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Lynx Twins with Linking Lynx: raising in captivity, capture, transport and introduction to the wild of the two Carpathian lynx (Lynx lynx carpathicus) born in the Domaine des Grottes de Han Animal Park as part of a European conservation programme

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Summary:

The following paper describes the conditions - observed by me or of which I was informed of - and protocols relating to the two young lynx, Graveli and Grandera, that were born in Grottes de Han wildlife park, Belgium, in mid-June 2023 and were transferred to Germany for reintroduction purposes in April 2024. The Grottes de Han park was, after a careful selection process, chosen to participate in a European lynx conservation program under the Linking Lynx Sourcing working group as one of the ex-situ breeding sites, which are managed by the European Association of Zoos and Aquaria (EAZA). I was taking part in the park's activities as an animal keeper intern for the duration of two months (late March to late May 2024) as a part of the Bachelor of Animal Care course in the University of Padova. During the two months I witnessed regularly the implementation of protocols for raising lynx for reintroduction, witnessed their capture, medical interventions, quarantine isolation and transportation to a coordination enclosure in Germany, after which I was only informed about their progress indirectly. This paper is a critical evaluation of those conditions and events. It can also be used to set expectations for nuances and achievable results in raising lynx as an ex-situ site for the Linking Lynx working group.

Introduction:

Eurasian Lynx (Lynx lynx) is the third biggest carnivore and the largest feline to populate Europe (Encyclopaedia Britannica, 2024). It is a solitary animal with huge home ranges (at least a hundred square kilometres on average) and is recognised by the round head with tufted ears and flared facial hair, long legs with large feet, short black tail (Hočevar et al. 2021). Among its representatives is the carpathian lynx (Lynx lynx carpathicus) - one of the three subspecies of

the Eurasian lynx commonly recognised in Europe (Kitchener et al. 2017). It is the lynx with thick soft fur which protects it from harsh winters and has a most densely spotted hide among all other lynx (Dudley Zoo, 2024). Linxes exhibit sexual dimorphism with males being on average larger than females (Gomerčić et al, 2010). They are a nocturnal ambush predator, talented at climbing, stalking and hiding but not at long distance pursuing (Krofel et al. 2013). They usually kill their prey by pouncing at them after a period of stalking and performing one precise bite to the throat (Černe et al. 2019). Roe deer is the preferred prey (Herfindal et al. 2005).

The Eurasian lynx, while categorised as Least Concern by the IUCN Red List, does not have equally stable populations across its range (Chapron et al. 2014). It has been driven to extinction in many parts of Europe by the end of the 19th century, with even the 'stronghold' population of the lynx in the Carpathian Mountains now presenting more of a 'metapopulation' of isolated animal groups (Bonn Lynx Expert Group, 2021). The 20th century sporadic reintroduction efforts have led to the establishment of new isolated populations that are currently in a dire need of reinforcement if they are ever to become viable (Chapron et al. 2014). The issue of isolation is further complicated by the fact that females exhibit philopatry - the practice of staying not far from their place of birth (Krojerová-Prokešová et al, 2019). Sometimes the daughters' home ranges would overlap with their mother's home range, and male lynxes although settling further - are trying to have an overlap with female ranges, so the lynx population expansion happens mostly along the edges of the resident populations, preventing rapid colonisation of new lands (Zimmermann et al. 2005; Janečka et al. 2006). This serves as one of the reasons due to which the

reintroduced populations in Europe remain isolated (Krojerová-Prokešová et al, 2019).

In 2019, the Bonn lynx conservation meeting between the lynx experts took place in Germany establishing several recommendations on the Eurasian lynx conservation topic that were later adopted by the Standing Committee of the Bern Convention as recommendation No. 204 (Council of Europe 2019). Part of these recommendations was to create working groups, each tasked with a different aspect of lynx conservation in Europe. The proposed working groups were:

- 1) The Sourcing Working Group, organising the lynx places of origin and coordinating them for animal translocation;
- 2) The Genetics Working Group, genetically monitoring and assessing lynx from all sources;
- The Health Working Group, taking care of the veterinary aspects of lynx projects;
- 4) The Monitoring Working Group, studying and evaluating the actual distribution of lynx;
- 5) The Policy Working Group, linking the experts and the policy-makers;
- 6) The Engaging with People Working Group, finding ways to improve connection with the public.

The first ones to be born during the year 2021 were the Genetics group named the CElynx consortium and the Sourcing working group bound by the Memorandum of Understanding.

While the works of the Sourcing group would be touched upon in more detail further in the text, I would like to briefly highlight the importance of the CElynx consortium creation. A paragraph from the report on the Bonn meeting caught

my eye describing all populations of animals that have historically experienced bottlenecks, are small, located in isolated groups or have been reintroduced, as needing genetic monitoring (Bonn Lynx Expert Group, 2021). In other words, the report continues, all European lynx need genetic monitoring. That is especially true considering early reintroduction efforts mostly did not concern themselves with places of origin of the lynx and so have created populations of extremely mixed lineages and for further conservation efforts it is important not only to know the origin of a certain animal, but also its genetic heritage.

