LIFE Lynx

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

LIFE16 NAT/SI/000634

Protocol for capture, transport and quarantine in the Romanian Carpathians

C.2 Live-capture and translocation of lynx from the Carpathian population in Romania for reinforcement of the Dinaric-SE Alpine population

March,2018

Introduction

The present document is required by the need to ensure a technical framework for the LIFE Lynx project team, to capture the planned number of Eurasian lynx individuals, from the Romanian Carpathian Mountains, and to safely translocate them to Slovenia and Croatia. It`s goals are to develop a framework for the project members/partners, detailing the use of the requested equipment and the work that has to be done to maximise the capture success, handle with low impact the captured individuals and ultimately safely reinforce the Dinaric – Alpine *Lynx lynx* population. This protocol is required to obtain necessary permits for lynx capture from the competent authorities, Romanian Academy, Ministry of the Environment, Environmental Protection Agencies, custodians of protected areas (where necessary), game district managers, Veterinary Agencies and Border Police, as well as to agree among partners, about procedures to be adopted. After each winter, i.e. the main capture season, activities will be evaluated and this protocol adapted upon need.

The principles that governs this protocol are: (1) *avoid common mistakes that can lead to low capture success,* (2) *prevent any injuries of the captured lynx, and (3) prevent injuries of people*. The protocol contains in detail all techniques, methods, and procedures necessary for the safe capture, quarantine, isolation, and transport of lynx to selected reinforcement areas, in respect to all legal requirements and best practice experiences from previous wildlife capturing by the project personnel.

We like to mention that this document is derived and adjusted for Romania from the KORA protocol **Dokumentation Fang, Narkose und Markierung von Raubtieren (2013)** from Urs Breitenmoser, Andreas Ryser and Marie-Pierre Ryser-Degiorgis.

Content

Section 1 Capture

1.1. Capture season

The most appropriate period for lynx capture is from February to April before and during the mating season, when lynx move more actively (and are probably less cautious). This period has two other benefits: (1) the young lynx are large enough to be marked with a GPS/GSM collar; (2) young lynx can survive without their mother and therefore we are able to capture and translocate adult females, as well as their offspring.

To avoid kittens being captured/injured and bycatch of juveniles/bear cubs the main rules governing the capture season are:

a. Main capture season mid-December to mid-April (kittens are big enough, bears are less active)
b. Previously identified individuals (e.g. males) can be captured year-round.
c. Box traps are placed in the field starting mid-September, before the snowfall, when capture sites are more accessible, but will only be activated after December.

1.2. Capture site selection

1.2.1. Main rules for selecting a capture site:

a. Site selection is based on results from telemetry, snow tracking and camera-trap monitoring.
b. Roads in steep and rocky areas have segments which "forces" traveling animals to use the road, such natural "force crossings" on a lynx path are ideal trap sites.
c. Recommended to be on a forest road, wildlife path or other linear structure.
d. If possible, use old houses, wood cabins or other abandoned infrastructure visited regularly by lynx for territorial marking.
e. The site should have a low to zero human activity and/or owner of the infrastructure is informed and agreed to the lynx capture on the site.
f. They have to be accessible by the field team within a max. 3 hours (considering both driving and walking) during the day.
g. The walk to the capture site should be preferably less than 30 minutes. For very good spots maximum one hour of walking is acceptable.
h. The capture site should be chosen in order to allow a team of 4-5 persons to move safely around the box.

1.2.2. Rules for managing the capture site:

a. At least one camera trap per site will be used. Video recording is recommended
b. Work should be done considering the minimum disturbance of the site
c. Camera trapping monitoring starts in mid-September.
d. The box size (larger or small) is to be determined according to each capture site accessibility and habitat structure (e.g. larger box traps at more accessible sites and those in more open habitats).

1.3. Equipment (KORA protocol)

1.3.1. Box traps

Box traps are constructed in a way that minimises the risk of injury to any animal caught. They should be smooth on the inside (e.g. made of plane wood or round logs) and dark when closed. The doors should be padded to avoid injuries when falling or closing. The size of the traps must be large enough

that an animal is completely inside the trap when it releases the trigger. All box traps are equipped with an alarm system (satellite, GSM of VHF).
For the trapping activity there will be available two types of box traps:

- Small box trap: ca. 740 x 840 mm, length 2000 mm
- Normal box trap: ca. 1200 x 1200 mm, length 2500 mm

Building instructions are available in the Annex 2.
Box traps are made of waterproof plywood and a steel/aluminium frame. Paddle doors are moving in metal rails and provide fast closing of boxes that are generally light-tight with sufficient air ventilation as well. The bottom edge of the door is lined with a thin rubber band. Boxes are triggered/activated by a nylon 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/activated.
A small lockable window is left in both paddle doors for visual inspection. When captured, lynx is narcotized through these windows with blow pipe.

Fig. 1 Small box traps built using KORA recommendations and measures, but with bear break-out pins and additionally the box trap can be folded.

1.3.2. Snare traps (on killed prey)
Foot snares can be used to capture lynx at fresh kills, but any use of holding device for captures needs to be done in accordance to national legislation and regulations. Foot snares must only be used by trained personnel and have to be set in a way to minimise the risk of injuries to any animal caught and need to be permanently surveyed by an alarm system. **An animal caught in a snare trap must be reached within max. 5-15 from the start of the alarm.**
Special caution has to be taken with regard to the risk of capturing a (young) bear. The trap should be seen from a safe distance. The surveillance of snare traps by means of an (infrared) photo or video trap is highly recommended.

Fig. 2 Snare traps developed and recommended by KORA to be used at kill sites

Foot snares resemble the "classic" traps used by Canadian fur trappers, but they are fundamentally different in their function. The round base (Figure 2) represents a throwing mechanism that does not have any capture function and is completely separated from the tube 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 6 x 9 mm stainless steel wire, which is placed in the groove on the jaws. The wire/snare extends to the throwing mechanism, which throws it over the animal´s limb after the trigger mechanism was initiated. 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 limb injuries as well. Foot snares are efficient and largely safe capture method. Exceptionally, scratch or local wounds of the hind legs can occur due to a great jump strength or try to release through the forelegs. The wires used to capture are replaced by new ones after each capture.

