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Article

Age and reproduction of wild-born and escaped mink (*Neovison vison*) caught in Danish nature

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Simple Summary: Previous studies of wild caught mink in Denmark show that 30-80% have recently escaped from farms. Therefore, it is debated whether a self-sustaining feral mink population is established in Denmark or whether the population rest upon a continuous contribution of captive-born farm mink. Knowledge about the reproduction and mortality of mink adapted for living in nature is important knowledge for management of feral mink. In this study, we have separated wild-born from captive-born mink caught in the Danish nature. The mean litter size ± SE of wild-born female mink was 7.6 ± 0.9 (range: 5-11 kits). The annual turnover of mink caught in nature is estimated to 66% and the yearly mortality to 69%, thus the population is slightly declining. Data confirm that the wild-born mink population in Denmark is reproducing and self-sustaining without continues influx of captive-born mink escaped from farms. The effect of escaped mink on the feral mink population will depend on the ability of captive mink to survive in nature. A model for mink age was developed based on the width of pulp cavity (% of tooth width) in the canine teeth. This is the first age determination model for mink.

Abstract: The feral mink population in Denmark consists of two groups of animals; mink born in the wild and mink that have recently escaped from farms. The aims of this study are; 1) to estimate the reproduction and mortality of wild-born and captive-born mink and 2) to estimate the age of mink based on the width of pulp cavity (% of tooth width) in the canine teeth. During 2018, 247 wild caught mink were sent for necropsy at the Danish National Veterinary Institute. Of these mink, 112 were determined as captive-born and 96 were determined wild-born. The mean litter size ± SE of wild-born females was 7.6 ± 0.9 (range: 5-11 kits) and for captive-born females 5.9 ± 0.9 (range: 1-10 kits). The best fitting regression line for mink age (in months) based on pulp width was y=0.42x2-11.52x+104.7, $R^2 = 0.77$, $p < 0.0001$. Individuals with a pulp cavity width <35% was found to be younger than one year. The turnover of mink caught in nature was estimated to 66% and the yearly mortality to 69%, therefore the population is slightly declining. In conclusion, a feral reproducing mink population in Denmark persists, besides the continues influx of captive-born mink escaped from farms.

Keywords: placental scars, demography, litter size, fecundity, turnover

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1. Introduction

Both accidental and intentional introductions of alien species into nature may have a large impact on native ecosystems [1]. The American mink (Neovison vison) is a semi-aquatic medium sized carnivore, native to North America [2]. The population of American mink in Denmark remained relatively small until the late 1980s, where after the annual hunting bags of mink increased from around 1000 to 8000 mink near the millennium. Today, the national game bags of mink are less than 2000 mink [3]. Mink have been bred for their fur in Denmark, since the mid-1920s [4]. During the years of study, there was around 1,300 commercial mink farms in Denmark producing around 17 million pelts per year [5]. Mink are known to escape from Danish farms and mink can be found in the wild in most parts of Denmark due to farm escapes [6]. In previous Danish studies, 80% mink in the years 1998–2000 and approximately 30% mink caught in nature during the winters of 2014 –2018 were found to be mink recently escaped from farms, leading to a question whether a truly feral mink population exists in Denmark [7-10].

Studies of reproduction in wild-born mink inside and outside its native domain are scarce. No studies from Scandinavia have previously documented the litter size of wild-born mink. Previous efforts to determine litter size from placental scars in mink showed that scars disappear few months post-partum [11-13]. However, when using an iron staining technique on the uterine tissue, the implantation site becomes visible, up to 7 month after parturition, i.e. from females caught during autumn and winter [13]. The litter size in free ranging mink from France, Spain and Belarus [13,14] are determined to be 4–7 kits. In Scotland, the mean litter size was 5.5 kits and the ratio of reproducing free-range females was 81% [15].

In Denmark, mink kits on farms are born between April 20th and May 15th with an average weaned litter size of 5.5 kits and a maximum litter size at birth of 17 kits [5,16,17]. Mink kits born in nature in Denmark are likewise born in April and May [18]. In southern latitudes (Belarus), most females caught by the end of March were with embryos, and new born mink kits were found in late April to the beginning of June [19].

