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Relocation shortly after mating does not have a major impact on stress responses and reproduction in female farm mink

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ABSTRACT

On farms, female mink are exposed to acute stressors multiple times due to handling, capturing and transport with restricted movement being a typical part of the mating procedure. We hypothesised that no relocation (group NON; i.e. omitted from one trapping and one transport to a novel cage) or direct relocation after second mating (group DIRECT; i.e. omitted from one capturing) reduces female mink's stress responses (indicated by cortisol and stereotypy) and thereby influences maternal care (nest building, nest climate and offspring retrieval) and consequently potentially improves the reproductive output (litter size Day 0–7, offspring mortality and growth) compared to a regular farm relocation procedure (group REGULAR). In numbers, group NON had lower faecal cortisol metabolite (FCM) concentrations, but relocation did not with statistical significance influence the FCM concentrations prior to (NON: 66 ± 8.4 ng/g vs. DIRECT: 115 ± 47.5 ng/g vs. REGULAR: 96 ± 20.0 ng/g; $F_{1,112} = 0.7$, $P = 0.49$) and after parturition (NON: 65 ± 7.2 ng/g vs. DIRECT: 79 ± 14.6 ng/g vs. REGULAR: 84 ± 13.6 ng/g; $F_{2,109} = 0.3$, $P = 0.72$); nor did the relocation procedure elicit a different performance of stereotypic behaviour (6 ± 2.1 vs. 3 ± 1.4, $F_{2,121} = 1.2$, P = 0.30). The nest score differed between groups ($F_{2,201} = 6.9$, $P = 0.001$;) as group DIRECT (3.2 ± 0.04) had a significantly higher nest score than group NON (2.9 ± 0.07; $P < 0.001$) and a tendency to a higher nest score than group REGULAR (3.0 ± 0.06; $P = 0.070$). Likewise, there was a tendency that REGULAR had a higher nest air temperature compared to NON ($P = 0.055$). NON (19.3 ± 0.10 °C) had a significantly higher daily mean nest air temperature compared to DIRECT and REGULAR (17.9 ± 0.13 °C and 18.3 ± 0.14 °C; $P < 0.001$). The difference in nest score and nest air temperature could be due to group NON being in a shed with a slightly higher temperature (8.9 ± 0.21 °C) compared to the shed containing most of the animals in groups DIRECT and REGULAR (8.3 ± 0.19 °C). The three relocation strategies NON, DIRECT and REGULAR did not affect litter size or offspring growth. We conclude that relocation of mink dams shortly after mating is not a major stressor, as we can only report a minor effect on FCM concentrations during the gestation, and detected no effect on the performance of stereotypic, maternal care or reproductive outcome using alternative procedures.

1. Introduction

Capturing, handling and fixation in traps are known to elicit stress responses in farm mink, as demonstrated in studies by increased concentrations of blood plasma cortisol (Hansen and Damgaard, 1991) and faecal cortisol metabolites (Malmkvist et al., 2011). Such repeated handlings during gestation may potentially impair the reproductive output; at least one study reported that repeated trapping and immobilisation procedures during gestation increased the number of offspring dying and reduced the litter size measured 6 weeks after delivery in mink (Jeppesen and Heller, 1986). On commercial mink farms, female mink are exposed to capturing and handling as acute stressors multiple times as part of the mating procedure, as natural mating trials are used (cf. Malmkvist et al., 1997). During the attempts of first and second mating, the female mink is captured in her home cage either by hand or in a trap and transported to the nearby male cage, then returned to the home cage after mating or end of an unsuccessful trial period of several hours. After the conclusion of the yearly mating season, the successfully mated female is again captured and transported to the maternity unit by transport trap or tube. During this transport, the mink is restricted in movement, but the immobilisation is milder than that used in some experimental studies eliciting marked cortisol

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responses. However, many mink react when in the trap by scratching and turning, and some individuals scream (personal observation), thus we expect the capture and the transport to be acute stressors. Furthermore, a previous study has documented that the timing of the relocation of mated females into the maternity unit influenced both their faecal cortisol metabolite (FCM) concentrations and their reproduction (Malmkvist and Palme, 2015). In the later study, relocation of mated dams close to implantation – i.e. around 10 April (Sundqvist et al., 1989) – increased both FCMs and reduced the reproductive outcome compared to mated females relocated earlier (23 March: Day -36 relative to day of birth, 0) or later during the gestation (25 April: Day -3, Malmkvist and Palme, 2015). Based on these results, the best-known practice is currently to relocate mated females relatively early after mating (i.e. around 23 March). In the cited study, the timing of relocation to the maternity unit was confounded with time for access to extra nesting material for nest building (being important in mink, cf. Malmkvist and Palme, 2008). Thus, the positive effect of the early relocation may not only be due to a less stress-sensitive period after mating but could also be due to early access to nesting material.