1.4. Preparing the site for capture

1.4.1. Box traps

a. Forest roads, wildlife path or other suitable site

Local authorities, hunters and land owners must be consulted and asked for permission. Approximately 50 meters away from the box trap, signs and panels are placed with information to alert public. Phone numbers, addresses (inc. email).as well as web page, are included for further questions and information on the project.

The box trap should be placed in the middle of the selected linear structure. The rest of the forest road must be blocked by installation of an optical and or physical barrier out of e.g. wood /branches/ camouflage net. The team finally checks the functionality before leaving.

After a capture signal a team must check the trap as soon as possible. In bear areas, special caution has to be taken with regard to the risk of capturing a (young) bear. In the case of the capture of a large bear, the box trap is designed with pins that will break upon strong pressure. The trap should be seen from a safe distance. The surveillance of a trap by means of an (infrared) photo or video trap is highly recommended.

The path leading towards the trap should be regularly cleaned. In winter when snow cover is present, trail in the snow should be made and regularly maintained.

b. Old wooden houses, abandoned structures

At the old wooden houses or abandoned structures it is important to identify the marking places (corners,doors other) and the path around/inside the infrastructure. The box (preferably large) will be build based on this information, preferably inside the building.

Rules for managing the box traps:

a. A team member will be responsible for each box trap. When the person is not available, he/she is responsible to find the replacement person. In the event that no other persons is available to assume responsibility, the box trap will to be blocked.
b. The box should be fully stable and horizontal.
c. The bottom of the cage will be covered with leaves or other natural material present on the ground at the location.
d. Once the box trap is active the alarm will be activated. If the cage is not activated for longer time period, the alarm should be removed to avoid damage or theft.
e. For alarm equipment there will be available satellite, GSM or VHF alarms, the selection will be made based on the site conditions (accessibility, GSM signal). VHF alarms on box traps have to be checked every morning.

f. Trigger mechanism and quick falling of doors will be checked at each visit.
g. The wire from the trigger mechanism should be high enough not to be triggered by small mammals and low enough not to reach the brisket or the head of the animal.
h. All the ice/mud from the doors will be removed at each visit.
i. For each trap, the responsible will have to keep an activity log book/form proposed in Annex 3 (when it is active, how many and which animals are caught, how often checked, other remarks).
j. At each visit, the box trap will be checked to be fully functional and refurbishment should be undertaken on the spot. If damage occurs and the box is not useable any more it will be removed from the field.

1.4.2. Snare traps (on killed prey)

If a fresh lynx kill site is discovered:

a. The kill is tied to a tree or earth screw.
b. 2 - 4 camouflaged foot snares are set near the lynx kill.
c. The tubes are anchored depending on the situation with steel wire to the trees, or with earth screws.
d. Traps are continuously monitored.
e. 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 max. 30 minutes, and immediately responds to the alarm.
f. The captured lynx is fixed by the catch net and narcotized by syringe, or directly by the blow pipe.
g. During the placement of individual snare traps, the extent of the movements is taken into account in order 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. Only few animals may approach to the lynx kill, and therefore non-target captures are very rare.

1.5. Captured animal (activated alarm)

IMPORTANT!! All members of the capture team need to know the protocol and need to be trained before each capture. Simulation will be carried out with all members of the project and existing

volunteers. Each member of the team must know what to do in advance, and a clear command line is fundamental for a safe capture. The team leader is responsible for the coordination of the field workers.

Checking traps

No checking of box traps during the night. When there is an alarm the field team controls the trap early in the morning. With satellite alarm, a message is sent every day to test that the alarm system is working.

The equipment for checking the trap consists of: string, tools, shovel, extra mousetrap, cord for holding the door (at least 4 m), batteries, door padding, blanket for covering the box trap if a lynx is caught, bear spray, saw, and first aid kit.

When approaching a trap the team first listens from a distance, next the existing sensor camera will be checked to identify the animal inside the box. Once the animal is identified decisions are made accordingly and in the framework of this protocol by the team leader.

Box traps

The person responsible, for the box trap site, checks trap after an alarm signal and contacts the handling team when a lynx is captured. Lynx remain generally calm in a dark box trap after a while. While waiting for the capture team, the person responsible for the box trap should stay at a distance whereby they are close enough to observe the situation, but not to scare the animal. Animal immobilisation in the box trap is best done by blow pipe.

Snare traps

Snare traps are checked immediately (within max. 30 min) after the alarm.

A lynx in a foot snare even if safely anchored has a certain freedom of movement. Capture teams must be aware of this and take the precaution to minimise the risk of injuries to people and animals. Immobilisation is best done by means of a blow pipe gun or by fixing the lynx with a net and manual injection.

All non-targeted animals caught in any of the traps have to be released immediately at the site, with no other supplementary interventions. Bycatches must be handled in a way to minimise the risk of harm and injuries and should be freed without immobilisation whenever possible. Capture teams have to take special precaution to be able to handle the capture of bears without risk to the people or animal. As a principle, capture teams include only trained personnel and are as small as possible. Local hunting ground manager presence should be considered for the safety of capture team. Noise, bright light and hasty movements are a stress factor for all animals caught and need to be restricted even if an animal is immobilised.

If a bear is caught and the release mechanism of the box is not functional, the field team will try to release it considering the following solutions:

- Check the area for other people and give warning.
- Try to use a rope to open the door from a distance. If this is not possible wait, there is no hurry.
- If the bear cannot be safely released tranquilize the bear.
- If a cub is caught and the release mechanism (planned for the doors) did not function, an option is also to wait for the female to go away.
- Bear spray is safer than gun.

IMPORTANT!! The presence of spectators (e.g. media people, officials) is restricted.

1.5. Tranquilising procedure (KORA Protocol)

For the lynx the most suitable anaesthesia is performed with a combination of sedatives and narcotics. Experience has shown that, an initiation with **medetomidine** followed by an injection of **ketamine** leads to a reliable, low-risk anaesthesia of lynx in all age groups, including animals in poor health.

1.5.1 Narcotics

Medetomidine is a sedative and can be applied intravenously, intramuscularly or subcutaneously. For the lynx, medetomidine preparation Domitor® (1 mg medetomidine hydrochloride / ml), has mostly been used. After intramuscular injection, medetomidine is rapidly absorbed. For Domitor® it is stated that its kinetics in the blood plasma after intramuscular injection are very similar to those after intravenous injection; the maximum plasma concentration was reached within 15-20 minutes and the elimination half-life was about 1.5 hours.

