Effects of cage-cleaning frequency on laboratory rat reproduction, cannibalism, and welfare

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Running title: Cage-cleaning frequency effects on breeding rats

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Abstract

Regular cage-cleaning is important for health, but for breeding rats it disrupts the nest and removes olfactory signals important for parental care. To investigate how different cage-cleaning frequencies affect breeding rats' health and welfare, we monitored reproductive output, pup mortality, pup sex-ratios, parental chromodacryorrhoea and in-cage ammonia levels for rats in a commercial breeding facility. Cages were cleaned twice-weekly, once-weekly or every two weeks (18 cages/group), replicated in two buildings, for the entire 36-week reproductive period. Frequent cage-cleaning had no clear benefits or major negative effects, showing no significant reductions in ammonia levels, or affects on health or overall pup mortality. However, frequent cage-cleaning slightly but significantly increased cannibalistic behaviour. This was because: (i) vulnerable 0-2 day old pups were more likely to be exposed to a cage-cleaning event in the more frequent cage-cleaning regimes, physically disturbing them, and disrupting the nest and scent marks; and (ii) in the twice-weekly and weekly-cleaned groups, pups under 2 days old at their first cage-cleaning were more likely to be cannibalised. Possible mechanisms behind these effects are discussed, including that cleaning might induce premature births, or stress the parents through noise or olfactory and physical disturbance. Finally, the cage-cleaning frequency producing most pups differed between the two buildings – an interactive effect corroborating previous findings that same-strain rodents' phenotypes can differ with environment. Overall, we suggest that for breeding rats, cage-cleaning regimes should minimise noise, dissemination of unfamiliar conspecific odours, and physical disturbance during very late pregnancy and the first few days following birth.

Keywords: animal welfare; husbandry; hygiene; cannibalism; reproduction; rodents
1. Introduction

Cleaning of animals’ cages or enclosures is necessary for almost all captive animals, whether they are kept in laboratories, farms, or zoos, or as pets, to prevent waste products accumulating to harmful or aversive levels. More frequent cleaning of rodent cages can reduce ammonia levels (Carissimi et al., 2000; Reeb-Whitaker et al., 2001; Burn et al., 2006a) and microbial counts (Borrello et al., 1998), and increase rodent health (e.g. Cisar & Jayson, 1967; Van Winkle & Balk, 1986). However, cage-cleaning almost always involves temporary displacement of each animal, physical and olfactory disruption of the ‘home’ environment, and human contact, which could each cause stress. Cage-cleaning does indeed cause acute increases in arousal and possibly transient stress in diverse species including rhesus macaques (Line et al., 1989), mice (Gray & Hurst, 1995; but see Blom et al., 1993), hamsters (Conn et al., 1990; Gattermann & Weinandy, 1996), rats (Saibaba et al., 1996; Schnecko et al., 1998; Duke et al., 2001; Sharp et al., 2002; Burn et al., 2006b) and snakes (Chiszar et al., 1980).

Whether frequent cage-cleaning causes chronic or cumulative stress is far less certain. In mice, two studies found that cleaning mouse cages more frequently increases pup mortality (Chantry, 1964; Reeb-Whitaker et al., 2001). Breeding mice whose cages were cleaned more frequently also tended to have higher corticosterone concentrations (Reeb-Whitaker et al., 2001). Even merely disturbing breeding mice, by regularly lifting the cage-lid and inspecting the hidden individuals, caused a strong trend towards poorer breeding success (Peters et al., 2002).

In our own previous studies, we investigated whether frequent cage-cleaning affected the welfare of male experimental Wistar and Sprague-Dawley rats, and found
little evidence that it is a cause for concern in those animals (Burn, 2006; Burn et al., 2006a; Burn et al., 2006b). However, one small-scale study of breeding rats did find indications that cage-cleaning has long-term effects. That work involved six Osborne-Mendel females per treatment (Cisar & Jayson, 1967). More pups were successfully weaned, they weighed more, and fewer needed to be culled by the experimenters in the twice-weekly cage-cleaned group than those in the weekly cleaned group. However, cannibalism was more frequent in the twice-weekly cleaned group.

