LIFE Lynx

Preventing the extinction of the Dinaric-SE Alpine lynx population through reinforcement and long-term conservation

LIFE16 NAT/SI/000634

Protocol for Eurasian lynx (*Lynx lynx*) capture, narcosis, transport, and quarantine in the Slovak Carpathians

This protocol is based on Breitenmoser U., A. Ryser, M. Ryser-Degiorgis (2013) *Dokumentation Fang, Narkose und Markierung von Raubtieren*, as well as protocols and materials from KORA (Raubtierökologie und Wildtiermanagement), FIWI (Zentrum für Fish- und Wildtiermedizin) and the LIFE project "Wiederansiedlung von Luchsen im Biosphärenreservat Pfälzerwald" (LIFE13 NAT / DE / 000755).

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Content

1. Methodology for capturing Eurasian lynx

1.1. Box traps

Box traps are made from a waterproof plywood (Figure 1) and a steel frame. Paddle doors move within metal rails and provide fast closure of boxes and are generally light-tight with sufficient air ventilation. The bottom edge of the door is lined with foam. Olfactory attractants e.g. lynx scat and urine or valeriana (*Valeriana officinalis*), catnip (*Nepeta cataria)* and castoreum may increase the likelihood of lynx capture. Boxes are triggered/activated by a silon string that is stretched in the middle of the inner part of the trap. This trigger mechanism (string) is connected with the KIEFERLE latch lock that holds the paddle doors and releases them when triggered. Box trap parameters are: width: 75 cm, height: 80 cm, length: 200 cm.

Small lockable windows are left in both paddle doors for visual inspection. When captured, lynx is narcotized through these holes with a blow pipe.

Boxes are used particularly in winter. The most appropriate period is from February to April before and during the mating season, when lynx move more actively. This period has two additional benefits: (1) the young lynx are large enough to be radio collared; and (2) young lynx can survive without their mother. Therefore, we are able to capture and translocate adult females.

The boxes are typically located in narrow and direct sites or forest and wildlife walkways. The passage is blocked from both sides by vegetation or camouflage nets (Figures 1, 2). Traps are installed only after consultation and approval from the appropriate authorities (rangers, game managers, foresters, hunters, stakeholders or forest owners). Furthermore, info panels are placed on both sides approximately 50 meters from the boxes. The capture team and local authorities phone numbers or/and mails as well as web page info are included on the info panels.

Box traps are monitored throughout the day with GSM transmitters. The alarm system is tested daily to test its functionality via a control SMS. Triggering the system will send an info SMS to the capture team. In addition to the capture team, the info SMS is also sent to a local person (ranger, hunter or forester) who will check the trap as quickly as possible and release any non-target species (baggers, foxes etc.). When lynx are captured, the capture team will be informed as soon as possible. Depending on the site and accessibility, the capture team has to be in place within 2-4 hours. Boxes are monitored by infra-black/red video cameras to record the behaviour of lynx and of other animal species in the immediate vicinity to the traps. Box traps are regularly checked once every 5-7 days. After snowfall the traps are checked daily to maintain their functionality.

Boxes are generally maintenance-intensive, but provide an effective lynx capture method with a clear distortion in favour of males: 70% of all captured lynx are male (Breitenmoser & Breitenmoser-Würsten 2008). Closed, plywood boxes with smooth walls are safe, no injuries were recorded except of a few broken claws. Metal boxes are easy to camouflage and therefore more efficient, however the lynx have often bounces, or broken claws and teeth. In light-tight wooden boxes the animals behave considerably more peacefully and are even protected against cold or weather. Lynx generally react well when the window on the paddle doors are opened. From these windows it is possible to narcoticize lynx using a blow pipe.

Figure. 1. Box made of the waterproof plywood on a forest ridge. Wooden traps do not have features that could injure lynx.

Figure. 2. Record from the moment the lynx enters the box trap.

1.2. Foot snares

Foot snares resemble the "classic" traps used by Canadian fur trappers but are fundamentally different in their function. The round base (Figure 3) represents a throwing mechanism that does not have any capture function and is completely separated from the bars and wire. The jaws of the throwing mechanism consist only of weak springs and can only be closed to 10 cm. The lynx is captured by a 6 x 9 mm stainless steel wire, which is placed in the groove on the jaws. The wire extends to the throwing mechanism, which throws it over the animal´s foot after the trigger mechanism was initiated (by stepping on it, Figure 4). The spring in the bar accelerates the pull effect of the wire, but acts also as a shock absorber when the captured lynx tries to escape. Moreover, it prevents immediate foot injuries as well. Two - four camouflaged foot snares are set near the lynx kill (Figure 5, 6). The bars are anchored depending on the situation with steel wire to the trees, or with earth screws.

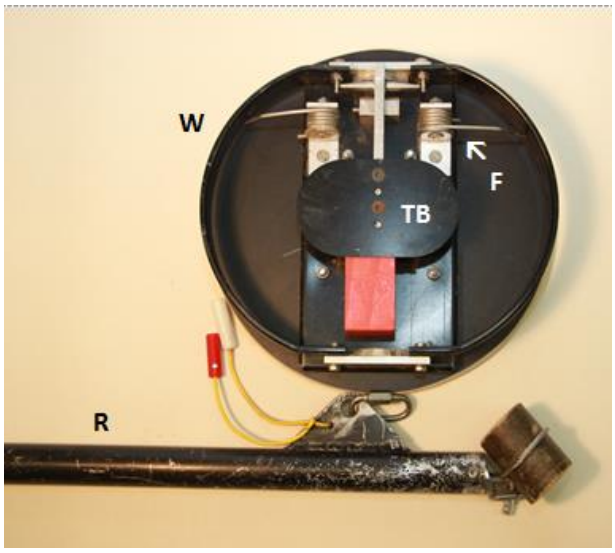


Figure. 3. The KORA 08 foot snares consist of a steel bar (R), and a split groove that acts as a throwing mechanism (W) and throws a wire on the animal's foot as it steps on the trigger mechanism (TB). However, the jaws (F) cannot be closed.