The age of the individual carnivores including mink are generally determined using the widths of the pulp cavity and/or incremental lines in the canine teeth [20-22]. Growth of pulp cavity and incremental lines in the canine teeth of carnivores is species specific, and incremental lines in mink are challenging to interpret. For example, in a study of wild-born mink from Hokkaido, no incremental lines were observed in several individuals, assessed as adults [23]. Recent papers on age determination of mink refers to either or both the paper of [21] and the Matson's Laboratory (PO Box 308, Milltown, MT 59851, USA) [15,24-26]. However, the model by Helldin (1997) is based on the width of the pulp cavity in teeth of Eurasian pine martens (Martes martes). A model describing the relation between pulp cavity growth rate and the age of the individual has not previously been presented for mink.

To reveal if mink born in nature can reproduce and survive in nature it is important to be able to separate captive-born i.e. mink born on farms from wild-born i.e. mink born in nature.

The aims of this study were to: 1) Estimate reproductive performance and mortality of the Danish mink born in nature (wild-born) compared to mink escaped from farms (captive-born). 2) To discuss the likelihood of a self-sustaining population of wild-born mink in Denmark. 3) Estimate the relation between pulp cavity width and the age of mink, as a species-specific tool for ageing mink caught in the wild.

2. Materials and Methods

Mink from different regions of Denmark were submitted for necropsy at the National Veterinary Institute of Denmark from 1st of March to November 2018. Few mink in the sample were culled and stored by hunters before this period. The carcasses were kept at -20°C until necropsy. At necropsy, sex was recorded, the body length from nose tip to first vertebrae of the tail was measured, and the uteri horns with ovaries were removed as well as two canine teeth for age determination. All samples were kept at -20°C until examination. Fur colours were determined by the use of colour templates with colour types known from farms [16]. Hunters were asked to provide date of mortality of individual mink. In a previous study of Danish mink both univariate analyses and Gaussian mixture model analysis demonstrated clear divisions between the body length of farmed mink and wild-born mink. Mixture analysis identified two groups within each sex of the wild caught mink, one assigned to farmed mink (born in captivity) and another group of smaller mink suspected of being born in the wild [7]. Hence, mink sampled for the present study were separated in two groups: Mink born in nature (wild-born) and mink born on farms (captive-born) according to [7].

The groups wild-born and captive-born can from 1st September (> 4 month old) be separated by body length; adult males and females longer than 43 cm and 39 cm, respectively were grouped as captive-born.

2.1 Method for age determination of mink

The age was estimated using the width of the pulp cavity, and dental lines of the canine teeth bearing in mind that mink are born in April to May [22][21]. As dentine is deposited inward into the pulp cavity, gradually filling the cavity (centripetal growth) a wide pulp cavity is only observed in young animals [21]. Cementum, which forms the dental lines, is deposited over the root dentine (centrifugal growth). In mink, the first age line is normally observed when mink are 6–10 months i.e. from October to December. Hereafter, a line is produced each year. Hence, it is feasible to determine age with an interval of 1 year per age category [20,22,23].

To determine mink age, the canine teeth were extracted, fixed for 36 hours in 4% w/v formaldehyde solution, dried overnight at 40°C, before embedded in cold-polymerizing metylmethacrylate-based resin. Saw sections of 70-100 μm were prepared 2 and 3 mm from the apex of the tooth root with a Leiden saw (Meprotech, Holland Heerhugowaard). The unstained saw sections were examined with a fully automated Olympus BX61 microscope (Olympus Ltd, Ballerup, Denmark) equipped with a DP80 camera (Olympus Ltd, Ballerup, Denmark).

To correlate pulp width in mink teeth versus age, the width of the canine tooth and width of pulp cavity was measured at the widest place (2-3mm from apex) of the canine, using Olympus cellSens Dimension software measuring to nearest 1/1000 mm. Pulp width in percent of tooth width was plotted against mink age (estimated from on mortality date). Two farmed females, known to be 2 years old, were used to estimate the width of the pulp cavity in mink more than one year of age.

A model for pulp cavity width versus age (month) was estimated through two steps: 1) The overall best fitting regression line was made 2) A new best fitting regression line was made removing mink considered to be from the previous season (seen as outliers).