The administration of medetomidine leads to sedation (lowering of consciousness), analgesia (pain insensitivity) and muscle relaxation. When fully effective, the animal is relaxed and no longer responds to external stimuli. But medetomidine can lead to a number of side effects and possible complications. Of particular importance to wildlife is that medetomidine interferes with thermoregulation (especially the lynx risk of hypothermia) and may result in decreased performance of the respiratory system and blood circulation.

Medetomidine is decomposed in the liver and its metabolites are excreted mainly via the kidneys (in the urine). The effects of medetomidine can be reversed by the injection of the specific antagonist atipemazole (see below). Without antagonization, ataxia may occur during the recovery phase

Ketamine (Ketasol-100, 100 mg Ketamine / ml) is used to completely eliminate the awareness in addition to medetomidine. It is an anesthetic (cyclohexane) with hypnotic properties that causes a functional and electrophysiological "dissociation" between the thalamus and the limbic system. It has a fast but relatively short effect (about 30-60 minutes, depending on dosage). Important potential side effects include an increase in blood pressure and heart rate (which, in combination with medetomidine, partially compensates for the sedative side effects), initial suppression of respiration and, if used alone, a high risk of convulsion. Metabolism occurs in the liver and urinary excretion. Ketamine can be injected intramuscularly, intravenously or subcutaneously. There is no antagonist for ketamine but this substance is characterized by a high therapeutic index. In case of overdose, the convulsions can be attenuated by the administration of diazepam (Valium®).

Atipamezole is an α2-antagonist. This drug abolishes the sedative effect of α2-adrenergic agonists (medetomidine). It can be administered intramuscularly, intravenously or subcutaneously and is excreted through the urine. Both Antisedan® or the generic drug Alzane® can be used. It is stated that atipamezole exerts its effect after 5-8 minutes after intramuscular administration, but that there are species-specific differences. High doses can cause increased heart rate (tachycardia), increased or decreased blood pressure, anxiety, shaking and cramping. If medetomidine has been injected in combination with a cyclohexane (ketamine, tiletamine), atipamezole should not be administered until at least 30-45 minutes after the last cyclohexane injection (i.e., after the cyclohexan drug has stopped working).

1.5.2 Anesthesia

Introduction phase. If a trap is used for the physical immobilization of the animal, the approach to the trapped animal is made as soon as possible after capture and with as little disturbance as possible (quietly and with the minimum necessary number of persons), so that the stress condition of the animal unavoidable remains at a minimum.

An animal to be anesthetized is first injected with the dose of anesthetic calculated on the basis of the estimated body weight. It is aimed at the thigh muscles.

The dose used is identical for subadult and adult lynx of both sexes: 2.8 ml Domitor® and 0.8 ml Ketasol-100®. Based on the actual weight, this results in a range of 0.11-0.16 mg / kg medetomidine and 3.2-5.5 mg / kg ketamine (Ryser et al., 2005). It is difficult to determine the age and sex of a full-grown lynx who is in a trap. Although lighter animals tend to sleep deeper than heavier animals, the standard dose has proven to be efficient and safe in all cases. In juvenile animals, the dose is adjusted to the estimated or known weight. After the Domitor® injection wait about 20 minutes until the lynx shows signs of deep sedation (lying down, head on the floor, no reaction to slight noises). This first dose is administered either by hand or with the blowpipe depending on the method of capture. If it is possible to observe the respiratory rate, this parameter will be measured from this point in time. When the lynx is sedated, it receives the ketamine 100® injected by hand into the thighs. Upon entry of the actual anesthesia, a decrease in the respiratory rate is detected. After 2-5 minutes, the lynx's reaction is checked for acoustic signals or contact.

Typically, the following six stages are expected. If the anesthesia is as expected, all animals will be in Stage 6 when they are first manipulated. If an approach / manipulation occurs too early (e.g., stage 4), there is a great risk that the animal regains full consciousness; in this case subsequently the chemical immobilization of the animal is more difficult and is also associated with greater anesthetic risks.

1. movement-incoordination, balance disorders,
2. lying down,
3. lying, head still held up,
4. lying, head down, gets up again when irritated,
5. lying, head down, barely reacts when irritated,
6. lying, head down, no reactions to stimuli (slight sound, touch).

If no more reactions are detectable, the animal is taken out of the trap. The respiratory function is briefly checked before it is moved for further manipulation (depending on the weather, terrain and possibilities in the field: a flat open place for the optimal storage of the animal and better conditions for the manipulation, sheltered place to protect from rain or snow and / or to reduce the risk of hypothermia).

Monitoring phase. The monitoring of the anesthetized animal shall be recorded using the form in Annex III. For the manipulations, the animals are placed in a lateral position. The limbs are tied together. The head is stretched cranially so that the upper airways are exposed. It is checked that the mouth contains no food remains or foreign bodies and the tongue is stretched out. A protective eye cream (Viscotears®) is applied to the cornea and the eyes are covered with a cloth. In cold conditions, animals are placed on a sheepskin and tarpaulin and covered with blankets. Depending on the body temperature, these are removed or hot bottles are placed to the animal. The equipment that can be carried in the field for anesthesia is limited. Indispensable are a thermometer, since the risk of hypo-

or hyperthermia is particularly great in field conditions and body temperature (see section 1.5.3) must be inspected periodically, and a watch. We also use a stethoscope and a portable pulse oximeter (to measure oxygen saturation in the blood and monitor the pulse).

Vital signs (at least respiratory rate, heart rate / pulse, mucous membrane color, capillary filling time) and reflexes (especially pupillary, corneal and ear reflexes) and body temperature are monitored as soon as possible and throughout the duration of anesthesia; a member of the team is responsible for ensuring that the investigations are regularly carried out and recorded. The doses of the administered agent (in ml), the times of all interventions, the observations (fall asleep, wake up, etc.) and measurements are recorded in the anesthesia protocol.

Canceling the anesthesia and post-release observation. After all the necessary manipulations have been completed, the anesthesia is antagonised as soon as possible. The effect of medetomidine is antagonized with atipamezole (5 mg atipamezole per mg medetomidine, ie 1 ml Antisedan® / Alzane® per ml Domitor® / Dorbene®). In a trouble-free catch the antagonist is given at least 60 minutes after the last injection of ketamine into the thigh muscle so that the animal is fully functional after waking up.