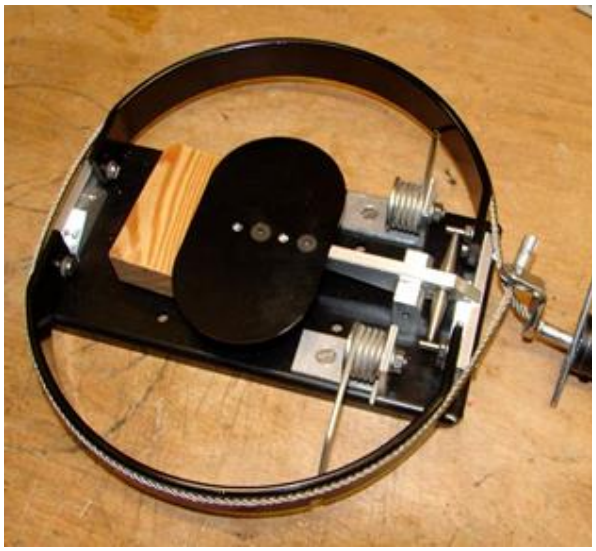


Figure. 4. The foot snares type „KORA 08“ with a wire set on the throwing mechanism.

Figure. 5. Foot snares „KORA 08“ set in close proximity to the lynx kill.

Figure. 6. Foot snares „KORA 08“ preferably in a similar position and camouflaged.

Traps are continuously monitored with VHF transmitters or with the GSM system used in the box traps. The capture team will wait at a suitable location sufficiently distant to avoid disturbance (several hundred meters), but close enough to reach the capture site within 5-15 minutes, and immediately responds to the alarm. The captured lynx is fixed by the catch net and narcotized by syringe, or directly by the blow pipe.

Foot snares are efficient and are generally a safe capture method. The device itself did not cause any injuries, particularly because the capture team comes to the animal in a few minutes. In only exceptionally rare cases, scratch or small wounds of the hind legs were recorded. The wires used to capture are replaced by new ones after each capture, whether or not there were any complications or injuries observed.

During the placement of individual snare traps, the extent of lynx movement should be considered to prevent the animal from getting into a dangerous position. It is often necessary to drag the kill a few meters in order to obtain appropriate conditions for capture. Foot snares are very species-specific. Since few animals will approach a lynx kill site, non-target captures are very rare.

1.3. Capture protocol

Data filled in by: _____

Date: _____ Time: _____ GPS coordinates: _____ / _____

Place & locality: _____ Height above sea level: _____m

Type of trap: _____ Lynx ID: _____ Lynx name: _____

Ear tag number: _____ Microchip number: _____

Collar number: _____ Frequency: _____MHz

Narcotic substance / substances: _____*

Photo: ___ no ___ yes Author: _____

Video: ___ no ___ yes Author: _____

Participating persons: _____

_____

Gender: _____ Weight: _____kg Age: ___ adult ___ juvenile exact: _____

Length: Body: _____cm Tail: _____cm Height in shoulders: _____cm

Tarsus: left: _____cm / right: _____cm
Ear: left: _____cm / right: _____cm

Ear bristle: left: _____cm / right: _____cm
The distance between canini teeth: upper: _____mm / lower: _____mm

Neck circumference: _____cm
The distance between the anal and genital holes: _____mm

Sampling	**Sample archiving**
__ blood quantity: _____ml	how/where: _____
__ teeth which: _____	how/where _____
__ hair which: _____	how/where _____
__ other: _____	

Notes:

* **Details of narcosis:**

1. Injection: Time: _____h Quantity: _____ml substance / substances: _____

2. Injection: Time: _____h Quantity: _____ml substance / substances: _____

3. Injection: Time: _____h Quantity: _____ml substance / substances: _____

4. Injection: Time: _____h Quantity: _____ml substance / substances: _____

Heart pulse (H) and Respiratory frequency (A)

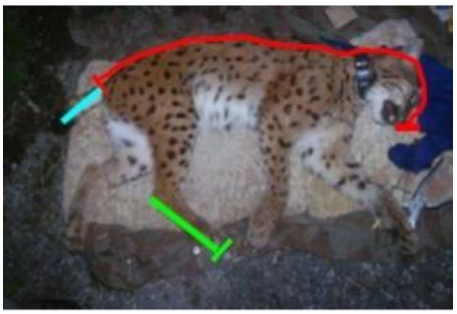
- Time: _____h H = _____/min A = _____/min
- Time: _____h H = _____/min A = _____/min
- Time: _____h H = _____/min A = _____/min
- Time: _____h H = _____/min A = _____/min
- Time: _____h H = _____/min A = _____/min

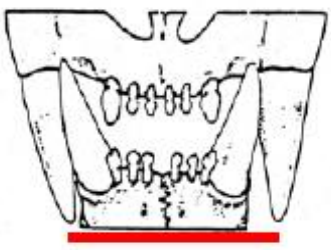
Time: awakening: _____h standing: _____h

Specifications for measurements:

- Body length: From the tip of the nose to the root of the tail *
- Tail: From the root to the tip of the tail *
- Height in shoulders: From an approximate bend of the foot´s joint to the upper scapula
- Tarsus: Link of the tarsus to the furthest toe of the joint
- Ear: The outer edge of the ear after anthelix *
- Ear bristle: From the tip of the ear to the tip of the bristle
- The distance between dentes caniniThe distance between the toes of dentes canini *
- The distance between the anal and genital holes Distance between centers of both holes

* Pictures:





2. Narcosis and veterinary health surveillance

2.1. Narcotics and antidotes

For lynx, narcosis is conducted through a combination of sedatives and narcotics. Prior experience has shown that this combination leads to a reliable, low-risk narcosis in all lynx age groups, including animals in poor health condition.

2.2. Administration, monitoring and termination of narcosis

Administration phase. If a trap was used for capture of the animal, the capture team has to approach as soon as possible with minimum disturbance (silence and minimum number of persons) to minimize lynx stress levels. First, anaesthetic is administered to the animal´s femoral muscles.

Within the framework of lynx tranquilization or manipulation in Slovakia, it is often difficult to determine the exact weight of the captured animal by visual means. Therefore, the following three animal-weight categories are used to classify an individual:

1. Small-sized lynx 3-8 kg,
2. Medium-sized lynx 9-16 kg,
3. Large-sized lynx 17-25 kg.

Most adult lynx in Slovakia weigh between 13,8-29 kg (the heaviest male was 35 kg). When tranquilizing captured wild lynx, only Medetomidine is used in doses of 0.15-0.2 mg/kg. After the animal is in a lying position (generally within 15-30 min) Ketamidor® is used intramuscularly in doses of 1-5 mg/kg.

