



Providing ‘get-away bunks’ and other enrichments to primiparous adult female mink improves their reproductive productivity



Misha Buob^a, Rebecca Meagher^a, Lauren Dawson^b, Rupert Palme^c,
Derek Haley^b, Georgia Mason^{a,*}

^a Department of Animal and Poultry Science, University of Guelph, 50 Stone Road East, Guelph, ON, Canada N1G 2W1

^b Department of Population Medicine, University of Guelph, 50 Stone Road East, Guelph, ON, Canada N1G 2W1

^c Department of Biomedical Sciences/Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

ARTICLE INFO

Article history:

Received 13 November 2012

Received in revised form 13 May 2013

Accepted 14 May 2013

Available online 28 June 2013

Keywords:

Mink

Infant mortality

Welfare

Bunks

Enrichments

Nursing sickness

Cortisol

ABSTRACT

This study investigated whether simple, cheap enrichments – ‘get-away bunks’ (a wire mesh semi-cylinder attached to the cage ceiling) and small manipulable objects (balls and suspended chewing items) – could improve welfare and productivity in nursing mink dams (*Neovison vison*) in commercial farm conditions in southern Ontario (Canada). Experiment 1 replicated a study conducted the previous whelping season on the same farm. It evaluated whether providing bunks to multiparous dams ($n = 164$) could decrease their morbidity and mortality or boost kit weaning weight, and assessed dams’ overnight use of these structures to compare day and night utilization. Overnight bunk-use proved to be no different from daytime use, and night- and day-use co-varied; bunk use recorded in daylight is thus a good proxy for overall use. Bunk use did not, however, influence kit weights at weaning, nor reduce dam deaths from nursing sickness (replicating the previous year’s findings), nor significantly improve subjectively scored teat health (unlike the previous year’s findings). Experiment 2 reassessed bunk effects using larger sample sizes, and investigated their interaction with enrichment objects. Focusing on primiparous dams ($n = 318$), it evaluated whether providing balls and items to chew, along with bunks in a cross-factored design, could decrease stereotypic behaviour, glucocorticoid output (assessed via faecal cortisol metabolites: FCM), kit losses, and, again dam mortality. Objects were provided c. 10 months earlier (since the previous July) for approximately 60% of the object-enriched dams, and c. 5 months earlier (since January) for the remaining 40%. Analyses showed that effects of bunks and enrichment-objects did not significantly interact for any variable. Bunks significantly reduced kit mortality: kit losses/litter were reduced by c. 0.3 infants, resulting in negligible levels of mortality, and bunks tended to reduce dam stereotypy levels by about half (from approximately 12.5% to 6% of time spent active). However, bunks had no significant effects on FCM or dam mortality rates. Bunk-use also significantly co-varied with litter size, being greatest in dams with bigger litters. Enrichment objects tended to increase weaning litter size, an effect caused by dams provided with these objects for 5 months weaning 0.9 more kits per litter than females without these items. However, this type of enrichment again had no significant effects on FCM or dam mortality. Instead, which farm animals lived on appeared to be the major determinant of FCM and dam mortality: both significantly varied between

* Corresponding author. Tel.: +1 519 824 4120x56804; fax: +1 519 836 9873.

E-mail address: gmason@uoguelph.ca (G. Mason).

farms, with one farm showing notably higher levels of both. Also, even controlling for farm, females who died tended to have had elevated glucocorticoids when sampled 2–3 weeks prior to death. In conclusion, bunks and manipulable enrichment objects seem ineffective against nursing sickness, but had independent, additive effects on the productivity of young adult females, possibly acting by improving primiparous dams' welfare.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Mink on North American farms typically live in small wire mesh cages, each with a nest box: environments providing limited stimulation or opportunities to exert control. Several European studies demonstrate welfare and productivity benefits from giving farmed mink environmental enrichments, including structural changes to cages (e.g. shelves), manipulable objects, and improved nesting materials (e.g. Hansen et al., 2007; Jeppesen, 2004; Malmkvist and Palme, 2008; Vinke et al., 2004). Our aims in this and related studies (Dawson et al., 2013; Mason et al., 2012) are to evaluate similar housing changes on commercial Canadian farms, with their different genetics, cage designs, climates and feeding regimes. One such manipulation involves providing items that can be chased and/or chewed, such as balls and hanging plastic chains. A Danish study found that objects of this type reduce cortisol levels, tail-chewing and stereotypic behaviour (Hansen et al., 2007). Another is structural, involving semi-cylindrical wire mesh 'bunks' attached to the cage ceiling. In some European countries, e.g. Denmark, bunks, shelves or suspended cylinders allowing mink an elevated area to climb into and rest may be incorporated into all cages for year-round use (e.g. Hansen et al., 2011). However, North American cages for paired juveniles and single adults typically have a 'drop-in' nest box that fills part of the cage, greatly limiting the space available for such additions. Only whelping cages (for nursing families) here have the nest box attached outside the cage, so leaving room for additional structures: fortunate since, as reviewed below, such furnishings seem particularly beneficial for nursing dam welfare.

Mink are polytocous, raising a litter of altricial offspring annually. After c. 90 generations of artificial selection for large litters, farmed females produce more infants ('kits') than their wild counterparts (Dunstone, 1993; Malmkvist et al., 2007), and by 30 days the mass of the litter is typically heavier than the dam (Jørgensen, 1985). Furthermore, kits are typically not separated from the dam until more than 2 weeks after they are mobile enough to pursue her into all parts of the cage (Brink et al., 2004; Brink and Jeppesen, 2005); and during the last 2 weeks of the whelping period kits do not just suckle (albeit it at reduced levels), but also drink their mother's saliva (Brink et al., 2004; Brink and Jeppesen, 2005). This situation may cause the dam health problems, especially mastitis and nursing sickness (e.g. Clausen et al., 1992; Rouvinen-Watt and Hynes, 2004; Schneider, 1996). It may also elevate stress, because dams cannot remove themselves from kits as they would in the wild (Hansen, 1990; Pedersen and Jeppesen, 2001). Cage furnishings can help mitigate some of these problems. Danish research shows that dams provided

with shelves or similar structures that they can climb into but their kits cannot, perform less stereotypic behaviour than controls (Hansen, 1990). Furthermore, dams use such elevated structures increasingly over lactation, although abandoning them once kits can climb up to them (Hansen, 1990; Jeppesen, 2004). One Canadian study (Dobson and Rouvinen-Watt, 2008) further found that amongst dams stressed by research procedures (e.g. blood sampling), those given suspended plastic bunks were better able to wean larger litters, in terms of kit number and total litter mass; while mortality rates in 'high weight loss' females (losing more than 20% body weight over lactation) also appeared reduced. Recently, we confirmed that elevated bunks on commercial Canadian farms are increasingly used by dams as their litters mature (Dawson et al., 2013); the bunks also reduced stereotypies, and in multiparous females, visually scored teat health problems.