The other groups would not commence until finally in the Harz Mountains, in the year 2023, they too were forged. The one group to rule them all was created there and then too and proclaimed to be the "Linking Lynx - Carpathian Lynx Working Group". Linking Lynx was meant to be the permanent working group to supervise and coordinate the others in the realm of the Carpathian lynx, as was foreseen by the Recommendation No. 204 (Lanz and Breitenmoser-Würsten, 2024). It is a network of professionals and a platform for coordination of the Carpathian lynx conservation effort. Their dedicated website is https://www.linking-lynx.org/en (available in English and German) and it contains publicly available protocols, which are referenced in this text extensively, as well as other publications concerning their program.

Opened on 13th of July 1970, the Wildlife Reserve of the Domaine des Grottes de Han was the extension of the Caves of Han tourist attraction. The park was first proposed by Jean Herman and inaugurated by Jules de Montpellier, managing director of the Société des Grottes (Grottes de Han, 2024). The park is located in South-Eastern Belgium, not far from the borders with France and Luxembourg, in the French-speaking Walloon Region, Province of Namur, Rochefort Municipality. The closest settlement is the village of Han-sur-Lesse,

where all the park's administrative buildings, intern residence and the depot are located. The park has a large 250 hectares territory, not visibly demarcated from the surrounding natural reserves (as it is located within the vast forests of Ardennes) which allows for its remarkable biodiversity of flora and fauna. Separately from the wildlife, the park houses around 650 animals of 36 different, almost all natively European, species in its enclosures. It is a full member of the European Association of Zoos and Aguaria (EAZA). EAZA is also the organisation in charge of the ex-situ breeding programme for the Carpathian Lynx, i.e. it supervises all the facilities that are a source of lynx for the Linking Lynx programme that are outside of the lynx natural habitat, for example places such as zoos and wildlife parks. To the best of my knowledge it is through EAZA that Grottes de Han Wildlife Park has joined the Linking Lynx conservation effort (personal communication from the animal keeper in charge of administration at the park) and was given a new female named Bogusia (born on 28/05/2021 in the Torun Zoo, Poland) to join the park's male Wald (born on 20/05/2016 in the wildlife park of the German National Park of Bayerischerwald) in hopes that they would form a breeding couple. In terms of conservation coordination the order of charge would therefore be the following: Linking Lynx -> Sourcing Working Group -> EAZA managing the ex-situ breeding programme (EEP) (as they had already been doing that since 2017 by the time Linking Lynx was created) -> Grottes de Han Wildlife Park.

In July 2024 the introduction of the female had come to fruition when it was discovered that she had given birth to two cubs: Gralevi and Grandera (the park tries to name animals born there beginning with "Gr").

I arrived at the park on the 25th of March 2024, as an animal care intern for a period of two months. The park participates in numerous conservation projects

but during my stay major development occurred only in two of them: arrival of the tree frog eggs for a Belgian tree frog repopulation project (10/04/2024) and the departure of the young lynx twins from the park into a coordination enclosure in Germany as part of Linking Lynx working group (24/04/2024).

The activities and conditions associated with raising those cubs in the Grottes de Han Wildlife Park for them to be suitable for the reintroduction into the wild and reasoning behind those activities and conditions is the subject of the main part of this text.

The carpathian lynx present at the park weren't the only lynx there, as not far from their enclosure was the enclosure of the two Northern lynx (Lynx lynx lynx) - a mother and a son - that would not be suitable for introduction to the wild even if needed be, due to their high response rate to and interest in humans and reliance on farm-produced meat, which would hinder their survival chances in the wild. The comparison between the animal care provided for the two different types of lynx I think would be a nice occasional supplement to the critical revision of the implemented protocols for the carpathian lynx. I will also expand on the reasoning behind these protocols using a research performed on the cognate species of Canada Lynx.

Critical Assessment of the Activities:

By the time I arrived at the park on the 25th of March 2024 the carpathian lynx twins were already 9 months old, still in their parents' enclosure but were soon to be transferred to the coordination facility in Germany.

The Enclosure

According to the Linking Lynx protocol, an enclosure for a breeding carpathian lynx pair should be at least a 1000 square metres in area (Lengger, 2024). The minimal spatial density indicated in the EAZA guidelines for lynx husbandry is 20 square metres per animal, increased by 50% for each additional animal (Krelekamp, 2004). Both of those minimal standards are met and greatly exceeded in the park's enclosure which stands at 1845 square metres. However, both the Linking Lynx protocol and the EAZA guidelines say that in a perfect situation the enclosure should be designed with separate parts (2 parts at least 600 square metres each according to Linking Lynx) providing a possibility to isolate animals from each other (Mellen and Wildt, 2003), which is not the case for the carpathian lynx at Grottes de Han Wildlife park, which are housed in a single space enclosure. This is, however, the case for the Northern Lynx enclosure of the park for a mother and her son, with two interconnected parts of equal area that can be closed off from each other. In this aspect the northern lynx enclosure follows more closely to the standards recommended for raising lynx in captivity than the carpathian lynx enclosure, which in most other aspects follows those standards more thoroughly and provides better conditions for the animals inside. The guidelines emphasise that space provided for the lynx is less important than the complexity of the enclosure itself (Mellen et al. 1998) complexity defined as a number of visual barriers present in the enclosure - and the ability to use vertical space. The carpathian lynx enclosure is set on a side of densely forested hill with plenty of vegetation providing an undisturbed location with numerous hiding spots and a visual barrier from the rest of the park. A rocky outcrop in the middle of the enclosure provides an ideal perching place for the lynx who prefer to survey their territory while remaining unseen. Mellen,

2003, specifically says that an elevated platform in the lynx enclosure should provide a good view of the horizon, which is provided for perfectly by the natural rocky outcrop. The outcrop can be seen on Figure 1 Left and Centre.



