EUROPEAN MINK, MUSTELA LUTREOLA LINNAEUS 1761,

CAPTIVE BREEDING AND HUSBANDRY PROTOCOL

Foundation LUTREOLA 2006

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A. Introduction

The European mink is one of the most critically endangered carnivores in Europe and is in need of urgent conservation action. The general decline of the species has been alarmingly rapid in Europe and is thought to be due to a number of anthropogenic factors including habitat loss, pollution, hunting & trapping and the introduction of the more robust and opportunistic American mink. All known wild populations are near to extinction and there is little hope for the survival of the species without intensive conservation management, both in the wild and captivity. Till now the conservation measures in the wild have had a limited outcome. They are more and more likely to depend on the strong and sound conservation breeding operation. Thus, the importance of a co-ordinated captive conservation programme as part of an overall conservation strategy to aid the species recovery is hard to overestimate.

Several attempts to breed the European mink in captivity have been made; for commercial, scientific research and conservation reasons. Unfortunately the results of these attempts have not always been successful (for more details see Maran T. 1994: Studbook for the European mink, *Mustela lutreola* Linnaeus, 1761, vol.1).

As the European mink is highly endangered, the well-being of the captive population is of vital importance to the conservation of the species and it must therefore be handled with utmost care and in the safest possible way. Therefore, the various protocols set out in these guidelines must be stringently adhered to wherever possible. However, there is always space for improvements in our captive husbandry and that will necessitate some modification and even experimentation to determine more effective management techniques. So the institutions are encouraged to be open and creative in their approaches. Although this may appear to be in direct contravention of each other, adoption of these two approaches will ensure best practices in a continuous and evolving programme

To achieve this goal all the participants should follow the protocol as much as possible and, where improvements are proposed, it should first be consulted with the species co-ordinator. The latter in discussions with the Species Committee will decide over the applicability of the proposed changes (first as test) and the modifications proven its validity it will be incorporated into the guidelines.

The guidelines place an emphasis on maintaining the European mink in large numbers in off-show breeding facilities as a core of conservation breeding. However, details of facilities on view to the visiting public in zoos and wildlife parks are also discussed.

The life history parameters of the European mink, such as short reproductive lifespan, relatively short breeding cycle and relatively large litter size will more or less define the most appropriate approach to its conservation breeding from point of view of institutional organization. Small number of animals (2 - 4) in most of the participating facilities would result in high number of animal transfers bringing along lots of additional paperwork, but also costs. Transfers can not be avoided because the genetic and demographic wealth of the captive population will demand this. These frequent transfers of animals will annoy the member institutions and are likely result in reluctance of the participating zoo to continue with the program and finally will lead to regression of the program itself. Also, the facilities with smaller number of animals tend be less efficient in breeding as the choices for alternative mating combinations are limited. There are two ways to escape such situation:

- (1) Establishment of sub-regional groups. This will help the institutions with smaller number of animals to exchange the animals inside of sub-region decreasing thus the costs for transport and management. The exchange will be performed in accordance of the coordinator and each sub-region has its own coordinator. In this way the sub-regions can be managed as larger groups of animals.
- (2) To make the conservation breeding more stabile and efficient there must be a small number (eg 4 5) facilities with larger number of animals around 50 100 animals. These facilities are more focussed for "real" conservation breeding and not so much for keeping animals only for display.

Such an approach would make the joint breeding operation more flexible and less cumbersome for members of the program. Forming of sub-regional groups is easier and depends mostly on the will of participating facilities to contribute to the joint action. The establishment of the larger subpopulations in single institutions will require, along with the motivation and willingness to contribute to *ex situ* conservation of this species, also a major financial contribution for conservation of this species. Though, it has to be emphasized that the costs of special breeding facility for European mink will be far less costly than construction of any modern zoo exposition.

The facility willing to maintain few animals (eg. few pairs) must keep in mind that for this purpose it needs in addition to enclosures for each animal also a temporary facility to maintain the litter of average 4 young per breeding pair till the beginning of next year after the birth of litter (that is 4 temporary enclosures). This is because there no possibility for the EEP coordinator to provide the recommendations for translocations of its surplus before the data on the yearly change in the overall stock has arrived from all participating institutions.

Since the publication of the 1st edition of the guidelines in 1996 the EEP conservation breeding program of the European mink has remarkably advanced though still not far from the formulated objectives. Several new institutions have contributed to the ex situ conservation breeding of this species and doing so they have brought along plenty of new knowledge and experience. For that purpose the revision of the guidelines was discussed in detail during the first species committee meeting of the European mink EEP in 2005. Current version largely bases on the comments, critics and additions proposed during this meeting. It is greatly changed and contains more detailed and update information on the management of this species. However, the current followup guidelines should not be seen as a definitive or final document, but as an evolving document where it will be subject to continuous change in the course of improving the captive conservation programme. Any comments or constructive criticism about the guidelines are welcomed.

The first edition of the guidelines has been revised by the European mink EEP Animal Committee and the changes have been incorporated by Tiit Maran and Sisco Manas. This husbandry and breeding protocol has been prepared in the frame and with financial support of European Union LIFE Coop project No LIFE 2003NAT/CP/E/000002.

B. EEP membership and exchange of information

For European mink EEP membership, the institution must have a

(1) proper enclosures for maintaining and breeding European animals,

(2) it must be ready to communicate information about keeping the European mink in its facility,

(3) it must be accept and follow the recommendations of the coordinator and the species committee,

(4) it should whenever possible and appropriate promote the conservation of European mink,

(5) it should be prepared to support the *in situ* conservation and conservation research of the European mink.

An institution willing to become a member shall send informal membership request with details about their facility to the co-ordinator. Once the coordinator decides that the facility is suitable for European mink EEP membership, the standard membership form will sent to institution for signing. If the institution does not have EAZA membership, the coordinator will contact EEP Committee for approval.

Coordinator (or studbook keeper) asks twice in a year information about the events taken place with the European mink in the facilities:

(1)After breeding season in early September co-ordinator asks for data about the breeding success. The data will be used in analyses and in preparation of transfer and

breeding recommendations for the next year. The results of the analyses will be

discussed in Species Committee and during the European mink EEP meeting. The

final recommendations will be made available for the end of the year. The Population

Management Plan and breeding recommendations will be made available only for

members of EEP and, as working documents, they are not for public distribution.

(2) <u>In the beginning of the year</u> the ARKS report (or similar report) will be asked to get the final status at the end of the year for studbook keeping and EEP reporting. The studbook will be updated yearly in the website <u>www.lutreola.ee</u>.

In addition, the co-ordinator may ask for help with specific questionnaires or information on the specific features in the biology of the species The member institutions are expected to share in timely manner the information about their animals and breeding in their institution, as this will favour better management the whole EEP population and secures survival of this species. The result of these requests of information will be made available for European mink EEP members.

C. Staffing

Every breeding facility should designate one member of the senior staff who is fully responsible for breeding and keeping of European mink. This person should preferably also be the contact person for the European mink EEP. Following current guidelines the number of keepers necessary to provide adequate cover at all times, especially during the breeding season, days off and holiday periods must be determined, along with their daily routine.

D. Disease Prevention Control

1.

Other animals

Other animals (i.e. feral cats) should be kept away from the vicinity of the European mink facility as much as possible. Live-traps should be maintained around the surrounding fence and trapped animals removed immediately on inspection. In addition an electric fence may be used to accompany the exterior fence to discourage unwanted animals climbing into the enclosed area.

Considering the danger of infecting the captive European mink with the incurable Aleutian disease, any animals brought from the wild must not be kept in the vicinity of the captive European mink stock and the quarantine requirement must be followed stringently.

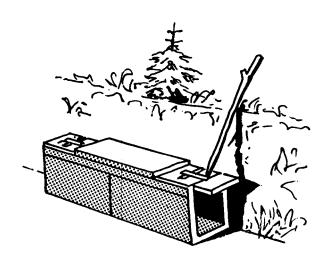


Figure 1.(from Behnke, 1982)

2. Other precautions

Excessive noise or uncontrolled artificial light could be detrimental to the animals well-being, particularly during the breeding season (March - May), parturition and the initial rearing period of pups (late-May to late-July). **Therefore the immediate area surrounding European mink facility must be kept as private as possible.** Disturbance, especially irregular and during breeding season (noise, visits etc) has to be avoided.

E. Housing Management Protocol

The European mink is considered to be highly solitary species. Therefore, there should be one enclosure for each specimen. The exception is the breeding season when the male and female until birth, and later female and pups till weaning, can be kept in the same enclosure.

1. Enclosure for public viewing

AREA: $25 - 30 M^2$

1. A minimum area of $25 - 30 \text{ m}^2$ is recommended to meet the spatial requirements of the female with cubs. The smaller enclosures could be used for single males or females, however, whenever possible larger enclosures

should always be encouraged. The large enclosure (on-show to the public) must be equipped with a diverse and enriched natural environment. This type of design promotes normal behavioural expression in a highly active and inquisitive species, and is equally attractive to both the mink and for the visiting public. The rotation of individuals in each of the enclosures adds further stimulation especially in scent-marking behaviour a highly important means of social communication.

Extra space has to be available for temporary maintenance of animals before translocation. As the suggestions for translocation of surplus can only be provided for the beginning of the next year, the facility should be able to maintain temporarily 4 additional animals in separate enclosures per every breeding pair.

WATER/LAND RATIO

The ratio of land to water should be > 4:1. It is important to make the bank-line of the water-body as long and intricate as possible. Small islets, stones and bridges are good in this respect.

OUTDOOR POOL

Although a semi-aquatic species, the European mink is not a deep water inhabitant. The pool depth should be no more than 0.5 m with running water to typify the preferred natural habitat, with various cascades and rapids. The banks should also be as diverse as possible with logs, stones and dense vegetation for cover. In addition, submerged and/or partially submerged logs and stones are also recommended. Mink also enjoy wallowing in a mixture of mud and sand, therefore, a muddy depression would enrich the behaviour of the mink. The water in the pool should be renewed constantly, or filtered, as reduced water quality has a marked effect on the protective ability of fur.

It is preferable to position the pool to the public side of the enclosure, so that public could have a good view of swimming animal and the pool will be additional barrier between the public and the animal.

It would be preferable to have water in the pool throughout the year, and where freezing may occur running water may help to prevent ice-formation.

The use of stagnant water is discourages, but where it is used for mink, the water has to be changed and the pool has to be cleaned frequently during warm season as the water becomes putrid. And this might be dangerous for animals. Cleaning the pool might be quite laborious task for keepers.

OUTDOOR ENCLOSURE(LAND AREA)

A grassed enclosure with good plant coverage is preferable, with areas containing sand and gravel. Natural rocks, stones, tree trunks & stumps, provide shelter and facilitate scent-marking behaviour. Various types of running tunnels should be provided for animals. Some tunnels could be partly opened for public viewing of animal moving in the tunnel.

In order to prevent the mink digging out from the enclosure the ground under the soil should be covered with mesh or concrete at least half a meter inside from the fence.

