Decreased litter size in inactive female mink (*Neovison vison*): Mediating variables and implications for overall productivity

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Meagher, R., Bechard, A., Palme, R., Díez-León, M., Hunter, D. B. and Mason, G. 2012. **Decreased litter size in inactive female mink (*Neovison vison*): Mediating variables and implications for overall productivity**. Can. J. Anim. Sci. **92**: 131–141. Farmed mink vary dramatically in activity: very inactive individuals rarely leave the nest-box, while others spend hours active daily, often performing stereotypic behaviour (SB). SB typically correlates with increased reproductive output, and inactivity, with decreased output. Our objectives were to determine whether SB or inactivity best predicted litter size (LS), and to test three hypothesized reasons for inactive dams' reduced LS: H1, excess fat; H2, chronic stress (potentially underlying inactivity because fear motivates hiding); and H3, health problems. We assessed time budgets pre-breeding, scored body condition visually, conducted health exams, and assessed stress using faecal cortisol metabolites (FCM) and "glove tests" for fear. Results did not support H2 and H3: inactive females were no more fearful than active females ($P > 0.10$), they excreted lower levels of FCM ($P = 0.033$), and were considered healthy. As predicted by H1, inactive females had higher body condition scores ($P < 0.0001$), which predicted decreased LS ($P = 0.040$). However, path analysis determined this was unlikely to mediate the inactivity–LS relationship. Compared with SB, inactivity more consistently predicted both LS (negatively, $P \leq 0.038$) and kit weight (positively, $P \leq 0.037$). Therefore, decreasing inactivity in farmed mink, rather than increasing their SB or decreasing their body condition should most improve productivity.

Key words: American mink, *Neovison vison*, inactivity, reproductive success, stress, body condition

Meagher, R., Bechard, A., Palme, R., Díez-León, M., Hunter, D. B. et Mason, G. 2012. **Réduction de la taille de la portée chez la femelle inactive du vison (*Neovison vison*) : variables médiatrices et implications sur le plan du rendement**. Can. J. Anim. Sci. **92**: 131–141. L'activité des visons d'élevage varie considérablement : des sujets très inactifs quittent rarement le nid, alors que d'autres consacrent quotidiennement des heures à un comportement stéréotypé (CS). Les CS sont habituellement corrélés à un taux de reproduction plus élevé et l'inverse est vrai pour l'inactivité. Les auteurs voulaient établir lequel, entre les CS ou l'inactivité, prévoit le mieux la taille de la portée. Ils souhaitaient aussi vérifier trois hypothèses susceptibles d'expliquer la plus petite portée des femelles inactives : (1) l'excès de graisse, (2) un stress chronique (éventuellement à l'origine de l'inactivité, la crainte incitant l'animal à se terrer) et (3) des problèmes de santé. À cette fin, ils ont évalué la répartition du temps avant l'accouplement, noté l'état corporel par un examen visuel, procédé à un examen médical et évalué le stress à partir des métabolites du cortisol dans les fèces (MCF) ainsi que la crainte en utilisant des gants. Les résultats n'appuient pas la deuxième ni la troisième hypothèse : les femelles inactives ne sont pas plus craintives que les actives ($P > 0,10$), elles excrètent moins de MCF ($P = 0,033$) et ont été jugées en bonne santé. Comme le présumait la première hypothèse cependant, les femelles inactives obtiennent une note plus élevée pour l'embonpoint ($P < 0,0001$), ce qui prédit une diminution de la taille de la portée ($P = 0,040$). L'analyse causale révèle néanmoins qu'il est peu probable que ce facteur soit à l'origine du lien entre l'inactivité et la taille de la portée. Comparativement aux CS, l'inactivité prévoit de manière plus uniforme la taille de la portée (négativement, $P \leq 0,038$) et le poids des jeunes (positivement, $P \leq 0,037$). Par conséquent, réduire l'inactivité chez les visons d'élevage, au lieu de rehausser leurs CS ou de diminuer leur embonpoint, a plus de chances de déboucher sur une hausse de la productivité.

Mots clés: Vison d'Amérique, *Neovison vison*, inactivité, réussite de la reproduction, stress, état corporel

Mink (*Neovison vison*) on fur farms show dramatic, consistent individual differences in activity levels (e.g., Mason 1993), with the most inactive doing

Abbreviations: **AIC**, Akaike Information Criterion; **BCS**, body condition score; **d.f.**, degrees of freedom; **FCM**, faecal cortisol metabolites; **GLM**, general linear model; **LS**, litter size; **PND**, postnatal day; **SB**, stereotypic behaviour

little but lie in the nest box, while the most active spend much time out in the cage, often performing stereotypic (abnormal, repetitive) behaviour (Bildsøe et al. 1991; Svendsen et al. 2007a). Previous research has sometimes found that females that perform a lot of stereotypic behaviour (SB) have greater reproductive success, manifest as lower rates of barrenness, increased litter size, and decreased kit mortality (e.g., Jeppesen et al. 2004). Complementary findings have come from our own research (Mason 1992; Meagher et al. 2011): inactive females give birth to fewer kits (i.e., have decreased litter sizes). However, not all differences in their reproductive performance are negative, as they are also more attentive to their male kits in retrieval tests, and their kits (especially males) grow more quickly.