1.5.3 Complication 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.

All capture team members are informed on the emergency measures and actions, as well as on the contents of the medical preparations and equipment before the capture season. 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 till 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, it may be that an animal vomits the contents of its stomach and inhales the foreign object, or gets convulsions respectively. Over the last 15 years of captures in Switzerland, there were only minimal complications recorded. The lynx 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; complication can be prevented by 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 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 or absenting respiration: leads to insufficient oxygenation of the tissues (hypoxia) and can cause a cell damage or even lead to mortality.

Symptoms: inadequate or absenting 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 of food, 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 Respirot® can be given on the tongue or in to the nasal holes (not too large animals only).
3. In the case of a complete respiratory failure: lateral position, chest compression (strong chest pressure 15-20 times/minute), "pumping" with the forelegs, or resuscitation mouth-to-mouth, respectively mouth to the nose. At the same time checking for colouring of the mucosa - if it is naturally coloured (pink), break in the resuscitation and check for the animal´s respiration, if there is no respiration registered continue with the resuscitation.

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, foots), rapid or shallow respiration, rapid or irregular heartbeat.

Causes: physical exertion, high temperature, direct sunlight, disturbance of thermoregulation centres due to narcotics, 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 of the pulse is very difficult (low blood pressure), cold or stiffened legs (frostbite).

Causes: disturbance of thermoregulation centres due to narcotics, low temperature, lack of thermo-isolation (wet coat, cachexia, too long positioning on the same body side), misfunction of the blood circulation (e.g. shock).

Precautions:

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

The risk of hypothermia is real particularly during the 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 return to normal temperature may be delayed. If necessary, prolong the narcosis until a normal body temperature is reached.

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

Symptoms: bruising, shortness of breathing, 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). The animal may die a few days after capture, although it appears to have recovered from the narcosis. The aspiration of a large number of vomits has a 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 ringero 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 (too little sugar in the blood).

Treatment:

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

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

1.6. Handling of the animals

In situ examination of immobilised lynx

The examination at the capture site (Annex 4) is important (1) to decide if the lynx caught fulfils the requirements (satisfactory health status, judged suitable for relocation), capture and anesthesia before transport), and (2) to check if the animal is fit for transport to the quarantine station. Lynx, which are not adequate for immediate translocation, are depending on the circumstances released at the capture site or brought to the quarantine center, either with or without radio-tagging.

Responsibilities of the vet

a. Prepare the narcotics according to the protocol described at 1.5
b. Tranquilizing the animal
c. Conduct a clinical examination to evaluate the health.
d. Take a blood sample for necessary blood tests - antibody titer against rabies, FIV, FeVL, parvovirosis.
e. Make the vaccination against rabies at the capture site.
f. Mark each animal with a microchip (officially recognized method) before the blood sample is taken.
g. Treat the animal against parasites with macrocyclic lactones and praziquantel before translocations occur.
h. Take/store genetic samples (blood, hair) from each lynx.

Responsible of the field team leader

a. Coordinate the action and give clear instructions to all involved people.
b. make sure that all field personal know how to behave and has a role during the action
c. check the collar functionality (VHF signal, all magnets removed)
d. collar the lynx

e. re-set or deactivate the trap
f. Photograph the animal from both sides for future identification purposes.

1.7. Transport to quarantine centre

From the capture site to the field car and then to the quarantine centre, the transport of the lynx will be made in small transport box (90x60x50 cm, length, width, height). To keep the animal calm the transport should be as smooth as possible and silence needs to be maintained by the team. Skis, wood slide or other means can be used.

During transport to the quarantine station all precautions mentioned bellow need to be carefully respected in order to minimise the risk:

a. Animals are loaded and transported by car when they are fully recovered from the narcosis;
b. The transport case and vehicle must have appropriate ventilation. The car must be adequately tempered;
c. Transport should be as smooth and as quick as possible (no stops, smooth driving and silence);
d. The animal must be surveyed, but in a way to avoid additional stress.

Section 2 Quarantine

Keeping wild animals in quarantine is requested by the international and national legislation and its main purpose is to avoid any disease spread caused by any movement of sick animal into a new environment. Depending on circumstances the animals will be kept in the quarantine in Romania or/and in release countries (Slovenia, Croatia)

Within other secondary objectives keeping the animals into a quarantine enclosure can address the following three purposes:

- ✓ to "break the homing-effect", in order to prevent a return to the original home range;
- ✓ to obtain sufficient time to catch a lynx pair and to organise its release;
- ✓ to be able to observe the animals in order to ensure that all animals are in a good health.

The duration of quarantine depends on:

a. The success/results of the blood test (antibody titer against rabies, FIV, FeVL, parvovirosis).
b. Health and behaviour of the animal
c. The time when rabies vaccine was given
d. Obtaining all the legal documents/approval requested for the international transport.

IMPORTANT!! Efforts should be made to fulfil all the conditions and translocate the lynx as promptly as possible. If the lynx is very stressed, the animal should be released at the capture site.

2.1. Quarantine centre

The plan of the quarantine centre is presented in Annex 5. The quarantine station is isolated both visually and acoustically from people and other animals. The station includes access to individual enclosures. Climbing and elevated parts as well as hiding possibilities are included in all three enclosures along with the inner shelters (boxes) for protection against rain and cold.

In the quarantine station an animal keeper should take care of the captive animals. The enclosures should be cleaned at least once a week from faeces and uneaten food.

Main rules:

a. Contact between quarantined lynx is strictly prohibited. The enclosures have adequate space

for keeping two lynx separated.

b. The quarantine station will be secure to prevent lynx from escaping and minimize the risk of injuries to humans and lynx during the quarantine period.
c. Each enclosure should provide structure to enable the lynx to climb and hide..
d. Discrete observation of lynx in the enclosure must be possible,
e. Contact should be kept to a minimum to avoid habituation and reduce stress.
f. Cleaned once a week from faeces and of food waste.
g. For release, all animals are again immobilised, weighed and examined.
h. Anthropogenic (human-caused) noise will be maintained at a minimum.
i. After transportation to SLO/CRO, the whole enclosure will be disinfected.

During quarantine, the tranquilising action should be maintained at a minimum, and will be considered only in the case of an emergency, and for preparing the animal for transport. A system for getting the animal safely into the transport box trap should be considered and devised.