FENCING

The height of the boundary fence should be no less than 1.5 m (NB! snow-coverage should also be taken into account). The fence can be either solid or welded wire mesh (25 x 25 mm with ~2 mm diameter). A mesh fence must have its upper part covered with ~30cm-wide of sheet-metal. The lower section of the sheet should be set apart from the fence (Figure 2). The use of an electric fence is recommended. The electric fence can be positioned under the apart standing part of the metal sheet to prevent the falling leaves etc to short circuit it. The corners of the fence should be examined with particular care as they may provide an easy way to escape (wherever possible it would be better to design the enclosure without sharp corners). Trees in the enclosure must be at least 1m away from the boundary fence, as any overhanging branches may provide another means of escape. Although the European mink, as a rule, do not climb on trees, few specimens have been observed in facilities eagerly climbing on trees.

The electric fence with voltage of 7000 V has been used for mink.

A closed service corridor for the keeper with a door for security is essential.

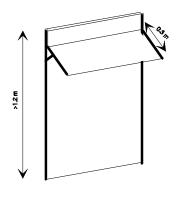
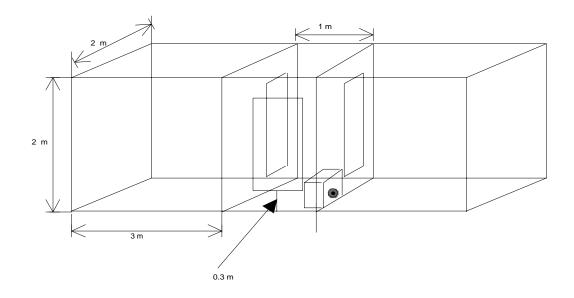


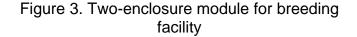
Figure 2.

2. Enclosure for breeding facility (off-show) SPACE :

A minimum area of 8 m² per individual animal should be provided. The design should be based on module of two rows of enclosures with service corridor in between. Also the service corridor must be closed and covered with net to avoid accidental escape of animals.

A space of this size is clearly enough for the maintenance of the species over winter. However, there are growing evidence that the males inadequacy behaviour in mating has something to do with the size of the enclosure and also with the arrangement of the interior at the time of raising the young, especially before weaning. Therefore, although it is far from full understanding, it seems advisable to have several additional larger enclosures with diverse interior for breeding purposes.





WATER/LAND RATIO

The ratio of land to water should not be more than 4:1(preferably 8:1).

OUTDOOR POOL

Depth of pool should not be less than 0.3m. Water should be renewed constantly or filtered.

It would be better to have the water in pool throughout the year, but freezing must then be taken into account (e.g. keeping the water running will help to prevent iceformation).

LAND PART(OUTDOOR ENCLOSURE)

A suitable substrate is smooth small pea-gravel. The bottom of ground should be covered with mesh to prevent mink digging themselves out. Cage furniture should include sand areas, rough stones, tree trunks & stumps and stones used for fur-rubbing (especially after swimming) and scent-marking.

Hollow tree trunks & stumps or pipes provide suitable denning sites for seclusion, resting and shelter. However, it is necessary for the keeper to be able to view these areas adequately.

FENCING

The enclosures must be fenced on all sides with mesh (25 x 25 mm, diameter ~2,5 mm) including the floor and roof. As the acid urine of animals will corrode the metal mesh (even when covered with zinc), it is recommended to use a mesh covered with plastic. Other ways can be used to construct the floor (e.g. concrete) as well, but always the digging habits of the animals have to be kept in mind. The height of enclosure should be approximately 1.8 - 2 m to allow easy access for the keeper. The door to the enclosure as well as the door to service corridor, must open inwards, and should be `stepped' with a threshold no less than 0.3m from the ground to prevent the accidental escape of an animal while opening the door.

The walls between the enclosures should be non-transparent to avoid the animals to see each other – this will reduce the stress and stereotypic movements of animals.

In regions with heavy snow-coverage in winter, roof damage may occur if left unattended, especially to the mesh-panels, due to the weight burden from the collection of snow.

There must be connecting doors for animals between the neighbouring enclosures. That provides an opportunity for additional space for the female with litter respective to the size of the litter.

3. Nest Box Requirements

Nest boxes should be situated in the outdoor enclosure. The standard sleeping-boxes (Figure 4) have been used. The nest box and enclosure should be connected with sliding shift door. The nest box(s) should consist of two compartments. As a rule the animal uses one of the compartments as sleeping box and the other as a latrine. In milder climate also the next boxes with one chamber can be used. This help to avoid the extra work of cleaning the latrine chamber, but might reduce the insulation of the

nest box in colder climate. Also, the handling of animals is likely to be little more complicated with one chamber solution.

A connecting door between each of the two compartments helps in the manipulation of the animal. In all doors the slider tracks should stop short at the edge of the entrance holes to the boxes to make cleaning easier. The size of the shift door should be of equal size in all entrances as this will allow using the same door for all entrances and thus will ease the management.

The nest box should be light and portable. This facilitates the nest box being moved with the animal to another enclosure or facility. The next box can be fixed to the wall as shown in Figure 3.

With larger number of animals it is recommended to fix the nest box to the height suitable for keepers to clean its interior. The animal reaching the nest box can be eased by connecting the entrance and the surface with small wooden pathway.

It is advisable to prepare the nest boxes from material with makes it breathable and avoids the collection of humidity and moisture into the nest box. Ventilation holes (0.7cm) drilled all around the top and bottom sides of the boxes may be advisable, especially in warm climates. However, it is important to remember that in continental climates at least the high temperatures in summer will accompany the very low temperatures in winters. Therefore, the holes have to be drilled in the way that it will not bring along draught in the cabin.



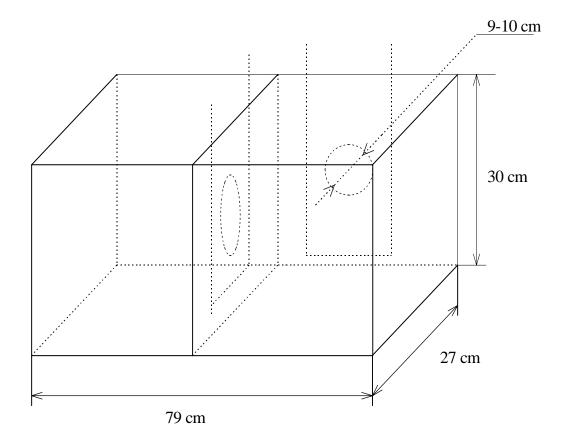
Figure 4: Two-chamber nestboxes in breeding facility in Pont de Suert (Spain)

MEASUREMENTS

The overall dimensions should be $27 \times 28-30 \times 70-80$ cm. The diameter of the entrance holes should be c. 6-10 cm. The box should be built of plywood or deal. Both compartments should have separate roofs. Ideally the box should be situated at the

farthest distance away from the water. In regions with extreme winter conditions the use of thermostatically controlled floor heating or the additional insertion of a smaller sleeping box (c. $25 \times 20 \times 31$ per compartment) may be appropriate.

In Germany, Association Euronerz uses smaller one-chamber nest boxes with inside measures of 25x 20x15 cm.





BEDDING

Dry moss (except sphagnum), hay or leaves (dry) could be used for bedding. Woodshavings are a good bedding material for use in the latrine. During the winter period unlimited bedding material should be offered to the animal(s). The unlimited extra bedding material for the animal will be important component of behavioural enrichment, but it might increase the working load for keeper as the animal usually wants to scatter the excess bedding around in the enclosure.

4. Breeding Box Requirements

All facilities should provide sufficient hiding places especially during the breeding season. However, it may be necessary to offer additional `denning sites' in the form of one-camber breeding boxes. Additional box could be used by male during mating attempts when the female is still unreceptive. Later the additional chamber provides more space for the litter.

5. Environmental Enrichment

Environmental enrichment is an important consideration in the design of the enclosure to encourage the normal behavioural repertoire of the mink. Though essentially static, it is therefore of equal importance to provide additional "behavioural" enrichment in the form of "movable" objects, such as floating objects in the pool. The positioning of different types of pipes and other tunnel-like objects would provide interest where mink like to move and hide themselves. Further re-positioning or renewal of movable objects is also desirable as it stimulates additional interest.

Foraging behaviour can be encouraged by hiding food that will also increase the overall daily activity. Live food in the form of invertebrates (locusts, crickets) and fish in the pool will stimulate hunting behaviour. However, the European mink are very conservative in relation to new food items and it might be very difficult at first for the mink to accept new food.

Different ways for environmental enrichment has been tested in Tallinn Zoo breeding facility. The results of these tests are provided in Annex 9.

6. Dietary Requirements¹

Due to the mainly crepuscular and nocturnal nature of the European mink, with a peak daily activity period occurring during these periods, it is better to feed mink in the late afternoon. If fed too early the food may be spoiled. A complete balanced captive diet has yet to be developed, nevertheless, the captive diet developed for the Black-footed ferret can provide a good starting point(Appendix 5).

A suitable captive diet comprises of around 33% fish (freshwater cyprinids & salmonids, or seawater species, such as, cod, haddock and whiting; but not oily fish like herring or mackerel) and the remainder comprising of day-old chicks, mice and occasionally amphibians and invertebrates (crayfish). Where legally permissible live food is recommended with appropriate supervision, or alternatively, freshly killed food can be offered.

A recommended feeding regime is as follows: ground meat (50%) and Mazuri Carnivore Feline Meat F (50%) for three days; fish diet for two days; mice & day-old chicks for two days - all presented on alternative days. The amount of daily food ration is dependent on individual consumption though it must be increased during the breeding season (see Breeding Protocol).

Vitamin/mineral food supplements may be of benefit, especially where fresh food is not always available. Commercially prepared supplements are available or preparations can be added to the food ration (see appendix 5)

7. Restraint

TRAPPING-BOX

Two types of trapping-boxes are recommended:

a) <u>Handling box</u> Constructed of wire-mesh (weld mesh) with a trap door (Figure
6) . This design is perhaps best for transfer of mink from the nestbox for removal and/or examination. The measurements of the box should be slightly less than that provided for the compartment next to the sleeping chamber of the nestbox. Place the trapbox against the `end' compartment (latrine) with sufficient cover (i.e. towel or sack) to darken the interior, then by lifting off the roof of the sleeping area the mink will almost always seek refuge in the darkened compartment containing the trapbox.

 $^{^{\}rm 1}$ The provisional study of European mink nutrition is underway and this chapter will be replaced in the nearest future.



Figure 6: Handling box in Tallinn Zoo.

b) <u>Live-trap</u>. In Tallinn Zoo wooden live-traps has been used. Constructed of wood for better protection, warmth and seclusion (Figures XX – XX). In Pont de Suert in Spain the breeding facility uses the commercially produced live-traps. This design of trap box is best deployed in the event of an accidental escape of an animal from its enclosure. As mink usually choose to run along the edge of a `wall' it will try and seek refuge in a dark tunnel-like place from which to hide, even without bait.







Figure 7-13: Wooden livetrap used in Tallinn Zoo.

Box for taking vaginal smear. Various types of box have been used for taking the smear. The box as in figures 14 - 16 has proved to the best for this task.







Figure 14 - 16 : Handling cage for taking vaginal smears.

8. Daily Husbandry Routine

It is important that a daily routine is established and maintained in the facility. A husbandry protocol must be established by each facility (based on the European mink Guidelines) and posted in the area.

Individual records must be maintained on each European mink kept in the facility, with the exception of kits whose records will be incorporated initially with the mother until they reach independence and are separated from her; whereupon they will be issued with their own individual records.

All European mink have to be individually checked at least once daily, the only exception being females with kits during their first 7-14 days after parturition.