Active and inactive mink may also differ in several other relevant ways, the first of which is that inactive individuals typically have higher body weights and likely more body fat (Jeppesen et al. 2004; Hansen and Møller 2008). This is relevant since fatter mink (i.e., those with higher body condition scores) often show signs of reproductive impairment, such as smaller litters and more stillborn kits (Jørgensen 1985; Baekgaard et al. 2007; Malmkvist and Palme 2008). Reduced body weight was also found to explain the increased reproductive success in female mink exhibiting higher levels of stereotypic behaviour; SB was not a statistically significant predictor after controlling for body weight (Jeppesen et al. 2004). Research on obesity in other species has shown that such reproductive impairments can be caused by adipokines, signalling chemicals released by fat cells (e.g., Gosman et al. 2006); at least one adipokine, leptin, is known to increase with fat levels in mink (Mustonen et al. 2005). Although a certain level of leptin is hypothesized to be necessary for reproductive readiness in humans and perhaps other species (Moschos et al. 2002), high concentrations of this and other adipokines can inhibit ovulation, implantation of fertilized eggs, and embryo development in humans and rodents (Moschos et al. 2002; Gosman et al. 2006). Another adipokine, tumour necrosis factor-α, also inhibits copulatory behaviour (Wingfield and Sapolsky 2003). Thus, decreased reproductive success in inactive mink might be due purely to fat-related physiological effects.

Alternatively, there are welfare-relevant hypotheses that might explain both profound inactivity and associated reproductive impairments. Since most inactivity occurs in the nest box, which is the only shelter available, it could conceivably be a form of hiding, reflecting chronic fear. Where activity and temperament co-vary, inactive individuals are more likely to be fearful than are their stereotypic counterparts (Hansen and Jeppesen 2006), although this relationship is not always detected [e.g., Svendsen et al. (2007a) in lines differentially selected for SB]. Relatedly, inactive phenotypes in mink have been associated with increased baseline cortisol levels, indicative of chronic stress (Bildsøe et al. 1991),

although, again, this is not always the case (e.g., Svendsen et al. 2007a). Like excess fat, chronic stress can impair reproduction at various stages, from gametogenesis and pregnancy (Hemsworth 2003; Wingfield and Sapolsky 2003) through lactation (Rushen et al. 1995; Lau and Simpson 2004). Finally, sickness (Hart 1988) or injury (Rutherford 2002) can elicit inactivity, and could interfere with reproduction (e.g., European Commission 2001). Although the increased maternal care and kit growth observed in our second experimental group (Meagher et al. 2011, 2012) render the possibilities of widespread health problems or severe physiological stress unlikely, we wished to rule them out directly.

Our aim here was to determine the practical consequences of the observed differences in reproductive output, and to identify which of the associated traits best predicts these differences. In exp. 1, we collected pilot data on one farm to confirm that inactive mothers in our population do raise fewer kits to weaning successfully. We then conducted a larger-scale experiment, exp. 2, in which we assessed overall differences in productivity between active and inactive dams, as indicated by both the total number of kits raised and the kit weights at day 21. We then investigated the various hypothesized variables that might mediate a decrease in litter size. Finally, given the inverse relationship between inactivity and stereotypic behaviour, and the fact that most research has focused on the latter, we aimed to determine which behaviour was most tightly linked with the observed effects and is therefore the best target for selection to improve productivity.

MATERIALS AND METHODS

Experiment 1

Animals and Housing
The subjects were 239 female mink on a commercial farm in Ontario, Canada, of two common colour-types, Black and Pastel ($N = 141$ and 98, respectively). Of these mink, 105 (42 Black and 63 Pastel) were approximately 10 mo of age at the start of the study (i.e., entering their first breeding season); the others ranged from 2 to 4 yr of age. The mink were initially housed individually in conventional wire mesh cages (minimum 61 cm × 19 cm × 46 cm, maximum 30 cm wide) with an elevated square wooden nest box at the back, the same width as the cage. Black and Pastel mink were housed in separate sheds, and there may therefore have been a confounding effect of shed on behaviour. Then, following standard practice on North American farms, all mink were relocated to larger whelping cages in other sheds (ranging from 61 cm × 32 cm × 46 cm to 61 cm × 46 cm × 46 cm). In these cages, the nest boxes (32 cm wide) were attached to the front of the cage, and wheat straw was provided for nest building. Black mink and Pastel mink were again housed in separate sheds, although each colour-type was now split between

multiple sheds, reducing the likelihood that differences between colour-types were due to shed effects. Water was provided ad libitum via nipple drinkers. Food was provided once a day according to standard practice on North American farms, with amounts reduced during the winter months on an individual basis, with the objective of all mink reaching a body condition score of approximately 3 on the scale described by Hynes and colleagues (2004).

Pre-breeding Behaviour and Reproductive Success

Twenty scanning observations were conducted over a 4-d period in February, immediately prior to breeding. A modified form of scan sampling was employed, in which the first behaviour performed was recorded, but the individual was observed for up to 30 s on each scan to correctly identify the behaviour if necessary (e.g., to determine whether an action was repeated and thus should be classified as stereotypic). This type of observation is commonly employed in mink research, and has been validated against more frequent scans using video data (Bildsøe et al. 1990; Svendsen et al. 2007). All observations took place in the mornings, from approximately 0830 until feeding time (approximately 1330). Behaviour was recorded as stereotypic behaviour, non-stereotypic (normal) activity, or inactivity; definitions are given in Table 1. A subset of 56 of these mink (24 Black, 32 Pastel) was observed again the following November, using the same method, to investigate behavioural consistency.

After weaning, data were collected for the number of females that were barren, and, for those individuals that produced a litter, the number of live and dead kits on the day after birth, and the number of kits that died by the time of weaning. Litter size at weaning was then calculated for use in analysis. Litters in which farmers cross-fostered (added or removed) kits for any reason were excluded from the analysis because such manipulation could obscure biological effects on litter size.