Our first aims were to build on this last study, using animals on the same farm in the following whelping season (Experiment 1). Teat health was not previously scored blind to treatment (Dawson et al., 2013); this therefore needed replication with blinding. Bunk use had only been assessed between c. 09:00 and 17:00h, leaving nocturnal and 24h use unknown. Dawson et al. (2013) also found no apparent benefits for nursing sickness: to investigate this further, we now focused on multiparous dams with large litters, *a priori* likely to be more at risk of this disease (Clausen et al., 1992; Rouvinen-Watt and Hynes, 2004). In Experiment 2, we compared the relative effects of bunks and manipulable enrichment objects on stereotypic behaviour, kit losses, weaning litter sizes, dam mortality rates, and levels of faecal cortisol metabolites (FCM). We also investigated whether they are synergistic if used together, since ecological and human health studies reveal that combining stressors can cause non-additively heightened negative effects (e.g. Dragano et al., 2005; Sih et al., 2004), while rodent-based neuroscience research shows that combining diverse enrichments can be disproportionately beneficial, compared to supplying enrichments separately (Sozda et al., 2010). This experiment capitalized on an ongoing, large-scale study (Mason et al., 2012) in which thousands of animals were provided with objects to chase and chew, on the farm used in Dawson et al. (2013) and Experiment 1 plus an additional two farms.

1.1. Ethical approval

The University of Guelph Animal Care Committee, complying with the University of Guelph Animal Care Policy and Canadian Council on Animal Care, approved both experiments.

2. Experiment 1: teat health, mortality and bunk use in multiparous dams

2.1. Materials and methods

2.1.1. Research population

This research was carried out on the same southern Ontario farm as used by Dawson et al. (2013) the previous whelping season. Black mink were housed in wire mesh whelping cages typical of Canadian industry (cage sizes: 61 cm l [long] × 35 cm w [wide] × 30 cm h [high]). A nest box bedded with wood shavings was attached to the outside of each cage (interior dimensions: 27 cm l × 27 cm w × approx. 28 cm h). Water was available *ad libitum*, supplied by automatic nipple drinkers. Mink were fed a semisolid meat-based feed, prepared on farm and dispensed onto the wire mesh lids of nest boxes between 15:30 and 17:00 h daily. Approximately 30% of cages had leftovers the following morning, which were redistributed between 08:30 and 10:00 h. Weaning was carried out by the farmers at 41–42 days *post partum*, by means of removing mothers from kits and relocating them to a separate shed.

Multiparous mink ('multiparas') were selected with litter sizes greater than six (based on counts conducted 1 day *post partum*) and parturition dates between April 28 and May 3. Parity and litter size criteria were chosen to: (1) determine whether the previous finding that bunks improve teat health in multiparas could be replicated, and (2) improve sensitivity for determining whether bunks can reduce nursing sickness, by focusing on animals likely to be at increased risk (Clausen et al., 1992; Rouvinen-Watt and Hynes, 2004). Since large-littered females also benefited from bunks in terms of weaning litter size and total mass, albeit it only when stressed (Dobson and Rouvinen-Watt, 2008), we also opportunistically weighed kits at weaning. The narrow window of parturition dates was chosen to delineate the experiment's timing and duration: important because author MB was conducting this experiment and Experiment 2 simultaneously. This selection resulted in a sample of 148 dams, widely distributed across the farm (in 16 rows within eight sheds). Half were provided with bunks and the other half left as controls, treatments being alternated in the subjects along each row. Bunks were semi-cylinders made of single-walled wire mesh (35 cm l × 16.5 cm w × 10 cm h); thus similar to the bunks used in Dawson et al. (2013) but not double-walled. They were secured to the cage ceiling at 21–24 days *post partum* following our previous protocol (Dawson et al., 2013), and to reduce the possible risks of bunk-related peri-natal kit mortality suggested by Dobson and Rouvinen-Watt (2008).

2.1.2. Overnight bunk use recording

Nine apparatuses for automatically recording overnight bunk use were available; a subsample of nine dams was thus randomly selected for measurement of nocturnal bunk use. Their bunks were specially modified as follows (Fig. 1). The front of each was fastened to the cage ceiling as normal (this point acting as a fulcrum), while the rear was suspended from a spring-loaded micro-switch (Moujen, MV 3000 series, Morgan Hill, CA, USA). Bunk use was registered

whenever the dam's weight tripped the switch, connecting the circuit to a 9-V timer (five manufactured by Naidian [model SH-1, Wenzhou, Zhejiang, China]; four manufactured by ENM [model T4488A, Chicago, IL, USA]) measuring to an accuracy of 1/10th of an hour. Timer readings were recorded every evening (at 17:00 ± 1 h) beginning at bunk installation until weaning at 41 days *post partum*. To compare day use with crepuscular and nocturnal use (c. 17:00–08:00 h), readings were recorded in the mornings (08:00 ± 1 h) between days 31 and 33 *post partum* inclusive. Data were also collected by live scanning on these days, to validate the timers; bunk use (defined as occupying the bunk so that no limbs were in contact with the cage floor) was recorded by instantaneous scans (Martin and Bateson, 2007, pp. 53–54): c. 12/day were made between 7:00 and 16:00 h by an observer (MB) quietly walking along the neighbouring row. The live scanning of mink at c. 45 min intervals has been well-validated as a recording method: its values correlate well with those obtained in a denser sampling regime (every 10 min) from video recordings (Svendsen et al., 2007), and it reliably detects stable individual differences in stereotypy and enrichment use (i.e. individual differences that are consistent over weeks) (Dallaire et al., 2012).

2.1.3. Teat scoring, mortality assessment and litter weighing

Mink were scored for signs of mastitis 0–1 days after being removed from their litters at 41–42 days *post partum*: the period when mastitis is most prevalent (Schneider and Hunter, 1993a). Nine subjects were too ill with nursing sickness to disturb, reducing the sample to 139. The method of Dawson et al. (2013) was used, but this time both scorers (MB and LD) were blind to the dams' former housing conditions. A novel object was moved above each cage to encourage mink to rise on their hind legs and expose their bellies. Teat health was then scored using a visual system based on nipple redness and swelling, mink being categorized as either 'sore-looking' or 'normal'. Unlike as described in Dawson et al. (2013), all teats per mink were not always similar in appearance. Therefore a female was scored as 'sore-looking' if two or more teats were red and swollen.

Kits were also counted and individually weighed once, post-weaning, between 42 and 43 days of age. In total, 138 litters were weighed (ten being omitted by accident). There was also some variation in the age at weaning (40–43 days), as necessary to accommodate the teat health scoring as well as data collection on two other farms (see Experiment 2). However, this variation in kit age at weighing, time since weaning, and also litter size at weaning was balanced between treatments, and included in statistical models (see below). Finally, mortality rates were recorded 54–59 days *post partum*; all nursing sickness should have occurred by this time, the peak on North American farms occurring at 42 days *post partum* (Schneider, 1996).