2.2. Veterinary protocols for the quarantine centre

Main objectives:

a. All animals are tested for selected infectious diseases using established procedures:
b. If needed, a second test for check for antigens: feline leukaemia virus (FeLV)
c. If needed, a second test for check for antibodies: feline immunodeficiency virus (FIV).
d. Appropriate additional analysis will be performed in case of other clinical findings.
e. If requested by the loss of first samples or other legal/administrative problem blood samples can also be taken for haematology, blood chemistry, genetic research and archive purposes.
f. The first faeces excreted in the enclosure are collected for parasitology examination and for archiving.
g. If requested, lynx receive a second standard treatment consisting of long acting antibiotic and anti-parasitic drugs beside/supplementary from the treatment done at the capture site.
h. Wounds are disinfected and treated appropriately.

All treatment that can be applied without tranquilising the animal will be applied by the vet. If requested the procedure for tranquilisation is similar with those described at point 1.5 of the present protocol.

The protocol used and all veterinary intervention are written into the quarantine log as proposed in the Annex 6.

If the animal's behaviour suggests a low adaptation to the quarantine centre and risk occur for its life, two solutions can be considered:

a. Releasing the animal back into the wild close to the place of capture with or without the collar.
b. Transporting the animal to Croatia if all the legal/administrative papers are available (they intend a hard release)

2.3. Feeding during quarantine

a. Fresh water will be provided at least at 24 hour intervals.
b. Preferably fresh cadavers of roe deer or red deer will be provided at each 4-5 days considering also the rhythm of consumption of the carcass. For the storage of roe and red dear meat (if difficult to obtain), a freezer can be used for long time storage.
c. If no wild animal carcasses are available, fresh meat (preferably organs) will be provided.
d. Food will be provided with minimum human disturbance.

At the end of the quarantine period, alongside the transport paper, a quarantine log (Annex 6) and a medical report (Annex 7) will be provided by the vet and the quarantine centre administrator.

Section 3 Transport to the release site

Transportation and transit exerts an extraordinary stress on each animal, especially for wild animals, and can, for example, lead to stress-induced hyperthermia, and in worst-case scenarios, even death. Since possibilities for monitoring and intervention during transport are limited, special attention must be given to the transport infrastructure and the conditions during transport. In addition to the safety of the animals, the safety of people must be considered!

Transport types of captured lynx:
a. From the capture site to the quarantine station:
b. From the quarantine station to the place of release: usually 3-5 weeks after capture, good health, anesthesia for health check and radio-collaring before transport.

For reasons of animal welfare, the transport (moment of animal loaded into the authorised transport vehicle until the animal reaches the final destination that might be a soft release enclosure, a quarantine centre or the place of release) must be well planned, well prepared and effectively executed. During transport animals must be protected against bad weather, extreme temperatures and unfavourable climate changes. Transport can only be performed if an animal is suitable. Animals must have a clear healthy status to assure they are capable of undertaking the transport without any help. Female lynx must not be transported if there are at 90% or more of their expected period of pregnancy or if new born animals do not have fully healed umbilical cords.

Weather conditions, potential causes for delays (e.g. border waiting time, status of transport routes), legal obligations that may include live transport licenses, locations of fuel and repair services, etc. should be investigated and must be taken into account prior to the onset of transport. The transport box and vehicle must have air conditioning. Transport activity involves all stops. The transport is accompanied by two drivers including a person authorized for animal transport.

The national administrative procedures and permits for lynx export must be obtained 48 hours before the transport and checked with the custom official.

An emergency plan for the long transport will consist on:

a. Identifying zoo or rehabilitation centre from Romania, Hungary on the road that might accept the lynx in quarantine if problems occur.
b. Contact prior departure the veterinary department from the selected zoo.
c. Have a copy of all documents in case of the need of an emergency that require a use of a zoo on the transport route.

3.1. Transport vehicle

a. Transport vehicle must be officially approved by national veterinary authorities and be clearly marked with a visible sign that shows that an animal is being transported.
b. Transport condition should prevent any injury or escape of animal(s).
c. Transport condition should ensure security of the drivers.
d. Access to animals must be provided in a way that they can be easily checked, observed, and appropriately cared for.
e. Transport condition should facilitate check – ups (e.g. during transport or at the border) and if

requested appropriate care of the animal(s).

f. Animals must have enough space to stand in a natural body posture and turn around.
g. Transport condition should have adequate ventilation at all times.

Transport of the lynx will be made using boxes inside of the car. The boxes are secure and cannot slip (lash down if necessary), (2) sufficient access to the transport box is ensured, and (3) the supply of fresh air is good. The best ventilation holes are of no use if they are covered with material or if the car itself is not adequately ventilated. Boxes must be situated and built so that urine and faeces cannot fall on to the animals. Boxes must have clear signs that say that they contain live animals.

Transport and care during transport: Individual behavior during the journey ranges from calm to extremely stressed and restless. During transport, the following points are important:

a. Keep the animal as dark as possible (but note the air supply!). Night transports, however, are not necessarily an advantage because of strongly changing light conditions;
b. Do not disturb the animal during transport. The necessary monitoring must be discrete and should only be done regularly;
c. Provide a quiet environment. Avoiding noise (especially human voices), if possible also minimizing external noise by selecting a different route;
d. Carry out transport smoothly and only interrupt the journey if absolutely necessary (in the case of longer transports several drivers should travel).

Even if all the requirements are met, the animal must be observed regularly and, depending on its behavior, one or the other measure taken (for example, removal of the cloth to darken and optimize the air supply). However, the animal should not be disturbed by a constant invasive observation. An essential part of the monitoring will be done with a video system fitted on the box. Basically, an animal not anesthetized has no problem with cool temperatures, but overheating is a major threat! The interior of the vehicle should therefore be cool (e.g., 15-20 ° C).

A healthy lynx needs no food during transport. Water needs to be available. If the animal is hyperventilating and there is a risk of hyperthermia, the driver should stop, the box should be taken out of the car to a shady and cool place and a maximum air supply ensured. Continue driving when the lynx has cooled down.

3.2. Documents requested for the transport

During transport from the source country to the final destination country, in this case, Slovenia/Croatia, each animal must have a veterinarian certificate which will be agreed in parallel with competent veterinary institution in Romania.