Statistical Analysis

All statistical analyses were conducted in JMP 8 (SAS Institute Inc., Cary, NC, USA 2009). Welch ANOVAs were used to compare SB and activity levels between the top and bottom quartiles for inactivity, since the data did not meet the assumptions of parametric tests. General linear models (GLMs) were

used to analyse relationships between age, colour-type (which included the confounding effect of shed) and inactivity, normal activity or stereotypic behaviour (SB). GLMs were also used to analyse the predictive value of these behaviour patterns for litter size at weaning, for only those individuals that gave birth. These models included age and colour-type as blocking factors, and all of the independent variables were treated as fixed effects. Interactions between the variables were initially included but were removed if they had a P value greater than 0.25. The risk of being barren was assessed in a nominal logistic regression, with age, colour-type and behaviour as the independent variables. Again, interactions between behaviour and the blocking variables were included if they were $P < 0.25$. The conventional probability value of $P < 0.05$ was used to establish statistical significance; however, exact values are reported for any values where $P < 0.10$ since these effects may be worth further investigation. Effects of colour-type and age are not reported unless they interacted with the variables of interest.

Experiment 2

Animals and Housing

A total of 549 female mink were observed across three commercial farms: 149 Black mink and 144 Pastel mink at Farm 1 (the site of exp. 1), 143 Black mink at Farm 2, and 113 Pastel mink at Farm 3. Only 2- and 3-yr-old mink (entering their second or third breeding season) were used, as these mink are more likely to be very inactive than 1-yr-old mink are, and focussing on them allowed us to omit the control for age, since this group is relatively homogenous in terms of reproductive output. Housing conditions were very similar to those in exp. 1. The exact cage sizes varied slightly between farms, with the minimum being 61 cm × 19 cm × 46 cm. All cages included a square nest box approximately 18 cm wide, and a single nipple drinker. At Farms 2 and 3, the nesting material provided in the larger cages where the mink gave birth was aspen shavings, while Farm 1 continued to use wheat straw.

Pre-breeding Behaviour

Nine to ten days of behavioural observations were conducted at each farm in February, before mating began. The protocol was the same as in exp. 1.

Fear and Adrenocortical Activity

In April, a "glove test" was applied to determine whether some females were fearful and thus likely to be experiencing chronic stress (Morgan and Tromborg 2007). In this temperament test, mink are classified as fearful, curious, aggressive or unresponsive depending on their response to a human extending a gloved hand against the cage. The detailed methods and validation of this test are described in Meagher and colleagues (2011). April was chosen for this test because all mink were

Table 1. Ethogram used to record the behaviour of female mink during the weeks before breeding

Activity	Description
Stereotypic behaviour	Movement or sequence of movements repeated at least three times consecutively, with no apparent function
Inactivity	Lying still, whether eyes open or closed
Normal activity	Animal neither inactive nor engaged in stereotypic behaviour; includes eating, drinking and grooming

expected to be pregnant by this time, and therefore a minor stressor was not expected to interfere with reproductive results [as it might if presented between mating and implantation (European Commission 2001)]; temperament is expected to be relatively stable in adult mink (Kirkden 2010). For a subset of 30 of the least inactive ("active") and 31 of the most inactive ("inactive") females on Farm 1, 24-h faecal samples were also collected prior to the glove test to assess baseline faecal cortisol metabolites (FCM) as a physiological indicator of chronic stress. These samples were collected fresh at the end of the 24 h period and placed in a freezer immediately. They were then thawed, homogenized, and the metabolites were extracted using 80% methanol and analyzed using an 11β-hydroxyaetiocholanolone enzyme immunoassay (Frigerio et al. 2004), previously validated in mink (Malmkvist et al. 2011).

Body Condition

The farmers scored animals for body condition in late February, the week before mating began. Body condition scores (BCS) were given on a 5-point scale based on the one developed and validated by Hynes and colleagues (2004), where 1 represented a very thin mink and 5 was very heavy. Scoring was done visually in the current study, and thus the scale differed from that of Hynes and colleagues in the sense that no criteria were used that could only be determined by handling the mink. Body condition scores correlate with amount of body fat (Clausen 2005, cited by Baekgaard et al. 2007). At most times of year, 3 is considered an ideal score, although during late gestation, somewhat higher scores are expected (Hynes et al. 2004).

Dam body condition was scored again, by the researchers (RM and AB), when the kits were counted on the day after birth. To ensure consistency between farms and scorers, in addition to verbal descriptions of the categories, scorers at the other farms used photos of each category from Farm 1 as a reference point. Inter-observer reliability was assessed after the first scores were assigned, and again at the end of the study in comparison with scores given to a subset of animals by a veterinarian (BH) using the original, similar but more in-depth protocol of Hynes and colleagues (2004) that involved handling the animals to feel for subcutaneous fat. Inter-observer reliability of body condition scores on the original five-point scale did not meet the conventional threshold of acceptability (a rank correlation of 0.7 or greater); however, agreement was typically good for animals at the extremes of the scale. For this reason, scores were pooled into three simpler categories in all analysis: thin, normal or fat (note that mink categorized as "fat" were not necessarily obese, but were merely judged to have more body fat than the other mink). With this modified scale, moderate to substantial agreement between observers was obtained, with kappa coefficients ranging from 0.42 to 0.65.

Reproductive Output and Kit Performance

Kits were counted, sexed, and weighed 21 d after birth. Weights were averaged by sex. Day 21 weights were used because this encompasses the period in which kits are fully dependent on their mothers for milk (Dunstone 1993; Mason 1994). Litter sizes from this time were used in place of those at weaning in this experiment, primarily for pragmatic reasons; however, because kit mortality is extremely low after this age (less than 0.1% of kits: Jørgensen 1985; European Commission 2001), these data should be representative of litter sizes at weaning. Litters where cross-fostering had occurred were again removed from all litter size analyses; however, presented kit weights are for all kits raised, whether or not they were biological offspring. Births began in late April and continued into early May on all farms.