2.1.4. Analyses

Categorical data were analyzed using chi-squared tests: thus effects of bunks on dam deaths; and comparisons of these subjects' mortality rates and mastitis prevalence with

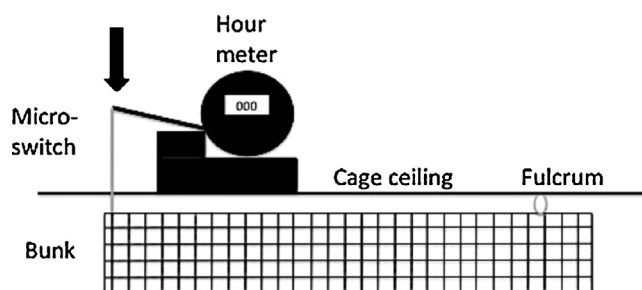


Fig. 1. Schematic of the bunk-use timer, designed to be triggered by the weight of the dam.

those of the multiparous females of Dawson et al. (2013). All other data were analyzed using general linear models in Minitab (v. 14.0), data being transformed where necessary. For non-orthogonal models, Type I (sequential) sums of squares were used, with the term of interest (treatment: bunk versus control) placed as the last main effect. Treatment effects on litter weight controlled for litter size at birth (because by chance this differed between groups, bunk females having given birth to slightly larger litters before treatments were allocated: $F_{1,136} = 3.90$, $P = 0.050$), and for kit ages at weighing and weaning. To analyze timer data, each dam's average diurnal bunk-use for that 3 day period was calculated (as a proportion of time or observations, respectively), for both timer data and direct observations; timer data for nocturnal and crepuscular use (c. 17:00–09:00 h; henceforth abbreviated to 'overnight') were also calculated as a % time, and averaged for each dam across the 3 nights.

2.2. Results

2.2.1. Overnight bunk-use recording

Direct observations of daytime use successfully validated the timer-recorded data: values obtained by the two methodologies over the same diurnal time periods did not significantly differ ($F_{1,8} = 0.47$, $P = 0.513$; proportion of direct observations in bunk [square-root transformed]: 0.127 ± 0.016 ; proportion of time in bunk recorded via timer [square-root transformed]: 0.111 ± 0.016), and they also significantly co-varied ($F_{1,7} = 26.71$, $P = 0.001$, +ve). One dam was once observed placing her kits in the bunk (setting off the timer), but this was not observed again, and her automatically recorded nighttime scores were all low suggesting this behaviour did not recur nocturnally. Timer-recorded daytime use was then compared to overnight use over those 3 days. There was no significant difference between daytime and overnight values ($F_{1,8} = 0.86$, $P = 0.380$). They also significantly co-varied ($F_{1,7} = 8.23$, $P = 0.024$, +ve). Automatically recorded data from 20 to 22 days after bunk installation through to weaning are presented in Fig. 2.

2.2.2. Teat soreness, dam mortality, litter weights and kit deaths

Mink with bunks did not appear to have improved teat health compared to controls. External symptoms of nipple redness and swelling occurred in 30/72 (41.7%) of the

bunk females, compared to 34/67 (50.7%) of control animals (two-tailed $\chi^2 = 1.152$, $df = 1$, $P = 0.282$).

Bunks also did not protect dams against nursing sickness: 11/81 of bunk animals and 11/83 of control animals died (two-tailed $\chi^2 = 0.004$, $df = 1$, $P = 0.95$). Furthermore, there was no significant difference in weaning litter weights between bunk and control groups (total litter weight: $F_{1,133} = 2.10$, $P = 0.150$; 2105 ± 38.40 g for bunk dams, 2184 ± 36.66 g for controls), nor average kit weight ($F_{1,133} = 1.74$, $P = 0.190$; 333.3 ± 6.06 g for bunk dams; 344.8 ± 5.78 g for controls). Kit loss rates (deaths per litter controlling for litter size) were also not significantly affected by bunks ($F_{1,135} = 0.79$, $P = 0.375$; 0.710 ± 0.135 for bunk dams; 0.543 ± 0.129 for controls). Comparisons between this study and the multiparous cohort observed on the same farm the previous year (Dawson et al., 2013) showed no differences in dam mortality (2011: 15/80 multiparous dams died, cf. 22/164 in 2012; $\chi^2 = 1.19$, $df = 1$, $P = 0.271$), nor in the prevalence of apparent mastitis (2011: 29/78 multiparous dams scored as 'sore', cf. 64/146 in 2012; $\chi^2 = 0.98$, $df = 1$, $P = 0.36$).

3. Experiment 2: kit loss and stereotypic behaviour in primiparous dams with bunks and/or enrichment objects

3.1. Methods

3.1.1. Research population

We used the same farm as in Experiment 1 (Farm A) plus two additional farms. At Farm B cage dimensions were 61 cm l × 38–45 cm w × 30 cm h; and at Farm C, 63 cm l × 38 cm w × 38 cm h. Nest box inner dimensions were as follows: at Farm B, 25 cm l × 25 cm w × 23 cm ± 1 cm h; Farm C, 24 cm l × 30 cm w × 25 cm h). Nest boxes on Farm C were divided widthwise into a 12 cm wide foyer area and an 18 cm wide nesting area. Again, water was available *ad libitum* via automatic nipple drinkers, and mink were fed a semisolid feed prepared on farm. On Farm B feeding occurred between 11:30 and 13:30 h and approximately 50% of cages had leftovers (redistributed between 08:30 and 10:00 h). Farm C fed between 11:30 and 14:00 h, and feed remained on 80–100% of cages the following morning (again being redistributed between 8:00 and 10:30 h).

Across all three farms, 442 females had already been provided (by RM) with enrichment objects for another study: c. 60% of them 10 months previously, from when

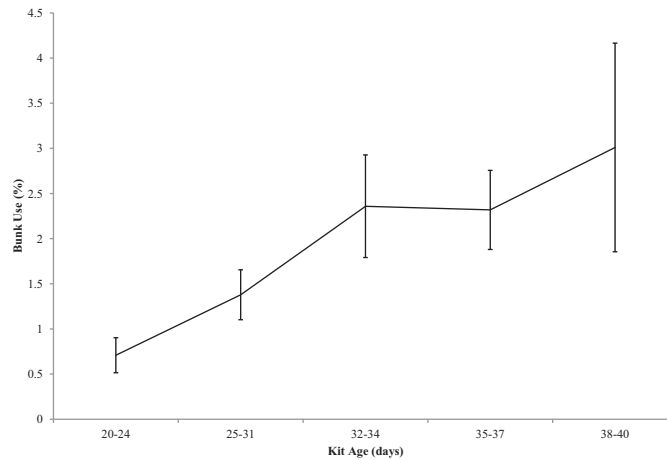


Fig. 2. Bunk use as a percentage of the overall 24-h daily time budget (\pm standard errors) from 20 to 40 days *post partum*. Data are presented as averages of three successive 24-h periods; $n=9$ for all except the first two periods where $n=7$).

pair-housed as juveniles the previous July (with matched non-enriched siblings as controls), and c. 40% of them, 5 months previously in January (without matched non-enriched sibling controls, since family identities were unknown by that phase of production). These enrichment objects consisted of either a plastic chain or a piece of rubber hose approximately 20 cm long, suspended from the cage ceiling (hose at Farm A, chain at Farm B and both, alternating one per cage, at Farm C), as well as a golf ball and a hollow plastic 'wiffle ball' (although all balls were removed temporarily between whelping and kits reaching the age of c. 21 days).