The veterinary certificate must be issued and signed from an official veterinarian from the country of origin (Romania). The official certificate must be sent to the final destination country (Slovenia/Croatia) using the electronic veterinary system TRACES, so that the medical history of each animal can be followed. The official veterinarian will examine the shipment in the final and complete the third part of the certificate. Through the electronic system (TRACES), the official veterinarian from the country of origin will be notified about the condition of lynx upon arrival. Veterinary certificate must be provided in electronic (TRACES) and paper version.

Together with TRACES, the following additional information must be provided:

a. Lynx passport (picture and information about the animal)
b. Quarantine protocol (logbook)

c. Medical report (official reports that document all medical treatments)
d. Permit for translocation from each individual animal issued by national authorities Romania.

A pre-approval of the initial veterinary certificate must be agreed before animal transport in tandem with the competent institution from source country (Romania) and destination country (Slovenia/Croatia).

Documents:

a. Health certificate signed by an official veterinarian with information about species, number of animals, identification (microchip), address of shipper and receiver, confirmation that the animals are healthy and fit for transport, free from communicable diseases and the transport case is disinfected.
b. TRACES notification, place of shipment, unloading site (coordinates or responsible forest office), vehicle registration number,
c. Route map: exact path of travel, driving and rest periods (replacement driver) are documented.
d. Notification that animals are wild, may be frightened, and can be dangerous.
e. Written instructions on feeding and required special care.
f. Proof of origin (certificate issued by the Management Authority)

3.3. Veterinary protocol for the transport

Minimum procedure:

a. The animal status will be checked periodically and a transport logbook (annex 7) will be kept.
b. Water will be supplied.
c. Ventilation will be checked.

Due to custom rules carrying of the drugs for tranquilisation is forbidden so any direct intervention on the animal is not possible so in the case of emergency the first zoo on the route will be announced by the project team and the lynx will be placed there. For this purpose a copy of all documents will be available for the emergency stop.

Annexes

Annex 1

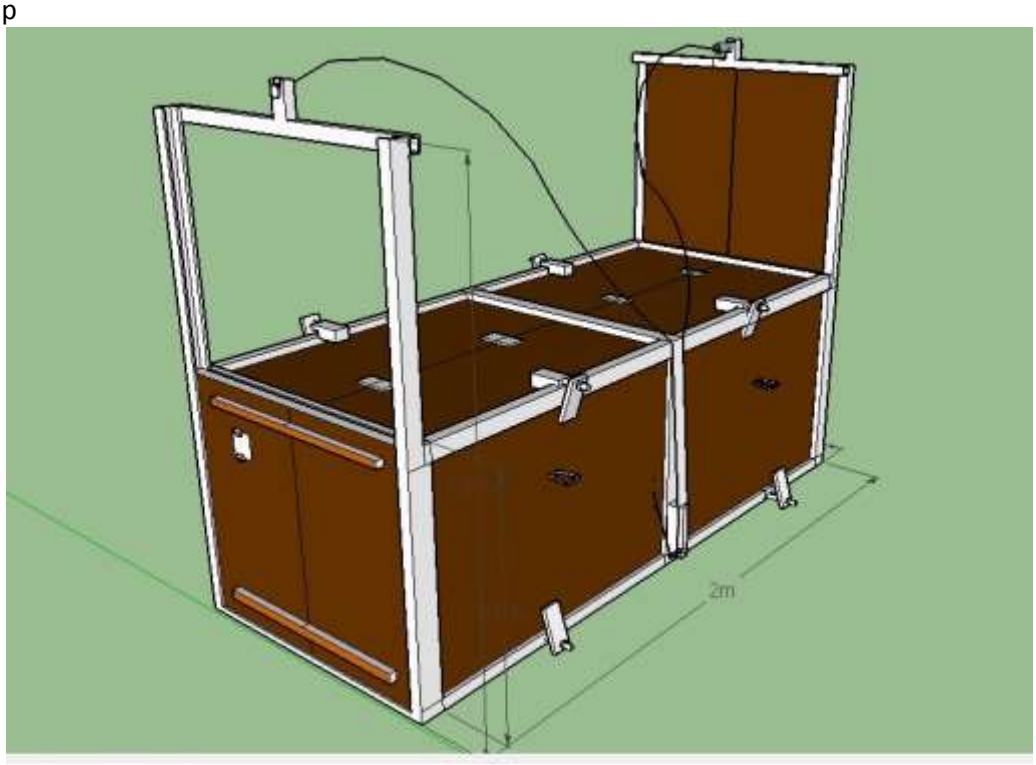
Safety rules for capturing and transport

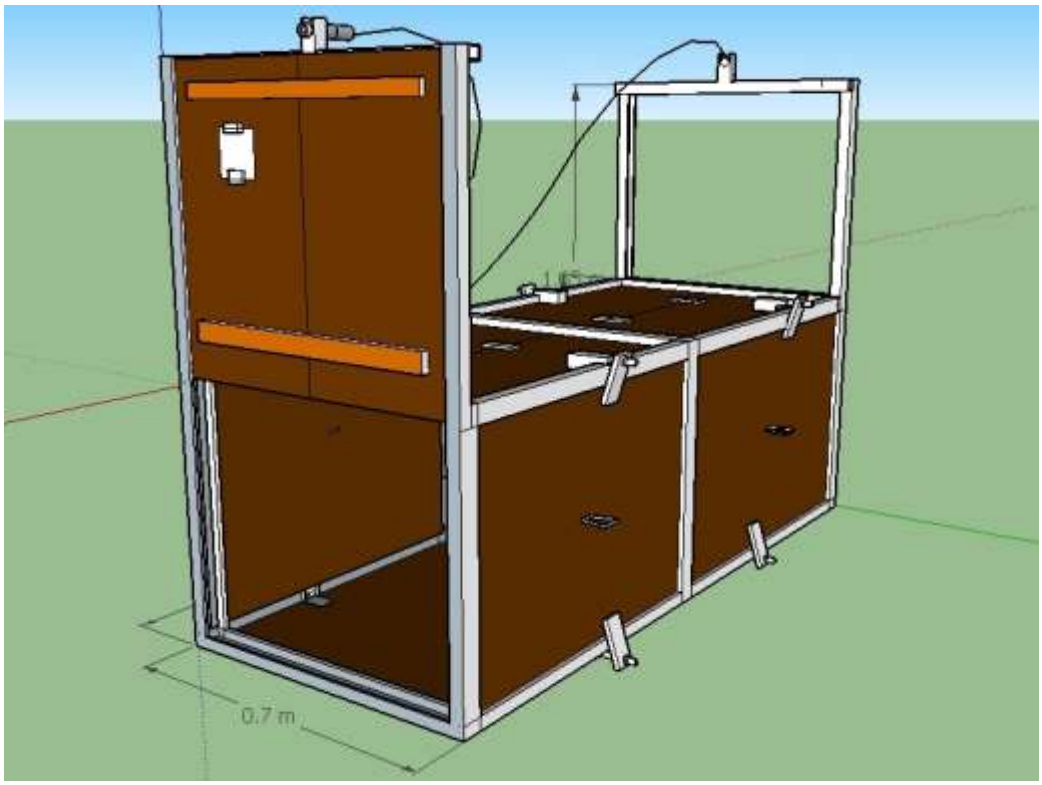
Principle: Safety of people and animals is priority!