Health

During all observation periods, it was noted if any animals did not appear to be eating normally or showed obvious signs of injury. At the conclusion of the study in June, a subset of animals at each extreme for inactivity levels were examined by a veterinarian (BH) to ensure that their previous inactivity was not caused by a physical illness ($N = 78$ total on two farms). This included checking for foot pad disease or old physical injuries that would cause discomfort when standing or walking, checking that the faeces appeared normal, and checking that there were no signs of respiratory illness or infection, such as discharge from the nose. Where possible ($N = 21$), the urine was also tested for pH and the presence of leukocytes, protein, glucose or blood/haemoglobin. Leukocytes would indicate infection, while more than trace amounts of protein would suggest kidney pathology (Zellen 1996), and glucose, a metabolic disorder (Hynes et al. 2004). Blood in the urine can be caused by kidney infections or other conditions (Hunter 1996).

Statistical Analysis

As in exp. 1, analyses were conducted in JMP 8, except for generalized linear models which were run using SAS 9.2 (SAS Institute, Inc., NC, USA 2008). The relationship between behaviour (inactivity or SB) and risk of being barren (a binary response) was assessed using generalized linear mixed models, controlling for farm as a random effect and colour-type as a fixed effect. Farm and colour-type were partially but not fully confounded and thus could be included separately in these models; however, only at Farm 1 could differences be definitively attributed to colour-type, and therefore the main effects of colour-type are not reported. Risk of barrenness was also compared between fat and normal or thin females using Fisher's exact test, split by colour and farm. All other relationships with inactivity were assessed using mixed model GLMs, because farm was treated as a random effect. Thus, the restricted

maximum likelihood (REML) method of estimation was employed (Searle et al. 1992). These GLMs also controlled for colour-type, and its interactions with the other variables; interactions were removed if they had no effect ($P > 0.25$). Adjusted (Type III) sums of squares were used unless otherwise specified. Normality and homogeneity of variance were assessed by inspection of the residual plots (Grafen and Hails 2002); Bartlett's test for equal variances was used where the effects of interest were categorical.

Litter size effects were again examined for those individuals that gave birth only, blocking by farm (as a random factor) and colour-type. Two outliers were excluded for male kit weights, because they were more than two standard deviations above the mean in absolute value, as were their residuals, and they were likely examples of measurement or recording error. Their exclusion corrected the non-normality of the original data, without altering the conclusions drawn from the analysis. In the kit weight models, total litter size on day 1 was also included as a covariate.

As in exp. 1, a Welch ANOVA was used to compare SB levels between mink categorized as active and inactive, since non-homogenous variance prevented the use of a GLM. To determine whether stereotypic behaviour or normal activity are better predictors of productivity than inactivity, the GLMs for litter size and kit weight were re-run with the alternative behavioural predictor added to the model. Sequential (Type I) sums of squares were used to determine whether each of these two behavioural variables was significant with or without controlling for the other. These models included farm and colour-type, as well as litter size when kit weight was the dependent variable, before the effects of interest.

Finally, relationships between inactivity and the hypothesized intervening variables of FCM, temperament, and body condition were then analysed using GLMs controlling for farm and colour-type, as above. A log transformation was applied to FCM to achieve normality of the residuals. Temperament was treated as the independent variable, and inactivity as the dependent variable to allow the use of a GLM. An arcsine square-root transformation was applied to inactivity. For body condition, a generalized linear mixed model was run instead of a GLM, using the multinomial distribution. If the variable of interest was significantly related to both inactivity and the measure of productivity, a path analysis was conducted using Amos 5 (Arbuckle 2003) to determine whether inactivity or the possible intervening variable best explained the litter size and kit weight data. In these models, it was not possible to include both farm and colour-type, and thus the two were combined into a single variable called "group". Model fit was assessed using the chi-square test, RMSEA (Root Mean Square Error of Approximation), NFI (Normed Fit Index), and AIC (Akaike Information Criterion) statistics. AIC and chi-square

values are reported here. Body condition scores from February were used in the litter size and barrenness models, since scores from that time were judged most relevant to conception and the gestation period, while scores from the day after birth in the kit weight models since they reflect condition at the beginning of the lactation period and thus stored energy available to invest in the kits. To assess the consistency of body condition over time, an ordinal logistic regression was used to compare scores in February with those on the day after birth, again controlling for farm and colour-type.

RESULTS

Experiment 1

Inactivity levels in February ranged from 0 to 94.7% of observations, with an overall mean of $40.3 \pm 1.7\%$. Very inactive females had significantly lower mean SB than active females did. They also had significantly lower normal activity. Detailed results of these analyses are presented in Table 2. Individual differences in inactivity levels were consistent over time: for the subset observed again in November, there was a significant correlation in their inactivity levels between these two periods ($F_{1,56} = 5.43$, $P = 0.024$, $r = 0.54$).

There were only six barren individuals; however, the likelihood of being barren was higher with increasing inactivity. Among individuals that successfully produced a litter, inactivity was significantly inversely related to litter size at weaning (Fig. 1a). Statistical details for both of these findings can be found in Table 3.

Individuals with high SB were significantly less likely to be barren than those with lower SB ($\chi^2 = 4.12$, d.f. 1, $P = 0.04$). However, SB did not have a significant main effect on litter size at weaning; instead, it interacted with both colour-type and age (SB × colour × age $F_{1,157} = 3.99$, $P = 0.048$). Analyses split by age showed no significant positive relationships between SB and litter size; among the very small group of 4 yr olds, there was even a trend for a negative relationship ($F_{1,9} = 4.00$, $P = 0.076$). Normal activity levels did not significantly predict risk of being barren ($P > 0.10$). For litter size, normal activity interacted with age ($F_{1,158} = 4.42$, $P = 0.037$); split by age, activity was positively related to litter size in 2 and 3 yr olds only ($F_{1,41} = 3.64$, $P = 0.064$ and $F_{1,29} = 6.55$, $P = 0.016$, respectively).