The aim was to collect behavioural data over a narrow kit age range, each litter being observed for 3 consecutive days between the ages of 30 ± 1 and 32 ± 1 days. This was partly to try and reduce disturbance and stress to the dams, as potentially caused by observation (Dawson et al., 2013). It was also because this is a crucial stage of kit development (kits are just transitioning to relying on solid food [Mason, 1994]; they start licking their dams to gain water; kit-kit aggression may occur; and dams start to show stereotypic behaviour [e.g. Brink and Jeppesen, 2005]), and one that our previous study identified as a period in which bunks have a particularly beneficial effect on dam stereotypy. To coordinate data collection, subjects on each farm were therefore selected to have whelping dates within a 3-day long window, and staggered across the three farms such that all farms could be observed in succession, with all litters at each farm being aged 29–33 days during data collection. Animals not previously provided with enrichment objects were selected (by whelping date) in the same sheds and rows as those that had been.

A final total of 318 black primiparous dams were included in the experiment (Table 1). Approximately 1 week before observations commenced, half our subjects were provided with bunks, alternating between object-enriched subjects and controls. Bunk provision to object-enriched females was skewed towards 5-month enriched dams rather than 10-month enriched ones, because for the large scale enrichment project they were part of (Mason et al., 2012), we wanted to avoid

manipulating object-enriched females who had living non-enriched sisters matched controls (all in the 10-month enriched group). Bunk design and installation were as in Experiment 1. Each farm contained all treatment combinations (Table 1). Experimental animals were widely dispersed on two of the farms: on Farm A they were located in 12 rows across eight sheds, while on Farm B, subjects were located in six rows across five sheds. In contrast, on Farm C, all object-enriched animals were located in one shed and as a result, all experimental animals were housed in two rows within one shed.

3.1.2. Data collection

Behaviour data were collected over 3 days on each farm. Dams were observed before feeding time (between 07:00 and 13:30 h on Farms B and C, and 7:00 and 16:00 h on Farm A) since feeding schedules are the primary influence on daily activity patterns (e.g. Bildsøe et al., 1991; Damgaard et al., 2012; Mason, 1993). Data were collected using instantaneous scan sampling (Martin and Bateson, 2007, pp. 53–54) and the ethogram in Table 2. This ethogram refined how stereotypic behaviour was scored (cf. Dawson et al., 2013), due to growing evidence that carnivore-typical locomotor and whole body movements differ in their correlates and underlying causal factors from the mink-typical repetitive scrabbling seen on some farms (Campbell et al., 2013; Dallaire et al., 2011, 2012; Meagher and Mason, 2012). Due to differences in farm layout and subject dispersion, there was variation in the number of scans between farms, with each individual animal being observed 12–30 times per day.

As in Experiment 1, observations were conducted from one row over whenever possible, so that dams under study were not exposed to unnecessary disturbance. Scans were conducted when approaching the cage as opposed to directly in front of the cage, to avoid interrupting the dams' behaviour. When observer presence disrupted a mink that was performing repetitive behaviour (so that it did not meet the 'three repeats' criterion; Table 2), the cage was passed and observed again 10 s later; the behaviour from the second scan was then recorded. Behaviour observations

Table 1

Distribution of subjects across farms and treatment groups; values in parentheses indicate the number that were sampled for FCM.

Group	Farm A	Farm B	Farm C	Totals
Object-enriched/bunk	35 (11)	15 (7)	34 (10)	84
Object-enriched/no bunk	28 (8)	4 (3)	48 (10)	80
Non-object-enriched/bunk	25 (8)	13 (6)	36 (8)	74
Non-object-enriched/no bunk (i.e. standard farm cage)	48 (13)	14 (10)	18 (7)	80
Totals	136 (40)	46 (26)	136 (35)	318

were halted when there was a major disturbance in the shed (e.g. a feed cart).

Approximately one third of females were also sampled for FCM (see Table 1). These were pseudo-randomly chosen on the basis of whelping date (their kits had to reach 32 days before MB had moved on to the next farm for data collection) and for an approximately even distribution across treatment groups. When these subjects' kits were 31 days old, mesh screens were laid down under the cages to catch faecal samples for 24 h; after 24 h, samples were stored in a -20°C freezer; then homogenized and processed (Palme and Möstl, 1997). Extracts were assayed (blind to treatment) for cortisol metabolite concentration using an 11β -hydroxyaetiocholanolone enzyme immunoassay (EIA) validated for mink (Malmkvist et al., 2011).

Litter size was recorded based on counts conducted by farm workers soon after whelping (0 or 1 day *post partum* on Farm A and B, and 10 days *post partum* on Farm C). Litter size was recorded for a second time when kits were weaned. On Farm B, farm workers carried out weaning on day 43 *post partum* by removing mothers from the kits and relocating them to a separate shed. Here, and at Farm A, experimenter MB tallied litter sizes at this time. On Farm C, weaning was carried out 59–61 days *post partum*. Dams were left in their whelping cages, and farm workers tallied kits and relocated them to cages elsewhere in the shed. In all cases, differences between the first and second tallies were used to calculate kit mortality (if litter sizes increased, presumably due to added fosterlings, mortality was assigned to be zero). As in Experiment 1, dam deaths were again recorded at 54–59 days *post partum*.

3.1.3. Analysis of data

Data were primarily analyzed using general linear models in Minitab (v. 14.0). Data were blocked by farm (fixed), and transformed where necessary. To determine the effect of enrichment objects, enrichment status was divided into

three categories: enriched for 10 months, enriched for 5 months, and controls. *Post hoc* Tukey's tests were used to test for differences in group means when treatment was significant or a trend ($P < 0.10$). Whether or not each female had a known living sister was also included as a blocking factor, since 10-month enriched non-bunk females were skewed towards such families (and might therefore have been higher quality, in a way that affected our dependent variables). To test for interactions between enrichment objects and bunks, the two enriched groups (5 months and 10 months) were pooled into one broader 'object-enriched' category, as models otherwise would not run. Kit loss analyses controlled for litter size at birth. Behavioural data were proportional (% of observations or % of normal activity) and so were arcsine square root transformed before analysis. Whenever models were non-orthogonal, they were re-run using sequential sums of squares with the term of interest last in the model. To explore behavioural data more thoroughly, models were then re-run including litter size and its interactions with the presence of enrichment objects/bunks as independent variables. Finally, the effects of enrichment objects and bunks on dam deaths were assessed via chi-squared tests. Chi-squared tests were also used to compare the mortality rates of these subjects at Farm A and the primiparous females, all at Farm A, of Dawson et al. (2013); as well as to compare these subjects with those of Experiment 1.

