-day period was found to be their least active. Restricting rabbits' opportunity to move 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 importance of allowing pet rabbits the freedom to exercise outside the mid-day period, even if they are kept in hutches larger than common practice. Hutches of commonly reported sizes of around 0.75 m^2 floor area should not be recommended for rabbit pairs, even with access to an exercise area for three hours per day during the middle of the day.

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 their 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 and 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,

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https://doi.org/10.1016/j.applanim.2023.105919
Received 15 November 2022; Received in revised form 27 March 2023; Accepted 31 March 2023
Available online 3 April 2023

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 and 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 and 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^2 to 1.33 m^2 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^2 including any permanently accessible runs and multiple floors (25th percentile=0.9 m^2, 75th percentile = 3.78 m^2). A total of 41% 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^2 to 36.84 m^2 with the most common size 1.0–1.2 m^2 floor area (Rooney et al., 2012). Access to an exercise area (run or other) was similarly variable. In Rooney et al. (2012) report, 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 hutch in the summer, but only 50% in the winter. Slightly over a fifth of pet rabbits in the UK had 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 h 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 h 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 and 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^2, 1.68 m^2 and 3.35 m^2, 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^2 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 (0.75 m) ceilings compared to low ceilings (0.45 m); and medium sized rabbits exhibited a rebound effect in hopping, rearing and, alert behaviours when moving from a low 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 (Buijs et al., 2011). Some of the variables are welfare-relevant as they are indicators of a rabbit's welfare state; examples would be binkying: a locomotory play behaviour ('frolicking', Held et al., 2001; Buijs et al., 2011) with raised levels indicating current good welfare or a motivational rebound after a period of behavioural restriction (Held and 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 and Loeffler, 1991; Rothfritz et al., 1992) or prolonged fighting as a negative social stressor (Bigler and 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 and 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 24 h.

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 × 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 25th and 75th percentiles of sizes recorded during previous visits to pet owners' homes (Held et al., 2012). They were 0.73 m^2 for the small hutches and 1.86 m^2 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 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 (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 and 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 0.2 m x 1.0 m strip of fresh turf, renewed at least once every two weeks. The run sides were wire mesh with a wooden frame (height 1.0 m), 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 (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 to 27° over the experimental period. All rabbits were fed Burgess Excel rabbit concentrate daily at 40 g 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.

2.1.3. *Data collection*

On arrival, rabbits were allocated to their hutches such that larger pairs (>2 kg kg per rabbit) and smaller-sized pairs (< 2 kg) 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 09:00–15:00 h.

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 24 h continuous access to their run.

2.1.3.1. Behavioural recording. Ten Sanyo VCC-6695 P 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 10 am, 11 am, 1 pm or 2 pm. 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 min before natural light faded; and one 30-minute period at midday, starting five minutes after the time at which rabbits with restricted access were