Figure 1. Photos of the enclosure highlighting its complexity and abundance of hiding sites

The lynx could most often be located in the area of the outcrop, surveying as they are being surveyed themselves, serving to advance the theory that provision of a platform with a good view increases their comfort.

The northern lynx enclosure however is almost completely flat, with not much hiding spots or perching places provided outside of the 2 man-made stilt wooden houses, atop or inside of which the lynx could usually be located. Again, Mellen's research, 2003, has shown that lynx should have access to at least 75% of the enclosure's vertical space - the greater height of the enclosure is associated with lower levels of faecal corticoids (hormones associated with stress). The northern lynx usable enclosure height is improved a bit by the presence of tall trees in the enclosure, which can be climbed by the lynx but was not observed to be so outside one occasion during the time of great stress for the lynx when the grass was cut in their enclosure. The carpathian lynx used the natural inclination of their enclosure plentifully however were not observed tree climbing by me. They may have done it when not observed. The tree vegetation in the enclosure is guite dense and not all trees are farther than the 3 recommended by the guidelines metres away from the fence, however they do not provide thick climbable side-branches that could be used to leave the enclosure. I must add here that in general, it was often that the carpathian lynx were not seen throughout the day. This, other than by the abundance of hiding spots in the enclosure, could also be explained by the fact that normally lynx lead a nocturnal lifestyle (Hočevar et al, 2021). I estimate that about a quarter to a fifth of the enclosure closer to the top of the hill would not be observable from the pedestrian path at the bottom of the enclosure. This is in addition to the tall grass, shrubbery, logs, bushes, trees and rocks that filled the enclosure throughout so the lynx could move around almost freely without being observed. Therefore the complexity of their enclosure could by all means be described as superb. Mellen (2003) has shown that in enclosures with 7 or more visual barriers pacing was reduced or non-existent. Morgan and Tromborg (2007) have shown that the ability to hide from both humans and conspecifics is important for the well-being and the ability to cope with stressors in many species inclusing lynx. Sunde et al. (1998) have shown that in nature lynx prefer terrain with an abundance of day-time hiding places. The adult lynx were also provided with a nesting box (see Figure 3, top) in the unobservable part of the enclosure to facilitate creating a den in it, as per EAZA regulations. The lynxes have ignored the box entirely (personal communication from the keeper). The 162 metre long and ~4 metre high wire mesh fence on the perimeter of the

enclosure is supplemented by electric wires kept at above 5 kV and an

inclination towards the inside of the enclosure on the top to discourage lynxes climbing out (see Figure 2 for more details). The electric fence was powered by a battery located in the holding pen. It rarely needed changing (as it was also kept passively charging by a solar panel on the roof of the building) and whenever the voltage dropped suddenly, it was usually because an unfortunate slug shortcutted the electric wires to the metal pole with its body.

The enclosure is accessible by a double door system with an antechamber (Figure 3, right). The keepers would enter the antechamber, close the door to it securely, and only then open the door to the enclosure, preventing the lynx from suddenly escaping. Access to the lynx enclosure is only allowed when two or more keepers are present.

EAZA guidelines cite a personal communication recommending a 1.5 metre distance separating the public from the fence as well which is not quite the case for the foot-path going past the carpathian lynx enclosure at the Grottes de Han park. However, for most of its stretch the foot-path is separated from the wire mesh by a waist-high wooden fence and some bushes providing about a metre distance, discouraging all but the most determined of visitors from touching the fence.



Figure 2: Lynx enclosure barrier. A photo (right) and the diagram indicating metal poles, wire mesh, attachment of electric wire and the concrete base (left)

On the animal side of the fence the electric wires prevent any possible attempt by the lynxes to reach out and attack keepers or visitors through it. No such attempt however was ever recorded and the lynxes never approached human beings (as a matter of fact, every time the keepers entered the enclosure the lynx disappeared from the view completely). This is an important pre-release condition for a lynx in the Linking Lynx working group as the lynxes in the wild should neither be too habituated to humans, nor be aggressive to them.



Figure 3: Top - the unused nesting box Right - enclosure antechamber and the camouflage net



Part of the wire mesh fence along the footpath is replaced by a wooden shed (~3 metres long) with a glass panel, providing the visitors a less obstructed view of the enclosure. While this doesn't strictly follow the EAZA recommendation for optimal fencing as being almost completely covered, leaving only peep-holes to look at the enclosure, this is negated by the amount of hiding space provided to the lynx. I must add however that I found it non-ideal that the lynx could openly see humans going past the glass. Here, however, the beauty of the park's trail design really made up for it. The park has two options provided for a visit: by foot and by a safari truck. The safari-truck road is the closest any vehicles ever come to the lynx enclosure as, once again, the park is located at the end of a single village road, surrounded by nature reserves of the Ardennes forests. And even the safari-truck road is located about 20 metres downhill from the enclosure. The pedestrian foot-path however, passes right by the enclosure, yet the foot traffic by it is mostly limited by a simple trick: before reaching the enclosure the path splits into two - one path continuing past the northern lynx on the same elevation as before, but the other path going upwards to the

carpathian lynx, whose enclosure is located closer to the top of the hill. Therefore, the natural traffic splits into two, with a preference for taking the lower path and reducing the disturbance to the carpathian lynx. The Linking Lynx guidelines specify that visitors should have access to no more than two sides of the enclosure (for enclosures under 2000 square metres), and this is fulfilled beautifully in the park design. On other sides the enclosure is surrounded by the forested part of the park with no regular proximity to other animals. The closest animal enclosure to the carpathian lynx is that of the northern lynx, which are located about 10 metres downhill. In a personal communication from a park keeper, I found out that the male northern lynx during the mating season tried to call out to the female he felt in the other enclosure, yet I am unaware of any other indirect influence their proximity might have on one another.