Our aim here was to investigate how cage-cleaning frequency affects the general health, welfare, and reproductive success of breeding rats and their pups on a large scale. Even if non-breeding rats are little affected by frequent-cage-cleaning (Burn, 2006; Burn et al., 2006a; Burn et al., 2006b), we were concerned that breeding rat populations could be more affected, for a number of reasons. Firstly, olfactory signals are very important in rat parental care. These include the pheromone, diodecyl proprionate, produced from the pup preputial gland to induce maternal licking (Brouettelahlou et al., 1991); odours produced by dams that prevent cohabiting males from killing pups (Mennella & Moltz, 1988), and that guide pups to the nipples (Porter & Winberg, 1999); and scents deposited in the bedding by the dams that reduce pup activity levels, keeping them in the nest (Porter & Winberg, 1999). Cage-cleaning could directly remove some of those signals (Mennella & Moltz, 1988; Porter & Winberg, 1999), and/or mask them with odours from human hands or gloves (including scents from previously handled rats). Furthermore, rodent cage-cleaning is accompanied by loud noise (Gamble, 1982; Voipio et al., 2006), physical displacement, human contact, and exposure to relatively bright light (Lane Petter, 1968; Busnel & Molin, 1978; Libbin & Person, 1979). It can also physically disrupt
the nest structure, and involves transferring pups to a temporarily colder environment (e.g. Chantry, 1964; Lee & Williams, 1975). Acute physical stressors such as these could trigger neglect and/or cannibalism of pups (Lane Petter, 1968). In the longer-term, if frequent cage-cleaning causes chronic stress to the parents, this could also reduce their fertility (Boice, 1972), reduce pup birth weights and increase mortality rates (e.g. Cabrera et al., 1999).

Rats comprise 20% of all laboratory animals used in Europe (Commission of the European Communities, 2003) and about 14% of those in Canada (Canadian Council on Animal Care, 2001); thus any findings relevant to their health, welfare, and productivity would have implications for a large number of animals, in both commercial and research facilities. Here, we assessed the effects of twice-weekly, weekly and fortnightly cage-cleaning on the rats of a commercial breeding company (Harlan, Bicester, UK). We used outbred albino Wistars, chosen because they are one of the most commonly used stocks in the UK, and because they are pair-mated (rather than harem-mated), enabling us to follow the lifetime breeding performance of individual pairs. Because of the biosecurity requirements of the commercial establishment, all measurements (except chromodacryorrhoea) were carried out by the animals’ normal technicians. Therefore, detailed behavioural observations were unfeasible, but measurements included as many rapidly observable aspects of rat health and welfare as possible.
2. Materials and methods

2.1. Animals and housing

The study animals were 108 pairs of Wistar (HsdBrlHan:WIST) rats, consisting of 54 pairs housed in each of two similar buildings (‘A’ and ‘B’) maintained under commercial, barrier conditions. The cages were polypropylene (51 x 32 x 20 cm, L x W x H) with a stainless steel mesh lid. Each cage contained autoclaved Lignocel 3-4 sawdust (J. Rettenmaier & Söhne, Holzmühle, Germany), to a depth of about 2 cm. They were provided with pelleted chow (2018S sterilisable, Harlan Teklad, Bicester, UK) *ad libitum*, and water was constantly available from an automatic system. The temperature and humidity were 19-21°C and 55 +/- 1 %, respectively. The light:dark cycle was 12:12 h, with 2 h dawn and dusk periods (only half the lights were on).

In Building A there were approximately 6200 adult rats plus pre-weanling offspring (BN/SsN, LE and LH) and in Building B there were 6000 (all HsdBrlHan:WIST). Obvious differences between the buildings were that the colony in A was populated in 2001 and all rats were kept in polypropylene cages, while B was populated in 2004 and rats outside the current study were kept in wire-bottomed cages. The individual staff also differed between the buildings. The study was staggered between the two buildings, with Building A being 1 month ahead of Building B. Pairs were formed at 10 weeks of age. The experiment continued for the commercial lifetimes of the pairs, which is 9 months (36 weeks). Pups were weaned between 19 and 26 days of age (depending on their weight) at cage-cleaning, as is standard practice.