Nest boxes should be cleaned in the morning. All soiled bedding material in latrines (shavings, wood chips etc.) should be replaced daily with fresh material. The bedding material forming the nest should be replaced when there is a need for this (for instance the material becomes soiled). Considering the importance of olfactory signals for the European mink it is important to avoid over cleaning and in this way removing the smells important for the animals too often. A scraper is used to remove faecal material from the sides of the latrine side of the nest box. Use of disinfectants and water for cleaning on a daily basis is discouraged as mink prefer their own scent.

Fresh food and water should be presented preferably in late afternoon.

F.

1.

European Mink Captive Breeding Protocol

Female European Mink - Detection of Oestrus

There are two overlapping ways to detect oestrus. For facilities with small number of animals, oestrus can be detected by checking the size of vulva and observing the behaviour of male and female (rather imprecise behavioural method); for facilities with larger number of animals taking the vaginal smears is recommended as more precise method (physiological method).

<u>Behavioural method.</u> The development of vulva size has to be observed since 15 of March. The development of vulva is shown in Figure 17.

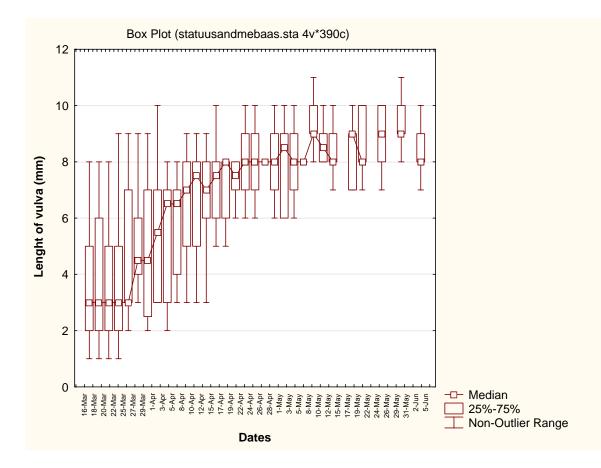


Figure 17. Development of vulva on European mink in Tallinn Zoo (n=10; Tiit Maran, unpublished data)

Once the length of vulva is more than 7 mm long, a female should be exposed to male through the welded mesh and the behaviour of both animals has to be observed. If male (known to be effective breeder) does not expose any aggressive behaviour, the behaviour of the female is calm and receptive and <u>the male (or also female) is</u> <u>emitting clucking sounds</u>, it is likely that the female is in oestrus.

Physiological method.

The walls of vagina are lined with epithelial cells and the onset of oestrus will cause these cells to become cornified. Non-cornified cells, which are quite small, round and with a distinct nucleus, are found when anoestrous vaginal flushes are taken and examined. A few cornified cells are found in pro-oestrus flushes. The onset of oestrus is characterised by a 90% vaginal cornification in general. Cornified cells are large, flaky cells without nucleus (Figure 19; see also Appendix 1: Reproduction). However, the testing in Tallinn Zoo breeding facility has shown that at least if the sample is taken by pipette, the count of cornified cells in the display does not reach often 90%, but will reach much lower values (figure 18). It was also revealed in Tallinn that the ability of counting the number of cornified cells depends upon staining method applied. Therefore, it is recommended to regard 80% of cornified cells in the sample as a sign of oestrus.

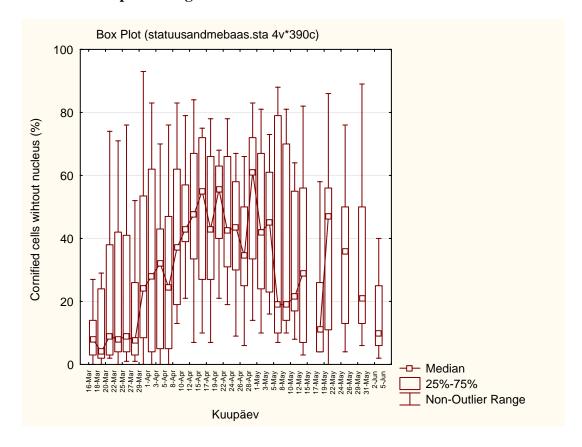


Figure 18. Number of cornified cells in the sample in Tallinn Zoo in 2004. (n=10; unpublished data)

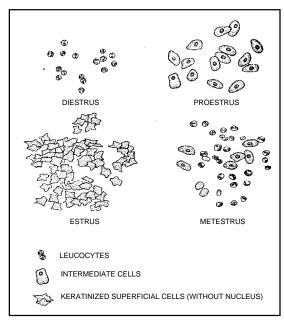


Figure 6. the cells in vaginal lavage of the oestrus cycle

Figure 19: Detection the estrus by vaginal smears

From March 15- 20th, females will be subjected to weekly checking of the size of vulva. The flushes will be taken whenever the length of vulva is larger than 5 mm. When the quantity of cornified cells is greater than 50%, then vaginal flushes must be conducted on every second day. <u>When 80% cornifications is achieved the female must</u> then be placed with an appropriate male for breeding².

Small pipettes or tampons with distilled water can be used for flushes. For flushes animal will be moved into a special handling cage as described under the chapter Restraint/Trapping box. With the help of pipette or tampon (Figure 20) the sample will be taken and smeared on the slide. The slide will be checked under microscope after staining. Commercially produced staining kits could be used. The best known identification power is provided by the diagnostic kit "Kit DIANOESTRUS by

 $^{^2}$ The protocol has been compiled mostly in accordance to the experience in Tallinn Zoo. The possible regional difference in the start of estrus cycle has to be kept in mind.

REACTIFS RAL (France). Very fast diagnostic performance, though with somewhat less diagnostic power provides a kit Hemacolor by Merck. The latter is recommended only if large number of females has to be tested for estrus.

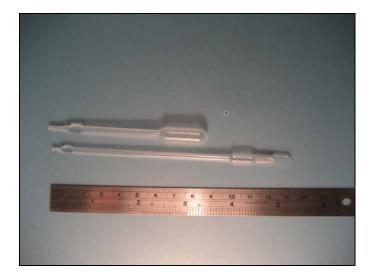


Figure 20. Pipettes used in Tallinn Zoo for vaginal flushes.

Measurements of vulva swelling and evaluation of vulva appearance will be conducted concurrently with vaginal flushes. It helps to predict a proper time for pairing, especially while having only a few breeding pairs of mink, or when the use of vaginal smears may seem to be too complicated a method to use.

The size of the vulva to certain extent indicates the start of estrus - when in full heat the vulva is swollen. In comparison, the size of the anoestrus vulva is minute, measuring a maximum 2 mm to 4 mm, but usually much smaller. When the female begins to come into heat, the vulva enlarges over a period of three to four weeks. Its maximum size may reach 10mmx10mm or more. The colour of the vulva also changes during heat from pale white during the anoestrus to pro-oestrus period and to a pinkreddish coloration during oestrus. The size and coloration of vulva can be easily examined and measured through the handling cage. However, it is important to note that size of vulva varies depending upon several factors including stress and nervousness of female. Therefore, it cannot be relied only on the size of vulva when detecting the start of oestrus, but it has to be used as complementary indicator along with the count of cornified cells. The vulva measurements should be at least 10mm x 10 mm and pinkish in colour before the introduction of the female to the male for breeding (Figure 21).



Figure 21: Size of vulva at the time of estrus.

Behavioural changes exhibited by the female may also provide a good indicator of heat, where the oestrous female becomes more active and less timid.

2. Male European Mink - Detection of Fertility, "male problem"

Starting from February 1st, the testes should be measured every now and then. The testes may grow up to 18mm x 16mm. If the size of the testes remains relatively small by the beginning of April, it might be a sign of poor breeding quality.

Nevertheless, large testes are not always indicative of a good breeding male, as very often males with relatively normal size testes are of equal breeding potential.

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Along with the physiological fertility also the behavioural fertility of males has to be detected. Accumulating experience in conservation breeding of the European mink has revealed that large number of captive-born males behave inadequately in mating situation: usually the males are overly aggressive towards female and may even kill the partner if not timely separated; some of the males, vice versa, do not show any interest in females. The proportion of abnormal males is rather large in captive population. As on wild-born males such a behavioural abnormality has not been observed, it is obvious that this is a result of mismanagement. Unfortunately, until the cause of such abnormal behaviour is not revealed, it is not possible to provide recommendations for proper adjusting of our management procedures.

It is highly important that each participating facility tests the males (in accordance of the breeding recommendations) and to provides to the EEP coordinator information on the mating behaviour of their males. This will help the coordinator to analyse the data and hopefully also to reveal any management-dependant patterns in the mating behaviour of males making, thus, it possible to discover the cause of their abnormal behaviour.

3. Breeding Protocol

A)

ANIMAL WEIGHTS

The weights of adult animals are variable within the sexes and change throughout the annual cycle. Males are considerably heavier than females, with males also exhibiting a greater seasonal weight change. The following recommendations should be taken as general guide and its application may also be dependent on individual variation. The animals usually tend to loose their weight themselves before the start of breeding season though a special dietary treatment may not be often even needed.

Adult females older than one year of age should lose 15% of their January weight prior to breeding. Their weight should then be maintained or slightly increased (2-3%) during gestation. Weights should be taken at a minimum of twice weekly to monitor weight loss during February and March.

Adult males, older than one year of age, should lose 10% of their January weight prior to breeding. Their weights should then be maintained during the breeding season by increasing the weight/volume of food ration provided.

Juvenile males and females should lose about 10% of their February 15th weights prior to March 15th.

PAIRING OF ANIMALS

B)

Pairing will begin once females are determined to be in oestrus (based on the vaginal smear samples, on vulva appearance and/or on behaviour of females).

Whenever possible, pairings will be based primarily upon genetic considerations determined through the recommendations provided by the species co-ordinator of the breeding programme. When ever needed, and possible, the co-ordinator will provide to the facilities the list of alternative choices for mating.

Handling and moving the animals should be carried out by the mink keeper or by other staff with whom the mink are familiar with.

Females in oestrus will be paired with successive males until successful copulation is achieved. If the male is aggressive or the female is not receptive to the male, the animals should be separated. However, if the temperament and behaviour of the pair(s) is extremely conducive then receptive animals can be left overnight and separated during daylight hours. If the copulation has not been observed and the animals have been left together overnight, a good indication of successful mating is the couple staying in the same sleeping box. It is desirable to take a vaginal smear to check for the presence of sperm, but it has to be noted that if the copulation occurs in the evening, it is unlikely to discover the sperms in the flushes taken in the morning. Each receptive female should be placed with the same male on three successful nights. Thereafter no further introductions to the male will be necessary or should be attempted.

A single male will be allowed to inseminate no more than one female per night. The males should be rested one or two nights between introductions to different females, if possible.

If the pair is found to be incompatible they will be separated and the female returned to her cage. A second introduction can be tried the following night and if this fails no further introductions to this male should be attempted.

It is important to note that although the oestrus usually lasts for more than three days, in some cases its duration may be less than 2 days. That is important to keep in mind while trying to mate genetically highly valuable females.

4. Whelping Management

The breeding enclosure must be isolated from visitors during the breeding period. Having only the keeper(s) with which the mink are familiar, to be allowed to care for pregnant or whelping females.

Exchange of keeper(s) during pregnancy or whelping is highly inadvisable, as it may danger of successful breeding.