One of the corners of the enclosure had a two-storey viewing tower for the visitors, providing a covert viewing place and lynx informational signs on the first floor and an open observation platform on the second. The tower did not exceed the natural height of the hill, and part of the enclosure was still not visible from it, yet it could potentially provide a distraction for the lynx. However, this distraction was once again minimised by the flow of the people: while people could walk past the carpathian lynx enclosure and continue on their way through the park, the tower was located on a dead-end appendage of the path, once again at a slight elevation from the main path, so a visitor, after walking for a few kilometres through the hills at that point should not only take a conscious decision to walk up to the carpathian lynx but then take another decision to walk an extra distance up to the tower and then come back, which passively discouraged people from climbing it. According to my personal

observations, about two thirds of visitors would not go up to the lynx enclosure and even fewer of them would climb up the tower, which did actually give a fantastic view of the beautiful lynx enclosure, without necessarily exposing the lynx themselves. In comparison the northern lynx enclosure could be observed from all sides and was surrounded by both the main pedestrian path and the safari-truck road, which I believe exposed them to a higher stress level than the carpathian lynx and certainly provided a much higher exposure to humans. It is also worth mentioning that both the mother northern lynx and her son approached the keepers when they were close to the fence, the mother was expecting food and on one remarkable occasion I heard her purr when I walked right towards her. On a different occasion the son also tried to attack me. On both occasions we were on the different sides of the fence as the trainees were not allowed inside big carnivore enclosures (lynx, wolves, and no one was allowed to go into the enclosure with the bears inside). Therefore, both animals were completely unsuitable for reintroduction to the wild due to their relation to humans, partly stemming from the location and properties of their enclosure. The other factor was played by the feeding procedures. Discussed in the next subchapter.

The aforementioned lack of proper separable living zones with equal quality of life conditions for each individual lynx, does not mean that there wasn't a safe way to isolate animals from each other, as there was a separate holding pen attached to one side of the enclosure, with an automatic guillotine type door separating it from the enclosure - the preferred type of shift doors for such purpose (Mellen & Wildt 2003). The holding pen had two separate cages with individual access to the enclosure and a separate keeper area. It will be described in more detail in the subchapter on capturing the young lynx.

The combination of all the factors discussed in this subchapter on the lynx's enclosure design helped to reduce the disturbance for the newborn lynx - preparing them for the subsequent rewilding - as much as reasonably achievable within the limits of Grottes de Han Wildlife Park, which is still primarily a commercial company which needs to exhibit animals.

The Feeding

The Linking Lynx protocols do not indicate specific food or the amount of it that should be fed to the animals that are participating in the program. The Linking Lynx representative told the park that they guessed it would depend on the individual needs of an animal (personal communication). The park's keepers kindly provided me with the ration records for before and after the birth of the lynx twins and I provide the summary of those details here:

- Diet: carnivorous
- Presentation: pieces
- Container: large stainless-steel bucket
- Delivery method: scattered in the enclosure

Field (1998) mentions that scattering the food in a well-planted enclosure serves a two-fold purpose of keeping the lynx active and increasing their visibility time (helping the keepers and the visitors). Since the introduction of the female to the enclosure and prior to the birth of the cubs, the adults were fed twice a day on the following schedule:

- Daily: 600 grams of chicken or beef heart alternating in the morning
- Tuesday: 2.5 kg of beef heart in the afternoon
- Thursday: 2.5 kg of chicken in the afternoon
- Saturday: 2 whole-carcass rabbits in the afternoon

- Sunday: 2.5 kg of chicken/quail

This diet is similar in its assortment to the other carnivores in the park (a carpathian lynx is an obligate carnivore who feeds by hunting (Hernandez, 2002)) and is quite unlike the diet an animal might encounter in the wild. As the adults are not meant to be released in the wild it is a perfectly acceptable diet, however the same diet cannot be given to the animal being prepared for rewilding, mainly for the two following reasons: the animal will have little idea of how its natural prey looks like if it is fed with beef hearts and, due to the associated smell, the animals are more likely to hunt livestock, invading the farms. Both of those outcomes are unacceptable for rewilded animals as the first one lowers its survival chances and the second one causes tensions with local population which also reduces its survival chances and the survival chances of its conspecifics. Therefore, the diet was switched after the birth of the young:

- No food in the morning
- 6 kilograms of wild prey + whole-carcass brown rabbits on Tuesday,
 Thursday, Saturday and Sunday in the afternoon (later on the portions were increased as the children were growing up)

The wild prey, while still presented in pieces, would not teach the young lynx to associate the smell of livestock with a source of food, reducing the chances of them invading local farms. However, presenting wild prey as a whole carcass would be ideal. In a personal communication from the keepers, I was informed that a whole chamois carcass was given to the lynx once or twice, hopefully giving them some idea of the essential skill of disassembling a carcass. Mellen, 2003, has shown that many felines exhibit the entire stalk-rush-kill sequence or at least a part of it even when presented with parts of a carcass. In the coordination enclosure in Germany, where the lynx went from the park before

being released into the wild, such skills would be assessed and would impact the decision to release the lynx into the wild or not.