In the present study, we aimed to enhance the understanding of whether the short-term transport and relocation of mated females to the maternity unit influenced their stress responses (cortisol and stereotypic behaviour), maternal care (nest building, nest climate and offspring retrieval) and reproductive outcome (litter size Day 0–7, offspring mortality and growth). We hypothesised that no relocation (i.e. avoiding one trapping and one transport to a novel cage) or avoiding one capturing would be favourable compared to normal farm procedure as it reduces female mink's stress responses and increases maternal care, consequently potentially improves the reproductive output.

2. Methods

Animal, housing, management and experimental set-up were similar to those described in Schou et al. (2018), but for the reader's overview methods are summarised in the current paper.

2.1. Animals and housing

This study was performed on 126 double-mated mink first time breeders born the previous year (primiparous) of the colour type brown. The mink were housed individually in European standard production cages (length × breadth: 90 × 30 cm, and 45 cm high, Hedensted-Group, DK-8722, Denmark) connected to a wooden nest box (l × b: 23 × 28 cm, and 20 cm high). Each female was mated with the same male twice in the period from 2 to 18 March. The animals were fed 200–230 g of standard commercial wet feed daily through birth once a day (11.00, Hvalpsund mink feed factory, Hvalpsund, Denmark). Straw was provided ad libitum on top of the nest box lid (mesh size: 2.5 × 2.5 cm). Water was available ad libitum via a drinking nipple in the cage. The cage units were placed in a housing facility with natural light and climate conditions at the mink research farm at Aarhus University, Denmark.

2.2. Experimental design and treatments

The study was performed on three treatment groups with different relocation strategies to the maternity unit: (1) No relocation (group NON; $N = 44$), these females were kept in the same cages from November to the end of the experimental period 7 days after birth, (2) Relocated to the maternity unit directly after second mating (group DIRECT; $N = 41$) and (3) Relocated to the maternity unit 23 March (group REGULAR; $N = 41$) on average 10 days after second mating. The group size was chosen to reach a minimum of 40 double-mated females for all three groups. This group size has previously been found sufficient to test effects on FCM, reproduction and fur-chewing between experimental treatments (Malmkvist et al., 2013), which also was the case in Schou et al. (2018).

Random selection into groups was done by use of the function 'Randbetween' (Microsoft excel 2010) by numbering each place within each group. Animals and data included in the current study as group NON (referring to relocation) are identical to group REGULAR (refer to allocation of nesting material) in Schou et al. (2018).

Animals in group NON experienced one capturing and one transport (related to relocation) less compared to regular farm practice. Group DIRECT animals experienced one capturing less compared to regular relocation practice as they were captured by trap in the male cage after second mating and relocated directly to the maternity unit. REGULAR female mink were mated and relocated in accordance to regular farm practice: after second mating the female mink was captured by hand or trap and inserted into her home cage where she stayed until capturing and relocation into the maternity unit on 23 March, identical to the best-known relocation practice found by Malmkvist and Palme (2015). Duration in the trap during relocation was 20 min, believed to be representative for the common relocation duration at Danish mink farms.

In the maternity unit, each cage unit was additionally equipped with 1) a whelping net to prevent the offspring from falling through the wire cage floor, 2) a paper sheet (30 × 30 cm) in the first third of the cage floor in front of the nest box under the whelping net to prevent nesting material from falling out of the cage and 3) a building brick fitted into the base of the nest box. For group NON, these changes were added to the home cages already on 15 January. From the 23 March, all groups had free access to nesting material, delivered as a loose pile of 80 g shredded barley straw placed in the cage close to the nest box entrance. Afterwards, access to nesting material was observed three times a week (Monday, Wednesday and Friday). If approximately 75% of the straw was removed from the cage by the mink, an extra 80 g of straw was added to the cages, thereby ensuring that the mink had continual free access to straw. Easy-strø (from Easy-AgriCare, Nykøbing Mors, Denmark; fine chopped 1–1.5 cm, heat-treated straw 85% wheat and 15% rape, 300 g) was added on 23 March to all nest boxes as bedding material.