1. Each field team is formed by minimum two persons (for all field activities)
2. Each team has at least a bear spray.
3. The project responsible from ACDB will be notified about the exact position of the box trap.
4. The project responsible of ACDB will be informed about the persons that are involved in the action.
5. The box traps will be checked only during the day even if the alarm is activated.
6. The minimum team to be present on the field from ACDB side, when an animal is inside the trap, should be composed by two of the field workers and the vet.

Annex 2

Box trap





Annex 3

Box trap log book

Action	**Details**
Filled by	
Coordinates	
Altitude (m)	
Trap type	
Alarm type	
Code of the alarm	
Frequency of the alarm	
Photo (who)	
Video (who)	
Participants at the trap set	
Date when set on the field	
Date when first activated	

Activated alarm	
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:
Date	Check results: Non-target species trapped:

log		
Date	Lynx: present at the box trap Other observations:	YES/NO
Date	Lynx: present at the box trap Other observations:	YES/NO
Date	Lynx: present at the box trap Other observations:	YES/NO
Date	Lynx: present at the box trap Other observations:	YES/NO

Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:
Date	Lynx: present at the box trap YES/NO Other observations:

Annex 4

Capture checklist & field form

Checked		Action	Details
YES	**NO**		
		Filled by	
		Date	
		Date of capture	
		Time of capture	
		Time of arrival of the capture team to the trap site	
		Coordinates	
		Altitude (m)	
		Trap type	
Tranquilizing			
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).			
		POI	
		IR	
		IM	
Marking equipment/details			
		ID Lynx (collar ID +number of the lynx)	
		Name lynx	
		Microchip series and number	
		Frequency of the microchip	
		Collar ID/frequency	
		Colar activated	
		Collar drop off activated	
		Photo (who):	left side right side head back teeth-front teeth-side
		Video (who):	
		Team Leader Vet Participants	
		Sex:	
		Weight (kg)	
		Age (adult/juvenile ; exact)	A J Exact:
Status			
		Body condition	
		Teeth and claws condition	
		Injuries	
		Objects in the mouth	
		Behaviour in the trap	
Narcotics			
		1. Injection	Time: Quantity: Product:

		2. Injection	Time:	Quantity:	Product:
		3. Injection	Time:	Quantity:	Product:
		4. Injection	Time:	Quantity:	Product:
		5. Injection	Time:	Quantity:	Product:
Samples					
		Blood quantity			
		Tooth (which one)			
		Hair (which one)			
		Others			
		Who is responsible for sample			
		First storage (place)			
		Deliver to (lab)			
		Date of deliver			

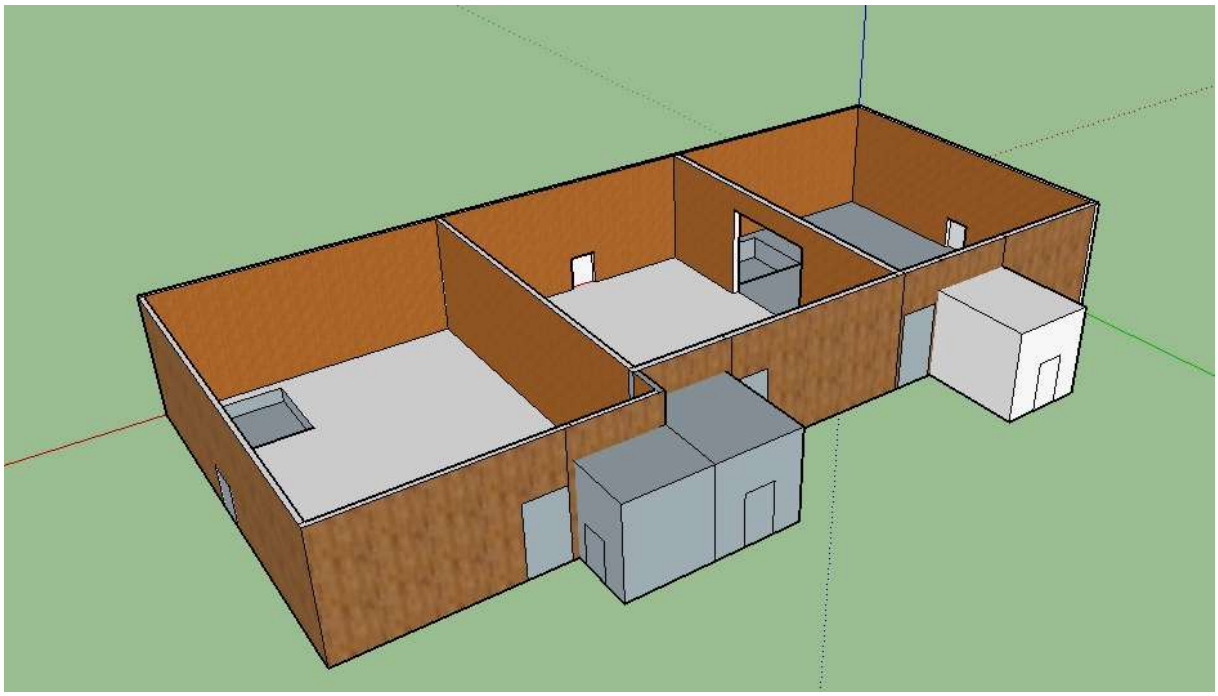
Heart (H) and respiratory (A) rhythms								
Frequency of respiration (FR) and **Heart frequency** (HF) are listed in heart rate and respiration per minute. The pulse can be detected, for example, on Vene Femoralis. Check for the heart rate (absent = pulse deficit), strong, pulsating or weak. (normal, strong, no deficiency).								
		1. Check	Time:	H:	/min	A:	/min	Pulse
		2. Check	Time:	H:	/min	A:	/min	Pulse
		3. Check	Time:	H:	/min	A:	/min	Pulse
		4. Check	Time:	H:	/min	A:	/min	Pulse
		5. Check	Time:	H:	/min	A:	/min	Pulse
		Time Waking up:						
		Time standing:						
		Behaviour during anaesthesia	**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 celzia 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!*							
		Time (hour/minute)										