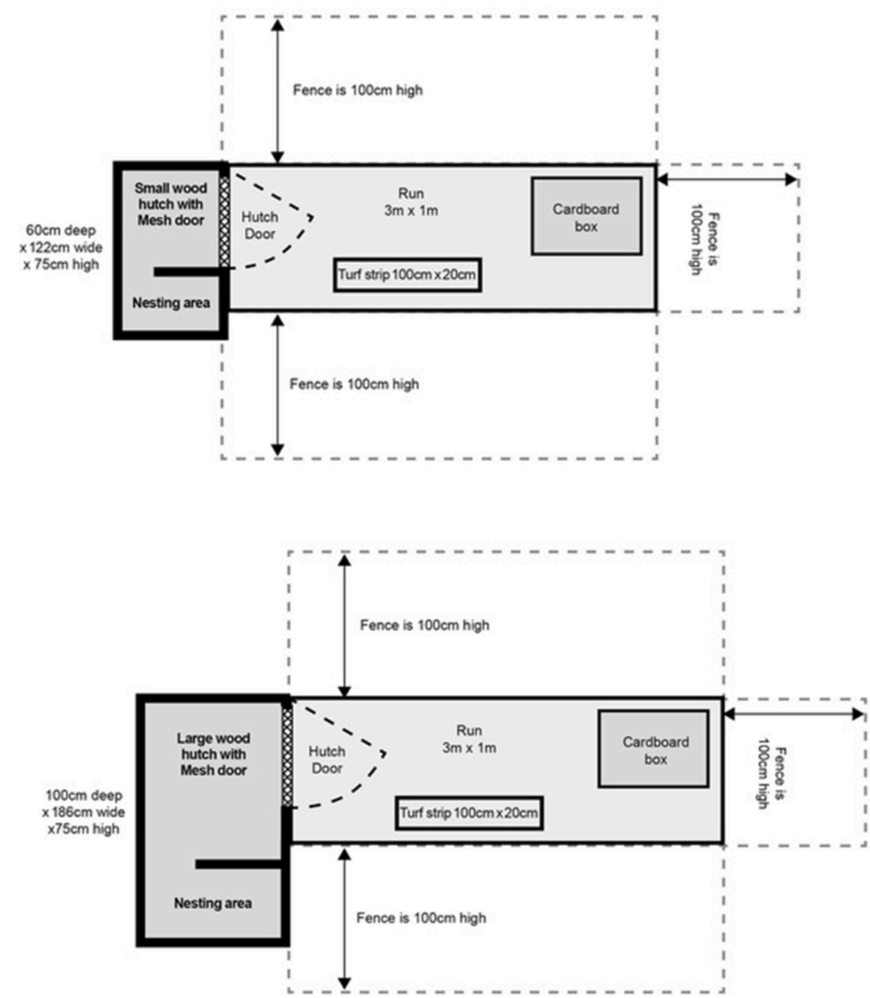

Fig. 1. a and b Small and large hutch and run set up.

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 (in the large hutch group) was excluded from the behaviour analysis due to ill health.

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 1500 h and placed in a plastic 20 ml 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 min observed per 24 h 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 (‘24 h′ and ‘mid-day only’), hutch size a between-subjects factor with two levels (0.73 m^2 and 1.86 m^2). Analysis of their interaction allowed us to determine whether any effects of run access depended on the size of the pairs’ hutch. 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.

Table 1
Behavioural variables recorded in Studies 1 and/or 2; definitions as in Gunn and Morton (1995), Held et al. (2001), Dixon et al. (2010).

Behavioural Variable	Type	Behavioural Variable	Type
Eat	state	Play social and locomotory	state
Drink	state	Attack	state
Walk	state	Manipulate object	state
Hop	state	Chase	state
Jump	state	Circle	state
Explore	state	Groom conspecific	state
Sitting Hunched	state	Nudge	event
Sitting	state	Head Flick	event
Lying outstretched sternal	state	Head Sway	event
Lying outstretched lateral	state	Chin Rub	event
Self-Groom	state	Binky/’Frolick’	event
Groom conspecific	state	Tail up	event
Scratch	state	Stretch	event
Dig	state	Body roll	event
Rear-up	state	Retreat from conspecific	event
Gnaw	state		
Mount	state	In box	state
Object Play	state	On box	state

2.2. Results

2.2.1. Behaviour

More overall time was spent in locomotion when run access was restricted to 3 h (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: $F_{2,24} = 20.56$, $p < 0.001$).

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 binky-ing 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: $W_{standardized} = 2.95$, $N = 19$, $p = 0.03$). 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: $W_{standardized} = 3.19$, $N = 19$, $p = 0.001$).

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: $T_{1,17} = 8.09$, $p = 0.046$).

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

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

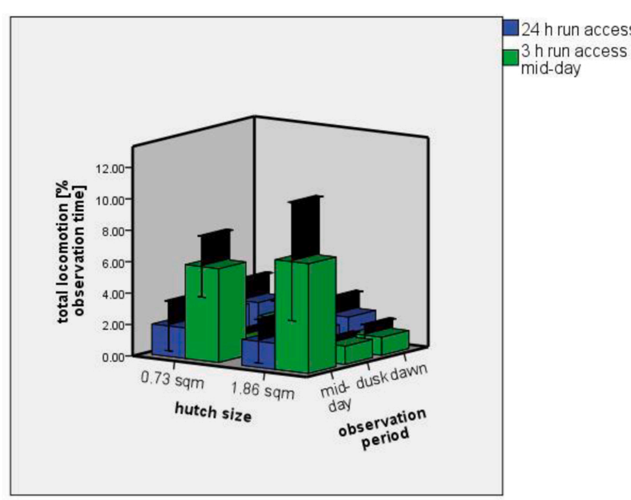

Fig. 2. Total locomotion by hutch size, run access and time of day; shown is total % of observation time spent by each rabbit in the pair, averaged for the pair. Black bars indicate 95% confidence intervals.

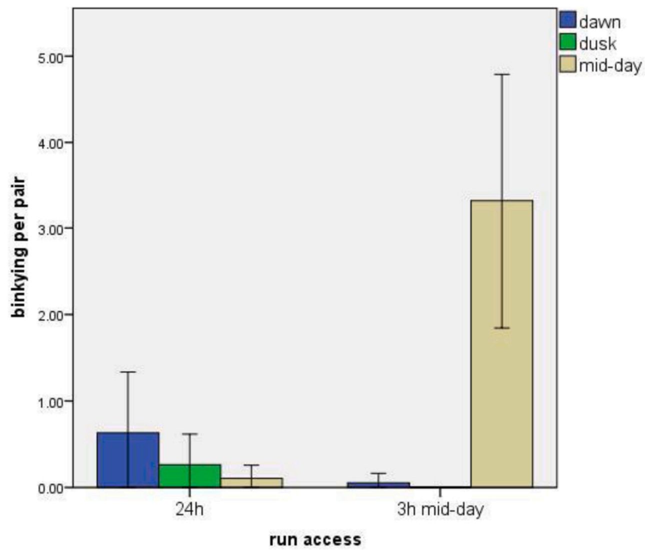

Fig. 3. Average number of binkies per pair by time of day and run access.