The source for the wild prey was mostly animal culling within the park. Breitenmoser and Breitenmoser-Würsten (2008) analysed the proportion of prey consumed by the lynx in Switzerland and came to the following results: roe deer - 60%, chamois - 25%, 18 other species - 15%. The park has a variety of deer and other ungulates roaming in the park and their population is controlled by culling, meat from which is then preserved and given to the lynx and other carnivores of the park, mostly already cut open into pieces but occasionally also as a whole carcass. A keeper informed me that they ran out of the wild meat stock only once since they started supporting the carpathian lynx on it and had to order game meat from a vendor which ended up being very costly and caused the park to freeze more of the culled animals' meat for the future. Food supplements under the brand name Sofcanis were also provided with the meat three times a week.

The switch to the once a day feeding schedule was in order to minimise the number of animal-keeper interactions so as to avoid young lynx habituating to the presence of humans.

Another mandatory protocol associated with feeding was to mask the approach of the keeper carrying food by covering a part (external) of the fence in camouflage net (Figure 3, right) and to throw the food from behind it, ideally when no lynxes were in sight as to avoid the kind of habituation that occurred in the northern lynx that were fed freely and could see the keeper twice a day throwing food to them. While sound in principle, the main keeper for the area of the park where lynx were located remarked that it can be improved upon significantly, as the lynx could still see the keeper from their rocky perch as he

was coming up the hill from the road (no alternative path existed as the enclosure was in a remote part of the park) and the smell of the keeper would always arrive simultaneously with the food, not to mention that falling from the sky was usually an unnatural way for meat to arrive in the wild. Unless it was a bird but birds are not the main source of food for the lynx. The cameras in the enclosure however captured an almost successful attempt by Graveli, the male cub, to catch a bird that was flying by, confirming the presence of basic hunting skill in the lynx cubs.

The drinking water was provided to the lynx in a wide (~1 metre diameter) green plastic drinking basin, the water in which was changed twice a week manually by the keepers. The basin was at the bottom of the enclosure, not far from the antechamber but completely hidden from the visitors' view. The protocols mention that it is common among the felines to defecate into water, fortunately no such problem occurred at the park.

The Medical Interventions

The lynx twins were born sometime in the middle of June 2023, were first spotted at about 4 weeks old on 13/07/2023, and the female was first captured on 17/08/2023, was measured at 2.4 kg, given a Bravecto Plus dewormer, chipped, and had a coproscopy sample, hair sample and saliva sample taken. Then the male cub was caught on 22/08/2023, measured at 3 kg, also given Bravecto Plus, chipped and had the same samples taken.

Similar to all other procedures concerning the animals intended for reintroduction, it was necessary to reduce the number of medical interventions requiring the handling of the young lynx to a minimum. The EAZA guidelines advise to leave the rearing of young to their mother, unless signs of aggression

have been detected or the young have been observed to be neglected (which quickly becomes apparent in the unweaned lynx by levels of dehydration). However, at 6 months old (the lynx cubs usually wean at 3 to 5 months of age) the female became sick and had to undergo regular treatment and observation. This happened prior to my arrival to the park, and so I don't have the full details of the medical procedures taking place but I have an access to medical records, which I summarise below:

28/12/2023: the female (6 months old) was found to be underweight, weighing 4450 grams, was administered Marbocyl (0.1cc), Shotapen (0.5cc), Cydectin (0.1cc), Ultra B (0.5cc). Radiography performed. Ringer lactate administered with perfusion. Temperature: 39.1°C. Two nasal swabs and a coproscopy sample taken for analysis.

01/01/2024: temperature decreased to 37°C, 250 g weight gained. Medication administered: Trymox LA (0.5cc), Ultra B (0.5cc), Estocelan (0.5cc), AD3E (0.5cc), Cerenia (0.47cc).

11/01/2024: the female now weighs 5500 grams, ringer lactate administered by perfusion. Bacteriological and PCR swabs collected.

27/01/2024: Weight: 6750 grams. Another swab collected. Rabies vaccine administered. Feligen(c) vaccine (a combined vaccine against Feline calicivirus infection, rhinotracheitis, panleukopenia and feline leukaemia) administered. This was the last medical intervention on the young lynx prior to their capture for quarantine and transportation.

From a personal communication with the keepers I found out that the female was weak for a prolonged period of time, suspicions of Feline Immunodeficiency Virus were raised and that euthanasia was proposed but the female ended up recovering.

On 17/04/2024 the young lynxes were transferred from the holding pen (capture described in the next subchapter) to the park's quarantine facility and the last main medical check was performed. The procedure went as follows:

- Two anaesthetic preparations were made using medetomidine (40 microgram per kilogram), ketamine (3 milligram per kilogram), and midazolam (0.1 milligram per kilogram). This is consistent with anaesthetic administration described in the Linking Lynx protocols used for lynx with potential heart conditions. A preparation for 15 kilograms was used on the female and a preparation for 20 kilograms was used on the male. The dose could've been adjusted based on the reaction but proved to be sufficient in both cases. In case additional anaesthetic would be required during the medical examination in the park's veterinary facility, sevoflurane was available.
- The female was anaesthetised with a dart at 11:17. 5 minutes later the vet entered the cage, checked the subcutaneous microchip, added moisturiser to the eyes to prevent them from drying out and placed the lynx in the carrying cage. Faeces in the cage were collected for a coproscopy.
- In the carrying cage the lynx was placed in one of the animal keepers' vans and delivered to the park's vet facility (Figure 4, top) located next to the quarantine facility immediately in order to avoid further anaesthesia.
- Female weight: 11450 grams. Pulsemeter was placed on her tongue, and the parameters reported in table 1 were measured. A stethoscope was used to assess the breathing and the heartbeat and a thermometer (cleaned before use) was used for measuring the temperature.