Within each building, cages were allocated to the following cage-cleaning regimes: twice-weekly, once-weekly, or every two weeks – ‘fortnightly’ (n = 18 pairs
per treatment in each building). Cages were cleaned by replacing the cage bases and bedding with fresh ones, but the cage lids were retained. Cage emptying was carried out manually on an unventilated table. The rats were always handled by the same technician. Due to the scale of the technicians’ work in this commercial facility, randomising the positions of cages or the cleaning sequence proved to be logistically unmanageable, so cages were instead arranged within same-treatment racks. The tier-positions of cages within the racks, which are known to affect rat behaviour and physiology (e.g. Rao, 1991; Reinhardt, 2004; Izidio et al., 2005), were balanced across treatments, but the replication across the two buildings was especially worthwhile to help control for unknown rack effects.

The animals were cared for in accordance with the code of practice for the housing and care of animals in designated breeding and supplying establishments (Home Office, 1995), and was approved by the local ethical review process at the University of Oxford.

2.2. Data collection

The breeding females (in Building A only) were weighed at the beginning and end of the study. Breeding parameters were measured, including the number of pups born in each litter, the number and sexes of the pups at each weaning, and for the first and third parity the body weights of one male and one female pup. The ages of the pups when cage-cleaning first occurred was noted for each litter. The rate of reproductive decline of the mothers was measured using the total number of litters they produced, and also as the ratio of the number of pups in their ninth litter (80% of the pairs produced at least 9 litters) against the number in their second litter (the first one often being atypical). Adult and pup mortalities were noted, and the causes of pup death
were recorded as ‘stillborn’, ‘eaten by the parents’, ‘missing’, ‘found dead’ or, in the

case of cage-flooding, ‘wet’.

After 4 months in each building, ammonia concentrations were measured in
each cage. A pump with Gastec glass measuring tubes with a range of 0.5–60 ppm
were used (Anachem Ltd., Bedfordshire, UK). These were inserted in through the
door in the top of the cage, and the measurements were taken in the centre of the cage
just above the sawdust. Measurements were taken on the day before all cages were
cleaned, so the fortnightly cleaned cages had 13 days of soiling, contrasting with the
twice-weekly cleaned ones, which had only 2–3 days of soiling.

Both parents’ noses were scored for chromodacryorrhoea – ‘red/bloody tears’,
a Harderian gland secretion that increases in a variety of aversive situations in rats
(Harkness & Ridgway, 1980; Mason et al., 2004; Burn, 2006) – by an experienced
experimenter, wearing a powered respirator due to rodent allergy. This took place 8
months into the project because older rats produce more chromodacryorrhoea than
young ones (Harkness & Ridgway, 1980), and hence the secretion is more visible.

Scoring was carried out in Building B alone, because it was not possible to enter both
buildings within the necessary timescale due to potential disease transfer (particularly
since the respirator could not be completely sterilised). The observer tapped gently
with a pen on the front of each cage to encourage the rats to present their noses for
inspection. The method used for scoring was on a 6-point scale (0-5), as described
elsewhere (Burn et al., 2006a; Burn et al., 2006b). Chromodacryorrhoea
measurements were taken the morning before cage-cleaning, when the soiling of the
different cages was at its most contrasting, and again at the same time on the day of
cleaning (about 2 h after cage-cleaning).
2.3. Statistical analysis

For the lifetime breeding parameters and ammonia measurements, general linear models (GLMs) were used, with cage-cleaning frequency and building, and their interactions as fixed factors. For the pup weights from the first and third litters, the sex of the pups and their cage (a random factor, nested in cage-cleaning frequency and building) were also included. For chromodacryorrhoea, the cage, the order in which cages were observed, and whether the measurement was taken before or after cleaning were included alongside cleaning frequency, and their interactions. Data were square-root, log, or arcsine square-root transformed as necessary, and the model residuals were inspected graphically to verify whether they met the assumptions of the model. For those variables that did not meet the assumptions of GLMs, logistic regressions were used. Models were improved by removing redundant non-significant interactions. The statistical program used was Minitab™ version 14 (Minitab Ltd, Pennsylvania, USA).