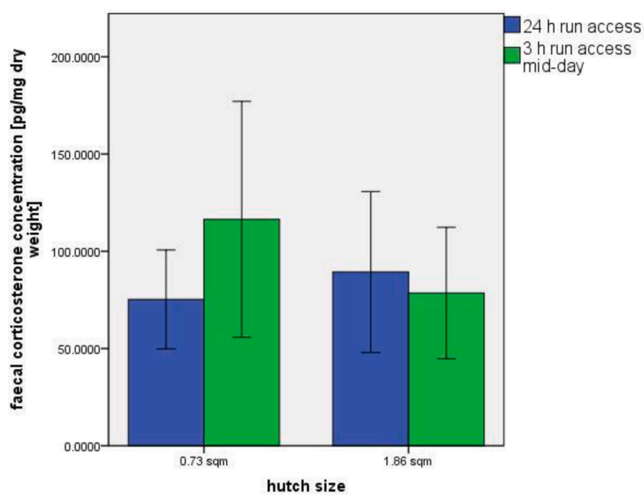

Fig. 4. Faecal corticosterone concentration by hutch size and run access in co-housed pairs of pet rabbits.

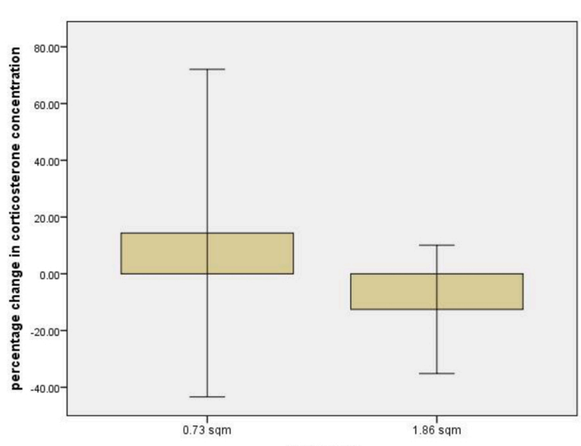

Fig. 5. Percentage change in faecal corticosterone levels between unrestricted and restricted run access in rabbit pairs in small and large hutches.

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 24 h: 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 selected at random. These included five smaller and five larger individuals.

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 Table 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).

3.2. *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).

3.3. *Results*

3.3.1. *Space use and proximity over 24 h*

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 ($F_{1,8} = 13.073$, $p = 0.007$). Fig. 6 shows that rabbits in small hutches spent on average over 80% of 24 h in the run, whereas the

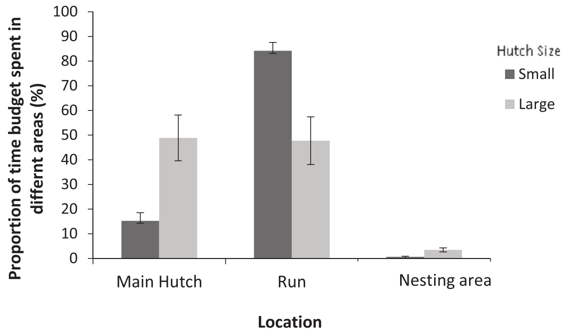

Fig. 6. Significant differences in the proportion of time spent in different locations over a 24-hour period between pet rabbits with small and large hutches. Data are averages (+/- SE).

rabbits in the large hutches split their 24 h 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 Fig. 7, totalled over 24 h. They show that all pairs spent a large amount of their time 2–5 body lengths apart.

3.3.2. *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 eating, drinking, inactive, self-grooming, alert and hopping (Table 2).

Fig. 8 shows representative peaks in the 24 h 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.

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.

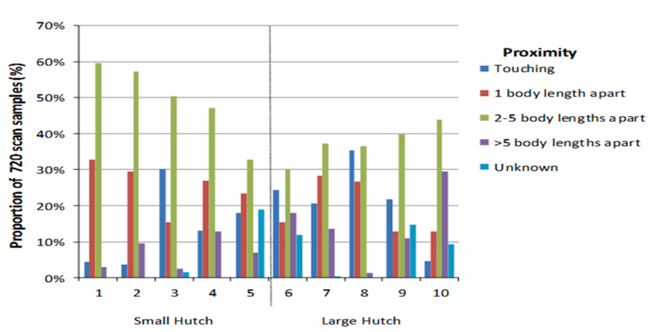

Fig. 7. Percentage of observations that individuals within a pair spent at different proximity over a 24-hour period.