2.3. Sampling and observation procedure

2.3.1. Faecal cortisol metabolites (FCMs)

Faeces were collected non-invasively after mating: on 9, 16, 23 April and again Day 3 after birth. Collection of fresh faecal samples was performed continuously for three hours following feeding. Collected samples was stored in cool boxes for maximum one hour during sample collection and transport to a freezer. Subsequent analysis of FCMs were performed using the methodology previously validated for mink (Malmkvist et al., 2011).

2.3.2. Behavioural observation

Direct observations were carried out by scan samples with the observer facing the cage from the feeding aisle 1 m in front of the cage units, with a maximum of six mink at each sample interval of 1 min (Martin and Bateson, 2007). Within the first 15 s of an observation, the animals habituated to the observer. To exclude behaviour performed as a reaction to the observer, active behaviour within the first 15 s was not registered. Each animal was observed for five scans on 17 April between 09.00 h and 11.00 h (before feeding). Only one type of behaviour was registered for each observational scan sample by one-zero score (Martin and Bateson, 2007), as stereotypy overruled active behaviour, which in turn overruled being inactive out in the cage/in nest. Stereotypic behaviour was defined as a monotonous repeating movement pattern (minimum three times of repetitions) without any apparent function or goal.

The data collection was done by trained observers using the predefined ethogramme. After relocation, the treatment group was obvious for the observers. The observers were distributed across the experimental housing and treatment groups. Further, the hypothesis behind

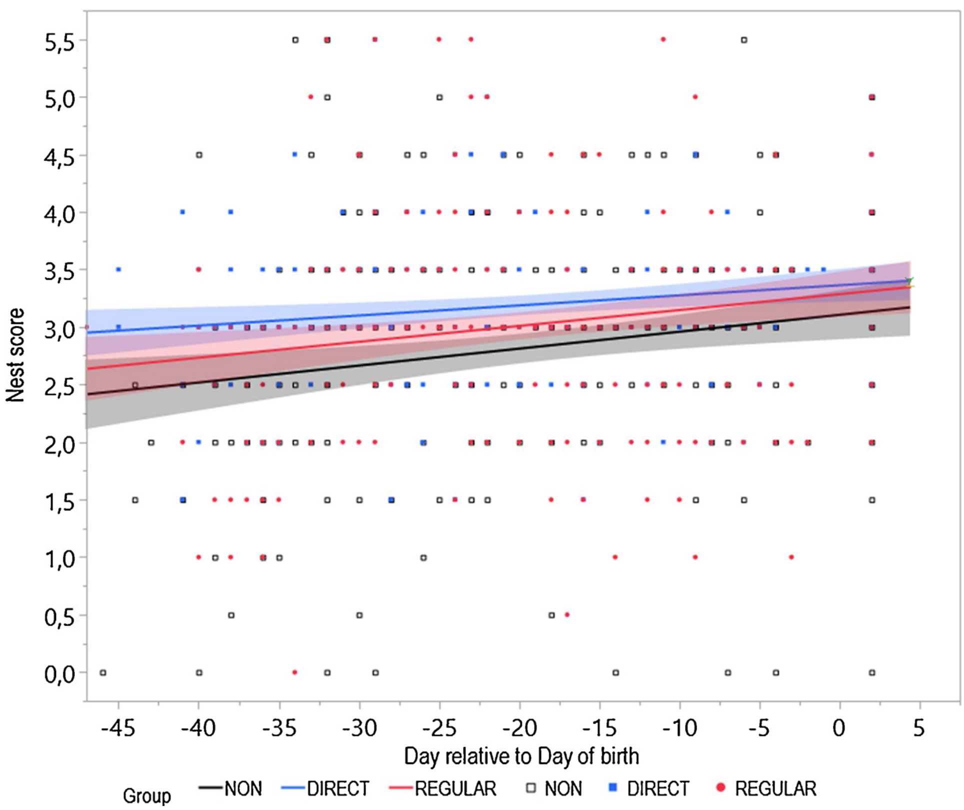

Fig. 1. Nest score, due to maternal nest building, increases towards delivery ($P < 0.001$). The line of fit illustrate per group the daily mean nest score relative to day of birth (Day 0) during the period from 24 March to Day 2 after birth. Group NON: not relocated, DIRECT: relocated to maternity unit directly after second mating, REGULAR: relocated to maternity unit March 23.

the study was not stated for the personnel responsible for the daily care, the capture and handling of the experimental mink.