In the quarantine/rehabilitation station, an alternative approach may be used:

1. Butorfanol 0.04 mg/kg + Medetomidine 0.08 mg/kg + Ketamine 5 mg/kg,
2. Butorfanol 0.04 mg/kg + Midazolam 0.2 mg/kg + Medetomidine 0.08 mg/kg + Ketamine 5 mg/kg,
3. Butorfanol 0.04 mg/kg + Midazolam 0.2 mg/kg + Ketamine 5 mg/kg.

As an anthagonist, Atipamezol®, Naloxon® and Flumazenil® are used. The substances used include Domitor®, Narcostart®, Cepetor®, Butomidor®, Ketamidor®, Antisedan®, Narcostop®, Midazolam®, and Flumazenil®.

As a standard, the following six narcosis stages can be expected, which are not usually observable, because the animals are left calm until they fall asleep. If the narcosis is successful as expected, the animals are in the sixth stage when the capture team starts to handle them. If manipulation is conducted too early (e.g. stage four), there is a high risk that the animal will gain full awareness; the tranquilization is then more difficult and also associated with a higher risk during the narcosis.

1. Uncoordinated movements, loss of coordination,
2. The animal begins to lie down,
3. Lying, the head is still held up,
4. Lying, the head is held down, but when stimulated, head rises up again,
5. Lying, the head is held down, when stimulated, the animal barely reacts,

6. Lying, head is held down, no reaction to external stimuli (sounds, touch etc.).

When reactions are no longer recorded, the lynx is released from the trap and his legs are fixed. The respiratory function is briefly checked before any further manipulation. Depending on weather conditions and terrain, a flat and open space should be used for optimal animal placement and handling. Tranquilized lynx must be protected from rain, snow, and/or hypothermia).

Monitoring phase. Monitoring of a tranquilized lynx is recorded into both, capture and narcosis protocols. For manipulation, the animals are placed in a lateral position. Legs are fixed together away from the torso (Figure 7). The head is stretched to the cranial position so that the upper airways are free. The mouth is checked for food remains or foreign bodies with a free-lying tongue. The protective eyedrops (Viscotears®) is applied to the cornea and the eyes are covered (Figure 8). During cold conditions, animals are placed on an insulating substrate and covered with blankets. Depending on the body temperature, these are removed or used together with additional heater bottles. The equipment that can be used for a field narcosis monitoring is rather limited. However, a thermometer is crucial, because the risk of hypo or hyperthermia in the field is higher and the body temperature needs to be checked regularly. Hours with a second pointer, a stethoscope and a portable pulse oximeter (for measuring oxygen saturation in the blood as well as pulse monitoring) are also needed (Figure 8).

Figure. 7. The animal´s feet are fixed together away from the torso, the head is stretched and mouth is checked for food remains or foreign bodies.

Figure. 8. Setting of the portable pulse oximeter and application of the protective eyebrow.

The vital functions/parameters (minimum frequency of respiration, heart pulse, mucous membrane colour and capillary filling time) are checked along with reflexes (especially rat, cornea and ear) and body temperature as soon as possible and throughout the duration of narcosis; one member of the capture team is responsible for a regular check and recording of these operations as well as for doses of administered substances (in ml) and exact times of all

actions or observations (sleeping, awaking, etc.). All measurements during a narcosis are recorded in the narcosis protocol.

Termination of narcosis and monitoring after the animal´s release. After completion of all necessary actions, the narcosis will be terminated as soon as possible. The effect of anaesthetic is antagonized (5 mg of atipamezol per mg of medetomidine, i.e. 1 ml of Antisedan®/Alzane® per ml of Domitor®/Dorbene®). If Midazolam was administered, Flumazenil® is used in doses of 1 mg per 13 mg of Midazolam. Midazolam is administered therapeutically only if necessary, e.g. in the case of convulsions. This is however possible only after 60 minutes of the last ketamine injection into the femoral muscle, so that the animal is capable of full movement after awaking. Injection to the shoulder muscles may lead to faster absorption of the substance; however, experience has shown that the animal awakes faster, nevertheless its stability and coordination are unsecure. After injection into the femoral muscle, convalescence is slower, smoother and the animal moves and recovers generally better. Therefore, in cases of a complicated capture, the antagonist is given to the femoral muscle. If there are dangerous conditions in proximity of the capture site (cliffs, frequented roads, forest creeks), the tranquilized animal is moved for at least a few hundred meters to a safer place. In box traps, the wake-up phase can take place inside and the box is open after the animal´s recovery. After an intramuscular injection of antagonist, the animal usually starts to move after 5 minutes and a full recovery is reached after 10 minutes. At this stage it is important that the lynx is not disturbed (especially no sounds or voices). After submission of the antagonist, the capture team moves away from the animal with all the equipment. Only 1 or 2 persons will monitor the situation. If the lynx is awake and well, this person/persons will move away from the animal.

2.3. Complications during narcosis

Each narcosis is associated with a risk of partly species-specific side effects and complications (for details see Kreeger & Arnemo 2007). However, the possibilities of dealing with complications in difficult field conditions are very limited. Therefore a great emphasis is placed on animal monitoring and incident prevention. If, however, a problem occurs, priority is given to the animal´s health and other actions are cancelled/discontinued.

Prior to the capture season, all capture team members are informed about the emergency measures and actions, as well as on the contents of the medical preparations and equipment. Important emergency measures are summarized in the narcosis protocol. Potential complications in capturing wildlife are different. Firstly, the animals can be injured in a trap or until they fall asleep. Second, there are several possible situations that may arise in relation to narcosis. The most common cases are associated with body temperature failure, insufficient respiration, or problems with the circulatory system. In addition, animals may vomit the contents of its stomach, inhales the foreign object, or have convulsions. During the last 15 years of lynx captures in Switzerland, only minimal complications were recorded. Injuries occurred occasionally but never had a serious character. There is a risk of hypothermia, especially in winter; but it never caused animal mortality. In just one case, insufficient respiration was observed, but it was during the transport of a seriously ill animal. Complications can generally

be prevented by taking appropriate measures. Many of the potential problems are due to excessive stress caused by capture, inappropriate placement or handling, and side effects of narcotics. Although stress cannot be completely eliminated, it is possible to minimize it by means of the appropriate approach and behaviour of the capture team and a proper placement of the animal. If a problem occurs directly related to narcotics (e.g. overdose), it may be partially or totally antagonized with medetomidine (depending on the situation and the time elapsed since the last injection of ketamine).