Pregnant females should have the choice of at least two nest boxes. Food must be provided without restrictions to the female with kits.

The European mink is a mono-oestrus species that typically have only a single litter each year. If the female resorbs her embryos or looses her first litter within a few days of birth, a second oestrus may occur and then a second breeding attempt may be made.

This pattern of reproduction may prove advantageous, at some stage, as a management tool to increase population size, where a second litter may be produced by removing live young shortly after birth and placing them with foster mothers. Such a propagation technique however must first have the approval of the species co-ordinator.

In very few cases also a secondary oestrus has been observed in captive European mink. Then the mating will take place in late May or early June and the litter is produced in July.

5. Cross Fostering of Kits

In the case of very large litters (5-6 young) or very small litters (1 young) the crossfostering may be necessary. Weak or stunted kits should be selected for crossfostering.

Cross-fostering can be attempted when the kit(s) are one month of age, prior to opening of the eyes and onset of hearing. The foster female to whom the kits are to be introduced should be locked out of the nest box and the foster kit(s) should then be placed into the nest with other kits. It helps to scent the newcomers by rubbing the introduced kit(s) with the existing bedding material removed from the nest. The operation should be done quickly to reduce the stress of the female being kept apart from her young.

The introduced youngster(s) can be temporarily marked for identification by dyeing or cutting away some of the fur or with microchips.

6. Hand Rearing of Kits

Hand-rearing of kits is not desirable or encouraged. Whenever possible, crossfostering is the preferred choice. Abandoned or orphaned kits reared by foster mothers are exposed to social interactions that are often absent or impossible to reproduce in animals reared in isolation.

7. Kit Care

A)

NEW KITS

Newly-born kits should not be disturbed for 7-14 days after birth. Any form of disturbance may result in the loss of young, especially by inexperienced primiparous females.

B) NESTBOX CLEANING

The female should be locked outside of the nestbox before cleaning commences. Everything must be cleaned as quickly and quietly as possible. If separation of the female causes aggression or trauma to the kits, then cleaning should be minimal or stopped. No chemicals or solvents can be used for cleaning.

C) SEPARATION OF YOUNG

There is no clear understanding what is the best time or methodology for separation of young. The weaning in the wild is observed to occur in late August and September. In captivity, a good indication for time of separation is increasing aggression in litter. The young should be separated when the first signs of serious aggression appear in litter.

The best method for separation is so far not clear, but is seems that it is the best to separate from the litter the animals which display highly aggressive behaviour. It is advisable to separate entire litter for the end of September.

G.Veterinary Care

European mink, like most wild animals, often do not show any obvious clinical signs of illness until disease has reached to more advanced stage. Information about health requirement of the species in captivity is limited and originates from single described cases. The importance of a strong preventive health care protocol is essential. It program must include proper housing, quarantine, nutrition, parasite control and regular physical examinations.

It is assumed that the local health protocols will be established in accordance to the requirements listed in present guidelines though the local features may require some adaptations of general requirements. Systematic and detailed medical records have to be kept. This helps to learn and better understand the medical problems specific for this species. The medical records should identify the history, physical findings, treatments and diagnosis. When a mink is sedated for any reason, the opportunity should be taken to carry out a full veterinary examination, blood collection, weight, body temperature, heart and respiratory rate, etc. to obtain baseline physiological parameters.

1. Physiological parameters

species co-ordinator for update this document.

Little pathological and physiological information is available about European mink; especially in comparison to piles of information on American mink.

It is important that physiological data (respiratory rate; heart rate; rectal temperature; haematological parameters etc.) are recorded when ever possible during routine veterinary examination.

Rectal temperature: In free ranging minks, the mean rectal temperature is 37,7°C (range 37,2°C to 38,4°C; data from Pont de Suert) 5 minutes after injection of anaesthetics (ketamine and medetomidine) The temperature declines during anaesthesia in larger parts of body an average 1,26°C (range +0,4 to -2,2 °C). All anesthetized animals must be kept in warm (eg. on electric warming pillows) or some other procedure has to be employed to avoid hypothermia. In few cases hyperthermia has also been observed during anaesthesia.

2. Haematological and biochemical parameters

The haematological and biochemical parameters of free ranging European mink in Spain are provided in Table 1.

Table 1.	Haematological	and ser	um bi	ochemistry	data	for	adult	European	minks.	Mañas	et	al.
unpublish	hed data.											

Parameters	Units	n	Mean	(SD) ^a		Range
Blood cells						
Red blood cells	$10^{6}/mm^{3}$	44	7.47	(1.06)	4.63	- 9.15
Hemoglobin (HB)	g/dl	44	15.13	(2.31)	7.70	- 18.30
Packed cell volume	%	44	47.75	(7.41)	26.10	- 68.60
Mean corpuscular volume	fl	44	64.05	(3.64)	56.00	- 76.60
Mean corpuscular HB concentration	g/dl	44	31.74	(1.67)	24.20	- 35.50
Mean corpuscular HB	Pg	44	20.30	(1.13)	16.70	- 22.30
Total white blood cells (WBC)	number/mm ³	44	9,477	(2,273)	5,500	- 16,000
Band neutrophils	number/mm ³	44	22.41	(56.48)	0	- 240
Band neutrophils	% WBC's	44	0.23	(0.57)	0	- 2
Segmented neutrophils	number/mm ³	44	5,205	(1,911)	2,090	- 10,240
Segmented neutrophils	% WBC's	44	54.70	(13.96)	28	- 82
Lymphocytes	number/mm ³	44	3,383	(1,459)	792	- 6,270
Lymphocytes	% WBC's	44	36.16	(14.87)	11	- 67
Monocytes	number/mm ³	44	600.64	(577.04)	88	- 2,340
Monocytes	% WBC's	44	6.11	(5.34)	1	- 20
Eosinophils	number/mm ³	44	265.45	(222.85)	0	- 1,170
Eosinophils	% WBC's	44	2.80	(2.01)	0	- 9
Basophils	number/mm ³	44	1.64	(10.85)	0	- 72
Basophils	% WBC's	44	0,002	(0.15)	0	- 1
Platelets	number/mm ³	43	545,627	(175,344)	159,000	- 895,000
Total plasma protein	mg/dl	44	64.91	(6.17)		- 77
Serum	-					
Total serum protein (TSP)	mg/dl	46	63.87	(6.68)	52	- 84
Albumin ^b	mg/dl	46	29.19	(2.80)	23.1	- 35.0
Albumin ^b	% TSP's	46	45.98	(4.99)	33.7	- 54.1
Globulin ^b	mg/dl	46	34.67	(6.24)	25.3	- 56
Globulin ^b	% TSP's	46	54.01	(4.99)	45.6	- 66.3
Alpha 1 ^b	mg/dl	46	4.37	(3.04)	0.6	- 11.9
Alpha 1 ^b	% TSP's	46	6.99	(5.01)	1.0	- 19.5
Alpha 2 ^b	mg/dl	46	9.49	(3.23)	4.0	- 16.6
Alpha 2 ^b	% TSP's	46	14.90	(4.85)	7.0	- 27.2
Beta ^b	mg/dl	46	12.80	(4.97)	4.0	- 31.0
Beta ^b	% TSP's	46	19.79	(6.50)	6.9	- 37.6
Gamma-globulin ^b	mg/dl	46	7.98	(3.87)	2.0	- 19.2
Gamma-globulin ^b	% TSP's	46	12.32	(5.36)	3.9	- 27.0
Albumin/globulin ratio	1:	46	0.86	(0.16)	0.51	- 1.18
ALT ^c	U/l	46	191.46	(325.73)	18	- 1,656
ALP ^c	U/l	46	71.91	(27.46)	35	- 147
AST ^c	U/l	46	89.57	(76.12)	30	- 391
Calcium	mg/dl	46	9.82	(0.85)	8.3	- 11.9
Creatinine	mg/dl	46	0.585	(0.284)	0.2	- 1.8
Glucose	mg/dl	46	154.54	(46.79)		- 321
Phosphorus	mg/dl	46	6.82	(1.31)		- 12.2
Potassium	mEq/l	41	5.09	(0.68)		- 6.9
Sodium	mEq/l	41	149.71	(5.10)		- 162
Urea nitrogen	mg/dl	46	78.54	(36.84)		- 194

^a SD: Standard deviation ^b: based on serum electrophoresis ^c: ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase.

3. Blood sampling

Blood can be taken from different parts depending upong the experience of the veterinarian and also the volume of blood sample required.

For very small sample in field by person little experience in veterinary, a tail cut and use of capillaries might be most suitable for a single small serological sample.

Sample from the vein cephalica is useful when not a big amount of blood is required and the haemolyses is not important.

If large samples are required, the jugular vein while the animal is in dorsal recumbence is recommended to obtain sample for haematology, serology or bioquemistry studies.

The bleeding of animals from jugular vein needs hair cutting on skin around jugular vena and moistening the area with alcohol (with low air temperature the hair cutting is not recommended). Often the vein is not clearly visible (especially with overweight animals) and veterinary is expected to know the exact location of the vein). Further, the vein tends to slip aside when penetrating the skin with needle (Figure 22).



Figure 22. Bleeding of European mink from jugular vein in Tallinn Zoo

4. Infectious diseases

Very little information on pathology is available about the European mink; especially in comparison to the American mink.

In general, therapy is quite similar to the domestic cat.

The most important diseases the species can suffer from are described below.

A) VIRAL DISEASES

Aleutian Mink Disease (ADV)

ADV was first described in 1956 in American mink as a syndrome causing chronic disease and mortality. Later the virus was characterized as an autonomous parvovirus.

Antibodies against this disease in free ranging European mink were first described in 1999 in Spain. Sure that the virus was established several years ago in the wild population of European mink, probably originated from farms or feral populations of American mink. The ADV is known to causes different syndromes on American mink. Classical ADV is a persistent infection associated with circulating infectious immune complexes, high titers of antiviral antibodies, hypergammaglobulinemia, plasmacytosis and immune complex disease. Syndromes ranged from decreased fertility and abortion, through acute pneumonia in neonatal mink to persistent infection and chronic immune complex-mediated glomerulonephritis and arteritis in adult mink. It was also shown that a persistent infection is maintained until death. **There is no vaccine or any effective treatment against ADV.**

Until now, the disease hasn't been detected in captive European mink but it seems to be quite common in free-ranging animals in both the western and eastern populations. The only four samples checked from Danube Delta gave negative results on serology, but tiny sample size does not allow to make any final conclusion about the presence of this disease there.

Mink Enteritis Virus (MEV)

Mink can be affected also by another autonomous parvovirus, the mink enteritis virus. It causes acute enteritis in affected American mink. The virus is closely related to canine parvovirus and feline panleucopenia virus. Mink enteritis is an acute highly contagious disease characterized by severe gastroenteritis. The general spread of infection is via the faecal oral route and in American mink non-vaccinated herds result in a drastic transmission, causing an epidemic disease of high morbidity and moderate mortality.

Evidence of seropositive animals was detected in European mink in free and captive animals but not evidence of disease has been reported.

Modified live vaccines should <u>not</u> be used for vaccination of European mink, as they have caused vaccine-induced mortality (check the chapter about vaccinations).