To assess effects on cannibalism, pups that were known to have been cannibalised, which is rarely witnessed directly, and those who were noted as ‘missing’, were combined into a single category (similar to Mohan, 1974). The effect of the age of the pups at their first cage-cleaning on the likelihood of cannibalism was analysed using a Generalized Linear Mixed Model (glmmPQL, MASS library, R freeware, version 2.4); cannibalism was the binary response, and the treatment group, mean age at first cage-cleaning, mean litter size, and their interactions were the predictors. Cage (nested within the treatment group) was included as a random factor.
3. Results

Cage-cleaning frequency had no significant main effects except that more pups were cannibalised when cages were cleaned more frequently (Figure 1). Other recorded mortalities did not show this pattern. More frequent cleaning increased cannibalism in terms of both how many pups were eaten or missing ($z = 2.15; n = 106; P = 0.032$) and the percentage of pairs that did or did not eat or miss pups ($z = 2.06; n = 106; P = 0.039$). An interaction between age at first cage-cleaning and the treatment group meant that, in the twice-weekly and, to a lesser extent, the once-weekly cleaning treatments cannibalism was more likely to occur if cages were cleaned sooner after birth (i.e. when the pups were younger), but this was not observed in the fortnightly treatment ($t = 2.85, DF = 882, with 106 clusters, P = 0.005$) (Figure 2). The absolute percentage of pups that were cannibalised was only $2.60 +/- 0.04 \%$ of all pups born, but at least one pup was cannibalised from $10.9\%$ of litters. Also, cannibalism was the largest recorded type of pup mortality (stillborns: $0.38\%$ of pups born; individuals found dead: $0.77\%$; and deaths from cage-flooding: $0.76\%$). The weaning weights of surviving pups from litters that had included some cannibalised pups did not differ significantly from other pup weights.

Cage-cleaning frequency had no other main effects, not even on ammonia concentrations (twice-weekly mean +/- S.E.: $13.5 +/- 0.5 \text{ versus fortnightly: } 13.9 +/- 0.6; P = 0.642$). However, it interacted with the building the rats were housed in (Figure 3): the fortnightly cleaning group had the fewest pups born and weaned in Building A, but in Building B the most were born and weaned in that treatment group (number born: $F_{2, 100} = 4.47; P = 0.014$; number weaned: $F_{2, 100} = 4.30; P = 0.016$). This effect was also apparent for the mean litter sizes, with rats in fortnightly cleaned cages
having the smallest litters in Building A, and the largest in Building B (F$_2$, 100 = 7.86; $P = 0.001$) (Figure 3).

Significantly more births were recorded on the cage-cleaning day itself, compared with non-cleaning days (F$_1$, 107 = 37.79; $P < 0.001$) (Figure 4). Thus, births on the day of cage-cleaning appeared around twice as likely as expected by chance, in the weekly and fortnightly cleaning groups. For the fortnightly cleaning treatment, Figure 4 suggests that births also peaked one week after cleaning.

Building also had a significant main effect (Table 1) on every variable tested, except for the pup sex ratios and mortality from cannibalism or stillbirths (parental chromodacryorrhoea and maternal weight were only tested in one building). In Building A more pups were born and weaned and there were more litters in total, but each pup was lighter than in Building B and a higher proportion of them were found dead. The weight difference between male and female pups (males being heavier) was significantly more pronounced in Building B than in A for the first litters.

4. Discussion

We investigated whether cage-cleaning frequency affected the reproductive performance and welfare of breeding rats. Overall, we found no significant main effects of cleaning frequency on any health and fertility measures (mortality rates and numbers of pups born and weaned), on other indices sensitive to stress (chromodacryorrhoea, maternal reproductive decline and weight gain, and pup sex ratio), or on ammonia levels. Furthermore, in a follow-up study, we found no significant effects on pup handleability or anxiety profiles in adulthood (Burn et al., in press).
However, we found that cannibalism increased with more frequent cage-cleaning, which is consistent with the small-scale study by Cisar and Jayson (1967), and with common perception (e.g. Home Office, 1995; Hansen et al., 2000). Cannibalism was rare, affecting about 2.6% of all the pups born, which is approximately consistent with previous studies (Chantry, 1964; Reynolds, 1981; DeSantis & Schmaltz, 1984), so cleaning frequency had no significant effect on pup mortality generally. Nevertheless, under these clean conditions and with this outbred strain, other causes of mortality were even rarer. Therefore, on a large scale or with more susceptible rat strains, reducing cannibalism might lead to significant increases in the numbers of pups weaned.