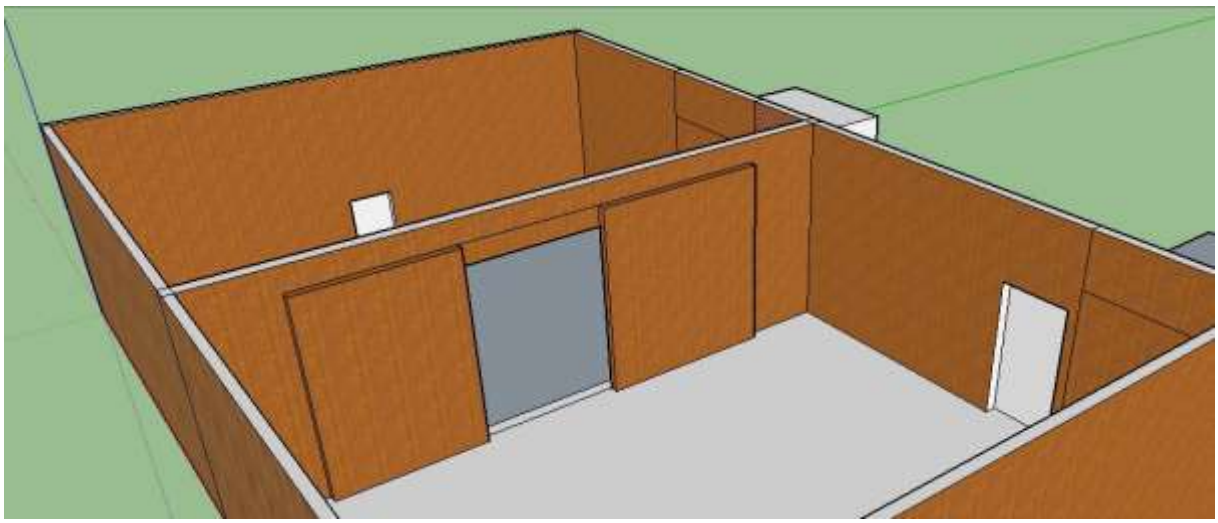
		CM-colour										
		CFT										
		O2-volume										
		T°										
		ROE										
		ROC										
		RE										
		ROL										
		Gasification of bark										
Measurements												
		Body length: tip of the nose – root of the tale										
		Tail (root of the tail – tip of the tail)										
		Neck size:										
		Shoulder height: Proximal edge of the toe ball to upper end of the blade bone (scapula)										
		Tarsus: Tarsus joint to outermost toe ball										
		Ear: Outermost joint to outermost toe ball										
		Ear tuft: tip of the ear to tip of the tuft										
		Distance b. canini: Measure tip to tip of the canini										
		Anus-genital aper. : From centre to centre of the holes										
		Footprint width										
		Foot print lenght										

Annex 5

Map of the quarantine centre

Left cage: 96 sqm
Middle and right cage: 88 sqm (they can be use alone or they can be used together forming a cage with 176 sqm)
Total surface: 272 m





Annex 6

Quarantine log

Action	**Details**
Registration number:	
Filled by	
Date of entrance into the quarantine	
Date of capture	
ID Lynx (square number + number of the lynx)	
Name lynx	
Microchip series and number	
Frequency of the microchip	
Photo (who)	
Video (who)	
Sex:	
Weight (kg)	
Age (adult/juvenile ; exact)	A J Exact
Entry Check	
First examination date	
Clinical Examination/Health assessment	
Treatment/Medication applied before entrance or at the entrance	
During quarantine	
Problems:	

Treatment*	Time:	Quantity:	Product:
Treatment*	Time:	Quantity:	Product:
Treatment*	Time:	Quantity:	Product:

Monitoring during quarantine:
Day 1.
Day 2.
Day 3.

Day 4. Day 5. Day 10. Day 15. Day 20. Day 30.	
Conclusions:	
Medical leaving check	
End date of the quarantine	
The duration of the quarantine (days)	
Clinical exam:	
Next location:	
Name of the vet:	Signature:

*if requested based on lynx health or administrative/legal measures.

Annex 7

Medical report

ID Lynx (square + number of the lynx)	
Name lynx	
Microchip series and number	
Frequency of the microchip	
Photo (who)	
Video (who)	
Sex:	
Weight (kg)	
Age (adult/juvenile ; exact)	A J Exact

Blood test

Rabies results:	
FIV	
FeVL	
parvoviruses	
Other	

Medical history

Day	Actions

Name of the vet:	Signature:

Date

Annexes:

Annex 8

Transport log

Action	**Details**		
Car ID			
Filled by:			
Date of exit from quarantine			
ID Lynx (square + number of the lynx)			
Name lynx			
Microchip series and number			
Frequency of the microchip			
Car loading photo (who)			
Sex:			
Weight (kg)			
Age (adult/juvenile ; exact)			A J Exact
During transport			
Check …..	Time:	Place:	Problem:
Check …..	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Check ….	Time:	Place:	Problem:
Conclusion:			

*if requested based on lynx health or administrative/legal measures.

Annex 9

Check list for transport documents and travel planning
(to be filled up before travel)

Documents	**Responsible**	**Checked**
TRACES data base		
TRACES documents		
Lynx passport (picture and information about the animal)		
Quarantine protocol (logbook)		
Medical report (official reports that document all medical treatments)		
Permit for translocation from each individual animal issued by national authorities Romania		
Route map and planning		
Written instructions on feeding, powering and required special care		
Results of the blood analysis (copies and originals)		
Actions		
Contact Slovenia/Croatia authorities prior travel)		
Check the documents with the custom authority		
Check the documents with the veterinary authority		
Check all the car paper/status		
Prepare the car for transport		

Annex 10

Transport box