The main potential complications, their causes and recommended measures (for more information see e.g. Kreeger & Arnemo 2007):

Shortness of breathing, insufficient respiration, or no respiration: leads to insufficient oxygenation of the tissues (hypoxia) and can cause a cell damage or even death.

Symptoms: inadequate or absent respiration, blue, greyish or "dirty" mucosa (cyanosis); possible loud sounds while breathing (whistling, etc.).

Causes: suppression of the respiratory system by narcotics,

Blocked respiration (nose, trachea), e.g. by tongue, twisting of the throat, vomiting, or pressure on the membrane (for example, due to pregnancy or bloating).

Precautions:

1. Inspection of the airway's accessibility: neck in strait position, tongue outside, absence of food remains or foreign bodies in the oral cavity, correct body position (for carnivores, side or sternal).
2. Depending on the state and stage of narcosis: administration of the doxapram injection intramuscularly or intravenously (1 till 2 mg / kg) and/or the antagonist intramuscular or intravenous. In the case of a slightly lower respiratory rate, droplets of Respirot® can be given on the tongue or into the nasal holes (only for animals that not too large).
3. In cases of complete respiratory failure: maintain animal in a lateral position, conduct chest compressions (strong chest pressure at 15-20 times/minute), "pumping" with the forelegs, or resuscitation mouth-to-mouth, respectively mouth to the nose. At the same time check for colouring of the mucosa - if it is naturally coloured (pink), pause the resuscitation and check for the animal´s respiration. If there is no respiration registered, continue with the resuscitation.
4. If the oxygen saturation drops below 70%, oxygen from a 2L portable oxygen bottle is given into the animal´s nose. The oxygen flow is set on 1-2L / min. If the respiration problems continue or worsen, the animal must be intubated with the endotracheal cannula. The ambu bag is then used for artificial respiration at the right rhythm until the oxygen saturation is greater than 90%.

Hyperthermia: an increase in body temperature until the demand for oxygen exceeds its availability due to increased metabolism.

Symptoms: increased rectal temperature (> 40 ° C), very hot legs (ears, feet), rapid or shallow respiration, rapid or irregular heartbeat.

Causes: physical exertion, high temperature, direct sunlight, disturbance of thermoregulation centers due to narcotics, or presence of infections (bacterial, viral).

Precautions:

1. Cooling the lynx: shade, watering (especially the throat and belly) and aeration, or even immersion in water if necessary, etc.

2. Administration of the antagonist intramuscularly or intravenously.

Severe hyperthermia (> 41 ° C) is an emergency situation. Temperature monitoring must be done as soon as possible and throughout the duration of narcosis. At temperatures > 42 ° C, regardless of animal survival, permanent health problems may be expected. Mortality at temperatures > 43 ° C.

Hypothermia: Body temperature reduced to cell death due to the restricted metabolism, freezing water in the cells, and/or blood vessel damage.

Symptoms: Decreased rectal temperature (<37 ° C), fever, reduced heart rate, recording a pulse is very difficult (low blood pressure), cold or stiffened legs (frostbite).

Causes: disturbance of thermoregulation centers due to narcotics, low temperature, lack of thermo-isolation (wet coat, cachexia, allowing body to remain in one position (on one side) for too long), misfunction of the blood circulation (e.g. shock).

Precautions:

1. Heat the animal (with hot water bottles, blankets, body heat, etc.).

Hypothermia is a real risk particularly during winter captures. It is necessary to carry warm water in thermos or to handle the animal in a warm place. Body temperature < 24° C can lead to mortality. The administration of the antagonist is not recommended under cold conditions, because the return to normal body temperature may be delayed. If necessary, prolong the narcosis until normal body temperature is reached.

Vomiting, aspiration (reversal of the stomach contents via the oesophagus and the oral cavity).

Symptoms: bruising, shortness of breath, sneezing, bloating, mucous blue, greyish or "dirty" (cyanosis), foreign material in the throat, in the trachea and/or in the nasal holes, respiratory disfunction.

Causes: side effect of narcotic substances, stress, excitement, head located lower than stomach.

Precautions:

1. Free airways, elimination of stomach and mucous membrane rejection if possible, sternal position, head and neck stretched downwards (if possible, the animal should be lifted upside down to allow vomiting).
2. Safety precautions same as in “absenting respiration” if necessary.
Administration of long-acting antibiotics.

Vomiting itself may not be problematic, but aspiration can lead to mortality, either directly (suffocation) or indirectly (with numerous vomiting and aspirated material, many bacteria can get into the lungs, which can lead to severe pneumonia). An animal may die in a few days after capture, although it appears to have recovered from the narcosis. Aspiration of a large quantity of vomited content has poor prognosis.

Cardiac arrest: (disfunction of the blood circulation due to arrest of heart function).

Symptoms: weak or absence of pulse, long capillary filling time (> 2 seconds), mucous blue, greyish or "dirty" (cyanosis), increased frequency of respiration, or its abnormalities, respectively arrest, pupils extended, cold skin.

Causes: stress, side effects of narcotic substances, acidobase imbalance: acidosis (acidification), alkalosis (acidification), electrolyte imbalance: hyperkalaemia, hypokalaemia, hypokal anemia, disfunction of the nervous system (sympathetic/parasympathetic), hypothermia.

Precautions:

1. Respiratory control, change in positioning of the lynx body to free the airways. Safety precautions same as in “absenting respiration” if necessary.

2. Administration of etilephrine (Effortil® Drops).

3. External heart massage: animal in lateral position, pressure on heart. Number of compressions 60-100 cycles per minute. At the same time, someone should have to focus on a femoral pulse to check the performance of the massage.

Shock: a clinical syndrome characterized by insufficient blood supply to the tissues, leading to oxygen deficiency in the cells. Shock is often observed in animals experiencing stressful capture. Many mortalities during capture are caused by shock or stress. However, there is often no clear, definitive diagnosis. The possibilities of shock treatment are very limited - for this reason, preventive measures are more important.

Symptoms: Fast heart rate, slow capillary filling (lower blood pressure), disturbances of perception, muscle weakness, hyperventilation.