Canine Distemper Virus (CDV)

Canine distemper virus (CDV) is a contagious disease caused by a large RNA paramyxovirus. The infection causes in all species of Mustelidae an acute systemic disease involving multiple organ systems, including the respiratory tract, lymphoid system, and central nervous system. Signs can include a mild conjunctivitis, skin lesions, high fever, loose of appetite, lethargy, diarrhea, seizures and death in few days.

Modified live vaccines should not be used for vaccination of European mink, as they have caused vaccine-induced mortality (check the chapter about vaccinations).

Rabbies

All mammal species are susceptible to this disease. Cause an acute, invariably fatal, viral encephalomyelitis.

Modified live vaccines should not be used for vaccination of European mink, as they have caused vaccine-induced mortality (check the chapter about vaccinations).

B) BACTERIAL DISEASES

Leptospirosis

This disease is caused by spirochete bacteria classified under the Leptospira, of which there are ~17 species. Infections may be asymptomatic or cause various signs, including fever, icterus, hemoglobinuria, renal failure, infertility, abortion, and death. Incidence in mustelids is low.

Clostridium perfringens type A

Large number of minks dead in Germany between October 2003 and January 2004 by this spore-forming, Gram-positive bacterium (Wibbelt *et al.*, 2005).

Prevention and treatment of this disease is difficult, therefore controlled feeding management should help to improve the situation.

Proteus mirabilis

Before breeding season it is recommended to take samples for vaginal cultures from all reproductive females. In Spain, the following strains were detected from such samples: *Micrococcus* and *Streptococcus* and in a low percentage of samples also *Proteus mirabilis*. *P. mirabilis* was developed, which causes the infections in urogenital tract. These infections are also regularly detected on otters and other species. In Spain, the treatment with antibiotics before mating is used against of *P. mirabilis* before mating.

C) MYCOTIC DISEASES

These are very rare in mink. The number of yeast in vaginal smears is slow.

D) PARASITES

(1) Ectoparasites

The most common ectoparasites in free ranging animals are ticks –specially *Ixodes hexagonus* in Spain- and fleas. In captive population the fleas may occur. Ticks have not been observed.

Treatment with a suitable, typically-applied, "anti-parasite" spray or powder is usually sufficient. Products as selamectina (*Stronghold**) put over the skin during anaesthesia have been used with any negative effect at domestic cat dosage.

(2) Endoparasites

Wild European mink have been reported to have high richness and levels of helminth infestations. In Belarus, Sidorovich & Bychkova (1993) recorded 17 species of parasitic worms (6 digeneans, 2 cestodes, 8 nematodes and 1 acanthocephalan) after dissection of 41 specimens with overall prevalence of 93.7%. More recently, Torres et al (2003) analysed 28 specimens from Spain and reported 9 helminth species with a similar prevalence of 89.3%. Therefore, the helminth fauna of *M. lutreola* in these two countries is know to consist of following speices: in Spain *Metorchis bilis, Parametorchis sp., Pseudamphistomum truncatum, Euryhelmis squamula, Apophallus donicus, Taenia martis, Taenia taenuicollis, Aonchotheca putorii, Strongyloides mustelorum, Molineus patens, Crenosoma melesi, <i>Aelurostrongylus pridhami, Centrorhynchus ninnii*; in Byelorussia *Euparyphium melis, Rossicotrema donicum, Opistorchis felineus, Metorchis albidus, Pseudamphistomum truncatum, Alaria alata, Spirometra erinacei, Taenia mustelae, Capillaria mucronata, Capillaria putorii, Trichinella spiralis, Strongyloides martis, Skrjabingylus masicola, Capillaria putorii, Trichinella spiralis, Strongyloides martis, Skrjabingylus masicola, Capillaria putorii, Taina spiralis, Strongyloides martis, Skrjabingylus masicola, Capillaria putorii, Trichinella spiralis, Strongyloides martis, Skrjabingylus masicola, Strongyloides martis, Skrjabingylus masicola, Strongyloides martis, Skrjabingylus masicola, Strongyloides martis* spiralis, Strongyloid

Filaroides martis, Molineus patens, Ascaris devosi, Corynosoma strumosum. It is important to mention srticking similarity between Belorussian and Spanish helminthocoenoses despite of the distance and differences in climatic parameters. However, some differences can be highlighted as well: a) in Belarus the most prevalent digenean was *Euparyphium melis* (40%), a species that uses amphibians as last intermediate hosts, whilst in Spain, *M. bilis*, a species that needs fishes as last intermediate host, was the most prevalent (32.1%), b) two different species of Strongyloides are reported in Belarus (*S. martis*) and Spain (*S. mustelorum*) but they are probably synonymous and c) among metastrongylids *Filaroides martis* was cited in Belarus and *Aelurostrongylus pridhami* in Spain.

Parasite born disease are infrequent in mustelids. It might evidence that the natural parasite burden can be withstood by wild population consisting of healthy individuals. Endoparasites have not caused so far also any specific and serious problems for European minks captive populations). At Tallinn Zoo the following species were found from faecal sampling and during post mortem examinations: Taenia sp. (*T. sibirica?*), *Contracaecum* sp., *Capillaria mucronata*, *Capillaria putorii* and the coccidians (*Eimeria* sp. and *Isospora* sp.). However special attention should be paid to some helminths such as metastrongylids living in the lungs, helminths that can invade several organs in no healthy individuals (*Strongyloides spp.*) and the cranial species (*Troglotrema acutum* and *Skrjabingylus nasicola*).

In breeding facilities it is important to periodically collect scat samples and conduct classical corpological analyses for detecting any signs of parasites such as oocysts, eggs and larvae of several parasites.

5. Non infectious diseases

Several non infectious diseases have been observed on European mink, but these are not fully analysed and the information about them is rather scattered. The better review of these diseases still needs to be done.

6. Preventative health care. Disease prevention control.

All European mink should have an annual examination during which the following is done:

- Transponder should be checked and reapplied if they are not readable.
- Baseline physiological parameters, such as weight, body temperature, heart rate, and respiratory rate should be obtained and recorded, whenever possible.
- Physical examination with complete evaluation of the oral cavity, all dentition and reproductive tract should be evaluated.
- Annual serology against Aleutian disease is recommended.

- Serum should be banked whenever possible for the institution.
- Also an annual faecal examination should be performed to check for internal parasites.
- The external parasite should be inspected regularly, usually along with any standard physical examination.

At Tallinn Zoo faecal samples are collected at least three times per year:

March	-	before the breeding season
July or August	-	after the breeding season
October-November -	prior to	the winter period

The use of *Ivermectin* (Ivomec*) 0.1 ml orally in food per animal has been used successfully against helminths and sulfonamids against coccidians.

Other animals (i.e. feral cats) should be kept away from the vicinity of the European mink facility as much as possible. Live-traps should be maintained around the surrounding fence and trapped animals removed immediately on inspection. In addition an electric fence may be used to accompany the exterior fence to discourage unwanted animals climbing into the enclosed area.

7. Quarantine

All European mink should undergo a minimum of 30-day quarantine after arrival to institution. This allows time for the development of clinical signs of disease that may have been incubating before the animal was shipped. During the quarantine period the animal should be observed for signs that may be associated with disease, such as sneezing, coughing, vomiting, diarrhoea, ocular or nasal discharge, etc. Three faecal examinations for parasites should be performed.

CONSIDERING THE DANGER OF INFECTING THE CAPTIVE EUROPEAN MINK WITH THE INCURABLE ALEUTIAN DISEASE, ANY ANIMALS BROUGHT

FROM THE WILD OR FROM ANOTHER FACILITY MUST NOT BE KEPT IN THE VICINITY OF THE CAPTIVE EUROPEAN MINK AND QUARANTINE REQUIREMENT MUST BE FOLLOWED STRINGENTLY AND IN FULL SCALE.

There is no information available about the time required after ADV infection to obtain a positive result in serology in European mink. In the Spanish breeding program, three serological test are performed for each wild animal before introduction into breeding program: the first test is done in the day after capture and 15 days later the second is performed; with two negative results, the animal is moved into quarantine area; the third serology will be performed one month after the arrival to the centre. After it, one test is performed annually.

The diet should be slowly adjusted over several weeks if there is to be a diet change.

8. Vaccination

There is limited information on the susceptibility of European mink to infectious diseases or the routine use of vaccines to build up immunity. The European mink is thought to be susceptible to a number of infectious diseases found in other mustelids, in particular: **canine distemper, rabies, mink enteritis, feline panleukopenia, leptospirosis and toxoplasmosis.**

Vaccines are produced specifically for domestic species, therefore vaccinations of nondomestic species is always off-label. There are a few general recommendations for vaccinating non-domestic species, which also apply to European mink:

- In general, animals with active clinical illness or under corticosteroid therapy should not be vaccinated.
- Monovalent vaccines (which use one pathogen), are preferable to multivalent vaccines due to the possibility of immune suppression which may occur after interaction between the pathogens.
- Inactivated viral or bacterial vaccines are preferable to modified live virus (MLV) vaccines.

MLV vaccines are produced to be avirulent in domestic species. However, they may be insufficiently attenuated to be non-pathogenic in exotic species.

In certain cases MLV vaccines can be considered safe and efficacious in non-domestic species, after extensive experience in zoos. However, published safety and serology data

remains limited, and studies evaluating protection against virulent virus challenge typically are not done.

Farm-ranched American mink are routinely vaccinated against canine distemper, rabies, feline panleuokopenia and botulism. BIOCOM-DP (United Vaccines, USA), is a four-in-one vaccine, i.e. it protects against four diseases with one inoculation. The vaccine is sold in two parts, a liquid, BIOCOM- P, which protects against panleukopenia, botulism, and pseudomonas; and a solid, Distemink, which uses a modified live distemper virus produced on chicken embryos. At Tallinn Zoo, adult European mink have previously been vaccinated with this vaccine without clinical problems, however: MLV distemper vaccines have caused fatal disease in several non-domestic carnivore species, including European mink in two zoos.

Therefore: Modified live vaccines (especially canine distemper and rabies) should not be used for vaccination of European mink, as they have caused vaccine-induced mortality.

Canine Distemper Virus: No monovalent inactivated vaccines are currently commercially available. A commercially available recombinant canarypox-vectored vaccine for ferrets (Purevax, Merial, Duluth, USA) has been found safe and efficacious in European mink, and is recommended in zoos by the American Association of Zoo Veterinarians for exotic carnivores. However, EU GGO regulations do not allow its use in the EU. In an experimental study conducted in Tallinn zoo an experimental immunostimulating complex (ISCOM, ErasmusMC, the Netherlands) vaccine has been used which was safe, and produced high CDV-specific antibody titres that lasted at least one year.

Rabies. Vaccination should only be done with an inactivated virus. Imrab-3 (Merial, Duluth, USA) is a commercially available inactivated vaccine approved for ferrets, and is recommended in areas where rabies incidence is high.

Mink enteritis virus. Cross protection occurs after inoculation with closely related canine parvovirus-2. In Rotterdam Zoo the mink are vaccinated with Fel-O-Vax (Fort Dodge, Naarden, the Netherlands), which contains an inactivated feline virus.

Leptospirosis. Incidence in mustelids is low, so vaccination is recommended only in areas with high prevalence. The liquid component of Duramune Max/5/4L (Fort Dodge, Naarden, the Netherlands) contains bacterins of *Leptospira grippostyphosa*, *L. icterohaemorrhagiae*, and *L. pomona*. Immunity is shortlived, and vaccination should be given twice annually.