3.2. Results

3.2.1. Reproductive variables

Dams provided with bunks lost approximately 0.3 fewer kits per litter than controls (for Box–Cox transformed data: $F_{1,302} = 5.78$, $P = 0.017$; for raw data see Fig. 3). Enrichment objects also tended to increase litter size at weaning ($F_{2,303} = 2.82$, $P = 0.061$; Fig. 4), even though early litter size in enriched animals was not significantly greater ($F_{2,304} = 1.97$, $P = 0.14$), nor kit losses smaller ($F_{2,302} = 0.68$,

Table 2

Ethogram.

Locomotor stereotypies	Repetitive body movements or locomotor route-tracing, performed with three or more consecutive repetitions (Dallaire et al., 2011)
Stereotypic scrabbling	Scratching with forepaws, usually at the corners of the cage (Dallaire et al., 2011)
Other activity out in the cage	Activity performed out in the cage, including grooming, eating, drinking, defecating, standing, sitting vigilant and repetitive behaviour <3 repetitions
Not visible/assumed inactive	Mink cannot be seen
Visibly active in the nest box	Visibly active (non-stereotypic) with all limbs inside the nest box or nest box opening
Inactive out in the cage	Lying down motionless on the cage floor
Using Bunk	Occupying the bunk so that no limbs are in contact with the cage floor (only relevant for bunk treatment groups)

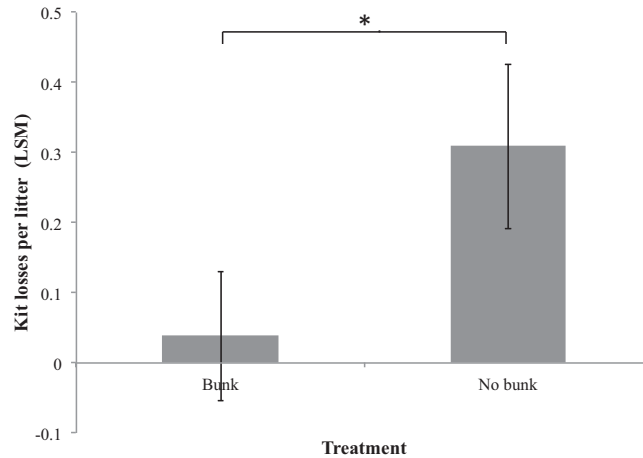


Fig. 3. Least squares means of kit losses (\pm standard errors) in female mink housed in standard cages with or without bunks (effects control for litter size in the early nursing period) (*indicates a statistically significant difference at $P < 0.05$). Raw data were used here to generate LSMs that are easy to interpret in terms of numbers of kits dying (GLM statistics for bunk effects using this variable: $F_{1,302} = 6.56$, $P = 0.011$). Results for Box–Cox transformed data are given in the text.

$P = 0.51$). *Post hoc* Tukey's analyses of weaning litter sizes showed that dams given enrichment objects for 5 months weaned significantly more kits than non-enriched dams ($t = 2.486$, $P = 0.035$, Fig. 4), the difference amounting to approximately 0.9 extra kits per litter. There were no significant interactions between the presence of bunks and enrichment objects for any reproductive variable ($P > 0.45$ in all cases).

3.2.2. Dam behaviour & FCM output

There was a trend for bunk provision to reduce locomotor stereotypies when activity levels were controlled for (locomotor stereotypies/all activity) ($F_{1,296} = 2.80$, $P = 0.095$); control females spent approximately 12.5% of their active time in this behaviour, whereas bunk females spent 6% of their active time performing stereotypic behaviour. In contrast, neither object-enriched treatment

significantly affected levels of stereotypies ($F_{2,304} = 0.36$, $P = 0.701$). This held true when normal activity was controlled for ($F_{2,296} = 0.45$, $P = 0.636$). Bunks and enrichment objects had no interactive effects on stereotypic behaviour ($P > 0.12$ in all tests).

Adding litter size and its interactions into behavioural models did not substantially change results; levels of stereotypy were not predicted by litter size, nor by interactions between litter size and either of the two enrichment types; and the trend remained for bunks to reduce locomotor stereotypy corrected for overall activity levels. However, within the bunk group, litter size did predict time spent in the bunks, females with larger litters spending more time there (litter size at the start of the nursing period: $F_{1,145} = 11.24$, $P = 0.001$; litter size at weaning: $F_{1,145} = 15.12$, $P < 0.0001$; see Fig. 5). Again there were no interactive effects on behaviour between

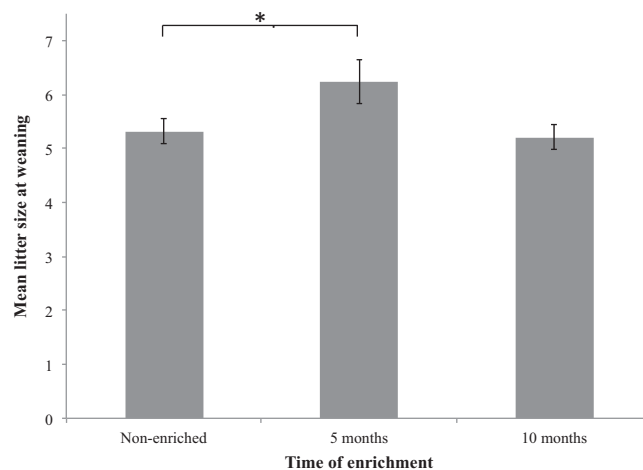


Fig. 4. Least squares mean litter sizes at weaning (\pm standard errors) of female mink provided with enrichment objects for the preceding 5 months, 10 months, or never. (*Indicates a statistically significant difference at $P < 0.05$ in *post hoc* tests; however, the overall treatment effect was a trend – see text for full statistical details).

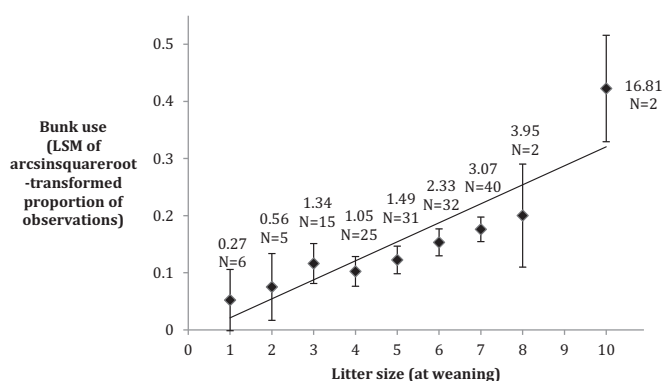


Fig. 5. Least squares means (LSM) for bunk use by dams with kits aged 29–33 days, and varying litter sizes (for statistics see text). Values on the y axis are arcsin square root transformed, but the additional value by each LSM shows the respective backtransformed figure, expressed as a % of observation time (along with the sample size in parentheses). For statistical details, see text.

bunks and enrichment objects (e.g. for locomotor stereotypies/observation: $F_{1,296} = 0.25$, $P = 0.617$; this also held true when overall activity was controlled for).