Causes: Longer physical exertion, delayed physiological or mental stress, severe blood loss.

Treatment:

1. Intravenous administration of ringer solution (30 ml/kg). In the case that the shock is caused by bleeding, the solution must be delivered to the central vein relatively quickly (the wound must, of course, be treated sufficiently).

2. Administration of dexamethasone (5 mg/kg) slowly, intravenously (more than 30 seconds).

Convulsions: (seizures, involuntary contractions of the muscles due to brain disfunction).
Symptoms: Muscle convulsions, convulsions of the whole body, stretched and stiffed legs, involuntary mouth movements.

Causes: Side effects of narcotics (e.g. ketamine), trauma, hypoglycaemia (low sugar level in the blood).

Treatment:

1. Slow administration of 10 mg diazepam intravenously (10 to 15 seconds, otherwise there is a risk of cardiac arrest). If necessary, repeat the dose.

2. Body temperature monitoring: If the convulsions last too long there is a risk of hyperthermia (see "hyperthermia").

2.4. Examinations and manipulation during narcosis

During the narcosis, it is necessary to carry out all the examinations and procedures on the animals for which the lynx was captured, namely (1) collaring/marking (2) sampling, (3) health examination, measurement and photography.

Collaring the lynx. The telemetry collar must be ready and checked: disconnection (drop-off), material fine, VHF frequency set in the receiver, GPS unit programmed, magnet removed. The collar is attached so the animal cannot remove it over its head, but does not press the throat (freely movable and rotatable). Moreover, the collar should not be attached too tightly so that the neck circumference can grow in the following season.

Applying the microchip: The microchip is applied to the subcutaneous part on the left side of the neck; it is necessary to massage the place well afterwards to ensure that the microchip does not slip out. The reader checks if the microchip transmitter is readable and the sticker with the corresponding identification number is stuck to the capture protocol.

Blood samples: Blood samples are collected for veterinary, medical and genetic analysis during the narcosis. Blood collection should be done at approximately the same time as the narcosis to avoid possible biases in the blood tests.

Blood sample is taken after appropriate preparation (air removal, disinfection, trumpet on the veins, Fig. 9.) from the vein on the forelegs (vena cephalica, Fig. 9.). The following samples are taken (approximate amounts are valid for adults, healthy animals depending on the size of the lynx):
- 4 ml of blood in tube with EDTA,
- 5 ml serum,
- 5 ml of blood in tube with Heparin.

Tubes with the blood samples must be labelled immediately. Immediately after the capture, the blood samples are centrifuged for the serum.

Figure. 9. Blood sampling.

2.5. Protocol on tranquilization of the FIWI

Protocol on narcosis

Date: _____ Place: _____

Data filled in by: _____

Weather conditions: _____ Air temperature/moisture: _____°C / _____%

Species: _____ **Gender**: _____ **Age**: _____

Weight: estimated: _____ weighted:_____

Animal´s name or ID: _____ Microchip number: _____

Status: O overweight O good O average O poor O cachectic

Health status: O good O poor O injured O questionable

Reason for tranquilization: O Collaring/marking and sampling O Health check O veterinary-medical treatment O transport Animal´s status before a tranquilization: O free living O captivity O quarantine

Capture method: O tranquilization weapon O box trap O foot snares O MICS O net trapping O blow pipe O capture net

Animal´s behaviour before capture: O calm O stressed O aggressive O scared O apathetic O active, but normal

Time (Hour)	Time (t)	
		Animal in the trap
		Calm/unstable on the legs (first symptoms of the narcosis)
		Lying with head up
		Lying on the side (narcosis)
		The animal is picked up
		Beginning of veterinary-medical examination/collaring
		Blood sampling
		End of veterinary-medical examination/collaring
		Weighing (capture site/quarantine station)
Microchip number	Microchip number	Microchip number
		Lifts head up (first symptoms of convalesce)
		Standing
		Moving away
		Released

Narcosis:

Substances	Dose	Injection administered to	Time (hour)	Time (t)	Effect	Duration

Additional Information/Notes (Complications, Problems, etc.)**:**

Narcotics and surveillance

Time (hour)												
Time (t)												
FR												
HF												
Pulse												
CM-colour												
CFT												
O2-volume												
T°												
ROE												
ROC												
RE												
ROL												
Gasification of bark												
POI												
IR												
IM												

Time (hour)												
Time (t)												
FR												
HF												
Pulse												
CM-colour												
CFT												
O2-volume												
T°												
ROE												
ROC												

RE												
ROL												
Gasification of bark												
POI												
IR												
IM												

Frequency of respiration (FR) and **Heart frequency** (HF) are listed in heart rate and respiration per minute. The pulse can be detected, for example, on Vene Femoralis. Check for the heart rate (absent = pulse deficit), strong, pulsating or weak. (normal, strong, no deficiency). **The colour of the mucous membrane** (CM-colour) is considered in the oral cavity: white/light/light-pink/reddish/bluish/violet/yellowish/etc. (normal: light-pink). **Capillary filling time** (CFT) is measured, by pressing the finger on the animal´s oral mucosa until it causes a bright spot, then counting for how many seconds it will elapse until it acquires a normal colour again (normally: 2-3 seconds). **Body temperature** (T°) is measured in degrees Celsius by a rectal thermometer (until the digital thermometer beeps) (normal temperature 38.0-39.0 ° C). **Reflex of the eyelid**: Touching with the finger on the animal´s lid leads to a blink (depending on the intensity: + / ++ / +++). In narcosis, reflex is absent (-). **Reflex of the corneas**: Touching the animal´s cornea with the finger leads to a blink (depending on the intensity: + / ++ / +++). In narcosis, reflex is absent (-). (ideal: reflex of eyelid absent, however corneal reflex is functional). **Reflex of the ears**: Touching the animal´s ears leads to their movements (+/-). **Reflex of the legs**: When pressing the skin between the fingers, the animal pulls the leg back (+/-). (In narcosis, reflex is absent) *ATTENTION: Reflexes are still present during the ketamine narcosis!*

The doses administered are in the liquid state. Place of injection (POI) = thigh muscle (T), shoulder (S), half to half (T/S) / injection route (IR) = intramuscularly (IM), subcutaneous (SC), intravenous (IV), oral (O) injection method (IM) = with hand (H), blow pipe (BP), tranquilization weapon (TW).