Cannibalistic behaviour is not a uniform phenomenon (Elwood, 1991); it can sometimes be a response to pups that are already dead or dying, and other times be a by-product of direct infanticide, which in turn can be a response to various different situations (Fox, 1975; DeSantis & Schmaltz, 1984). Here we found no indication that it occurred as a result of poor maternal health or welfare (Boice, 1972), since cage-cleaning frequency had no significant effects on maternal chromodacryorrhoea, weight gain, rate of reproductive decline, or litter sex ratios. Cage-cleaning also did not seem to affect litter size, so the increased cannibalism in the more frequent cage-cleaning groups was not a response to larger litters (Day & Galef, 1977; Gandelman & Simon, 1978).

It is possible that cannibalism was a response to a higher proportion of weak pups in the more frequent cleaning groups; for example, if the cleaning process itself triggered birth (Figure 4), this could have resulted in a higher proportion of premature litters. Alternatively, (or in addition) frequent cage-cleaning might have increased
cannibalism through more frequent olfactory (e.g. Mennella & Moltz, 1988; Moles et al., 2004) auditory, and/or physical (Lane Petter, 1968; Busnel & Molin, 1978) disturbances, or nest disruption and cooling (e.g. Chantry, 1964; Lee & Williams, 1975) at a time when pups were vulnerable. Noise certainly reaches a peak during cleaning and is a stressor (Gamble, 1982; Voipio et al., 2006), so cleaning cages as quietly as possible around breeding rats could help prevent cannibalism (Lane Petter, 1968; Busnel & Molin, 1978). Cage-cleaning in an unventilated area of the stock room also increases airborne levels of rodent urinary proteins (Thulin et al., 2002), potentially disseminating unfamiliar conspecific odours between cages – since unfamiliar male odours are known to stress mother rats (Moles et al., 2004), this olfactory disturbance should be avoided where possible. Figure 2 shows that, in more frequently cleaned cages, cannibalism was more likely to occur if cage-cleaning occurred on the day of birth (consistent with cannibalism of prematurely born pups), or during the first 2 days following it (consistent with cannibalism when cages containing very young pups are disturbed). Note that we had no information on when cannibalism events occurred, so even though cage-cleaning affected them, they were not necessarily on the same day as cage-cleaning.

In fact, the vast majority of cannibalism in rats has been reported to occur during the first week after birth, with some taking place in the second week, and virtually none in the third week (DeSantis & Schmaltz, 1984). Some rodent breeders already avoid cage-cleaning between birth and weaning (Libbin & Person, 1979), or at least within the first few days of birth (Peters et al., 2002), specifically to prevent cannibalism and neglect by the parents. Our data suggest that for twice-weekly and
possibly once-weekly cleaning regimes this might be an effective way of reducing cannibalism (Figure 2).

Overall, cage-cleaning frequency affected the mean litter size, and correspondingly the numbers of pups born and weaned, but the effect depended on the building the pups were born and raised in: fortnightly cage-cleaning was associated with the greatest number of pups in one building and the fewest in the other. These interactions between cage-cleaning frequency and building imply that if we had only carried out our study in Building A, we would have concluded that fortnightly cage-cleaning reduces the numbers of pups, while we would have concluded the opposite had we only used Building B. This could have been due to differences between the cage racks, the different humans working in the buildings, olfactory or vocal communications within the different animal populations, or any number of environmental differences. This supports and extends the finding that rats of the same strain differ between different breeding companies (Rex et al., 1996; Germann et al., 1998); here they differed within the same breeding company. Therefore, in agreement with previous studies (e.g. Crabbe et al., 1999; Wurbel, 2000; Wahlsten et al., 2003; Garner et al., 2006), our findings reveal difficulties regarding the feasibility of standardisation between different experiments, and reaffirm the merits of replicating research under different conditions.