Neither bunks nor enrichment objects had significant main effects on FCM output (bunks: $F_{1,84} = 0.01$, $P = 0.917$; enrichment objects: $F_{1,84} = 0.53$, $P = 0.592$), nor did they affect FCM levels when supplied together ($F_{1,84} = 0.24$, $P = 0.626$). The one significant influence on FCM was farm (Fig. 6: $F_{2,84} = 27.78$, $P < 0.0001$). *Post hoc* Tukey's tests showed that Farm A had higher FCM levels than both B ($t = 3.183$, $P = 0.0058$) and C ($t = 7.416$, $P < 0.0001$); and Farm B also had higher levels than C ($t = 3.89$, $P = 0.0006$).

3.2.3. Dam mortality

Dam mortality rates were not affected by the presence of bunks ($\chi^2 = 0.084$, $df = 1$, $P = 0.772$), nor by enrichment objects ($\chi^2 = 0.152$, $df = 1$, $P = 0.697$). However, they were significantly affected by farm ($\chi^2 = 11.989$, $df = 2$, $P = 0.0025$), because Farm A had significantly higher mortality rates than the others (for both comparisons, $\chi^2 > 4.7$, $df = 1$, $P < 0.03$; on Farm A, 13/136 subjects died, on B,

0/46, and C, 2/136). Furthermore, even controlling for farm (Farm A animals had elevated FCM as well as mortality) females that later died of nursing sickness had a trend to elevated FCM levels ($F_{1,94} = 3.10$, $P = 0.08$). However, note that the primipares on Farm A tended to have lower mortality rates than their equivalents in our study there the previous year (Dawson et al., 2013) ($\chi^2 = 3.40$, $df = 1$, $P = 0.065$).

4. Discussion

Experiment 1 built on the findings of Dawson et al. (2013), by collecting data on the same farm the following whelping season. Like the previous study, it failed to find evidence that bunks reduce dams' risks of dying from nursing sickness, even though we selected a sub-population at risk of this condition. Experiment 1 suggested no effect of bunks on kit losses either. This again resembled our previous findings (Dawson et al., 2013), but was contradicted by Experiment 2's larger-scale study (see below). Bunks also did not seem to enhance kit growth rates, again

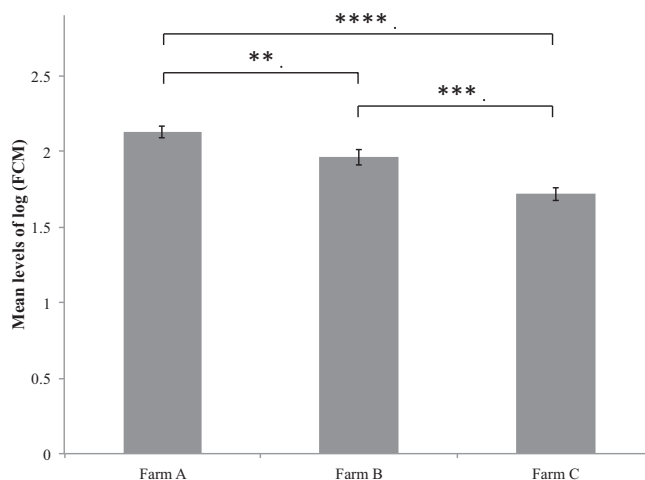


Fig. 6. Least squares mean levels of log FCM (\pm standard errors) at 32 days *post partum* according to farm (**** indicates a statistically significant difference at $P < 0.0001$; *** indicates a statistically significant difference at $P < 0.001$; ** indicates a statistically significant difference at $P < 0.01$). For statistical details, see text.

like the previous study. Dobson and Rouvinen-Watt (2008) had found that amongst dams exposed to research procedures (e.g. blood sampling), those given bunks were better able to wean larger litters, but perhaps such effects are special to nursing females exposed to stressors. Consistent with our results, Hansen (1990) likewise found no effects on kit growth or weaning weight, in otherwise unmanipulated families given an elevated wire mesh cylinder or experimental 'retreat den'. Collectively, these studies and our new data suggest that bunks at least do not affect kit growth rates adversely. In addition, Experiment 1 found no evidence that bunks improved teat health. This conflicts with Dawson et al. (2013) and it is unclear why. The previous data were not collected blind to treatment, and so unconscious bias could have generated non-replicable results. However, even in our new study the scoring method was still subjective, and so it could be that scoring inconsistencies led to Type II errors this time. Alternatively it could genuinely be that populations differ in whether or not bunks protect them against mastitis. After all, the two populations observed did differ in certain ways. First, we focused here on larger than average litters, in principle more at risk of mastitis (Clausen et al., 1992; Rouvinen-Watt and Hynes, 2004), although prevalence was not significantly higher than in the previous study's randomly chosen multiparas; second, subjects here were randomly selected with respect to barren females, and thus unlikely to have a non-reproductive neighbour, whereas the former subjects were all within two cages of a barren (thence unusually active) female; third, subjects here were only exposed to a human observer for a few days, not daily for the last 3 weeks of lactation; and fourth, at least in the nine mink from which behavioural data were collected, this study's females subjectively seemed to use their bunks less (peak use being approximately half the 7% reported in Dawson et al. (2013)). Overall, given the great importance of mastitis as a health and welfare issue for mink (Schneider and Hunter, 1993a), and the limitations of our subjective teat-scoring system, we urge for more research on the potential benefits of bunks for this condition, using larger sample sizes and, more importantly, objective veterinary indicators of mastitis (e.g. somatic cell counts measured from milk samples [Højsgaard and Friggens, 2010]).

Finally the automated nocturnal data collection in Experiment 1 revealed that nighttime and daytime bunk use was similar. Our previous work (Dawson et al., 2013) showed that daytime use correlated with how soiled the sides of the bunks became (seemingly with grease from the females' haunches), suggesting that use directly recorded during daylight did correlate with 24 h use. However, daily maximum usage during this time had only averaged about 30 min, and it was unknown whether bunk-use increased, decreased, or stayed stable over the hours of darkness. Our new automatically collected data reveal that nighttime use and daytime use are very similar: rates of use recorded during daylight thus give good estimates of the total time budget spent in the bunk. Subjectively, these data also illustrated how bunk-use increases as their kits mature (see Fig. 2; as also shown by Dawson et al., 2013; Hansen, 1990; Jeppesen, 2004).

Experiment 2 yielded further evidence that dams modulate their use of bunks according to how demanding their kits are: bunk use during our observation window (kit ages 29–33 days) was significantly greater for dams with large litters. Our previous work failed to find such a pattern (Dawson et al., 2013), but its sample sizes were much smaller than those used here increasing the likelihood of a Type II error. This response is consistent with the mothers with larger litters showing more avoidance of them. Furthermore, dams were also occasionally observed stashing their kits in the bunks (although too rare to be analyzed statistically: not witnessed on Farm B, rarely seen on Farm A, but recorded in 0.5% of scans on Farm C), and at the ages observed (29–33 days), the kits seemed unable to get down again independently. As well as using the bunks in these ways, dams with bunks showed a trend to reduce locomotor stereotypy relative to overall activity during the period observed. Despite this, bunks once again did not protect them against nursing sickness; nor did bunks reduce dams' adrenal cortical activity.