3. Eurasian lynx translocation

3.1. Lynx transport

Transport is stressful for any animal species and especially wild animals. During the transport, the possibilities for monitoring or intervention are limited and therefore it is necessary to conduct lynx transport carefully to ensure safety of animals and people.

Transport of animals can only be done if the animal is fit for the intended journey. The detailed requirements regarding the fitness for transport are described in Chapter I of the Annex I of the Council Regulation 1/2005, but the most important are:

The lynx must be transported in conditions that guarantee that they will not be injured or endure unnecessary suffering. The lynx must be able to move independently without pain and be able to walk unassisted. Sedatives shall not be used on animals to be transported unless strictly necessary to ensure the welfare of the animals and shall only be used under veterinary supervision.

Means of transport must be designed, constructed, maintained and operated in such a way to avoid injuries and suffering and ensure safety to the animal transported. Animals must be protected against bad weather, extreme temperatures and unfavourable climate changes. Floor must be non – slip and impermeable for urine and faeces. Access to animals must be provided in a way that they can be easily checked on, observed, and appropriately taken care for.

Transport vehicles must have the capacity to have overhead lighting so that lynx can be illuminated during transport so that check – ups and appropriate care can be provided to the animal(s). Animals must have enough space to stand in a natural body posture. The airflow must not be obstructed and there must be adequate ventilation at all times.

Transport is defined as the moment when an animal is first loaded into a vehicle until the moment when the last animal exits from transport vehicle to the final destination, or when animal is at the final destination for at least 48 hours. Transport time involves all stops during the journey.

The lynx will be transported in the specially designed transport boxes described below.

3.2. Transport boxes

The most detailed description of the requirements for the transport of wild animals can be found in Live Animal Regulations (LAR) from IATA[1] and CITES[2] guidelines.

The basic requirements for transport boxes are:

Size: When standing, the animal has a minimum space of 10 cm in each direction. In lynx (adult male body length is about 100 cm, height 55-60 cm and width 15-20 cm), it means

[1] The IATA Live Animals Regulations (LAR) is the global standard directive for safe animal transport, humane and cost-effective: http://www.iata.org/publications/pages/live-animals.aspx.

[2] www.cites.org/eng/resources/transport/index.php – CITES guidelines for air transport of live the wild animals and plants (access 07.03.2018).

an interior space of approx. 120 x 70 x 40 cm (L x H x W). The height and length are sufficient, but the width must be chosen so that the lynx can simply rotate and lie down. For this reason, two sizes are suitable for the transport of lynx: 120 x 66 x 60 cm (L x H x W; large boxes) or 90 x 60 x 50 cm (D x V x Š; small boxes).

A smaller version is intended for smaller animals, while a larger box for larger animals and longer shipment to the place of release. In both cases, it is important to provide relatively small holes that ensure optimal ventilation.

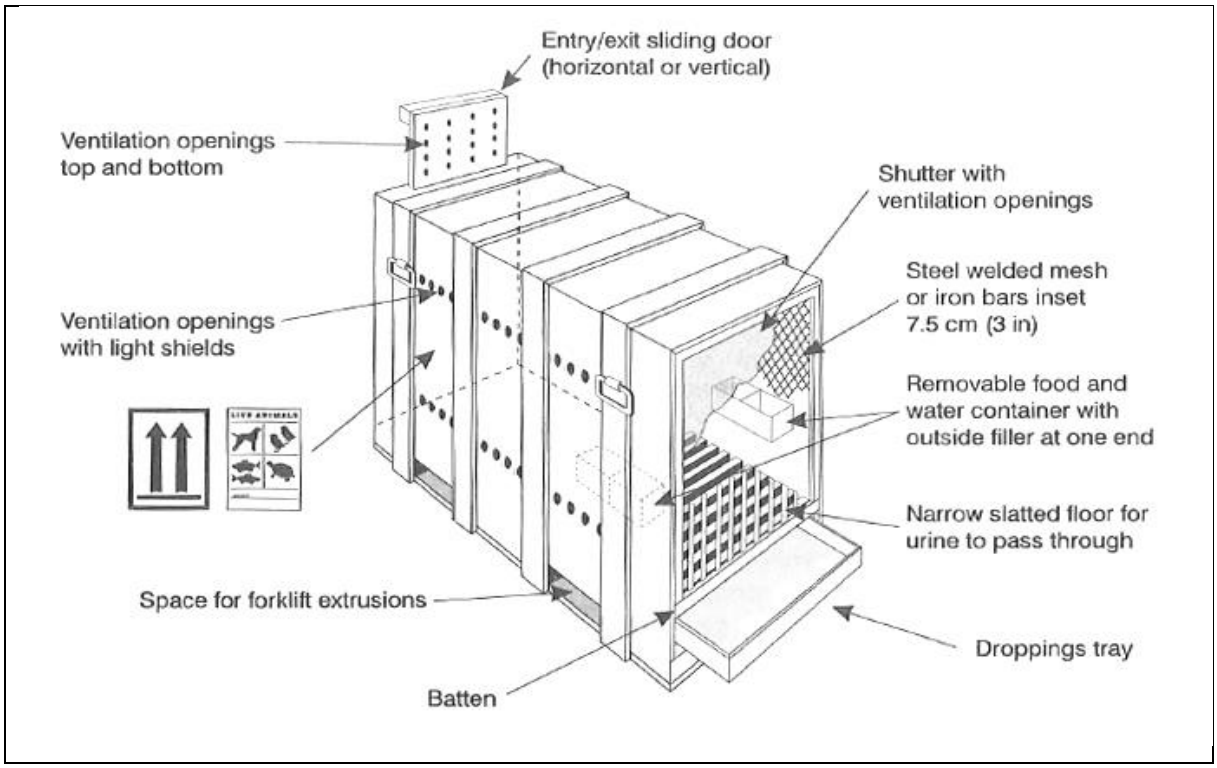


Figure. 10. Transport box for medium to large animals according to IATA requirements. Source: IATA Live Animals Regulation, 39th Edition, October 2012, page 340.

Material: strong enough to prevent attempts to escape. Wood and metal are the usual materials used. But plywood boards are also appropriate. Enhanced parts, handles, observation holes, sliding doors, etc. are made of solid wood or metal (iron, aluminium). Covers (holes, ventilation) of wire mesh and/or fabric (airy and non-resilient).

