



O.T2.2 Health, Husbandry and Management of Eurasian lynx (*Lynx lynx*)

Vet Handbook and Husbandry Guidelines



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1 Biology, distribution, conservation and ecology of Eurasian lynx in Western and Central Europe

1.1 History of Eurasian lynx in Western and Central Europe

Once, the Eurasian lynx inhabited forested areas throughout most of Europe, the Middle East, Central Asia and Russia, with the exception of some of the larger islands such as Ireland and Sicily and countries with insufficient forest cover. (Garman 2000; Breitenmoser 2000). The species was also absent from the Iberian Peninsula, where the smaller Iberian lynx (*Lynx pardinus*) occurs (Breitenmoser 2000).

Within the past 150 years, the species had been eradicated from most of the Western European sub-regions, surviving only in the north and the east. Human activities and development associated with a loss in forest cover and infrastructural development led to habitat fragmentation and played a central role in reduction of the populations in Western Europe to their lowest numbers in the 1950's (Breitenmoser 2000) (IUCN 2007). Additionally, numbers were drastically reduced by hunting and trapping, and intensified hunting pressure on the ungulate species that served as prey for the lynx meant that natural prey were increasingly rare. Remaining lynx were driven to prey on domestic animals and livestock leading to further hunting pressure.

In the northern and eastern sub-regions of Western Europe, numbers fell in the early 1900s but subsequently recovered, as ungulate populations rebounded following eradication of other large carnivores such as bear and wolf.

In Central Europe, lynx survived only in the Carpathian Mountains and a small area of the south Dinaric Mountains in Greece, Macedonia and Albania. In the 1970s, concern for the future of the lynx led to reintroduction of Eurasian lynx from areas where they were still abundant within Europe to forested and mountainous areas of Switzerland, Austria, Germany, Czech Republic, France, Slovenia, Croatia and Slovakia (Breitenmoser 2000). Reintroductions were moderately successful leading to partially self-sustaining populations with reproduction (e.g. Switzerland, Czech Republic). However, in many sub-regions, these populations remained fragile requiring active support by additional reintroductions (e.g. Austria, Italy, Slovenia, France) (Hernandez 2002).

In general, reintroduced lynx adapted well to settled and cultivated areas with sufficient forest cover. In Switzerland, the reintroduced population has now stopped expanding and is threatened by an imbalanced sex ratio. Because of unusually high losses of male cubs, possible congenital issues are being investigated (Breitenmoser-Würsten & Obexer-Ruff n.d.). Since the fall of the Iron Curtain, an increase in migration of Eurasian lynx into Central Europe of Eurasian lynx has been observed (Martin et al. 2008).

1.2 Classification and evolution of Eurasian Lynx

Classification - Subspecies

In 1758, the species was first described by Carl Linnaeus, who coined the scientific name *Felis lynx*. In the 19th and 20th centuries, the following Eurasian lynx subspecies were described and delineated according to their range and partially phenotypical and morphological differences.

- European lynx (*L. l. lynx*) Linnaeus, 1758: Scandinavia, Eastern Europe, Western Siberia
- Turkestan lynx (*L. l. isabellinus*) Blyth, 1847: Central Asia
- Caucasian lynx (*L. l. dinniki*) Satunin, 1915: Caucasus
- Siberian lynx (*L. l. wrangeli*) Ognew, 1928: Eastern Siberia
- Balkan lynx (*L. l. balcanicus*) Bures, 1941: Balkans
- Carpathian lynx (*L. l. carpathicus*) Kratochvil & Stollmann, 1963: Carpathians, Central Europe

Other subspecies (e.g. Irkutsk Lynx (*L. kozlovi*)) were also described but are no longer recognised.

The Eurasian Lynx in Western Europe is divided into different subpopulations, but not all of them are recognised (see above). The Northern lynx (*Lynx lynx lynx*) can be found in Norway, Sweden, Finland, Estonia, Latvia, Lithuania, Poland, Belarus, Ukraine and the Western Russian Federation. The Carpathian lynx (*Lynx lynx carpathicus*) has its range in the Carpathian Mountains (Slovakia, Poland, Romania, Ukraine), as well as the Czech Republic and mountainous parts of Hungary. The Balkan lynx (*L.l. balcanicus*) has its range in the South Dinaric Alps of Albania, Macedonia, Serbia, Montenegro and Kosovo (Krelekamp 2004). Nowadays, almost the entire population (Bohemian-Bavarian-Austrian-, (East-) Alpine-, Dinaric-, Jura- & Vosges-Palatinat Population) of Western Europe (within Austria, Croatia, France, Italy, Slovenia, Switzerland and Germany) is derived from captured and reintroduced individuals of *Lynx carpathicus* from the Carpathian Population in the 1970s and 80s (mainly from Romania and Slovakia).