2.3.3. Nest score

The nest score used included six primary scores: 0) No hollowing in the nest bottom layer, 1) Hollowing without built side walls, 2) Hollowing with built side walls < 5 cm, 3) Hollowing with built side walls ≥ 5 cm, 4) Side walls and top layer present in two thirds of the nest, 5) Nest with side walls and completely closed ceiling. Each of the primary scores was additionally given a secondary level of low (0) or high (+0.5) based on evaluation of whether it was in the lower or higher end of the primary scores. The nests were scored the day before and the day after providing free access to nesting material and thereafter weekly through to Day 2 after parturition.

2.3.4. In-nest box air climate

The in-nest box air climate and shed climate were measured every 15 min using iButton temperature and humidity loggers (Resolution: 0.6% RH; ≤0.5 °C; Maxim Integrated, San Jose, CA, USA), placed as described previously (cf. Fig. 1, Schou and Malmkvist, 2017). Loggers dislodged by the mink were re-installed, and data from the finding day and 24 h before were excluded from the data before analysis.

2.3.5. Offspring retrieval

Mink dams were tested on Day 5 for their reactivity towards an offspring out in the cage as described in Malmkvist and Palme (2008). Offspring selection was randomized, and we shifted between male and female kits within treatments. The offspring was placed in the middle of the cage. Mink dams' reaction was measured as the latency to touch the offspring and latency to retrieve the offspring to the nest. If the dam did not retrieve the offspring within 240 s, the test stopped, and the observer returned the offspring back into the nest.

2.3.6. Reproduction, weight and mortality

The nest boxes were checked during three daily rounds, i.e. in the morning between 08:00 h and 12:00 h, in the afternoon between 12:00 h and 16:00 h, and in the evening between 19:00 h and 20:00 h. Sound and other signs of offspring were used as indications of birth (Day 0). To determine whether dead offspring were stillborn or liveborn lung tissues was cut out and tested if it floated (=liveborn) or sank (=stillborn, Malmkvist et al., 2007). Litter size, offspring sex and offspring weight for each sex within a litter were registered on Days 1 and 7.

2.4. Statistical analysis

SAS software (version 9.4; SAS Institute Inc. Cary, NC, US) was used for statistical analyses and JMP software (version 13; SAS institute Inc.) was used to create the figures. The significance level was set to 0.05 and P values between 0.05 and 0.10 are reported as tendencies. In the models tested, covariates with no tendency to significance ($P > 0.10$) were excluded. The demands for dispersion and variance homogeneity were for ANOVA evaluated from plots of the model residuals for the full and the final models. Results are reported as mean ± standard error of the mean based on the raw data, unless otherwise stated.

The statistical testing was grouped in relation of the main hypothesis of our study of capturing and relocation of adult female mink, i.e. regarding the influence on (i) stress responses, (ii) maternal care, and (iii) reproductive outcome, including offspring growth.

2.4.1. Stress responses

2.4.1.1. Cortisol. Treatment effects on the FCMs (ng cortisol metabolites/g faeces) during gestation (9, 16 and 23 April) were analysed by ANOVA models with repeated measures (per animal ID) performed by using the Mixed procedure in SAS (Littell et al., 1996), testing for main treatment effects (NON, DIRECT and REGULAR), time and the treatment*time interaction. The time structure was analysed using an autoregressive first-order covariance matrix [ar(1) in SAS], as this resulted in the best fit (versus unstructured or compound symmetry) according to Bayesian and Akaike information criteria

(BIC and AIC in SAS). Day relative to birth (rather than calendar date) was used to take the influence of gestation and birth into account. FCM data sampled Day 3 after birth were tested separately. The FCM response was in all cases log transformed to obtain a better residual distribution.