- A total of 6 tubes of veinal blood samples from different legs were collected. An anal swab was performed as well. 0.3 cc of Cydectin (an antiparasitic medication) was administered.
- The female was then placed on some hay in the quarantine facility, her head positioned higher than the rest of the body to prevent possible suffocation. Atipamezole (0.6 cc) was then given to reverse the anaesthesia.
- At 12:03 the male too was darted with his preparation of the anaesthetic and followed a similar procedure (Figure 4, bottom). His parameters are outlined in table 1. 0.3 cc of Cydectin was given. He was then placed in the second quarantine room and given atipamezole at 12:34.

Parameter	Female (Grandera)	Male (Graveli)
Weight	11450 g	15400 g
Oxygen saturation	92%	86%, raised to 97% after oxygen mask administration
Temperature	39.7°C	39°C
Heart Rate	145 bpm	117 bpm
Medication	Cydectin	Cydectin

Table 1: Summary of the last major clinical examination before the start of the quarantine



Figure 4: the lynx twins under anaesthesia on the examination table in the veterinary room. Top - female, bottom - male.

The lynxes were anaesthetised one by one instead of together in order to remove the chance of a simultaneous emergency happening to both lynx and so that the vet could focus on one lynx at the time. It is worth mentioning that the behaviour for both animals was consistent between before and after being darted with an anaesthetic and before falling asleep: the female was pacing along the box at any time that humans were in the holding pen and continued to pace until she couldn't drag her feet any longer; the male was lying down and occasionally growling at the people in the building and remained motionless after being darted until he fell asleep. The female's pacing behaviour wasn't present to such an extent when they were held together in the quarantine enclosure.

The last medical check-up took place on the 25th of April prior to the lynx departure to the coordination facility in Germany. It was a minor check to collect additional blood samples and ensure the good condition of the lynx after the quarantine period. One blood sample accompanied the lynx to the facility in Germany, while the other one was sent directly to the Laboklin laboratory in Germany. Both blood samples were tested for common lynx pathogens, including FIV (Feline Immunodeficiency Virus) - a serious pathogen affecting lynx (Ryser-Degiorgis, 2021) - which if detected would most often result in exclusion from the Linking Lynx program and euthanasia. The result of the sample that followed the lynx was FIV-positive, however due to the fact that the sample was already quite old by the time it reached the laboratory from the German facility it couldn't be fully trusted to be a real positive (personal communication with the park's veterinary). The sample sent by the vet directly to the laboratory received a negative result for all tested pathogens including FIV. This test was deemed more reliable and so euthanasia was avoided and female lynx proceeded with the program. While personally I am very happy with this outcome it remains to be seen if further testing had to be done (in all probability it has been done in the German facility however I have no information of it).

The Capture and Quarantine Facilities

In order to safely isolate the lynx young and prepare them to be anaesthetised the following procedure took place:

 The usual food rations were placed inside the holding pen instead of being scattered in the enclosure. The food rations were also cut into smaller pieces in order to prevent the adult lynx from dragging the food out of the building for the young to eat.

- The enclosure was equipped with two interconnected cages (also referred to as Box 1 and 2 further in the text) of about 4 square metres each, each possessing an individual guillotine-type door to the rest of the enclosure. A camera was installed in the boxes programmed to notify the animal director of the park on the phone when a movement is detected. From his phone the animal director was also able to remotely close the guillotine doors, using a magnetic release mechanism.
- The set up involved closing the door to the enclosure of box 1, opening the door between box 1 and box 2, placing the food in box 1, and opening the door to the enclosure of box 2, so the lynx would have the incentive to go further from the entrance, making the door closing a bit safer.
- 13/04/2024, at 16:00 the system was activated, the food was placed in box 1 at 16:16 and at 16:39 both females (mother and daughter) were caught together. At 16:48 the mother was released, the daughter was locked in box 1, more food was placed in Box 2. At 20:46 the male young was caught too.
- The vet then arrived on 17/04/2024 to anaesthetise the lynx and retrieve them from the cage to place in the quarantine facility. Only three people were allowed to be present at the darting to reduce the stress for the lynx
 namely the vet, his intern and one of the keepers.

After the lynx had fully recovered from the anaesthesia, the door between their quarantine rooms was opened in order to house them in a larger common space (as they are a 'social unit of the same origin admitted at the same time' as per protocol), reducing the stress levels. On all observations the siblings were found huddled together during the quarantine period. Each of the two interconnected rooms was 16 square metres in area. Prior to the lynx arrival to the quarantine

rooms, the floor was covered in hay and wood shavings and logs were provided as an enrichment, however no box recommended by the EAZA quarantine guidelines was provided.