Nevertheless Experiment 2 did reveal for the first time that bunks bring benefits for kits: litters with bunks had slightly but significantly lower rates of kit loss. This suggests that previous failures to find such effects in smaller-scale studies (Dawson et al., 2013; Experiment 1) were Type II errors. Future research should seek to replicate this finding, and if confirmed, investigate how it is mediated. One possibility is that the presence of bunks enhances crucial aspects of maternal care, analogous to the way that giving mink dams access to straw for nest building results in faster kit-retrieval responses (Malmkvist and Palme, 2008). For instance, one paradoxical finding by Dawson et al. (2013) was that bunks seemed to enhance nursing, at least in multiparas. Both aspects of maternal care should now be studied in more detail in control and bunk-provisioned dams. Furthermore, Schneider and Hunter (1993b) determined that wounds were a leading cause of mortality in kits between 4 and 42 days of age, and thus effects of bunks on aggressive behaviour, both within a litter (cf. e.g. Brink et al., 2004; Drake et al., 2008) and from the dam (e.g. Martino and Villar, 1990, cited in Malmkvist et al., 2007) should be investigated too. Future research on bunks should also see if providing them from before parturition reduces kit losses to greater degrees than those observed here (and investigate suggested concerns that climbing in and out of bunks prior to parturition may cause trauma to unborn foetuses, contributing to increased peri-natal kit mortality: Dobson and Rouvinen-Watt, 2008).

The provision of enrichment objects months before breeding also appeared to benefit reproductive productivity, although not via reducing kit mortality. Again, FCM levels were not reduced, nor were enriched dams protected against nursing sickness; furthermore, enrichment objects did not have any detectable effects on stereotypic behaviour. Nevertheless, depending on their timing, they had potentially dramatic effects on reproductive output: 5-month enriched females weaned nearly one kit more per litter. This now needs replicating, since the overall treatment effect was a trend and the stronger pattern was only suggested by *post hoc* tests: it must yet be confirmed by analysis of breeding records for all the object-enriched

females, not just the sub-set used in Experiment 2. However, in the interim it raises questions for future research about how such effects could be mediated (a combination of reduced mortality and greater birth litter sizes, neither effect being significant on their own?). It also raises testable hypotheses about why having enrichment objects for 5 months appears beneficial, but having them for 10 months seems not, habituation being the most plausible explanation: many species, including farmed mink, become disinterested in toys and other enrichments over time, especially if they are never changed (Jeppesen and Falkenberg, 1990; Young, 2003). If mink are to be provided with enrichment objects long-term from the juvenile period (*cf.* e.g. Hansen et al., 2007), it would be worthwhile to chart their use over the following months, and to investigate the effects of adding new objects periodically, e.g. once animals reach young adulthood.

A final issue to discuss is dam mortality. In these experiments, the timing of dam deaths all strongly implicated nursing sickness: a metabolic condition that is the leading cause of mink dam mortality (Clausen et al., 1992; Rouvinen-Watt and Hynes, 2004; Schneider and Hunter, 1993c). It was disappointing that neither type of enrichment protected females from this: we had hoped that greater opportunities to perform naturalistic activities and to escape from infants at will, perhaps combined with interest by the kits in the objects, would help protect dams from nursing sickness by reducing both their stress levels and the demands that kits make on them. Instead we found evidence that, as has been suggested in previous work (e.g. Rouvinen-Watt, 2003; Wamberg et al., 1992), elevated cortisol is a precursor to the disease. We also found, just as in other studies (Rouvinen-Watt and Hynes, 2004; Schneider and Hunter, 1993c) marked farm effects, perhaps reflecting differences in feeding regime in the months before whelping (Rouvinen-Watt, 2003; Rouvinen-Watt and Hynes, 2004), and/or other factors that could affect cortisol levels (e.g. handling styles; or degrees to which calm temperaments are selected for). Finally, we found that mortality levels in our primiparas at Farm A showed a strong trend to be lower than same-age females at that location the previous year. This could merely be a year effect; or it could reflect two changes in our protocol: our attempts to minimize observer disturbance, and our selection of random dams instead of dams within two cages of a barren female (Dawson et al., 2013), animals which are more active than nursing dams and perhaps disturbing as neighbours. More research is therefore needed on why the devastating condition of nursing sickness varies so greatly in prevalence and severity between populations so that it can be more effectively tackled – with our data suggesting that enrichment is not a solution.

5. Conclusions

Our results indicate that providing dams with 'get-away bunks' improved both productivity and dam welfare by reducing dam stereotypy and infant mortality during the nursing period. Introducing dams to novel enrichment objects during the winter months, 5 months before nursing, also apparently increased the number of kits weaned.

Despite the benefits that bunks had on productivity and welfare, there was no evidence that they improved teat health or reduced nursing sickness, but farm identity and potentially cortisol levels both played a role in dam mortality. More research is needed on the mechanisms involved in the beneficial effects of both bunks and enrichment objects on reproductive output; into what the optimum timing is for providing adult female mink with both of these types of enrichment; and on the roles of management practices and glucocorticoid levels in population differences in nursing sickness.

Acknowledgements

We are very grateful to the owners of all three farms; to Francis Papillon for manufacturing the bunks; and to NSERC and the Ontario Ministry for Food and Rural Affairs, for funding.