Experiment 2

Reproductive Success and Kit Performance
Table 3 presents detailed statistical results for relationships between inactivity and the reproductive variables. Inactivity did not significantly predict risk of being barren. The predicted negative relationship between inactivity and litter size, however, was detected (Fig. 1b).

Inactivity predicted increased male and female kit weight at 21 d, even after statistically controlling for the decreased litter size of inactive dams (Fig. 2). For both sexes of kit, there was a significant main effect

Table 2. Relationships between mink females' inactivity in winter and possible mediators of its relationship with reproduction

Dependent variable	Descriptive statistics by inactivity category[z]		Inactivity effect statistics
	High	Low	
Experiment 1			
SB (% of observations)	10.8 ± 0.02	54.2 ± 0.03	$F_{1,70} = 157.1$, $P < 0.0001$
Normal activity (% of observations)	18.3 ± 0.02	27.2 ± 0.03	$F_{1,66} = 6.94$, $P = 0.011$
Experiment 2			
SB (% of observations)	13.4 ± 1.1	60.7 ± 1.8	$F_{1,219} = 487.9$, $P < 0.0001$
Normal activity (% of observations)	17.6 ± 0.8	20.1 ± 1.3	NS
Protein in urine (% of mink)[y]	14	29	NS
Blood in urine (% of mink)[y]	33	0	NS
FCM (ng g^{-1})[x]	69.0 (56.8–83.3)	93.4 (76.6–113.8)	$F_{1,58} = 4.79$, $P = 0.033$
BCS in February			Odds ratio: 0.950
			$\chi^2 = 53.1$, d.f. 552, $P < 0.0001$
BCS on postnatal day 1			NS

[z]Categories are top and bottom quartiles of the population for this variable, or a subset of those quartiles for urine and FCM measures. Data are mean $\pm$ SE unless otherwise specified.
[y]Values are percent of mink tested ($N = 21$) that had more than trace amounts of the substance in their urine at the end of the study (June). Statistics presented are from Fisher's exact tests.
[x]Means are back-transformed least squares means, with 95% confidence intervals in parentheses.
NS = not significant, $P > 0.10$; d.f. = degrees of freedom.

of inactivity (see Table 3), but also an interaction between inactivity and litter size (females: $F_{1,423} = 4.14$, $P = 0.042$; males: $F_{1,419} = 3.87$, $P = 0.050$) such that inactivity had a positive relationship with kit weight only in smaller litters.

Stereotypic Behaviour and Normal Activity as Alternative Behavioural Predictors
Inactive mink again had significantly lower mean SB (see Table 2). Contrary to expectations, average levels of normal activity did not differ enough between active and inactive mink to be detected by a Welch ANOVA. Activity was therefore not tested as an alternative explanation for the observed relationships with inactivity.

There was no relationship between SB and barrenness ($P > 0.10$). Neither was it a statistically significant predictor of litter size, regardless of whether it came before or after inactivity in the model. By contrast, inactivity was always a significant predictor of litter size, and this effect was actually stronger if a control for SB was included first ($F_{1,302} = 5.98$, $P = 0.015$).

As expected, high SB predicted lower kit weight, i.e., the relationship was the inverse of that with inactivity. For female kit weight, as above, SB had a significant main effect only if before inactivity in the model ($F_{1,417} = 19.7$, $P < 0.0001$; else $P > 0.10$). Inactivity, on the other hand, was significant regardless of its position in the model ($F_{1,413} \geq 4.40$, $P \leq 0.037$). For male kit weight, both SB and inactivity were always significant, but the effect of inactivity was
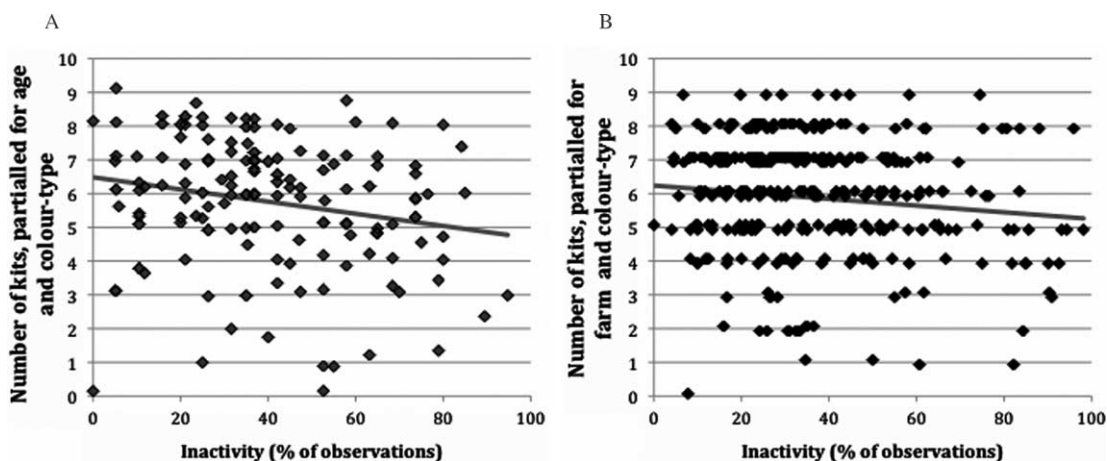

Fig. 1. Partial residual plots of litter size vs. pre-breeding inactivity levels in mink. Y-values are calculated as residuals $+ \beta_0 + \beta_1 x$ from the models controlling for colour-type, age (exp. 1) and farm (exp. 2). (a) Experiment 1: litter size at weaning. (b) Experiment 2: litter size at day 21.