Unlike Cisar and Jayson (1967), we did not find that overall weaning success was increased by more frequent cage-cleaning. This might be explained by the relative contribution of hygiene to pup survival in the two studies. In Cisar and Jayson’s study, rats were kept in a conventional animal unit in the 1960’s, when around 97.5% of pup mortality in similar units was due to diseases (Porter, 1968).
Therefore, frequent cage-cleaning might have been essential for preventing disease. In contrast, our study animals were kept in a barrier unit, in which there were no detected pathogens (Harlan’s Health Monitoring reports, 2005, unpublished) and ammonia levels were almost certainly lower (Table 1, cf. Perkins & Lipman, 1995; Carissimi et al., 2000).

Here, the cage-cleaning days themselves were associated with more births than non-cleaning days. This may have been an artefact of the way that births were recorded: pup births and ages were usually recorded and estimated during cage-cleaning, so very young pups might have been recorded as being born that day unless it was obvious that they were several days old. However, the opinions of the staff involved differed over whether this recording artefact would occur or not, so other explanations require consideration. Also, a recording artefact would not fully explain the peak in births midway through the cleaning cycle in the fortnightly treatment. An alternative explanation could be that the noise (Lane Petter, 1968; Busnel & Molin, 1978), circulation of conspecific odours (Moles et al., 2004), or physical disturbance associated with cleaning, even of neighbouring cages, triggered births perhaps through stress or increased activity in the mother. Physical strain, and chronic and acute stress, can shorten gestational duration under some circumstances in humans (Glynn et al., 2001; Hobel & Culhane, 2003) and domestic animals (Silver, 1990), but the effects are little understood, and differ between species. The mid-way peak in the fortnightly treatment also suggests that we may not have entirely effectively simulated the fortnightly cleaning rate, when all cages in a room would be cleaned fortnightly.
Apart from cage-cleaning effects, most variables differed between the two buildings. Interestingly, the differences between the buildings corresponded to the pattern of reproductive trade-offs predicted by theory, in that offspring ‘quality’ is balanced against the number of offspring produced (Smith & Fretwell, 1974). Building A produced more offspring (in terms of numbers born, weaned and the number of litters), but each individual pup weighed less, sexual dimorphism was reduced, and pup mortalities were higher than in Building B.

5. Conclusion

Here and in a follow-up study (Burn et al., in press), we found no clear benefits of frequent cage-cleaning to breeding rats, but it did increase the likelihood of cannibalism, and the cleaning process itself might have triggered parturition. We would therefore recommend that cages are not cleaned during the last few days of pregnancy (possibly avoiding premature induction of birth) or the first 2 days following birth (avoiding acute early disturbances). Also, more research is required about what aspects of cleaning trigger cannibalism, but until then, noise and the transfer of odours between cages are likely stressors, and should be kept to a minimum when cleaning the cages of breeding rats.

6. Acknowledgements

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Figure and table captions

**Figure 1.** The effect of cage-cleaning frequency on cannibalism of pups. More frequent cleaning increased (A) the mean (+/- S.E.) proportion of pups that were cannibalised in the two buildings, and (B) the percentage of parents that cannibalised pups at some stage during the project. *indicates the pairwise significant difference (P < 0.05) on graph A, but in graph B it was the covariate trend across cleaning intervals that was significant.

**Figure 2.** The relationship between the age of the litters when their cages were first cleaned and the likelihood of cannibalism. The fitted lines and shaded 95% confidence intervals show that for the twice-weekly, and to a lesser extent the once-weekly, cleaning group cannibalism was more likely if cage-cleaning occurred earlier in life. There was no significant trend in the fortnightly cleaned group. Note that the day of cleaning might not necessarily have been the day the pups were cannibalised; data on when the cannibalism occurred were not available, so the pups may have been eaten at a later date.

**Figure 3.** The mean (S.E.) lifetime number of pups (A) born and (B) weaned, and (C) the litter size for breeding pairs cage-cleaned at different frequencies in two different buildings. The fortnightly cage-cleaning decreased the number of offspring compared with more frequent cage-cleaning in Building A, but it increased the number of offspring in Building B.