Table 2
Effects of time-of-day on recorded behaviours; only significant effects are shown.

Behaviour	Effect d.f.	Error d.f.	F-value	p-value
eat	5.56	50.93	2.76	0.023
drink	4.94	44.32	2.70	0.033
inactive	5.82	52.43	2.63	0.026
self-groom	5.17	46.55	2.50	0.042
alert	5.43	48.91	3.15	0.013
hop	5.19	46.7	2.72	0.029

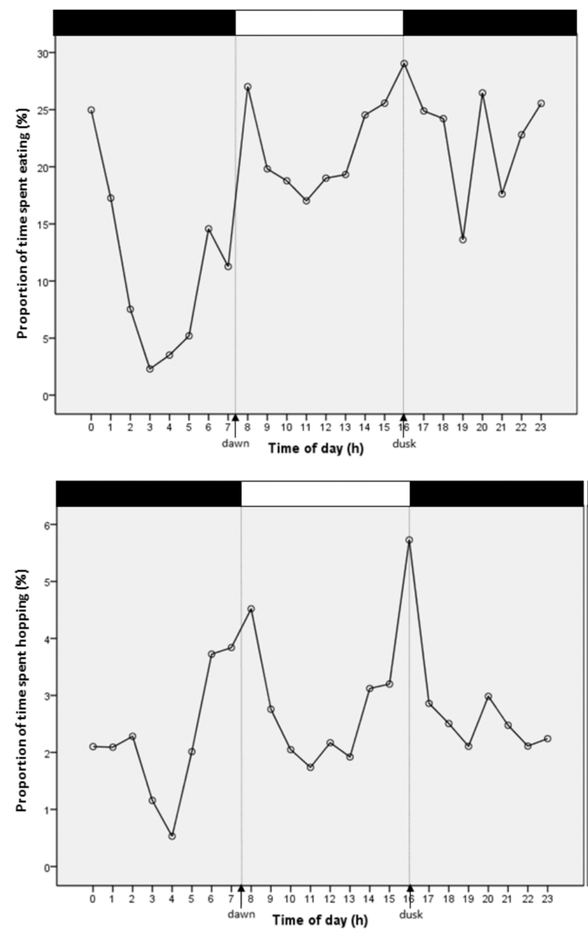

Fig. 8. Proportion of time spent on feeding and binkying per rabbit per hour over a 24-hour cycle. Data are averages. The upper white and black bars indicate the light and dark periods of the day.

The main study (Study 1) found that pet rabbit pairs with run access of only 3 h 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. A 'motivational rebound', 'behavioural rebound' or 'postinhibitory 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^2, 1.68 m^2 and 3.35 m^2. 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^2 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^2 or 1.86 m^2 and had permanent or restricted access to an additional 3 m^2 in the shape of a long run (1 m x 3 m). For pairs with permanent run access, the actual living spaces thus measured 3.73 m^2 or 4.86 m^2 (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, binkying is a form of locomotory play in rabbits. Increased levels of locomotory play can indicate current good welfare as well as previous earlier deprivation (Held and Špinka, 2011; Ahloy-Dallaire et al., 2018). 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 supports the need to incorporate the opportunity to exercise for more than 3 h mid-day into any future housing designs or guidelines for pairs of pet rabbits even where the main accommodation measures 1.86 m^2.

Pairs of rabbits with only 3 h 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). Monclús 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 min 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^2) with only 3 h of run access a day. In the rabbit pairs living in large hutches (1.86 m^2), 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 (Jimeno 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.

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 Fig. 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 and 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 and 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^2 floor space and a height of 0.48 m under an artificial lighting of 10.5 h dark:1 h dim:12.5 h light. While normal locomotion was impossible in these cages, rabbits still showed increases in adapted locomotory behaviours during the dark periods (Gunn and 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. This may have been expected if rabbits were using the space randomly, since for pairs with small hutches, the run represents a greater proportion of the total available area. On average they used the run for a slightly greater 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 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^2 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 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^2 or 1.86 m^2, locomotory activity including locomotor play increased significantly when the pairs with access only for 3 h mid-day were allowed into the run which increased the space available by 3 m^2. 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 24 h 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 importance of allowing 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^2 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the rehoming centres for kindly loaning us their pairs of rabbits – Windwhistle Warren Cottontails, RSPCA centres Taylor's Animal Rehoming Centre (West Dorset), Brent Knoll, West Hatch, RSPCA Little Valley Animal Shelter and Newport.

Thanks to the technicians Lionel and Andy who cared for the rabbits whilst at Langford.

This work was commissioned by the Royal Society for Prevention of Cruelty to Ansimsl (DFAS-RJ5432) by a competitive tendering process. We thank them for their financial support, and Jane Tyson for her comments on the manuscript.

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