2.4.1.2. Abnormal behaviour. For each behaviour observed (normal and abnormal forms), the percentage of scans in which the animals performed the behaviour was calculated and treatment effects tested in an ANOVA the April 17. Day relative to birth (−4 to −20) and its interaction with treatment were additionally included in the start model. The data for the variables 'In nest box' and 'locomotory stereotypic behaviour' was square root transformed as it resulted in better residuals. In case the transformation did not result in satisfactory residuals (true for four less common forms of stereotypic behaviour, but not for locomotory and total SB) data was analysed as binomial distributed (using the procedure Genmod in SAS), i.e. based on whether the behaviour was observed or not per individual mink.

2.4.2. Indicators of maternal care (nest building, nest air climate and offspring retrieval)

2.4.2.1. Nest score. Development of nest scores was tested in the period 24 March to Day 2 after birth.

2.4.2.2. In-nest climate. The daily air temperature parameters mean, maximum, minimum and standard deviation (temperature fluctuation) were tested in the period (23 March to Day 7 after birth).

Both indicators (nest scores, in-nest climate) were analysed by repeated measures model of the same type as used for the cortisol response with day relative to birth used to take the influence of gestation and birth into account.

2.4.2.3. Dam offspring retrieval response. In the offspring retrieval test, latencies for the dam to touch and retrieve offspring were analysed with methods for survival analysis (procedure Phreg in SAS) considering censored data (Klein and Moeschberger, 2003), as some animals did not touch or retrieve the offspring within the test time. The assumption behind using the used cox proportional hazard model was confirmed by approximate parallel lines between the treatment groups in plots of survival function versus the survival time and in the graph of the log (−log[survival]) versus log of survival time (using procedure Lifetest in SAS). Latencies to touch and to retrieve the offspring are presented as medians with 25% and 75% quartiles, and the proportion of non-responders is given in the results.

2.4.3. Reproductive outcomes

2.4.3.1. Litter size and offspring mortalities. The total litter size, 'totborn', was calculated as the total number of offspring born within a litter including both liveborn and stillborn offspring. The number of liveborn offspring dead within a litter is defined by the sum of collected dead offspring tested as liveborn post mortem plus any missing offspring on Day 7 which previously had been counted as alive. The number of liveborn offspring that died was tested for treatment effect using a Poisson distribution with the natural log of number of original liveborn offspring as the offset value (using the procedure Genmod in SAS). A similar statistical test was performed for the number of stillborn offspring in each litter, however, for which the natural log of total number of offspring born served as an offset value. The total litter size, and the litter size of liveborn D1, D7 were tested in as normal distributed in ANOVA with treatments (NON, DIRECT and REGULAR) as main effect.

2.4.3.2. Early offspring growth. Mean offspring weight was tested Day 1 and Day 7 by ANOVA (procedure Mixed in SAS). The mean offspring weight was calculated as the total weight of the litter divided by the number of offspring alive on that day. The total litter weight Day 7 was used as measure of total reproduction outcome of the dams. In order to truly include all litters of our study, we set the total litter weight to 0 g in litters where all offspring died. The sex ratio (ratio of males per litter, from 0 to 1) and treatment was included in the models. To include all litters in the statistical analysis, a mean sex ratio of all recorded litters Day 7 was assigned to litters with total offspring weight of 0 g at Day 7 after birth.

3. Results

3.1. Faecal cortisol metabolites (FCMs)

The FCM concentration increased significantly as the time of birth approached (days relative to day of birth: $F_{1,220} = 26.1$, $P < 0.001$). Generally, relocation did not significantly influence the FCM concentration prior to delivery (9, 16 and 23 April): NON (65.7 ± 8.36 ng/g), DIRECT (115.2 ± 47.45 ng/g) and REGULAR (96.1 ± 19.98 ng/g; $F_{1,112} = 0.7$, $P = 0.49$). However, when only testing 16 April, there was a tendency that females in group NON (52.5 ± 9.16 ng/g) had a lower level of FCMs compared to females in groups DIRECT (84.9 ± 21.99 ng/g) and REGULAR (84.4 ± 20.05 ng/g; $F_{2,109} = 2.5$, $P = 0.088$). On Day 3 after birth, no difference was evident in FCM concentrations between the three groups (NON: 65.2 ± 7.21 ng/g v. DIRECT: 79.0 ± 14.60 ng/g v. REGULAR: 84.2 ± 13.63 ng/g; $F_{2,109} = 0.3$, $P = 0.72$).