The incremental lines were counted in individuals more than one year of age (based on percent width of the pulp cavity). After testing different microscope setting, reflected light differential interference contrast (DIC) microscopy were found to be optimal for obtaining high-magnification images of mink incremental lines (DIC cube U-MDIC3, and high-contrast DIC prism U-DICTHC, Olympus Ltd, Ballerup, Denmark; Figure 1).

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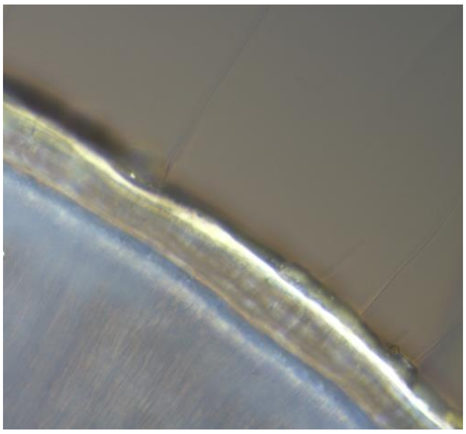

Figure 1. Saw sections (70-100 µm) of canine teeth of mink 2 and 3 mm from the apex under a microscope in reflected light differential interference contrast (DIC) microscopy. Three dark incremental lines are visible.

2.2 Determination of the reproductive performance in mink

Litter size, fecundity and proportion of reproducing/barren females were used as estimate for reproductive performance. Litter size was estimated from counts of placental scars (PSC) and embryos in the uterine horns (Figure 2).

Figure 2. Uterus from female mink with different staining intensity of placental scars (PSCs). Nine clearly visible PSCs (black arrows) and two pale scars (grey arrows).

In this study, the staining method described by [27] was used. The method identifies iron (from hemoglobin at the placental implantation site) in the uterine wall by dark blue staining (Figure 2). In short, the uteri were thawed in tap water, followed by lengthwise opening of the uterine corpus and horns with scissors. The uteri were immersed into a fresh 10% solution of ammonium sulfide (H8N2S) for 10 min and rinsed in tap water, followed by 10 min immersion in a 1:1 solution of 1% chlorhydric acid and 20% potassium hexacyanoferrate (K4[Fe(CN)6], 3H2O), rinsed in tap water and examined under a stereo microscope.

Litter size is given for the reproducing females only, whereas the measure for fecundity expressed as mean litter size includes barren females. Fecundity and percent producing females is based only on females known to have been caught within 7 month after mean season for parturition, to prevent an underestimation of fecundity and percent breeding females, due to disappearance of the PSC after 7 months post-partum [28]. Throughout, kits day of birth were set to 1st May and females were expected to conceive when 10 months old [15]. The fecundity and barrenness was based on adult females euthanized between 1st of March and 1st of November.

Females 0-10 months were classified as juveniles. Females 11-22 months old were classified as age class 1 (first season breeding females), whereas older females were classified as age-class 2, 3 and 4+. One year old or older females without reproductive activity, i.e. no PSC or embryos were characterized as barren.

The software program PAST was used for all statistical analysis: https://folk.uio.no/ohammer/past/

2.3 Ethics approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. No ethical approval was required from an institutional or national ethics review board. The study complies with current Danish laws. The research was carried out as part of the regular culling program for mink and surveillance of wildlife diseases by the National Veterinary Institute Danish Technical University, Section for Diagnostics and Scientific Advice, Copenhagen.

3. Results

3.1. Age determination

During 2018, 247 mink were submitted to the National Veterinary Institute of Denmark. Of these mink, 208 could be separated belonging to either of the groups wild-born or captive-born; 96 mink considered born in nature and 112 mink to be captive-born, which is in accordance to [7]. Six mink of 84 (7%) with known color in the wild-born group were recessive colored, while 43 of 93 (46%) of the captive-born group with known color were recessive colored.