Table 3. Relationships between mink females' inactivity in winter, reproductive success and kit performance

Dependent variable	Descriptive statistics[z]	Effect of inactivity[y]	Effect statistics
Experiment 1			
Proportion barren	0.035	Odds ratio: 0.009	$\chi^2 = 5.39$, d.f. 1, $P = 0.020$[x]
Litter size	5.5 ± 0.2	-1.801768	$F_{1,157} = 5.61$, $P = 0.019$
Experiment 2			
Proportion barren	0.081		NS
Litter size	5.9 ± 0.1	-0.09906	$F_{1,327} = 4.32$, $P = 0.038$
Female kit weight (postnatal day 21)	122.5 ± 0.1	0.1819041	$F_{1,424} = 24.4$, $P < 0.0001$
Male kit weight (postnatal day 21)	136.1 ± 1.3	0.2918679	$F_{1,420} = 47.9$, $P < 0.0001$

[z]Mean $\pm$ SE for continuous variables, proportion for binary variables.
[y]Regression coefficient, unless otherwise specified.
[x]Results of likelihood ratio χ^2 test.
NS = not significant, $P > 0.10$.

stronger: SB had $F_{1,415} \geq 4.64$ ($P \leq 0.032$), compared with $F_{1,415} \geq 7.25$ ($P \leq 0.007$) for inactivity. Because inactivity was thus the more consistent predictor of the reproductive variables of interest, it was the focus of our remaining analyses.

Potential Mediating Variables for Inactive Females' Reduced LS

Fear and Stress. Inactive females did not exhibit physiological signs of elevated chronic stress; indeed, they had significantly lower FCM than active females (see Table 2). Temperament was significantly related to inactivity ($F_{4,474} = 8.75$, $P < 0.0001$); however, the only category that proved significantly different (using Tukey's Honestly Significant Difference) was "unresponsive", in which inactivity was higher ($F_{1,474} = 24.5$, $P < 0.0001$). Furthermore, a contrast of fearful versus non-fearful (aggressive, curious and unresponsive pooled) mink showed no difference in inactivity ($P > 0.10$). Because neither fear nor FCM was elevated in inactive individuals, these variables could not mediate

Fig. 2. Mink kit weight vs. mother's pre-breeding inactivity. PND = postnatal day.

a negative relationship between inactivity and litter size, and thus they were not used in further analyses.

Post hoc analyses were conducted to determine whether the temperament category "unresponsive", rather than fearful, predicted barrenness or litter size. However, no significant relationships were found with either of these variables ($P > 0.10$).

Health. No signs of lasting illness or injury were evident in these mink during the behavioural observation periods, nor were serious chronic illnesses or injuries detected in the veterinary exams conducted on a subset of the animals. At most, small amounts of protein were found in the urine, but the occurrence of this did not differ significantly between the active and inactive females. Likewise, although the only females to have blood in the urine were inactive, this effect was not statistically significant with this sample size. Statistical details of these results are presented in Table 2. Even for mink that did have blood in the urine, because the amount of blood was typically small (urinalysis scores of $1+$ or $2+$) and these individuals did not show other signs of chronic kidney problems (e.g., urine-soaked fur), this was not judged to indicate a chronic condition that would have affected their behaviour in the winter or their reproductive ability. Supporting this conclusion, these individuals had litters of average rather than small size. No other abnormalities were detected in the urine of any mink tested.

Body Condition. BCS did not remain consistent over time ($P > 0.10$; data not shown). In February, it was significantly positively related to inactivity (see Table 2); however, after the birth of the kits, it was not ($P > 0.10$).

Role of Body Condition in Mediating Relationship with Reproductive Output

Being fat rather than normal or thin did not significantly predict risk of being barren, either overall or in any farm by colour group ($P > 0.10$). It could not therefore
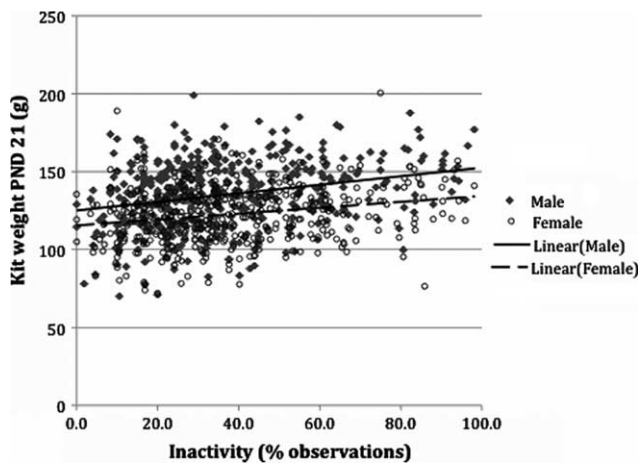
explain the tendency for reduced barrenness observed in inactive Black females at Farm 1. However, body condition in February interacted with colour-type to predict litter size when inactivity was not in the model ($F_{2,324} = 3.25$, $P = 0.040$). Among Black mink only, fat mink appeared to perform worse than did normal or thin mink, although the contrast was not significant in either colour-type (Black mink: $F_{1,323} = 32.43$, $P = 0.120$; Pastel mink: $F_{1,323} = 0.08$, $P = 0.777$; Fig. 3).

We therefore used path analysis to determine whether the relationship between inactivity and litter size was direct or was mediated by BCS. Fig. 4 shows the hypothesized pathways tested in the best-fitting model that included all of these variables; path analysis gives relative weights to each path, with a higher weight indicating that a particular path is more likely than the others to explain the observed effects. The direct relationship between BCS and litter size proved to be non-significant and was therefore removed. These results suggest that the relationship between inactivity and litter size is not a result of greater body condition; rather, inactivity predicts decreased litter size and increased BCS independently. Detailed results for the best fitting model are presented in Table 4.