**Figure 4.** The numbers of pups born on each day of the (A) twice-weekly, (B) weekly and (C) fortnightly cage-cleaning cycle. The cage-cleaning day, Day 0, is highlighted in black. The dotted lines show the numbers of pups expected by chance if they were distributed evenly across days. For the twice-weekly group there is a step
in probability, because half the cleaning events occurred on Day 2 of the cycle (Fridays) and half on Day 3 (Tuesdays), so pups had 50% less chance of being born on Day 3 than any other day. In general more births were recorded on the cage-cleaning day itself than on other days, but the fortnightly cleaning group also suggests a peak midway through the cleaning cycle, on the day when all neighbouring cages would have been cleaned.

Table 1. Significant differences between the two buildings. The statistics used were either GLMs (where an F-value is given), or for non-parametric data, ordinal logistic regressions (where a z-value is given). In Building A, significantly more pups were born and weaned, and there were more litters in total, but pups were lighter and more frequently found dead than in Building B. Ammonia concentrations were low in both buildings, but were higher in B than in A. Maternal weight and parental chromadacryorrhoea could not be compared between the buildings because measurements were only possible in one building.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistical values</th>
<th>Building A (mean +/- SE)</th>
<th>Building B (mean +/- SE)</th>
<th>Effect direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number born per pair</td>
<td>$F_{1,100} = 6.40; P = 0.013$</td>
<td>98.9 +/- 3.4</td>
<td>87.6 +/- 3.0</td>
<td>A &gt; B</td>
</tr>
<tr>
<td>Number weaned per pair</td>
<td>$F_{1,100} = 4.70; P = 0.032$</td>
<td>92.1 +/- 3.0</td>
<td>82.9 +/- 3.1</td>
<td>A &gt; B</td>
</tr>
<tr>
<td>Number of litters per pair</td>
<td>Odds = 3.59; $z = 3.39; n = 106; P = 0.001$</td>
<td>9.9 +/- 0.2</td>
<td>9.0 +/- 0.3</td>
<td>A &gt; B</td>
</tr>
<tr>
<td>Number found dead per pair</td>
<td>Odds = 5.41; $z = 2.10; n = 106; P = 0.036$</td>
<td>1.3 +/- 0.4</td>
<td>0.4 +/- 0.3</td>
<td>A &gt; B</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; litter pup weight (g)</td>
<td>$F_{1,96} = 19.77; P &lt; 0.001$</td>
<td>40.9 +/- 1.0</td>
<td>47.3 +/- 0.9</td>
<td>B &gt; A</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; litter pup weight (g)</td>
<td>$F_{1,95} = 16.97; P &lt; 0.001$</td>
<td>39.8 +/- 1.3</td>
<td>46.9 +/- 1.3</td>
<td>B &gt; A</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; litter male-female weight difference (g)</td>
<td>$F_{1,96} = 6.02; P = 0.016$</td>
<td>0.51 +/- 0.50</td>
<td>2.09 +/- 0.40</td>
<td>B &gt; A</td>
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<tr>
<td>Ammonia (ppm)</td>
<td>$F_{1,101} = 273.33; P &lt; 0.001$</td>
<td>10.9 +/- 0.3</td>
<td>16.5 +/- 0.2</td>
<td>B &gt; A</td>
</tr>
</tbody>
</table>
Figure 1

A

Percentage of pups cannibalised

Cage-cleaning interval (days)

B

Percentage of pairs that cannibalised pups

Cage-cleaning interval (days)
Figure 2

The graph shows the probability of cannibalism in a litter as a function of the age at first cage-cleaning (days) for three different cleaning frequencies: fortnightly, once-weekly, and twice-weekly. The probability decreases as the age at first cage-cleaning increases, with the once-weekly and twice-weekly cleaning frequencies showing a more pronounced decrease compared to the fortnightly frequency. The x-axis represents the age at first cage-cleaning in days, ranging from 0 to 14, and the y-axis represents the probability of cannibalism in a litter, ranging from 0 to 0.3.
Figure 3

A

B

C

Twice-weekly
Once-Weekly
Fortnightly
Figure 4

A

B

C

Days since last cleaning

Days since last cleaning

Days since last cleaning

Number of litters born

Number of litters born

Number of litters born