Ventilation: Good ventilation of the transport box is extremely important! In ideal cases, there are a series of ventilation holes on the top and bottom of the box that provide sufficient air intake. Sliding doors (one-sided) or (on both sides) should be designed with sliding bars for optimal aeration. Optionally, more massive sliding doors are constructed so that both open (sliding bars) or closed versions are available. The ventilation openings can be covered with airborne fabric.

Construction and equipment: Inner walls of the transport box must be smooth. In the case of untreated boards, these must be removed! The ventilation and observation holes must be designed so that lynx cannot damage their teeth or claws. Wire or metal parts that can be

reached by teeth should be avoided. The lower part of the boxes is covered with brass or wood shavings that can absorb urine and, in the case of a slippery floor, it is possible to add a strong rubber mat.

Doors on both sides are recommended because they provide more handling and ventilation options. Boxes have different variants of sliding doors that can be exchanged: (1) massive doors for 'normal' use, (2) metal bars that can be used if the animal has to be well observable or handled (3), massive with inner doors (small drop-down doors) which can be used to tranquilize/wake the animal in the box, and (4) cover or compression plates that can be used for door exchange.

Whenever possible, animals should get used to the transport boxes. For this reason, the transport box is placed during quarantine within the enclosure with doors open on one side to allow the lynx to enter.

3.3. Transport vehicle

Animals can be transported only in registered transporter TYPE 1 (up to 8 hours driving) or TYPE 2 (over 8 hours driving). Transporter (TYPE 1 or TYPE 2) must be licenced for animal transport. Both transporter types will be provided by the National Zoo Bojnice as a licensed organization.

Only transporter TYPE 2 (over 8 hours driving) documents must be carried next to the certificate of approval for a road transport vehicle.

Written instructions on feeding, powering and required special care – Movement document, are required. Journey log according to Regulation 1/2005 is not required.

3.4. Transport procedure

Lynx must be tranquilized according to the pre-transport rules (export medical examination, setting of the telemetric collar). However, the lynx must not be transported in narcosis! The transport box is closed only after full recovery from the narcosis and the transport starts only after the animals are fully conscious. Attention is to be paid to (1) the closure and stabilization/fixation of the boxes, (2) ensuring sufficient access to the transport boxes, and (3) sufficient ventilation. Even if the transport box has suitable ventilation this will not be sufficient if the load compartment itself is not properly ventilated!

Transport and procedure during transport: Lynx reaction and behaviour during transport may be very different, depending on the animal, from peaceful to extremely stressed and wild individuals. During the transport the following points are important:

• Keeping the animal in dark conditions (but with adequate ventilation!). Night transports are not necessarily beneficial due to enormous changes in the light conditions;

• Do not stress the lynx during transport. Necessary monitoring takes place discreetly and only when needed (ideal - a remote camera);

• Avoiding noise (including loud debate or laughing), minimizing external noise through appropriate route selection;
• Ensure transport equipment and fuel for the car as well as liquids (enough of cold water!) for both the animal and the car, breaks during transport are possible only when necessary (in long distances, two drivers are needed).

Since it is often not possible to meet all the requirements at the same time (e.g. good ventilation and darkness is often not easy to combine), the animal must be monitored. Concrete measures are depending on the lynx behaviour. However, the animal may not be stressed with invasive monitoring. An important part of monitoring can be done with remote infra-black/red camera or acoustically! Generally, the lynx in full consciousness does not have problem with the cold (unlike animal in narcosis!), but the main risk is hyperthermia. The interior of the vehicle should therefore be cool (e.g. 15-20 ° C). An air-conditioned vehicle must be used during higher outside temperatures.

A healthy lynx does not need any food during the transport for approximately 8-10 hours of travel, with mild temperatures. With a good transport progress, there is also no need for water. For transport over 8-10 hours water must be supplied. Moreover, if the animal is hyperventilated and there is a risk of overheating, there must be cold water available. In such cases, the transport should be also interrupted and the box placed in the shaded area with sufficient ventilation.

3.5. Documents required for lynx transport.

Each animal consignment must be accompanied by veterinary health certificate with the requirements defined by the competent veterinary authorities in the destination country and country of origin. Veterinary health certificate issued by an official veterinarian has to include: species, number of animals, definite identification (microchip), contact address of shipper and receiver, confirmation (at least approved by responsible veterinarian within 24 hours before start of transport) that the animals are healthy and fit for transport, free from diseases and the transport box is disinfected. Veterinary certificate must be announced in TRACES and provided in paper version and official veterinarian is in charge for this.

Movement document (part of the TRACES, also called as the Route plan) with indication of species, number of animals, definite identification (microchip), place of shipment, unloading site, vehicle registration number, last time of animal feeding, name and signature of the person in charge of the transport from the National Zoo Bojnice.

The notification of consignment must be sent to the final destination country using the electronic veterinary system TRACES, so that the Competent Authority at the place of destination is informed of the arrival of animal(s) in advance.

CITES certificate is provided by the responsible ministry (the Ministry of Environment of the Slovak Republic).

In addition to the above, the following additional information must be provided by the responsible person in charge of the transport from the National Zoo Bojnice:

- Lynx passport (picture and information about the animal),
- Quarantine protocol if the lynx was in quarantine (diary—e.g., lynx behaviour, feeding, observations, etc.),
- Medical report (official reports that documents all medical treatments including rabies vaccination and tests),
- Permit for translocation (included for all animals in the official permit on lynx capture, manipulation and translocation issued by the Ministry of Environment of the Slovak Republic).

4. Lynx quarantine

4.1. Infrastructure and animal handling

Between capturing and translocation, lynx must be in quarantine to carry out health analysis required for export documentation. Also, quarantine can help to break the homing-effect, in order to prevent the return of the lynx to its original home range. The minimum duration of quarantine in Slovakia, under the condition that animals are rabies-free, is 21 days. During this time, no physical contact to other captured lynx is allowed. If new lynx are placed in neighbouring enclosures in the quarantine station, the start of the 21 days period begins with the arrival of the second animal.