3.2. Behavioural observation

Relocation procedure did not significantly affect the performance of the different types of stereotypic behaviour tested, with some of the variables summarized in Table 1. However, there were a tendency that group DIRECT dams stayed less inactive out in the cage (NON: 8 ± 2.1%; DIRECT: 3 ± 1.2%; REGULAR: 6 ± 1.8%; $F_{2,121} = 2.6$, P = 0.080; Post hoc test: NON vs. DIRECT, P = 0.03; NON vs. REGULAR, P = 0.66; DIRECT vs. REGULAR, P = 0.09).

3.3. Nest score

Nest score increased over time towards Day 2 after birth ($F_{1,428} = 31.9$, $P < 0.001$; Fig. 1). The nest score differed between groups through the period ($F_{2,201} = 6.9$, $P = 0.001$;) as DIRECT (3.2 ± 0.04) had a significantly higher nest score than group NON (2.9 ± 0.07;

Table 1
Behaviour of group NON (not relocated), DIRECT (relocated directly after second mating) and REGULAR (relocated March 23) mink females presented as mean (± SE) scans for the observation day. Total SB (Stereotypic Behaviour) consists of several forms (Locomotory, Stationary, Scratching, Oral), see text for details.

Variable	NON	DIRECT	REGULAR	Statistical test value	*P* value
In the nest box, %	52 ± 5.4	52 ± 4.4	58 ± 5.2	$F_{2,121} = 0.4$	0.65
Active in the cage, %	34^{AB} ± 4.2	42^{A} ± 4.3	28^{B} ± 4.0	$F_{2,121} = 2.7$	0.070
Total SB, %	6 ± 2.1	3 ± 1.4	7 ± 2.5	$F_{2,121} = 1.2$	0.30
Total active[a], %	40 ± 4.7	45 ± 4.5	36 ± 4.7	$F_{2,121} = 1.0$	0.37

Different letters (A, B, C) indicate significant different values within rows in pairwise post test at P < 0.05.
[a] Total active = Active in cage + Total SB.

$P < 0.001$) and a tendency to have a higher nest score than group REGULAR (3.0 ± 0.06; $P = 0.070$). Likewise, there was a tendency that REGULAR had a higher nest score compared to NON ($P = 0.055$).

3.4. In-nest box air climate

NON (19.3 ± 0.10 °C) had a significantly higher daily mean nest air temperature compared to DIRECT and REGULAR (17.9 ± 0.13 °C and 18.3 ± 0.14 °C; $F_{2,145} = 10.1$, $P < 0.001$). Standard deviation (2.6 ± 0.03 °C v. 2.3 ± 0.04 °C v. 2.2 ± 0.04 °C; $F_{2,145} = 17.1$, $P < 0.001$) and maximum temperature (24.2 ± 0.10 °C v. 22.0 ± 0.15 °C v. 22.4 ± 0.16 °C, $F_{2,182} = 29{,}15$, $P < 0.001$) were also higher for NON. Minimum temperature was not affected by treatment (13.6 ± 0.13 °C v. 13.2 ± 0.17 °C v. 13.8 ± 0.17 °C; $F_{2,183} = 1.20$, $P = 0.30$). All temperature parameters were significantly affected by day relative to the day of birth, i.e. with an increasing mean, minimum and maximum temperature and in addition a decreasing standard deviation (all $P < 0.001$). Group NON were in a shed with a slightly higher temperature (8.9 ± 0.21 °C) compared to the shed containing most of the animals in DIRECT and REGULAR (8.3 ± 0.19 °C), which could be the main reason for the temperature difference found between groups.