Related species

In the western hemisphere, in addition to the Eurasian lynx, there are three closely related species of the lynx genus. The endangered Iberian Lynx (*L. pardinus*) is only distributed in a very limited area in southern Spain, and breeding has only been confirmed in Sierra Morena and Doñana coastal plains (Gil-Sánchez et al. 2011). The Canada lynx (*L. canadensis*), currently classified as a species of least concern, is found in northern and mixed boreal forests across Canada and Alaska, while the bobcat (*L. rufus*), also a species of least concern, is native from the border region of Southern Canada throughout the United States with few possible exceptions (Roberts & Crimmins 2010).

Evolution

The four species of the Lynx genus are assumed to have evolved from the Issoire lynx (*L. issiodorensis*), which was distributed in Europe and Africa during the late Pliocene to early Pleistocene.

1.3 Distribution and conservation status

Until the early 19th century, Eurasian lynx were quite common across Europe. By the middle of the 19th century, they had been extirpated in most countries of Central and Western Europe, due to hunting and human population growth with development leading to changes in land use and landscape alteration.

Consequently, the species was driven extinct in much of Western and Central Europe over the last two centuries. However, during the past few decades, as a result of conservation efforts, the status of the species has improved. Reintroductions have restored lynx within some areas of its former range, although many of these reintroduced populations remain fragmented and extremely small, relying on continuous reintroduction efforts to protect the fragile populations against genetic depression, inbreeding and decline. The lynx population within the EU remains small (below the population size threshold for Vulnerable listing under Criterion C, although it does not currently meet the subcriteria); if ongoing conservation action ceased, it is likely that the species would quickly start to decline again and could meet Criterion C1 in the near future (IUCN 2017).

The species has a wide distribution range and remains abundant in the northeastern part of Europe.



Figure 1. Eurasian lynx distribution range in Europe (adapted from Fernández-Gil et al. (2018))

Distribution & estimated population size of Eurasian lynx populations in Central Europe			
Name	Country	Size (km ²)	Approx. population size
Baltic Population	Estonia, Latvia, Lithuania, Poland, Ukraine	60.000	~1.600 individuals¹ Estonia: 600 - 800 ind. ⁸ Latvia: < 600 ind. Lithuania: 40-60 ind. Poland: 96 ind. Ukraine: 80-90 ind.
Balkan Population	Montenegro, Albania, Kosovo, Macedonia, (Serbia, Bulgaria)	1.600	40-50 individuals¹ Albania: 5-10 Serbia (incl. Kosovo*): 15-25 Macedonia: 23 Montenegro: ?
Bohemian-Bavarian-Austrian Population	Austria, Germany, Czech Republic	6.000	~ 110 individuals¹² Czech Rep.: 50 - 70 Germany: ~29 Austria: 5 - 10
Carpathian Population	Romania, Slovakia, Poland, Ukraine, Czech Republic, Hungary, Serbia & Montenegro, Bulgaria	104.000	2.300 - 2.400 ind.¹ Romania: 1200-1500 Slovakia: 269 ⁷ Poland: ~200 Ukraine: 350-400 Czech Republic: 13 Hungary: 1-3 Serbia: 50 Bulgaria: ≥ 11
Dinaric Population	Slovenia, Croatia, Bosnia and Herzegovina	10.000	50 - 80 ind.¹ Slovenia: 10-15 ind. Croatia: 30 - 40 ind. Bosnia-Herzegovina: 40(90?) Ind. ⁹
East-Alpine Population	Italy, Slovenia	3.400	~10 ind.¹
Jura Population	France, Switzerland	11.000	100 ind.⁶; 58 ind. (+/-13)^{1,2}
Karelian Population	Finland	not available	2.430 - 2.610 ind.^{1,4}
Scandinavian Population	Norway, Sweden	...	1.500 - 1.700 ind. Norway: 56 family groups (330 ind.) ¹¹ Sweden: ~200 family groups (1.200 - 1.300 ind.) ¹⁰
Vosges - Palatinian Population	France, Germany	2.800	~10 - 20 ind.⁶
Western-Alps Population	Switzerland, France	12.600 ⁵	134 ind. (+/- 9)¹; 15 ind.⁶