E) VACCINATION SCHEDULE

Primary vaccination:

- CDV, panleukopenia: 3 vaccinations at 3 week intervals. This can be started at the age of 10-12 weeks to avoid interference by maternal antibodies.

- Rabies: 1 vaccination at 4-6 months

Booster vaccinations: yearly with 1 dose. Leptospirosis twice yearly.

Adverse effects: Post-vaccine reactions with self-limiting fever and swelling at the site of injection may occur. Ferrets have been reported with post-vaccine anaphylactic reactions, and these may therefore also occur in European mink. The anaphylactic reactions usually respond to epinephrine, steroids, antihistamines and oxygen.

Other precautions

Excessive noise or uncontrolled artificial light could be detrimental to the animals well-being, particularly during the breeding season (March - May), parturition and the initial rearing period of pups (late-May to late-July). **Therefore the immediate area surrounding European mink facility must be kept as private as possible.** Disturbance, especially irregular and during breeding season (noise, visits etc) has to be avoided.

H. Management in captivity

9. Identification

Various methods of permanent and unique identification have been used in the form of ear tags, tattoos and microchip transponders. The best method for identification of European mink is the use of microchips.

10. Transportation

Animals should always be transported separately. Crates should be made of solid plywood resistant to biting. Where possible it is preferred to use for transportation the nest-box of the animal. Good ventilation through wire-netting is very important as there is a **risk of overheating**. During the long-distance transportation the animals should be provided adequate food and water. Transportation during hot weather should be avoided whenever possible.

Follow the International Air Transport Association (IATA) life animal regulations (1997) (www.iata.org).

11. Restraint Anaesthesia Immobilization / anesthesia (immobilisation)

Manual restraint

Whenever possible, manual restraint of mink should be avoided. A handling cage can be used for all procedures such as examination, vaccination, anaesthetising, vaginal smears, sample collection or for weighing. Measurements of the handling cage should be 12cm x 12cm x 30cm and include a sliding-door. The mink can be encouraged to enter the handling cage (located towards the latrine compartment) by carefully lifting the lid to the sleeping compartment from which it will leave. A towel placed over the handling cage makes it more inviting to enter.

Only **in extreme cases** should mink be manually restrained. When this is necessary the mink must first be caught in the handling cage. A darkened bag is then placed over the `mouth' of the handling box from which the mink usually will run into. The animal should then be

handled carefully with gloves through the sack. After use the sack should be washed and disinfect.

This procedure will help reduce the amount of stress caused by manual restraint.

Chemical restraint

Whenever an animal is sedated for whatever reason, the opportunity should be taken to carry out a full veterinary examination, blood collection for genetic and/or physiological data, weight and other morphological measurements etc.

Careful monitoring of anaesthetic depth and vital signs are imperative during any anaesthesia attempt. Body temperature, respiratory rate and depth, heart rate and rhythm, and mucous membrane colour should be assessed frequently during each anaesthetic procedure.

European mink are have not been observed to be more sensitive than the European polecat (*Mustela putorius*) or American mink (*Mustela vison*) to overdoses of anaesthetic agent. Vomiting may occur during induction and initial apnea. Respiration of the animals may stop and artificial respiration may be needed to save the animal.

Injectable anesthetic agents recommended for used:

• Medetomidine - Ketamine

The most suitable products to use in captive as well as free ranging animals is the combination of medetomidine (Domtor*) and ketamine hydrochloride (Imalgène 1000*). Use of Atipamezol (Antisedans*) to reverse the effects of medetomidine is useful and any adverse reaction have not been noted at a dose rate of five times the initial dose of medetomidine.

The dosages can vary in relationship of the stress of the animal and body condition:

a) Free ranging animals. In these animals and when the level of stress of the animals is reduced to the minimum (no visual contact with persons, no photographing, no weighting, no weight, no noise around) the following dosage is enough to do a basic management of the animal: $100 \mu g/kg$ medetomidine + 5 mgr/kg ketamine.

For animals with unknown weight the weight 400 gr for females and 800 gr. For males have been used to decide over the doses in Spain.

b) Captive animals. By the better body condition, a high little more anaesthesia is required: 150 μ g/kg medetomidine + 7.5 mgr/kg ketamine (see also Fournier *et al.*, 2003).

• Xylazin - Ketamine

Combination of 10% Xylazin (Rompun*) and 5% Ketamine Hydrochloride (Ketalar, Ketaset, Ketaject, Vetalar*) (1:1). This combination has a wide safety margin. It has been used successfully used previously in at Tallinn Zoo. In practice a dose rate of 0.2-0.5 ml per animal (dependant on body size), by intramuscular injection is usually sufficient. It is better to administer small doses at first, with additional doses added until the desired response is attained. Relaxation is good, usually within 5-10 minutes (in some cases after one minute) and it lasts between 10 to 30 minutes.

The use of Ketamine Hydrochloride as a sole anaesthetic agent is NOT RECOMMENDED having been known to bring on seizures and abnormal breathing.

Isofluorane

Another method of anaesthesia induction is to use isoflurane delivered via an anaesthetic chamber. European mink have a large respiratory reserve and can hold their breath for a relatively long period of time. Induction time may be as long as 10 minutes using this method and rectal temperature usually decrease more than with injected anesthetic agents. It makes this protocol less recommended than the combination of metedomidine and ketamine.

Regardless of the method of induction, anaesthesia can be prolonged with isoflurane anaesthesia for longer procedures.

12. Necropsy protocol

A thorough necropsy should be performed on all minks that die as soon as possible after death. All participants of the breeding program are requested to send post-mortem reports and results to the species co-ordinator. Please use the standardized necropsy report found in Appendix 6, also available in the pathology module of MedARKS; unless a more detailed autopsy report is available. In addition, where possible, tissue collection for taxonomic, genetic and pathological research is also important.

A copy of the mink medical report, gross post-mortem report, pathology report, cultures, and any related laboratory tests, as well as the extra set of fixed tissues should be submitted to the species co-ordinator, or veterinary advisor, once all the information is complete in order to identify medical problems in the population.

Formalin fixed tissues

Two sets of tissues should be collected and placed in 10% buffered formalin. All tissues (except ovaries and testicles) may be placed together in a single container as long as the volume of formalin is at least 10 times the total volume of the tissues collected. Tissues should be no thicker than 0.5 cm.

Frozen tissues

Store 3-5 cm section of the most important organs (at least of Liver, Brain, kidney, lesions.....) in plastic bags at -20°C to -70°C

Bank: also in neonates, stillbirths, abortions.

Germoplasm Bank

The institutions have to be prepared to send ovaries and testicles after death of the animals to germoplasm bank to store the reproductive material for future uses if agreed with and requested by speices coordinator.

I. Literary sources used in preparation of the protocol

Behnke, H. 1982: Jagd und Fang des Raubwildes: Anleitung zur gerechten Bejagung. Hamburg,Berlin:Parey. 106 pp.

Carvalho, C.F., Howard J., Collins, L., Wemmer, C., Bush, M., Wildt, D., 1991: Captive breeding of Black-footed ferrets (*Mustela nigripes*) and comparative reproductive efficiency in 1-year old versus 2-year old animals. J. Zoo. Wildl. Med. 22: 96-106.

- Devison, D.1992: Metro Toronto Zoo Black-Footed Ferret Captive Propagation Protocol. Metro Toronto Zoo. 15 pp. Manuscript.
- Maran, T. 1994: Studbook for the European mink, Mustela lutreola Linnaeus 1761. Vol. 1. EMCC & Tallinn Zoo, 50 pp. Manuscript
- Ternovskij, D. 1977:Biology of mustelids(Mustelidae). Nauka. Publ. Novosibirsk. (In Russian)
- Vogt, P. 199? : Husbandry Guidelines for Lutra lutra (EEP). Manuscript

J. Appendices

1. Reproduction.³

Unlike the majority of mustelids, the European mink does not display delayed implantation. Mating takes place in late March, April and in early May, but according to the breeding results at Tallinn Zoo the peak of the heat falls in the second and third week of April (Figure 23). The gestation lasts 42 days (Figure 24) and parturition usually occurs at the beginning of June.

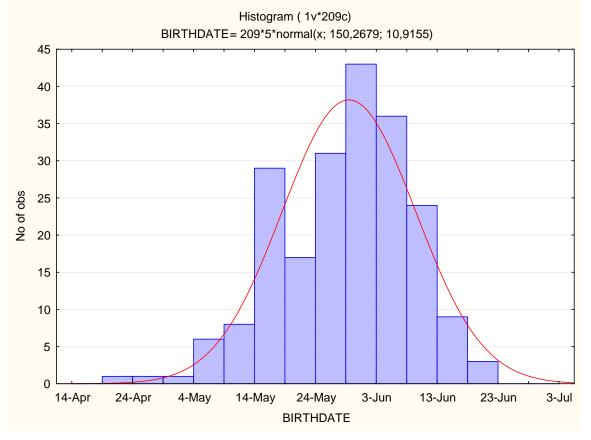


Figure 23. Birth dates of the European mink in the EEP population (Tiit Maran et al., unpublished data)

³ From: Maran, T. 1994: Studbook for the European mink, *Mustela*

lutreola Linnaeus 1761. Vol. 1. EMCC&Tallinn Zoo, Tallinn, 50 pp.

Appendix 1

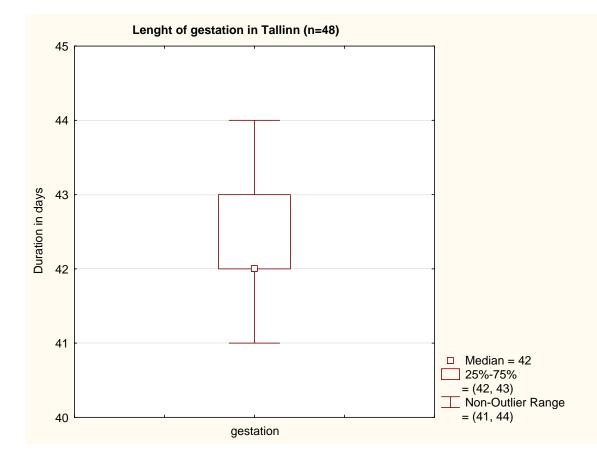


Figure 24. Gestation length in Tallinn Zoo (Tiit Maran et al, unpublished data)

The heat of the female is easily detectable by the size of vulva. The anoestrus vulva is minute, measuring 2 mm to 4 mm maximum, usually much smaller. When the female begins to come into heat, the vulva enlarges over a period of three to four weeks. Its maximum size may be 10 mm to 10 mm or even more. The colour of the vulva also changes slightly from pale white during the anoestrus to pro-oestrus period, to pink-reddish during oestrus. The size and colouration of vulva can be easily measured and checked through the handling cage.

Changes in the behaviour may also be a useful indicator of oestrus. The oestrus female becomes more active and less timid.

More detailed detection of heat can be achieved by histological investigation of vaginal smears. Samples can be obtained through vaginal flushing and collected in a small pippette while the animal is held in a handling cage. A small amount of sterile water is aspirated into the tip of the pipette. The tip of the pipette is then inserted approximately 10 mm into the vagina, the sterile water is injected and immediately aspirated back into the syringe. The sample is placed on a slide, and can be stained before examination under the microscope for cornified cells (Hamilton & Gould, 1940, Travis et.al., 1978, Doboszynska, 1976).