Role of Body Condition in Mediating Relationship with Kit Performance

BCS significantly predicted male kit weight at 21 d ($F_{2,382} = 3.19$, $P = 0.042$). It also predicted female kit weight at this age, but interacted with colour type ($F_{2,382} = 3.58$, $P = 0.029$). Using path analysis, both inactivity and BCS proved to have significant direct effects on kit weight for both sexes, although BCS was weighted more heavily. The best-fitting model did not include a correlation between inactivity and BCS, suggesting this path was unimportant in explaining our results. The final path diagram is presented in Fig. 5, and statistical results in Table 5.

DISCUSSION

It appears that mink that are inactive in the winter do have smaller litters. In practical terms, this effect was relatively small on an individual level, particularly in exp. 2. Thus, as seen in Fig. 1, the difference between the expected litter sizes for the most and least inactive individuals is just under one kit. Although litter size is the most economically important measure of productivity (Lagerkvist 1997), the increase that would be obtained by reducing inactivity in some instances might not be worth the cost of the associated decrease in kit weight. This will likely vary between farms depending on which aspect of productivity has the most room for improvement. The results for rates of barrenness are less clear, since it was linked to increased inactivity only in exp. 1. These findings therefore need replication, particularly given the low incidence of barrenness in our samples. Although SB continues to receive much attention in mink research, it appears that it is less relevant to productivity than is inactivity. Given the strong inverse correlation between SB and inactivity, the previously reported relationship between SB and reproductive success (e.g., Mason et al. 1995; Jeppesen et al. 2004) might actually have been driven by decreased inactivity rather than by this abnormal behaviour per se. However, the relationships may also differ between populations: thus, we detected no apparent relationship between SB and litter size even before controlling for inactivity, while others have (e.g., Jeppesen et al. 2004). Inactivity was also a more consistent predictor of productivity than was normal activity in exp. 1, and these two aspects of the time budget were uncorrelated in exp. 2, indicating that normal activity was not the true explanation for the observed variation in productivity.

None of our hypothesized mediating variables could fully explain the relationships between inactivity and our reproductive variables. Both chronic fear and poor health could be ruled out as causal factors, since neither
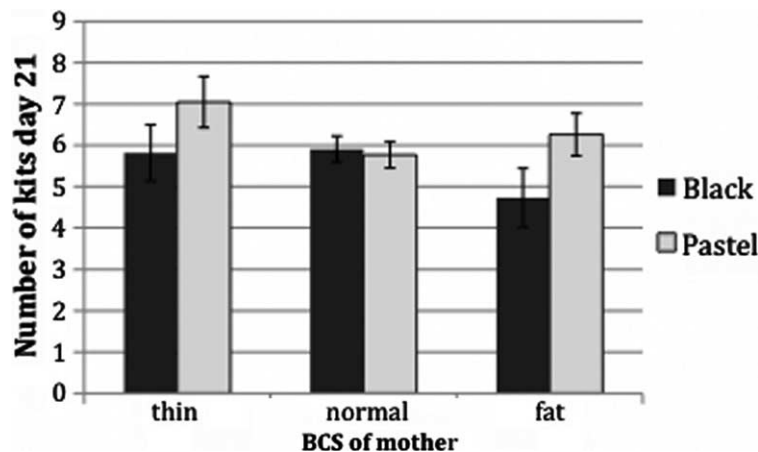

Fig. 3. Mink's litter size on postnatal day 21 vs. body condition score on postnatal day 1. Data are least squares means $\pm$ SE.
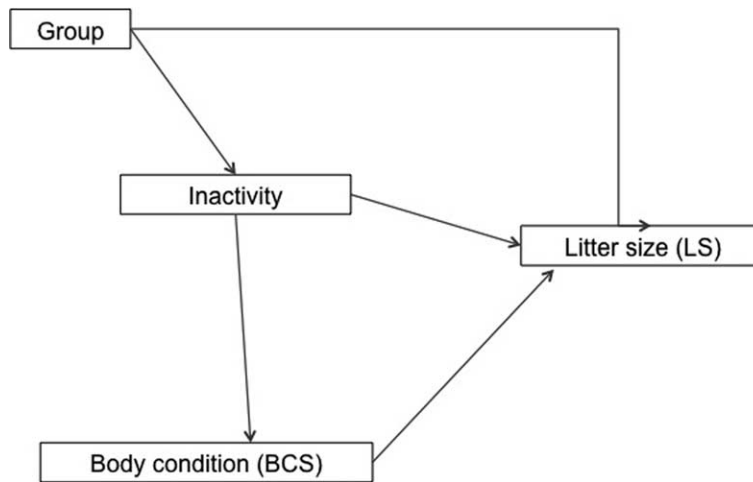
Fig. 4. Hypothesized path diagram for mink litter size in exp. 2. Group is a combination of farm and colour-type. Maternal body condition was assessed on the day after giving birth.

was elevated in inactive females. This was consistent with our findings that inactive dams in some ways appear to be better mothers than their more active peers (based on retrieval times and kit growth; Meagher et al. 2011). It remains possible that females who were inactive in February were experiencing transient illnesses that were not manifest in changes in feeding and had no lasting effects that could be detected by the time full veterinary examinations were conducted, but it seems unlikely that such short-lived conditions had an impact on litter size or kit weight. Our findings that very inactive, and thus non-stereotypic, mink tended to have lower concentrations of faecal cortisol metabolites and were no more fearful than were individuals that are more active contrast with the results of Bildsøe and colleagues (1991) mentioned above, but were consistent with more recent data using lines differentially selected for SB (Malmkvist et al. 2011; Svendsen et al. 2007). These FCM findings could reflect higher psychological stress in individuals with high SB levels, or merely their intense physical exercise (e.g., Girard and

Garland 2002). Moreover, our temperament findings are in line with those of Svendsen and colleagues (2007), who also demonstrated no difference in fearful responses between lines selected for high and low SB, but instead that the low SB mink were more likely to be "uncertain" (which was called "unresponsive" in this paper). The meaning of this category in welfare terms remains unclear (Meagher et al. 2011). Nonetheless, there is currently no evidence that the welfare of inactive individuals is compromised in comparison with that of others in the same population.