Table 1. Distribution and estimated population size of the Eurasian Lynx

(¹Kaczensky et al. (2013), ²www.kora.ch (2015), ³Vandel et al. (2006), ⁴www.luke.fi (2017), ⁵Zimmermann et al. (2011), ⁶Bauduin et al. (2018), ⁷Kubala et al. (2018), ⁸Remm et al. (2018), ⁹Kunovac and Omanović (2018), ¹⁰Hemmingmoore (2018), ¹¹<http://www.environment.no> (accessed September 2018)), ¹²Mináriková et al (2019)

Conservation status

Eurasian lynx conservation and hunting legislation

Under current hunting regulations, the Eurasian lynx is protected by year-round-closed-season hunting regulations and additional nature conservation acts within all European countries in which it is found apart from Estonia, Finland, Latvia, Norway and Sweden, where hunting is allowed during defined seasons and only to predetermined quotas, which are based on tightly controlled population monitoring.

International overview

Although the Eurasian lynx is not listed as endangered, threats such as illegal poaching, traffic accidents, habitat loss, as well as changes in prey base dynamics contribute to ongoing population stagnation or decline. (Kaczensky et al. 2013). Some populations (e.g. Dinaric-, East-Alpine- and Vosges-Population) have already shrunk to numbers insufficient to avoid genetic bottlenecks and inbreeding. These populations may not survive unless active measures (e.g. reintroductions, translocations) are taken to conserve them on a medium- to long-term basis.

In Europe, the Eurasian lynx is protected by the following conventions, directives and lists:

Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention)

The Eurasian lynx is listed under Appendix III. This obliges contracting parties to take appropriate and necessary legislative and administrative measures to ensure the protection of the Eurasian lynx, regulate any exploitation in order to keep the populations out of danger, and take measures including closed seasons and/or other procedures regulating the temporary or local prohibition of exploitation in order to restore satisfactory population levels. The directive also regulates the sale, keeping for sale, transport for sale or offering for sale of live and dead wild animals.

EU Habitat Directives

The Eurasian lynx is listed in Appendix II. This appendix includes animal and plant species of community interest whose conservation requires the designation of special areas of conservation. Eurasian lynx is not currently listed as a priority species. Additionally, it is listed in Appendix IV as well. This appendix includes animal and plant species of community interest that are in need of strict protection.

IUCN Red List

The species is listed in the category as Least Concern (LC) given its wide range and stable populations in the north and east of Europe and in large parts of its range in Asia. A recent assessment of the status of Eurasian lynx in Europe shows that some isolated subpopulations such as *Lynx lynx ssp. Balcanicus* remain Critically Endangered (CR) (IUCN 2017).

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

The species is listed under Appendix II, which contains species that are not necessarily threatened with extinction but may become so if trade is not controlled.

1.4 Biology, morphological traits and vital signs

This subchapter describes basic biological information of Eurasian Lynx in the wild with regard to morphology, physiology, and vital signs.

Measurements

The average measurements of male and female lynx are shown in the table below.

Measurements	Adult males	Adult females	Newborns
Head and body length (cm)	90 - 130	80 - 99	
Height (kg)	20 - 32	15 - 21	0,25 - 0,3
Height at shoulder (cm)	52 - 70	49 - 57	
Tail length	19 - 25	16 - 23	

Table 2. Measurements of Eurasian Lynx (Breitenmoser & Breitenmoser-Würsten 2008; Molinari 2000)

Morphology

The Eurasian lynx has a stout body with a soft thick pelt. The summer coat is shorter and less dense than the winter coat. The relatively long extremities, with hind legs longer than front legs giving the body a tilted forward appearance, allow for agile movement during close range ambush of prey. The front feet have five toes, while the hind feet have only four (Breitenmoser 2000). The absence of a clavicle permits more freedom of movement of the forelimb, and the ulna is well developed (Miller & Fowler 2015). The claws are sharp, strong and retractable, especially those on the front feet used for seizing prey (Breitenmoser 2000). The claws are covered by an epithelial envelope when retracted. Eurasian lynx have large, wide-spreading feet that are covered in fur. In winter, the fur at the bottom of the feet becomes very dense resulting in a „snowshoe effect“ that prevents the species from sinking into the snow. The animal has a prominently flared facial ruff around its neck and under its chin. As all felids, it possesses elongated whiskers for assistance in orientation and long (up to five cm) tufts of hair on the tips of its large pointed ears (Breitenmoser & Breitenmoser-Würsten 2008).

Male lynx are generally larger than females, which is the main obvious difference between sexes, apart from visible reproductive organs (testicles) and the os penis (baculum) in the male lynx, as is present in many feline species (Miller & Fowler 2015). However, a large female in an older age class can be confused with a small male in a young age class.