Human contact was limited to the area's keeper feeding and checking up on the lynx once a day to avoid either stress or habituation as advised by the Linking Lynx protocols. This procedure was strictly followed. The quarantine facility presented by itself a separate concrete and metal building close to the in-park offices. It had a metal door entrance, an antechamber for sterile conditions, a main hall for equipment and for access to all three quarantine rooms, thick metal doors between the rooms to provide a possibility for uniting them into a single space, and a metal gate for vehicle access to one of the rooms. No other animals were kept in quarantine at the time. The lynx spent a total of one week at the quarantine facility leaving for Germany on the 25th of April 2024.

The Transport

On 25/04/2024, the transport van arrived around 11:00. It was a specially designed large white van with an air-conditioned windowless holding place for medium-sized animals. It has brought with it two wood and metal transport boxes, measuring 160x70x90 cm, with feeders and drinkers provided in them. The lynxes were darted with doses of anaesthetic identical to those used during the previous capture for the transfer to the quarantine facility. As the clinical examination didn't take as long as it did the previous time, the vet had to wait after placing the lynx in their respective transport boxes and before injecting the antidote in order to avoid complications - at least 20 minutes had to pass between anaesthetic mixture and its antidote. After the antidote (Atipamezole) was injected, the boxes were closed and we waited for the lynx to fully recover

and stand up. During the recovery we had to interact quietly to once again avoid additional stress or habituation for the lynx. Once the vet made sure that the lynxes were well, the boxes were fixed within the van with special cargo belts, the doors were closed and the van set off away from the park.

The route to the German quarantine facility took about 6 hours. I was informed that the lynxes have successfully undergone their quarantine upon arrival and have entered a coordination enclosure - a large enclosure where their ability to survive in nature would be examined. From the emails I understood that they are currently in the coordination enclosure in Wildkatzendorf Hütscheroda (Thuringia), and there's a plan to release male Graveli in the Black Forest (Baden-Württemberg) and the female Grandera in Saxony, near the border with Poland, finally separating the twins in order to make the best use of their genetic inheritance in different areas. Upon the time of the submission of this paper the lynxes were still located in the coordination enclosure but I believe they have every chance of getting released into the wild soon.

Linking Lynx protocols and potential reduction of stress levels

Brought about by the reduction of reproductive success rate of the Canada Lynx in captive environments, a 2013 study conducted by KV Fanson and NC Wielebnowski was investigating stress levels in different captive Canada lynx using the measurement of faecal glucocorticoid metabolites associated with stress response. While not the same species, the Eurasian lynx (to whom lynx carpathicus belongs to) and the Canada lynx are closely related (Anderson and Lovalo, 2003) and similar results in stressors could be expected. Having those stressors present will negatively impact both the individual lynx and the conservation effort. The study has shown that the stress levels were most

significantly influenced by the presence and sex of the cage-mates, area of the enclosure, and the number of hiding spots available.

In regards to the amount of area and hiding possibilities provided, Sapolsky (2002) has shown that an individual's perception of control in an environment is important to their well-being and in a big enclosure of great complexity and high usability an animal has significant control over its actions, and therefore the stress levels are reduced. The area of great size and complexity provided for the lynx in the Grottes de Han park, answering to the Linking Lynx demands for their ex-situ sites, therefore increases the well-being of the lynx and helps the young to be more prepared for the wild environment. In regards to the group size, lynx, whether it be Canada lynx or the Carpathian lynx, are a solitary species and have the lowest stress levels when they are housed alone (Mellen 1991). In the Grottes de Han this is negated by the fortunate fact that the male and female adults have established a stable couple, else the lack of the possibility to isolate them in different parts of the enclosure would be highly detrimental to both their well-being and the safety of their off-springs.

Conclusion

As the nature of raising the animals intended for rewilding is to reduce human interference with them to the minimum, my role in the raising of the young lynx twins was limited to feeding and manually transporting them in an anaesthetised state. Yet I could observe the factual conditions the animals were in and compare them to the existing best-practice recommendations and regulations as well as witness first-hand their benefits. The procedures that are in place to secure the carpathian lynx's ability to be reintroduced into the wild and whose implementation I have described in detail in this paper are useful as we can see by comparison with the Northern Lynx at the park - who are highly affected by human presence - and are likely to reduce the stress experienced by the animals in captivity as shown by the research done on the Canada Lynx. Yet, both the Grottes de Han WIIdlife Park and the Linking Lynx protocols still have a space to improve to increase both the animal well-being and the reintroduction success.

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Bibliography:

'2019 Recommendations - Convention on the Conservation of European Wildlife and Natural Habitats - Www.Coe.Int'. Convention on the Conservation of European Wildlife and Natural Habitats, https://www.coe.int/en/web/bernconvention/2019-en. Accessed 10 July 2024.

Anderson, Eric M., and Matthew J. Lovallo. "Bobcat and Lynx." Wild Mammals of North America: Biology, Management, and Conservation, edited by Feldhamer et al., 2nd ed., Baltimore: Johns Hopkins UP, pp. 758–86.

Bonn Lynx Expert Group. "Recommendations for the conservation of the Eurasian lynx in Western and Central Europe." Cat News, Special Issue 14 (2021): 78-86.

Brandes, Florian. "Quarantine." www.linking-lynx.org, 24 Jan. 2024, www.linking-lynx.org/?action=get_file&id=24&resource_link_id=96. Accessed 10 July 2024.

Breitenmoser, Urs, and Christine Breitenmoser-Würsten. Der Luchs: Ein grossraubtier in der kulturlandschaft. Salm, 2008.