The quarantine station should be isolated both visually and acoustically from people and other animals (e.g. the forested background of the National Zoo Bojnice). The station includes a covered passage with access to individual enclosures. These enclosures are divided into small acclimatization parts and larger outdoor parts. Climbing and elevated places as well as hiding possibilities and the inner shelters (boxes) for protection against rain and cold are included in both parts. The quarantine station should prevent the lynx from escaping and the risk of injuries to both humans and animals during quarantine should be minimized.

Educated staff should be present daily to take care of the captured lynx. The enclosures should be cleaned at least once a week from faeces and food remains. Fresh water and food, preferably fresh roe deer and red deer meat should be provided. After the animal´s transport for release, the entire enclosure has to be disinfected.

Figure. 11. The quarantine station in the National ZOO Bojnice.

Figure. 12. The station includes a covered passage with access to individual enclosures, that are divided into small acclimatization parts and outdoor parts (Fig. 13).

Figure. 13. Four outdoor enclosures.

Figure. 14. Climbing and elevated places along with hiding possibilities and the inner shelters are included in both parts.

4.2. Veterinary procedures

At capture location animals will be weighed and examined clinically, with particular attention paid to their body condition, size and weight, teeth, claws and genitals. A microchip is administrated to the animal for further identification. Lynx in a normal body condition (considering that adult males may be thinner during the mating than in other seasons), aged more than one year (with the exception of females less than one year old) but not more than approximatively 12 years old (estimation based on body size, weight, appearance of the genitals and teeth), without significant clinical abnormalities, will be considered as adequate for translocation and will be transfered to the quarantine. Older-aged lynx, or animals with a nonlethal malformation of potential genetic origin will be released at capture site; may be radio-collared to follow the progress of their conditions and to eventually recover their carcasses for

pathological examination. Lynx younger than one year (with the exception of females) or with a disease or trauma with good chances of healing (e.g. mange after appropriate treatment) will be released on site with a collar to be re-captured at a convenient time. In some cases, a transfer to the quarantine station for more intensive care may be considered. However, animal welfare questions must be considered (e.g. stress induced by transport and captivity may have a negative impact on health), as well as the risk that an animal suffering from an infection may infect other lynx in the quarantine station.

At the capture location, all animals are marked with a microchip and those to be translocated must be vaccinated against rabies, with EU-approved vaccine for felids. In Slovakia Nobivac Rabies® is used. After one application, the antibody level is achieved for at least 3 years. A combination of other vaccinations (e.g. panleukopenia virus [FPV] and feline leukemia virus [FeLV]) is not recommended, because these are functional only after 2 applications (ranging from 2 to 4 weeks) and remain active just for 1 year. Another aspect of multi-vaccines is that they burden the animal´s system, which is still in the stress of capture and the new environment. The immune system has to create antibodies, however along with the metabolism it does not work properly in stressed animals. The FeLV vaccine is especially dangerous. If the animal ever came into contact with the virus, the vaccination will lead to a leukemia disease.

Blood samples are taken from all captured animals and immediately tested for Feline Leukemia Virus (FeLV), panleukopenia virus (FPV), Antigen and Feline Immunodeficiency Virus (FIV). Further tests include analysis for Corona, Parvovirus, Calicivirus, Herpes, Bartonella and Ehrlichia. FeLV, FPV and FIV positive animals will not be translocated.. Further actions with the positive animals will be discussed with the responsible authorities (the Ministry of Environment of the Slovak Republic, the State Nature Conservation and the National ZOO Bojnice.

Also, blood samples taken at capture will be analysed in the laboratory. Hematology and blood chemistry values will be compared with reference values obtained from clinically healthy free-ranging Eurasian lynx. Animals with blood values significantly different from reference data will be submitted to additional testing as appropriate.

Feces taken from rectum during narcosis at capture site or first feces found in the transport box/enclosure will be collected and analysed for lung and gastro-intestinal parasites. At capture, all lynx to be translocated will receive antiparasitic treatment. This treatment has to be done because the stress reduces the animal´s immune system and the parasites can proliferate (within 2 weeks), which can endanger the lynx. Antiparasitic treatment is administrated against macrocyclic lactones and praziquantel roundworms, tapeworm, protozoa (*Isospora*) and external parasites (fleas, ticks, mosquitoes).

Antibiotics or any other drug will not be administrated unless it appears appropriate based on the clinical findings.

Only above mentioned infections (FeLV, FPV and FIV) are considered as a criterion for not translocating the captured animal. Other agents such as Canine Distemper Virus (CDV) or

Parvovirus may cause disease in lynx but are also known to circulate in healthy populations. In such cases, the clinical status and blood parameters of the animals will be a more relevant criteria to evaluate than the health status of the animals from the infection per se. Finally, infectious agents such as *Cytauxzoon felis* and intestinal worms are widespread in clinically healthy lynx and can be considered as part of the lynx' "fauna and flora." Subsequently, their detection should serve as documentation for the long-term health monitoring of the source and reintroduced populations, but not be considered a criterion for translocation.

Samples for genetic analysis will be taken at capture locations and tested thereafter. In case of close relatedness with other animals already translocated or simultaneously kept in quarantine for translocation, the animal will be excluded from the translocation program and will be collared re-released in close vicinity of the capture site as soon as possible.

Samples for additional tests of scientific value but not relevant for the translocations will be compiled and stored and analysed at the end of the translocation period.

Between the 10th and the 14th day after capturing, the rabies vaccination blood samples must be taken to analyse rabies antibody titter by EU accredited laboratory. A neutralising antibody titration must be at least equal to 0.5 IU/ml. If the antibody titter is not high enough (less than 0,5 units/ml) the lynx must be vaccinated again and remain in the enclosure for another 21 days after a second vaccination and then be retested for the correct level of antibody titter (at least equal to 0,5 units/ml).

Before transport to the release site, lynx will be tranqulized and fitted with a radio-collar and undergo another clinical check before transportation. Blood samples will be taken for archive purposes, but unless there is a specific indication, no tests will be performed at this point. If at this point, a reason for exclusion not noticed earlier would be detected, experts in charge would consider three options: (1) release of the animal at the original capture site, (2) euthanasia, or (3) prolongation of the quarantine (with treatment as appropriate and subsequent re-assessment).

Information about the transport must be filled in electronic system TRACES by licenced veterinarian. The official veterinarian will examine the shipment and complete the third part of the certificate.

At the destination (location of the release of the animals) official veterinarian from the destination country must be present and must confirm the arrival of the animals through the electronic system (TRACES).

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