Inactive females did have higher body condition scores, and thus likely possessed more body fat, at least at the time of mating and conception, and this was negatively correlated with litter size in Black mink. However, the path analysis suggested that the variation in litter size was better explained by a direct relationship with inactivity than by the mediating effects of body condition. This analysis cannot definitively determine whether dam inactivity has a causal effect; however, it does indicate that body condition likely did not, in our populations. Body condition also did not fully explain the positive relationships found between inactivity and other aspects of reproductive performance: although it did increase kit weight, it did not fully explain the higher kit weights in offspring of inactive mothers, and it had no relationship at all with rates of barrenness in this sample.

The finding that body condition was not related to litter size independently of inactivity levels contradicts current beliefs; as discussed in the Introduction, previous studies have found an effect of body condition (Jørgensen 1985; Baekgaard et al. 2007; Malmkvist and Palme 2008), and this effect is usually attributed to direct physiological rather than behavioural mechanisms (e.g., mink: Jeppesen et al. 2004; humans: Gosman et al. 2006). There are several possible explanations for these conflicting results.

Table 4. Standardized regression weights and statistics from a path analysis of mink litter size, to determine whether direct effects of inactivity or effects mediated by BCS best explain the results (exp. 2)

Relationships	Regression weights and fit statistics
Inactivity →Litter size	−0.010*
Inactivity →BCS	20.837***
BCS →Litter size	Removed from model
Group →Inactivity	2.004†
Group →Litter size	−0.220*
χ^2	1.382,
	$P = 0.501$ (d.f. 2)
AIC	25.382

†, *, *** indicates $P < 0.10$, $P < 0.05$, and $P < 0.001$, respectively. Negative values indicate an inverse relationship, if the predictor is quantitative.
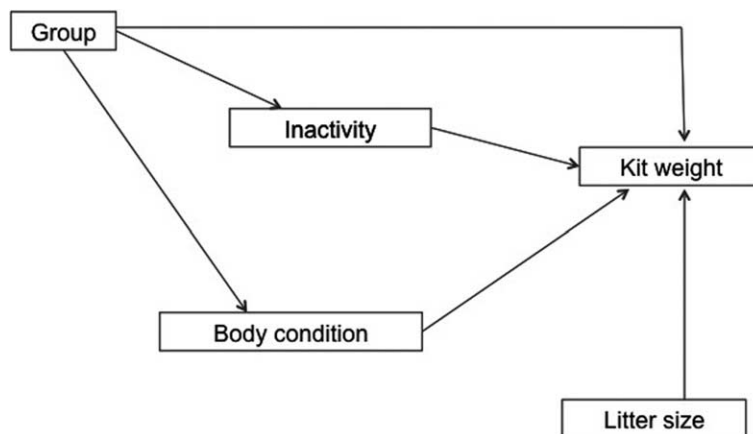
Fig. 5. Hypothesized path diagram for maternal effects on mink kit weight in exp. 2. Male and female weights were analysed separately but with identical paths. Group is a combination of farm and colour-type. Maternal body condition was assessed on the day after giving birth. Non-significant relationships were removed.

First, they may be due to prior studies not controlling for behavioural differences between animals of different body conditions, in which case the benefits of feed restriction might be mediated by the increased activity it elicits [e.g., Bildsøe et al. 1991; Damgaard et al. 2004; however, Hansen and Møller (2008) found no difference in total activity, only in circadian patterns of activity]. Second, they may reflect differences between populations, due to differences in the distribution of body conditions observed or the colour-types studied. Finally, our results may perhaps simply reflect lower statistical power for detecting body condition effects than relationships with inactivity in the current analysis, since inactivity was assessed with much greater resolution. These results do not eliminate the possibility that any physiological effects of body condition were present; for example, there may have been differences between active and inactive females

in the magnitude of the change in body condition during the slimming period prior to the beginning of this experiment that influenced their reproductive performance. However, the timing of body condition assessment here was similar to that used in other studies that did report an effect. Thus, any influence of fluctuation in body condition does not explain the discrepancy between these results and those previously reported in the literature.

Overall, it appears that inactive females are neither stressed nor ill, but that there is some other fundamental difference between the behavioural phenotypes that results in differences in reproduction. Reducing inactivity through selection or altered husbandry (e.g., the provision of running wheels or other objects to promote activity), rather than focussing on restricting feed to decrease body fat as is currently standard practice, would seem to hold the most potential for increasing reproductive output. Future work should look at the relationships between reproductive success and inactivity in the fall, prior to breeder selection, to determine whether selection will truly be effective. Any reduced inactivity, however, may come at a cost in terms of decreased kit growth and potentially increased incidence of barrenness. The overall effects on profitability may therefore vary depending on which factor most limits production on a given farm.

Table 5. Standardized regression weights and statistics from path analyses of relationships between maternal characteristics and kit weight in mink (exp. 2)

DV[z] Relationships and fit statistics	Male weight	Female weight
Inactivity →weight	0.235***	0.140***
BCS →weight	17.375***	14.845***
Number of kits →weight	−2.908***	−2.277***
Group →Inactivity	1.804†	2.146*
Group →BCS	0.068***	0.077***
Group →weight	−1.758†	
χ^2	6.561, $P = 0.161$ (d.f. 4)	9.565, $P = 0.089$ (d.f. 5)
AIC	38.561	39.565

[z]DV =dependent variable.
Values given for relationships are standardized regression weights.
†, *, *** indicates $P < 0.10$, $P < 0.05$, and $P < 0.001$, respectively. Negative values indicate an inverse relationship, if the predictor is quantitative. Small AICs indicate good fits.

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