Černe, Rok, et al. A fieldguide for investigating damages caused by carnivores: Brown bear, grey wolf, golden jackal, red fox, Eurasian lynx. Slovenia Forest Service, 2019.

Chapron, Guillaume, et al. "Recovery of large carnivores in Europe's modern human-dominated landscapes." science 346.6216 (2014): 1517-1519.

European Association of Zoos and Aquaria » EAZA. https://www.eaza.net/#map_home. Accessed 10 July 2024.

Fanson, Kerry V., et al. "Comparative patterns of adrenal activity in captive and wild Canada lynx (Lynx canadensis)." Journal of Comparative Physiology B 182 (2012): 157-165.

Field. Guidelines for Environmental Enrichment. Association of British Wild Animal Keepers, West Green, United Kingdom, 1998.

Gomerčić, Tomislav, et al. "Cranial morphometry of the Eurasian lynx (Lynx lynx L.) from Croatia." Veterinarski arhiv 80.3 (2010): 393-410.

Herfindal, Ivar, et al. "Prey density, environmental productivity and home-range size in the Eurasian lynx (Lynx lynx)." Journal of Zoology 265.1 (2005): 63-71.

Hernandez. "Species account - Lynx lynx - Eurasian lynx." animaldiversity.org, 31 July 2002,

animaldiversity.ummz.umich.edu/accounts/lynx/l. lynx\$narrative.html.

Hočevar, Lan, Urša Fležar, and Miha Krofel. Overview of good practices in Eurasian lynx monitoring and conservation. University of Ljubljana, Biotechnical Faculty, Department of Forestry and Renewable Forest Resources, 2021.

Janečka, Jan E., et al. "Rapid whole genome amplification of DNA from felids: applications for conservation genetics." Wildlife Society Bulletin 34.4 (2006): 1134-1141.

Kitchener, Andrew C., et al. "A system for designating taxonomic certainty in mammals and other taxa." Mammalian Biology 102.1 (2022): 251-261.

Krelekamp, C. J. "Husbandry guidelines Eurasian lynx (Lynx lynx sspp.)." European Association of Zoos and Aquaria (EAZA). Amsterdam, Netherlands (2004): 9-34.

Krofel, Miha, Tomaž Skrbinšek, and Ivan Kos. "Use of GPS location clusters analysis to study predation, feeding, and maternal behavior of the Eurasian lynx." Ecological research 28 (2013): 103-116.

Krojerová-Prokešová, J., et al. "Genetic constraints of population expansion of the Carpathian lynx at the western edge of its native distribution range in Central Europe." Heredity 122.6 (2019): 785-799.

Kvapil, Pavel, et al. "Anaesthesia." www.linking-lynx.org, 2 Feb. 2024, www.linking-lynx.org/?action=get_file&id=24&resource_link_id=a3. Accessed 10 July 2024.

Lanz, Tabea, and Christine Breitenmoser-Würsten. "Perspective of the Metapopulation of Carpathian Lynx." www.linking-lynx.org, 24 Jan. 2024, www.linking-lynx.org/?action=get_file&id=24&resource_link_id=98. Accessed 10 July 2024.

Lengger, Jochen. "Ex-Situ Management for Reintroducing Carpathian Lynx." www.linking-lynx.org, 24 Jan. 2024, www.linkinglynx.org/?action=get_file&id=24&resource_link_id=92. Accessed 10 July 2024.

'Lynx (Carpathian)'. Dudley Zoo and Castle, https://www.dudleyzoo.org.uk/animal/lynx-capathica/. Accessed 10 July 2024. Mellen, and Wildt. "Husbandry Manual for Small Felids." www.felidtag.org, 13 Oct. 2003.

Mellen, et al. "Captive Environmental for Small Felids." Second Nature: Environmental Enrichment for Captive Animals, edited by Shepherdson et al., Smithsonian Institution Press: Washington DC., 1998, pp. 184–201.

Mellen, Jill D. "Factors influencing reproductive success in small captive exotic felids (Felis spp.): a multiple regression analysis." Zoo biology 10.2 (1991): 95-110.

Morgan, Kathleen N., and Chris T. Tromborg. "Sources of stress in captivity." Applied animal behaviour science 102.3-4 (2007): 262-302.

Rafferty, John P. "Lynx" - Carnivore, Solitary, Fur | Britannica. 2 July 2024, https://www.britannica.com/animal/lynx-mammal/Eurasian-lynx.

Ryser-Degiorgis, Marie-Pierre, et al. "Management of suspected cases of feline immunodeficiency virus infection in Eurasian lynx (Lynx lynx) during an international translocation program." Frontiers in veterinary science 8 (2021): 730874.

Sapolsky, Robert M. "Endocrinology of the stress-response." (2002).

Sunde, Peter, Snorre Ø. Stener, and Tor Kvam. "Tolerance to humans of resting lynxes Lynx lynx in a hunted population." Wildlife biology 4.3 (1998): 177-183.

'The Wildlife Park: A Stunning Background to the Cave'. Grottes de Han, https://grotte-de-han.be/en/our-pledge/park. Accessed 10 July 2024.

Zimmermann, Fridolin, Christine Breitenmoser-Würsten, and Urs Breitenmoser. "Natal dispersal of Eurasian lynx (Lynx lynx) in Switzerland." Journal of Zoology 267.4 (2005): 381-395.