In case of early death of the litter, the potential polyestricity exhibited by European mink, may still provide the chance to provide further offspring. Six to nine days after the

death of the young it is possible for the female to come into oestrus a second time and for copulation to take place again (Moshonkin, 1977).

The reproductive physiology of the European mink is often confused with that of the American mink, *Mustela vison*. The latter displays delayed implantation and its gestation period may be up to 93 days (Ternovskij, 1977).

The size of the litter varies from 1 to 8. Figure 25. There is a slight difference in the mean litter size recorded at Tallinn Zoo, St.Peterburg Zoo, Euronerz and in Novosibirsk.

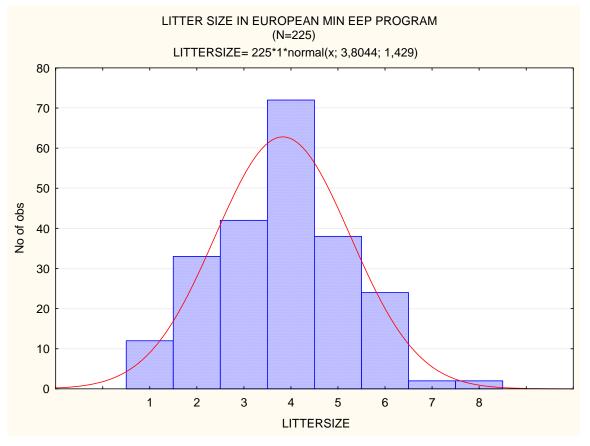


Figure 25: Littersize of the European mink in EEP Program (Tiit Maran et al., unpublished data)

Appendix 2

		FEMALE				MALE			
	n	mean	<u>±</u>		n	mean	<u>+</u>		
Weight of the animals ⁴	9	540.3	5.8	gr	17	814.6	30.4	gr	
Weight of liver	5	29.2	3.10	gr	8	39.9	1.03	gr	
Weight of heart	5	5.0	0.30	gr	8	6.0	0.21	gr	
Weight of lungs	5	7.5	1.10	gr	8	10.5	0.50	gr	
Weight of brain	5	7.9	0.40	gr	8	9.0	0.40	gr	
Weight of pancreas	5	2.6	0.60	gr	8	2.4	0.219	gr	
Weight of left kidney	5	2.5	0.10	gr	8	3.4	0.13	gr	

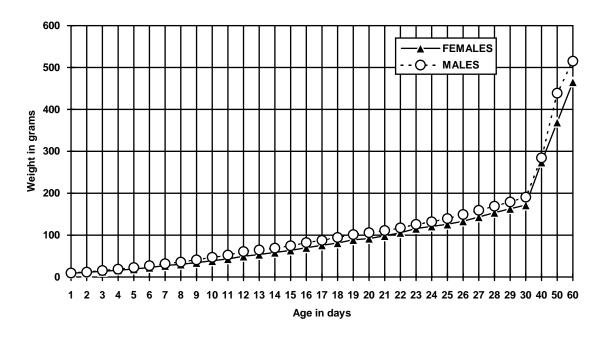
2. Morphological parameters(Ternovskij, 1977)

⁴ Weights from: Danilov, P.I. & Tumanov, I.L. 1976: The ecology of the European and American mink in the Northwestern U.S.S.R. -In: Ecology of birds and mammals in the Northwestern of U.S.S.R.. Akad. Nauk. Karelski Filial. Inst. Biol., Petrozavodsk, p. 118-143.

Appendix 3	
avalanment of the	

Age in days	Female			Male		
	n	mean	<u>±</u>	n	mean	±
1	8	8.8	0.24	8	10.0	0.22
2	8	11.3	0.27	8	11.9	0.55
3	8	13.6	0.43	7	15.7	0.31
4	8	16.1	0.51	7	18.6	0.37
5	8	19.2	0.91	7	22.7	0.41
6	8	22.7	1.00	7	27.2	0.39
7	8	27.4	1.14	7	31.5	0.52
8	8	30.4	1.47	7	35.7	0.53
9	8	34.2	1.17	7	41.2	0.52
10	8	38.9	1.60	7	46.6	0.80
11	8	43.3	1.85	7	52.2	1.12
12	8	49.5	2.30	7	60.6	2.01
13	8	53.9	2.21	7	64.8	1.71
14	8	58.7	2.27	7	69.2	1.71
15	8	63.6	2.55	7	74.9	2.00
16	8	70.0	2.50	7	81.8	2.39
17	8	75.7	2.62	7	87.6	2.63
18	8	81.3	2.85	7	94.4	2.98
19	8	89.0	2.86	7	101.6	3.36
20	8	91.8	3.60	7	106.2	3.57
21	8	98.5	4.30	7	111.1	3.88
22	8	105.6	5.15	7	117.0	4.20
23	8	115.8	4.27	7	126.1	4.70
24	8	120.7	4.43	7	131.4	4.43
25	8	125.8	4.71	7	139.6	5.80
26	8	133.2	5.86	7	149.2	7.00
27	8	143.3	6.15	7	159.2	7,96
28	8	153.5	6.47	7	169.2	8.94
29	8	162.9	6.38	7	179.1	9.97
30	8	171.7	6.69	7	190.6	10.53
40	8	274.0	8.10	7	284.4	13.42
50	8	369.5	21.06	7	438.3	21.04
60	7	465.1	14.24	6	515.3	45.15

3. Change in weight during the post-embryonic development of the young(Ternovskij, 1977):



DEVELOPMENT OF YOUNG EUROPEAN MINKS (by Ternovsky, 1977)

4. Development of fur coat.

- **3-7 days.** The new-born young have no proper fur and are covered with a fine natal down. The colour of the dorsal side of body is dark violet; and the ventral side varies from pinkish-violet to greyish violet. The markings around the mouth is barely noticeable. The growth of the mane appears on the neck which reaches a length of 5mm; on the back the fur is 3mm; and on the stomach and chest 2 mm.
- **10-16 days** General body coloration is similar to the previous development period but with the white area around the mouth becoming more distinguishable.
- **19-27 days.** A more uniform body colouration is developing with the dorsal side of the body a dark grey or dark violet and the ventral side approaches a dark violet.
- **29-40 days** Mane disappears. The juvenile fur darkens towards the pelage colouration of an adult mink. The appearance is similar to the young of the European polecat (*Mustela putorius*) until the appearance of the guard hair, after which the kits resemble the young of the American mink.

The eyes begin to open at 30-36 days of age. At 35-40 days the young are visually capable of following moving objects. However, the reaction to moving objects diminishes at 53-54 days of age.

45-80 days The fur of young resembles more and more the appearance of adult animal.

At 90 days old the juveniles are indistinguishable from adults.

Appendix 5 [For Helena to decide over this Appendix, perhaps to take out or to substitute with something else – eg. Feeding ration for Pont de Suert ?!]

5. Feeding Rations of the Black-Footed Ferret.

Diet of the BBF at CRC of the National Zoological Park (Carvalho et. al. 1991)			
~ · · · · ·	gr		
Commercial mink chow	1,275	62.3%	
Ground rabbit meat and bones	675	32.9%	
Blood meal	50	2.4%	
Bioliver	50	2.4%	
Total	2,050	100.0%	
Per one animal in a day Each adult ferret received 60 -90 g every day	68.33		
Diet at Laramie for <i>Mustela</i> eversmanni (Kwiatkowski 1989 in			
lit):	4 ==		
Liver	17%		
Fish	20%		
Bovid stomach	10%		
Chicken	25%		
Commercial mink growth ration	25%		
Black-Footed Ferret diet at Laramie (Kwiatkowski 1989 in lit):			
5 days a week			
Commercial mink ration	60%		
Grounded prairie dogs	40%		
+ liver(dry 6%)			
2 days a week hamsters or small rodents			
Black-footed Ferret diet at Toronto Zoo (Devison,1992)			
Mink chow	60%		
Ground rabbit with blood-meal, bio-	40%		
liver and vitamin E			
Ad. females lose 15% of their January weight prior breeding			

The weight should be maintained or slightly increased(2-3%) February - March daily ration 50gr/animal

Ad. males should lose 10% of their January weights prior to breeding. Daily ration in February, March 62gr/animal

Juv. males & females should loose 10% of their February 15th weight prior to March 15th.

Obligatory to add to the ration (Kwiatkowski 1989 in lit):

Vitamin E 100 IU/kg of ration Zinc 50mg/kg of ration Liver 20% (=6% of bioliver) Weights of BFF and the (Walker ,1991) **European mink** female male **Black-Footed Ferret** 809 1021 European mink 440 54% 739 72%

Appendix 6

6. European mink (Mustela lutreola) post mortem report

Studbook No:	IS	IS No:	Local ID:				
CaseNo:	Zoo:		Age:	Weight:			
Date of Birth	_//	Date of Arr	ival	_//			
Date of Death	_//	Date of PM	Report _	//			
CarcassConditio Fresh / Refrigerate		Decomposed /	Other				
Physical Condition Normal / Fat* /		Other					
Fat*: grade on	kidneys:-	1) 2) 3) 4)	some fat at	pletely covered with fat kidney showing poles or no fat			
Comment if obes	e:						

Gross Post Mortem:

skin /appendages	digestive	Urinary
Sensory	liver	Endocrine
Muscular	respiratory	Reproductive
Skeletal	cardio-vascular	Nervous
Adipose	lympho-ret	

Please write:

A - if abnormal; B - if normal; NE - if not examined

Gross Post Mortem Description:

Parasitology:

Arthropods:	Y/N	Protozoa:	Y/N	Helminths:	Y/N
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Results and comments:

Microbiology:

Bacteria:	Y/N	Fungi:	Y/N	Virus: Y/N	Other: Y/N
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Results and Comments (please give bacteria code A,B,C etc and indicate organ of origin):

Antibiogram

Antibiogram	Bacteria Code				
	Α	В	С	D	Ε
Chloramphenicol					
Kanamycin					
Streptomycin					
Sulphonamide					
Oxytetracyclin					
Trimetoprim + S					
Gentamycin					
Ampicillin					
Neomycin					
Colistine					
Spectinomycin					
Flumequine					
Others(specify):					

Code: + = sensitive; $\pm =$ some inhibition; - = resistant

Haematology:

Blood Smear: Y/N EDTA: Y/N

Heparin: Y/N Marrow: Y/N

Results and Comments:

oesophagus	kidney	lung
Stomach	adrenal	skin
Intestines	cerebrum	eye
Pancreas	cerebellum	lymph nodes
Liver	spinal cord	Others(specify):-
Thymus	pituitary	
Thyroid	heart	
parathyroid	muscles	
Spleen	urinary/bladder	
testes/ovaries	aorta	

Histology: please indicate which were studied

Results and comments:

Probable Cause of Death:

Final Conclusion about Cause of Death:

Veterinarian Signature NB PLEASE ATTACH FULL CLINICAL HISTORY IN ADDITION TO THE PM REPORT)

7. Tissue collection for pathological research

In addition to specimens submitted for diagnostic pathology, the following tissues should be preserved in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Sections should be no thicker than 1cm. All lesions should also be included. Tissues should be accurately labelled and stored at the collection of origin.