References

- Bildsøe, M., Heller, K.E., Jeppesen, L.L., 1991. Effects of immobility stress and food restriction on stereotypies in low and high stereotyping female ranch mink. *Behav. Process.* 25, 179–189.
- Brink, A.L., Jeppesen, L.L., Heller, K.E., 2004. Behaviour in suckling mink kits under farm conditions: effects of accessibility of drinking water. *Appl. Anim. Behav. Sci.* 89, 131–137.
- Brink, A.L., Jeppesen, L.L., 2005. Behaviour of mink kits and dams (*Mustela vison*) in the lactation period. *Can. J. Anim. Sci.* 85, 7–12.
- Campbell, D.L., Dallaire, J.A., Mason, G.J., 2013. Environmental enrichment reduces perseveration in the American mink, but enhances spontaneous alternation. *Behav. Brain Res.* 239, 177–187.
- Clausen, K.T., Olessen, C.R., Hansen, O., Wamberg, S., 1992. Nursing sickness in lactating mink (*Mustela vison*) I. Epidemiological and pathological observations. *Can. J. Vet. Res.* 56, 89–94.
- Dallaire, J.A., Meagher, R., Díez-León, M., Garner, J.P., Mason, G.J., 2011. Recurrent perseveration correlates with abnormal repetitive locomotion in adult mink but is not reduced by environmental enrichment. *Behav. Brain Res.* 224, 213–222.
- Dallaire, J.A., Meagher, R.K., Mason, G.J., 2012. Individual differences in stereotypic behaviour predict individual differences in the nature and degree of enrichment use in caged American mink. *Appl. Anim. Behav. Sci.* 142, 98–108.
- Damgaard, B.M., Dalgaard, T.S., Larsen, T., Hedemann, M.S., Hansen, S.W., 2012. The effects of feed restriction on physical activity, body weight, physiology, haematology and immunology in female mink. *Res. Vet. Sci.* 93, 936–942.
- Dawson, L., Buob, M., Haley, D., Miller, S., Stryker, J., Mason, G., 2013. Providing nursing female mink with get-away bunks improves their health and welfare. *Appl. Anim. Behav. Sci.*
- Dobson, J., Rouvinen-Watt, K., 2008. Seasonal body weight, body condition score, blood glucose and stress level of female mink (*Neovison vison*) with or without access to resting bunks. *Scientific 32*, 184–185.
- Dragano, N., Verde, P.E., Siegrist, J., 2005. Organisational downsizing and work stress: testing synergistic health effects in employed men and women. *J. Epidemiol. Community Health* 59, 694–699.
- Drake, A., Fraser, D., Weary, D.M., 2008. Parent-offspring resource allocation in domestic pigs. *Behav. Ecol. Sociobiol.* 62, 309–319.
- Dunstone, N., 1993. *The Mink*. T&A Poyser, London.
- Hansen, S.W., 1990. Activity pattern of lactating mink and the effect of water trays or wire netting cylinder in mink cages. *Scientific 14*, 187–193.
- Hansen, S.W., Malmkvist, J., Palme, R., Damgaard, B.M., 2007. Do double cages and access to occupational materials improve the welfare of farmed mink? *Anim. Welf.* 16, 63–76.
- Hansen, S.W., Møller, S.H., Damgaard, B.M., 2011. Feed restriction and tubes for environmental enrichment in growing mink – consequences for behaviour and welfare. *Appl. Anim. Behav. Sci.* 134, 193–200.
- Højsgaard, S., Friggens, N.C., 2010. Quantifying degree of mastitis from common trends in a panel of indicators for mastitis in dairy cows. *J. Dairy Sci.* 93, 582–592.

- Jeppesen, L.L., Falkenberg, H., 1990. Effects of play balls on pelt biting, behaviour and level of stress in ranch mink. *Scientifur* 14, 179–186.
- Jeppesen, L.L., 2004. Mink welfare improved by combined implementation of several small initiatives. *Scientifur* 28, 11–18.
- Jørgensen, G., 1985. Mink Production. Scientifur, Denmark.
- Malmkvist, J., Gade, M., Damm, B.I., 2007. Parturient behaviour in farmed mink (*Mustela vison*) in relation to early kit mortality. *Appl. Anim. Behav. Sci.* 107, 120–132.
- Malmkvist, J., Palme, R., 2008. Periparturient nest building: implications for parturition, kit survival, maternal stress and behaviour in farmed mink (*Mustela vison*). *Appl. Anim. Behav. Sci.* 114, 270–283.
- Malmkvist, J., Jeppesen, L.L., Palme, R., 2011. Stress and stereotypic behaviour in mink (*Mustela vison*): a focus on adrenocortical activity. *Stress* 14, 312–323.
- Martin, P., Bateson, P., 2007. *Measuring Behaviour: An Introductory Guide*, third ed. Cambridge University Press, Cambridge.
- Mason, G.J., 1993. Age and context affect the stereotypies of caged mink. *Behaviour* 127, 191–229.
- Mason, G.J., 1994. The influence of weight, sex, birthdate and maternal age on the growth of weanling mink. *J. Zool.* 233, 203–214.
- Mason, G.J., Bahlmann, K., Dawson, L., Dallaire, J.A., Meagher, R.K., Bowyer, S., Diez-Leon, M., Campbell, D., 2012. Simple year-round enrichments for mink. In: Larsen, P.E., Møller, S.H., Clausen, T., Hammer, A.S., Lassen, T.M., Nielsen, V.H., Tausen, A.H., Jeppesen, L.L., Hansen, S.W., Elnif, J., Malmkvist, J. (Eds.), *Proceedings of the Tenth International Scientific Congress in Fur Animal Production*, Wageningen Academic Publishers, The Netherlands. *Scientifur* 36 (3/4), 491.
- Meagher, R.K., Mason, G.J., 2012. Environmental enrichment reduces signs of boredom in caged mink. *PLoS ONE* 7, e49180, <http://dx.doi.org/10.1371/journal.pone.0049180>.
- Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Int. J. Mamm. Biol.* 62 (Suppl. II), 192–197.
- Pedersen, V., Jeppesen, L.L., 2001. Effects of family housing on behaviour, plasma cortisol levels and production in adult female mink (*Mustela vison*). *Acta Agric. Scand. Sect. A* 51, 77–88.
- Rouvinen-Watt, K., 2003. Nursing sickness in the mink – a metabolic mystery or a familiar foe? *Can. J. Vet. Res.* 67, 161–168.
- Rouvinen-Watt, K., Hynes, A.M., 2004. Mink nursing sickness survey in North America. *Scientifur* 28, 71–77.
- Schneider, R.R., Hunter, B.D., 1993a. A survey of the causes of mortality in adult mink with emphasis on the lactation period. *Can. Vet. J.* 34, 103–108.
- Schneider, R.R., Hunter, B.D., 1993b. Mortality in mink kits from birth to weaning. *Can. Vet. J.* 34, 159–163.
- Schneider, R.R., Hunter, D.B., 1993c. Nursing disease in mink: clinical and postmortem findings. *Vet. Pathol.* 30, 512–521.
- Schneider, R.R., 1996. *Mink – Biology, Health and Disease*. Canadian Mink Breeders Association, Rexdale, Ontario.
- Sih, A., Bell, A.M., Kerby, J.L., 2004. Two stressors are far deadlier than one. *Trends Ecol. Evol.* 19, 274–276.
- Sozda, C.N., Hoffman, A.N., Olsen, A.S., Cheng, J.P., Zafonte, R.D., Kline, A.E., 2010. Empirical comparison of typical and atypical environment enrichment paradigms on functional and histological outcome after traumatic brain injury. *J. Neurotrauma* 27, 1047–1057.
- Svendsen, P.M., Hansen, S.W., Jeppesen, L.L., 2007. Direct scan sampling reliably reflects video recorded differences in stereotypy in selected lines of mink. *Scientifur* 31, 19–25.
- Vinke, C.M., Bos Van Den, R., Spruijt, B.M., 2004. Anticipatory activity and stereotypical behaviour in American mink (*Mustela vison*) in three housing systems differing in the amount of enrichments. *Appl. Anim. Behav. Sci.* 89, 145–161.
- Wamberg, S., Clausen, T.N., Olesen, C.R., Hansen, O., 1992. Nursing sickness in lactating mink (*Mustela vison*) II. Pathophysiology and changes in body fluid composition. *Can. J. Vet. Res.* 56, 95–101.
- Young, R.J., 2003. *Environmental Enrichment for Captive Animals*. UFAW, Blackwell Science Ltd., London, pp. 150–151.