3.5. Offspring retrieval test

For NON, five of 31 (16.1%), for DIRECT, one of 31 (3.2%) and for REGULAR, one of 36 (2.8%) mink dams did not retrieve the offspring within the test duration of 240 s. Although the latency to touch and retrieve appears similar between reacting dams from the three groups (touch and retrieve: NON = 10 [5; 32.3] and 21.5 [10; 36,0]; DIRECT = 18.5 [7.5; 46.3] and 28 [13.8; 56.3]; REGULAR = 10.5 [6.3; 16,0] and 20 [11; 38.8]), the higher proportion of non-reacting mink in group NON leads to a tendency for significantly reduced reaction in the offspring retrieval (Survival analysis, Chi-sq = 5.5, $P = 0.064$ and Chi-sq = 5.4, $P = 0.067$).

3.6. Reproductive outcome

The three relocation strategies NON, DIRECT and REGULAR did not affect any single reproduction parameter, the overall reproduction and offspring vigour (Table 2). A large total litter size (‘totborn’) increased the risk of dams giving birth to stillborn offspring ($F_{1,80} = 3.06$, $P = 0.003$).

4. Discussion

4.1. Does relocation act as a stressor on mated females?

We report a non-significant difference in the level of FCMs between the three treatments (NON, DIRECT, and REGULAR) in the current study, a period with increasing levels of cortisol during the gestation period and after birth. However, some caution is advised when interpreting this finding. In numbers, group NON (i.e. dams staying in their home cage) had a lower mean level of FCMs throughout the gestation period compared to dams in groups DIRECT and REGULAR. The lack of overall significance could be due to the relatively high variation in FCM levels between individual samplings (from under 10 to above 40,000 ng/g) in relation to the number of mink used in our experiment. We have no biological explanation for the high variation in the FCM during the gestation period. We used different houses for the mink (group DIRECT and REGULAR moved away) which may have introduced some variation in FCM, although the cage conditions and management otherwise were identical between groups of dams. We cannot exclude the possibility that having a larger number of experimental mink would render the lower level of FCM in non-relocated mink significant; e.g. on 16 April there was a tendency that the females who were not relocated (NON) had lower levels of FCMs compared to the relocated females (DIRECT and REGULAR). Relocation has previously been found to affect the level of FCMs, but these findings were found on larger group sizes ($N = 60$), and the relocations were confounded with access to nesting material, which could enhance the effect (Malmkvist and Palme, 2015). The current results indicate that non-relocated mink perhaps experienced minimally less stress compared to the two relocation groups moved to a novel cage.

We did not see any behavioural differences, including in stereotypic behaviour, which could indicate differences in stress and/or frustration between the treatment groups. Thus, relocation appeared not to be a major stressor for the mated females.

One reason for finding a low impact of relocation during this period may be that events around and the mating itself induces stress responses, activates the HPA-axis and thus overrules the effects of our treatment groups. Mating in wild mink has been described as ‘fightful’, involving the male biting on the neck of the female during copulation (summarised by Dunstone, 1993) and the neck biting is also present under mating at farms (Malmkvist et al., 1997). Both in the wild and on farms, the mating trial procedure is repeated several times during the mating season of approximately 20 days. This intense mating (period) could mean that female mink are more stress resistant during the time around mating, e.g. due to a high motivation for mating or, on farms, also due to habituation to repeatedly being captured and transported to another cage. Changes in stress sensitivity are found in, for instance,

Table 2
Reproductive output, offspring survival and growth presented as group means (± SE) for NON (not relocated), DIRECT (relocated directly after second mating) and REGULAR (relocated March 23) sampled from birth until day 7 after birth.

Variable	NON	DIRECT	REGULAR	Statistical test value	*P* value
Totborn, n	8.2 ± 0.34	8.3 ± 0.33	7.8 ± 0.41	$F_{2,112} = 0.6$	0.58
Alive day 1, n	6.5 ± 0.35	6.7 ± 0.38	6.3 ± 0.48	$F_{2,112} = 0.2$	0.79
Stillborn, n	1.0 ± 0.19	1.1 ± 0.21	0.8 ± 0.27	LR Chi-SQ = 0.8	0.68
(% of Totborn)	(13.5 %)	(12.1 %)	(8.0 %)		
Litters with stillborn mortality, n	22 of 41	18 of 36	12 of 38	LR Chi-sq = 3.8	0.15
				LR Chi-sq = 10.5	Totborn: 0.001
Liveborn mortality Day 0-7, n	1.8 ± 0.30	1.6 ± 0.22	1.6 ± 0.26	LR Chi-Sq = 0.3	0.84
(% of Totborn)	(20.1 %)	(19.7 %)	(21.9 %)		
Mean kit weight Day 1, g	11.0 ± 0.34	11.4 ± 0.35	10.5 ± 0.30	$F_{2,106} = 2.0$	0.14
Mean kit weight Day 7, g	32.3 ± 0.96	33.1 ± 1.10	30.1 ± 0.96	*F2,104 = 2.0*	0.14
				F1,104 = 4.1	Sex-ratio: 0.045
Total litter weight Day 7, g	174.9 ± 13.40	187.3 ± 13.32	162.0 ± 15.10	*F2,111 = 0.7*	0.50
				F1,111 = 8.1	Sex-ratio: 0.005
Alive Day 7 n	5.4 ± 0.37	5.6 ± 0.37	5.4 ± 0.48	$F_{2,112} = 0.1$	0.87