The exact mortality date was provided for 141 mink. For these individuals, the pulp cavity width ranged between 15%-79% (Figure 3 A). Considering all individuals with pulp cavity width <20% as mink born in the previous years, and expecting individuals that had died before 6 month post parturition to have a pulp width of more than 30%, the best fitting regression line was $y=0.42x2-11.52x+104.7$, $R=0.77$, $p<0.0001$ (Figure 3B). The model-estimated pulp cavity at 10 months is 30%, ranging between 27%-34%. Hence, individuals with a pulp cavity width <35% is considered to be younger than one year old. The pulp width of two females, known to be two years of age were 15% and 19%, respectively. Thus, individuals with a pulp cavity <20% was considered to be in their second year.

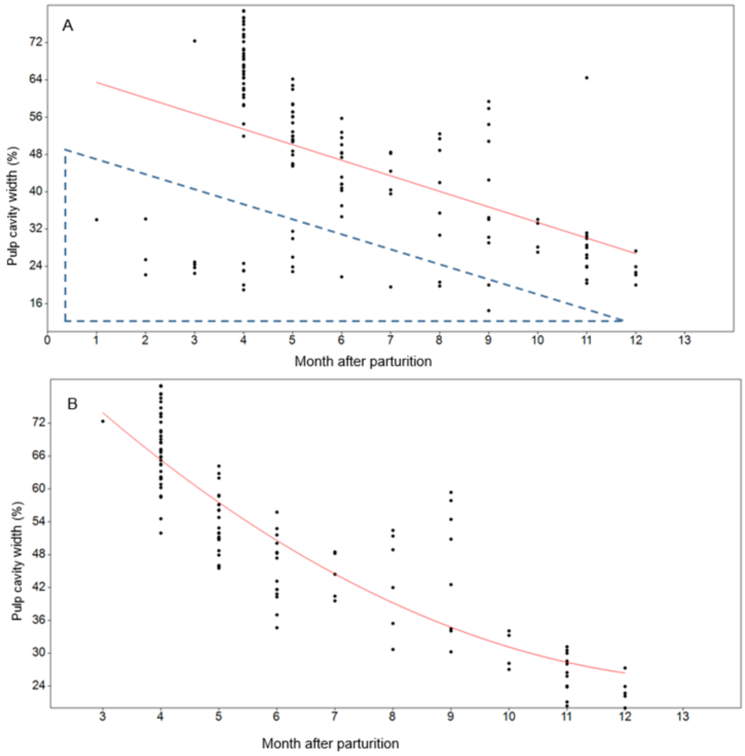

Figure 3. A) Pulp cavity width in relation to month after parturition of all mink with known death date (n=141). Dotted triangle marks outliers i.e. individuals with pulp width < 35% before June and individuals with pulp width less than 20% considered to be individuals born in the years before. B) Polynominal regression line modeling pulp cavity width of individuals < one year in relation to month after parturition was y=0.42x2-11.52x+104.7, R=0.77, p<0.0001.

3.2. *Reproduction*

The *uteri* from 105 females were analyzed. Based on pulp cavity width, 41were determined as adult females (<35%) and 64 were young females (<10 month, pulp width >34%). Of these 34 adult females were culled between 1st March and 1st November i.e. lack of PSC among these females is not expected to be due to degeneration of scars. The mean ± SE litter size of females determined as wild-born (n=7) was found to be 7.6 ± 0.9 and litter size of females considered to be captive-born (n=11) was 5.9±0.9 (Table 1). Fecundity of wild-born and captive-born was 3.8 ± 1.2 and 3.4 ± 0.9, respectively (Table 1). The percent reproducing wild-born females was 50%, and that of captive-born 58% (Table 1). No significant difference in either litter size or fecundity was found between wild-born and captive-born femlaes, however this may be due to small sample size and a large variance in litter size of captive-born females (Figure 4, Table 1).

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Table 1. Summary of reproduction of 41 adult mink females, of these 34 known to have died within 7 month *post partum* were used to estimate fecundity and percent reproducing females. Mean litter size ±SE (excluding barren), fecundity ±SE (including barren).