During post mortem examination much taxonomic information can be lost by careless technique. In order to avoid such problems, please make sure all skin incisions are as straight and neat as possible. Do not remove any more skin than is required for diagnostic purposes. Ensure that no skin is attached to the sets or skeletal muscle if samples of these tissues are removed. If it is necessary to remove the brain for examination, please make a straight saggital skin incision from the crown down the nape of the neck, allowing the skin to be peeled neatly away from the cranium.

Tissue	Area		Taken	
Adrenal	Entire gland with transverse cut	YES	NO	
Brain	Sliced longitudinally along midline	YES	NO	
Heart	Longitudinal section of atrium, venticle and valves from each side	YES	NO	
Intestines	Duodenum, jejenum, ileum, ceacum, colon; open along long axis	YES	NO	
Kidney	Section of cortex, medulla and pelvis from each kidney	YES	NO	
Liver	2 sections from two lobes with capsule and gall bladder	YES	NO	
Lung	Sections from several lobes including bronchus	YES	NO	
Lymph nodes	Cervical, anterior medisatinal, bronchial, mesentric and lumbar with a tansverse cut	YES	NO	
Pancreas	Samples from two areas	YES	NO	
Peripheral nerve	3cm section of sciatic nerve	YES	NO	
Skeletal muscle	Cross section of thigh muscle	YES	NO	
Skin	3cm length of full thickness of abdominal skin		NO	
Spleen	Cross section including capsule	YES	NO	
Spinal cord	Sections from cervical, thoracic and lumbar cord		NO	
Stomach	Cardia, antrum and pylorus	YES	NO	

Appendix 8

8. Reproductive parameters

Reproductive pattern	oductive pattern Male		Female		
	Mean	range	mean	range	
Age at first copulation					
Age at first conception					
Oestrus			4.5days	3-6days	
Gestation period			42days	40-43days	
Mating period			April	March	
				April	
				May	
Birth period			June		
Litter size			4	1-8	
Birth interval					
Reproductive life-span					
Life-time production of kits					

Appendix 9

9. Environmental enrichment for the European mink

European mink is a solitary animal. Therefore the captive animals have to be kept separately, except during breeding season. Such a life style combined with poor environmental diversity of enclosure is likely to results in behavioural and physical abnormalities. Too small size of enclosures is likely to strengthen the overall negative impact of captive conditions to the animal. These abnormalities include stereotyped behavioural moving patterns, but probably also the passiveness, boldness and increased aggressiveness of the individuals. The animals in poor environment tend to gain overweight and increased fat deposits, which will decrease the reproductive efficiency of the animals.

Decreased exploratory behaviour and passiveness of the animals will also reduce their value as exposition animals in zoo displays and consequently they can not be used as good bearers of conservation message for zoo public. Further, low level of activity as well as reduced behavioural repertoire will also reduce the chances for animals to survive after reintroduction.

In 2005 – 2006, various ways to enrich the environment for the European mink were tested in breeding facility at the Tallinn Zoo. Some of the aspects of the enrichment have been tried to implement in Tallinn Zoo also earlier. In the following the experience gained in Tallinn Zoo about the ways to enrich the captive environment has been summarized. The results should not be regarded as conclusions from scientifically sound experiments, but rather as the experience gained during the captive maintenance of the animals and also from little experimenting with different methods. Therefore, the topic of environmental enrichment needs further elaboration and the members of the European mink EEP are encouraged to experiment with this, though to extent which will not endanger the conservation breeding program and the animals.

Stereotyped movements.

Stereotyped behaviour easily appears in captive European mink. The stimuli triggering such a behaviour seems to be, at least in Tallinn breeding facility, the keeper's movements in the facility or the sounds of chicken (prey), both connected with the provision of food. The situation where the signal about the availability of food is in place, but the animal cannot reach it, is stressful for the animal and seems to be a factor causing stereotyped movements of the animal. Sensing the keeper in the neighbouring enclosures seems to initiate the stereotyped movements, but also having a visual contact with the other mink in neighbouring enclosures tends sometimes to initiate such behaviour.

Usually these movements cease once the animal has received the food, but these may result also in more continuous stereotyped movements without any obvious stimuli, as it has been observed in Tallinn.

In Tallinn Zoo, the possible impact of increasing and/or altering the diversity of captive environment to the frequency of the stereotyped behaviour was tried to evaluate in 2005 –

2006. According to our experience the diversity of the interior and the objects in enclosure do not reduce remarkably stereotyped behaviour. The only way to reduce the frequency of the movements is to limit visual contacts to the keepers in the facility and also to other mink as much as possible. This is an important aspect to be kept in mind while planning a new facility. In old facilities, the covers can be used between the enclosure and service corridors.

In deciding over what kind of enrichment to apply the following considerations were kept in mind:

- 1. Enrichment method should stem from species' natural behavioural repertoire.
- 2. The most important behavioural stimuli for mink are related to prey, conspecifics and environment/shelter. Therefore the object or procedures related to these are most likely to be effective.
- 3. The main stimuli have to be related mostly to olfactory senses as these are one of the most important channels of information for the species.

The following enrichment methods have been applied in Tallinn:

1. <u>Provision of live prey (partridge, chicken, rodents, fish, amphibians, snails and insects etc).</u> Provision of live prey is prohibited in number of European countries. Despite of this it is important element in the environmental enrichment for the European mink and has to be used as much as possible. Mink are the top predators and killing of the prey forms an important part in their behavioural repertoire. Inhibiting this behaviour by not making the live prey available is believed to have negative impact even to the health of the animals.

Live fish in the pool, make animals more active in the water and increases their movement in the water even in times when the fish is not in the pool. Also provision of any terrestrial live prey (like rodents, chicken, snails, insects) will enhance behaviour and activity pattern of the animals. It is recommended, whenever it is eligible and appropriate, to introduce the live prey into the enclosure with the European mink closed to the nest box. Time should be given to the prey to hide in the enclosures and then the animal has to be freed from the nest box. This is believed to trigger a seeking behaviour of the animal. Animals usually find the prey quite quickly, but despite of this the action of looking after the prey is believed to be important for the well-being of the animal.

Grayfish, snails and insects (crickets) have been given to mink in Tallinn. Most of the animals easily hunt and kill the grayfish, but not all the animals are interested in crickets and snails. Some of the animals will learn to hunt and eat the crickets and snails with time.

2. <u>Hiding of food items in enclosure.</u> The food is recommended to provide with animals closed into nest box. The food items has to be hided into various places in the enclosure, e.g. under stumps/branches, inside tubes. This forces the animal to look around in enclosure in looking for food. Animals usually discover the food quite quickly but despite of this it is an important was to diversify the animal's actions in enclosure. _

3. <u>Proving food inside of pet ball with hole (dead rat and mice)</u>. Every now and then the dead mice or rat is recommended to offer inside of the pet ball (look figures XX).



Animal will spend lots of time actively to get the rat/mouse from the ball but the animal also attempts to bring a ball into the nest box. This will increase the overall activity of the animal. The main argument against this method could be that it is not very "natural" way for mink to look for the prey in plastic balls. However, it seems likely that the wild animals have to try to tore out the prey from cavities in the wild (like grayfish for instance). Considering the amount of time the animals actively spend with this ball, it still seems important to use such enrichment methodology.

- 4. <u>Putting a bedding material moistured with urine and faeces (as smell sign) of rats and</u> <u>mice into the enclosure of mink.</u> This was usually combined with providing respective live food. The smell signs are regarded to be one of the most important sources of information for mink about surroundings, included also about the prey. However, providing smell signs (urine and faeces) of prey species (rats and mice) should always be combined with providing the same type of prey for food (for instance dead rat and mice), otherwise the smell signs are likely to loose their meaning for the animal.
- 5. <u>Diversifying the interior of the enclosure (pool, stumps, branches, tubes etc.)</u>. As the mink in natural environment use shelters and tend to avoid open areas, it is important to imitate

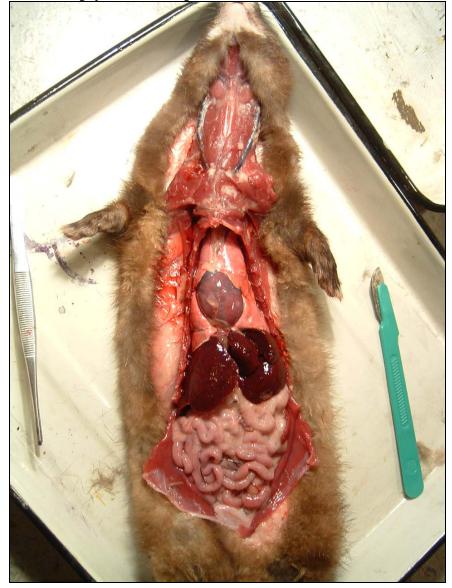
in enclosure the very same environment. Stumps, branches of trees, various tubes (like the tubes used for canalisation) – everything what can be used for hiding – will improve the quality of the enclosure. Usually open areas are avoided by the animal.

Shallow-water pools and shallow mud/dirt pools are very useful for keeping the animal busy. Especially shallow mud or dirt pools are attractive playground for mink and are used very often. The running water, like rapids or small falls, are additional attraction to the mink and are recommended to use as much as possible.

- 6. Moving the items (tubes, stumps etc as smell signs) from one mink enclosure to another. As noted previously, the olfactory signals are one of the most important sources of information for mink about the surrounding, including the conspecifics. In captivity, mink are kept solitary for most of the time (except only the breeding season and nursing of young). Therefore, the opportunities for recognize the olfactory signals of the conspecifics are very limited. For this reason it is recommended to move the items kept in the enclosure of one animal to the enclosure of another. The stumps, tubes used by one animal will contain smell signs for the other animal. The tests in Tallinn have shown that such items have been investigated by animal very thoroughly. The main argument against this method is that it could potentially form a vector for diseases to spread from one enclosure to another. First of all it is anticipated that sufficient precautionary measures in facility are in place any way. Secondly, considering the vicinity of enclosures in facility it is obvious that if such an unfortunate disaster happens that a contagious disease strikes the facility the likelihood of spread inside of facility is so high that the increase of danger caused by previous moving of items from enclosure to another is insignificant.
- 7. Providing surplus bedding material. The availability of surplus bedding material (hay, straw, moss, twigs of trees etc anything large and suitable for animal to bring into nest as bedding in the enclosure provides for the animal an opportunity take to care about the insulation and bedding of the nest-box itself. It does not mean that the cleaning of the nest-box is not needed, but only that the animal can "correct" the way of bedding material in enclosure may also cause problems for keepers, as the animals may start to distribute the bedding material all around the enclosure. Also, it has to be observed that the surplus bedding material is kept dry.
- 8. <u>Adding new objects into enclosure</u>. Addition of new objects into the enclosure will raise the curiosity of animal and motivates it to move around and investigate the new object: new stumps, branches, tubes, heap of leaves etc. However, usually the effect of new object is relatively short and the animal looses interest in it unless the object does have additional features which lock the interest of the animal to this item for a longer time.
- 9. <u>Putting floating objects (pieces of log, empty plastic bottles of beverages etc) into pool.</u> Pool and the floating objects are in a special position in the attempts to enrich the behaviour of the animals and its captive environment. The water in the pool should be relatively shallow with lots of easy entries into water. The animals like to spend time in

rolling in the muddy and wet areas close to water. Any floating objects will increase the activity of the animal in water quite remarkably. Our tests have revealed that 1 - 2 litre plastic beverage bottles are chased and played with in water for long periods.

Appendix 10



10. Autopsy of the European mink