birds breeding under harsh environments as they are less sensitive to acute stressors, such as weather changes or capturing (Reviewed in Wingfield and Sapolsky, 2003).

4.2. Does the relocation strategy of mated females influence indicators of maternal care?

The nest score was significantly higher in the relocated groups than in-group NON. We speculate that the increased nest building displayed by relocated female mink could be due to the new environment causing a higher motivation for establishing a covered safe place, which can be accomplished by elaborated nest building in the novel nest box. In rats, increased non-maternal nest building is seen when mated females are relocated into maternity units directly after mating, with the maternal nest building commencing 0–2 days prior to parturition (Denenberg et al., 1969). However, the difference in nest score could also be a response to the lower shed and nest box temperature in the maternity units for the relocated animals. Female mink are known to build more advanced nests when exposed to cold nest boxes (Schou and Malmkvist, 2018), although the (un-intentionally) environmental temperature difference in the current study was small compared to previous studies. Nest temperature increased through to Day 7 after birth, which was probably due to weather changes and the insulation of the advanced nests. It may also be due to the mink dams staying for longer inside the nest boxes in the time prior to giving birth and especially after having given birth, which is supported by female mink staying > 90% of the time in the nest boxes after having given birth to a litter (Malmkvist et al., 2007; Schou and Malmkvist, 2018).

After having given birth, there was no clear effect of treatment on offspring retrieval, but fewer NON dams (non-significant) retrieved their offspring in need out in the cage (offspring retrieval test). Latency to offspring retrieval was found to be positively correlated with offspring mortality (Malmkvist and Houbak, 2000), and mink with a barren nest box were found to have longer latency to retrieve, which could indicate that they are stressed (Malmkvist and Palme, 2008). In the present study, we had no indications that dams in-group NON experienced a higher level of stressors than the relocated dams.

4.3. Does the relocation strategy of mated females affect reproduction and offspring growth?

Relocation did not affect the reproduction results between groups for any of the response variables. This lack of effect suggests that there was no major difference in stress experienced between the relocation strategies. Strategies regarding the timing of allocation of nesting materials are known to affect the reproduction results in mink (Malmkvist and Palme, 2008, 2015; Schou et al., 2018), which is why similar results would be expected in the current study if relocation was a significant stressor. In the current study, we had to use different houses for the experimental group NON and groups DIRECT/REGULAR. We do not expect the shed difference to be the reason for the lack of difference in reproductive performance and offspring growth between treatments. Please note that the mink in the different sheds were managed and kept under identical conditions – except for the relocation treatment. The NON house was slightly warmer in air temperature, however, in light of previous findings of the minor influence of in-nest temperature on offspring growth and survival – in a large scale study across years and houses (Schou and Malmkvist, 2017) – we do not expect the shed difference within the farm to be large enough to explain the lack of difference between treatments. Even though capturing and restraining in traps elicit a stress response in mink (Heller and Jeppesen, 1985; Jeppesen and Heller, 1986; Hansen and Damgaard, 1991; Malmkvist et al., 2011), when relocation was conducted shortly after mating, no effect on the reproductive outcome was found.

5. Conclusion

We conclude that relocation of mink dams shortly after mating is not a major additional stressor, as we only reported a minor and non-significant effect on FCM concentrations during the gestation and detected no effect on the performance of stereotypic behaviour, maternal care or the reproductive success.

Conflict of interest

None.

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