	Wild-born females	**Captive-born females**
Litter size	7.6 ± 0.9 (range 5-11, n=7).	5.9 ± 0.9 (range 1-10, n=11)
Fecundity	3.8 ± 1.2, n= 14	3.4 ± 0.9, n=20
Reproducing females	50%	58%

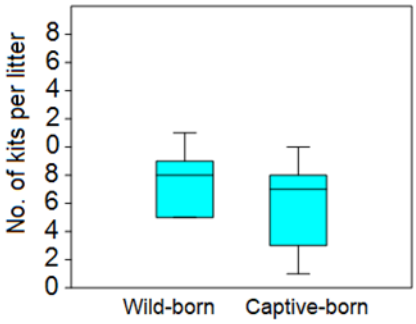

Figure 4. Boxplot of litter size (number of kits) for wild-born and captive-born females.

No females with pulp width > 35% showed any signs PSC. Among females considered to be adults (pulp width <35%), one female with pulp width of 34% contained PSC, while the remaining females with PSC all had pulp width below 28%. Among wild-born females six had pulp width between 21-26%, and one female a pulp width <20%. In the group of reproducing captive-born females six females had pulp width between 34% and 20%, and two females had pulp width <20%, two reproducing females had four age lines.

The mean amount of juveniles per wild-born adults, i.e. the fecundity divided by two, is 1.9. Accordingly, juveniles will make up 0.66 (1.9/2.9: juvenile generation divided by juvenile generation plus adult generation) of the population at the beginning of each generation defined as a turnover of 66%, i.e. the percentage that the total mortality has to correspond to in order to sustain a stabile population of wild-born mink [29].

3.3 Mortality and life tables

The age of 214 mink was determined. The overall juvenile/ adult ratio (J/A) in the culled mink population was 1.7 (136/78). Only mink more than 4 month old (from September) could be separated in wild-born and captive-bo

Table 2. Life table of mink born in nature and mink captive-born on farms. Danish mink: n = number (percent) of mink in each age class; qx=mortality rate. rn. Therefore the start generation (0) was calculated from the fecundity of females (Table 2).

	Nature-born n=80	Wild n=91	Nature-born q_x	Wild q_x
0 generation*	*61 (66%)*	*75 (63%)*	*0.70*	*0.77*
4 md-1 year	48 (60%)	47 (52%)	0.62	0.64
<1 year	18 (23%)	17 (19%)	0.56	0.41
<2 years	8 (10%)	10 (11%)	0.38	÷0.30
<3 years	5 (6%)	13 (14%)	0.80	0.69
<4 years	1 (1%)	4 (4%)	0.100	1.00

Both wild-born (qx= 0.70) and captive-born (qx=0.77) have high mortality within the first year, while the mortality rate in one-year-old and two-year-old individuals was relatively low; qx in wild-born mink ranged between 0.38 and 0.56 and in captive-born qx ranged between ÷ 0.30 to 0.41 (Table 2). Only five (2%) of aged mink, were determined as more than four years old. The oldest individuals had five age lines and were considered to be 4 ½ years.

The average proportion of individuals dying per year in wild-born mink was; 0.66x0.70 + 0.23x0.56 + 0.10x0.38 + 0.06x0.8 + 1x0.01 = (0.46+0.13+0.04+0.05+0.01) = 0.69. And average mortality per generation in captive-born; 0.63x0.77+0.19x0.41+0.11x0.30+0.14x0.69+0.04x1= 0.49+0.08-0.03+0.10+0.04= 0.68.

4. Discussion

In a previous investigation of mink caught in the wild in Denmark, comparison of body length of captive-born mink and wild-born mink revealed a substantial number of mink escapes from Danish mink farms [7]. Hence, newly escaped farmed mink may bias studies of wild-born mink reproduction and demography, and thereby the question of a self-sustainable population. This bias may lead to false conclusions about demography, reproduction and turnover and henceforward management actions of mink. In this study, wild-born and captive-born mink culled by hunters were separated in two groups before examination of reproduction and mortality.

Pulp width in canine teeth is used as a guideline for age and it is species specific [20,22]. However, in age determination of mink in previous studies authors have referred to pulp width of pine martens [15,21]. Also, the annual layers (incremental lines) which appear in the canine tooth cementum are useful as an age determining guideline, and is widely used in age determination of carnivores [20,22]. In mink, the dark incremental line is formed in the late summer and autumn, and the second line develops in individuals of approx. 1.5 years [22,23]. However, the development pattern of the incremental lines can be uneven among terrestrial mammals in different areas, and adult individuals may lack age lines [22,23]. In the present study, pulp cavity filling of showed a polynomial exponential growth, with stepwise filling of the pulp cavity, during the first 10-months followed by a flattening of the modeled pulp filling curve (Figure. 3). Compared to pine marten the pulp cavity of mink seems to have a slightly slower centripetal growth, dividing between juvenile and older pine martens at 10-months of age with a pulp cavity width between 16% and 32% (mean 24%). Danish mink at 10-month age showed a pulp cavity width ranging from 27 to 34% (mean 30%). Hence, using the pulp width of pine marten on mink, will assign a higher number of individuals to be juveniles compared to adults, than if the regression line for mink is used. Using both the actual mink pulp width versus age and incremental lines, the demography is considered to be more accurate. However, a detailed study of incremental lines in aging mink is needed for future studies.

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Normally PSC degenerate (faded) after 7 month [28], thus staining of PSC from previous years is not expected. Hence, all scars, both dark and faded were interpreted as marks from fetal attachments of the current reproduction cycle. The amount of barren females is relatively high (50% in wild-born and 42% in captive-born). The reproduction of Danish wild-born mink (mean litter size 7.6 and fecundity 3.8) is high compared to the litter size of wild-born mink ranging 4–7 kits observed in other European studies [15,19,28,30]. In a study from Scotland, evidence of a density dependent response in female fecundity was found, with a negative relationship between fecundity and relative density of females. Mink culling lead to a younger, more fecund mink population, which is considered to be a compensation for the culling [15]. The high number of kits per female observed in this study, may therefore be due to culling effect. High culling is also expected to reduce the number of barren females in a population [31]. However, this does not correspond with a relatively high ratio of reproducing females found in the Danish wild-born mink population (50%), compared to the studies in Scotland (81%) by [15].

The mortality rate of both wild-born and captive-born mink (qx>0.70) was higher within the first year, than in one year and two year old individuals. In two years old farmed mink even a negative mortality rate was found (qx=– 0.30) (Table 2). The negative mortality rate may reflecte an influx of escaping farmed mink, indicating that the demography of farmed mink may not reflect survival and mortality in the farmed population in nature, but most probably merely the escape rates of juveniles and adults from farms.

Only five (2%) of aged mink, were determined as more than four years old. The oldest individuals had five age lines and were considered to be 4 ½ years (Table 2). The low number of adults may reflect high hunting pressure or that older individuals are less likely to enter traps than younger animals [32]. The average number of individuals dying per year in wild-born mink population was 0.69.

Comparing the demography of wild-born mink populations on two islands in the UK, South Harris and North Uist with a mink population on an Estonian archipelago in the Baltic Sea the J/A ratio was found to reflected the hunting pressure [25]. High hunting pressure led to relatively high J/A ratio, while low hunting pressure led to low J/A ratio. Also, in a study of two mink populations in North America the harvest pressure affected the J/A ratio [24]. In Idaho with a moderate hunting pressure, the J/A ratio was 1.47. In Alaska with almost no hunting pressure, the J/A ratio was 0.8 [24]. The overall J/A in the culled population of mink in this study (1.7 indicated a moderate to high hunting pressure.

Based on the fecundity of wild-born females the turnover of wild-born mink was estimated to 66%. Compared to the yearly mortality estimated to 69%, the data point to a declining population of wild-born mink in Denmark, which is in agreement with national game bag records of mink. However, the yearly mortality is only marginally higher than the estimated turn over, and the balance between recruitment of the new generation and yearly mortality may tip with small changes in either reproduction or mortality.

5. Conclusions

The data show that the wild-born mink population in Denmark reproduce (litter size mean ± SE litter size of 7.6 ± 0.9, and fecundity 3.8 ± 0.9). Hence, that the population is self-sustaining, also without continuous influx of captive-born mink escaped from farms. However, escaping mink from farms may supply the wild-born mink population with additional individuals. The effect of farmed mink on the wild-born population depends on the survival rate and the ability of farmed mink to adapt to a life in nature, which at the time being is unknown.

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