Additionally, individuals from the species' northern and eastern geographical range tend to be larger than those from southern and western areas (Breitenmoser 2000).

Coloration

The primary colouration of the fur of Eurasian lynx varies from grey, rusty or brownish-yellow, to reddish-brown. Greyish colours dominate in the thicker wintercoat (Tomkins 1962). There are three main coat patterns within the European populations: spotted (rosettes or solid spots), striped and unicolour. These patterns are especially prominent during summer and barely visible during winter. Pelt colours also vary within and between the species' distribution range. The ventral midline, including ventral neck, abdomen and medial limbs, as well as the ears are usually coloured in a lighter cream colour (Breitenmoser & Breitenmoser-Würsten 2008). Spotted and striped individuals predominate in current reintroduced European lynx populations in Western Europe, originating mainly from the Carpathian population. Overall, individuals in the North tend to be greyer and less spotted than individuals in the South, which mainly have a reddish tint and more extensive spots (Breitenmoser & Breitenmoser-Würsten 2008). The ears have tufts of dark hair, and the caudal aspect of the pinna is black towards the tip and characterised by light central spots. Eurasian lynx have a short black-tipped tail. Their white whiskers frame their muzzle (Hernandez 2002). The irises are yellow brown to light yellowish green in colour.

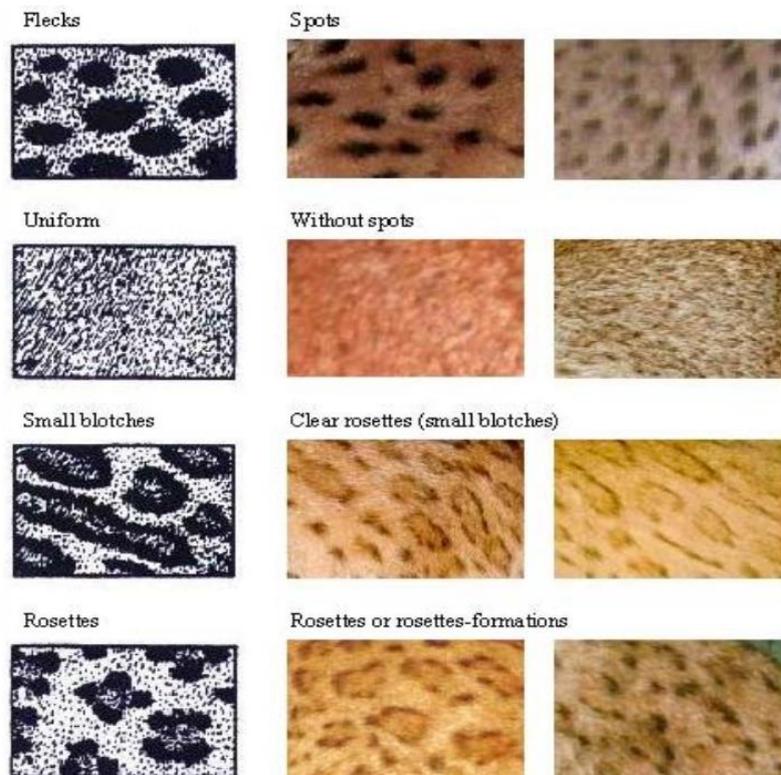


Figure 2. Four different types of Eurasian lynx coat patterns (adapted from Werdelin & Olsson (1997)).

Dentition

The skull of Eurasian Lynx has characteristics typical of other felids: a short rostrum, rounded cranium, small M1, and absence of M2. Their dentition features large well-developed canines to hold prey tightly by the throat and suffocate them after ambush. Six incisors assist, cutting meat from prey animals (Honders and Kuipers 1992). The dental formula for Eurasian lynx is: I3/3 C1/1 P2/2 M1/1.

Senses

Due to its foreshortened muzzle common to many felids, the lynx's sense of scent is rather limited (Schlexer 2008). Lynx depend more on their acute sense of hearing along with their eyesight to detect prey (Hernandez 2002).

Vocalizations

The Eurasian lynx and other smaller cat species have a bony hyoid and simpler vocal folds, which allow them to purr but not to roar; whereas bigger cats have an elastic ligament in the hyoid apparatus allowing them to roar but not to purr (Miller & Fowler 2015)). Absence of these long, fleshy, elastic vocal folds within the larynx is the main difference between vocal abilities of smaller cat species, such as the Eurasian lynx, and bigger cats. The otherwise very quiet male lynx can be heard calling in the mating season (Breitenmoser & Breitenmoser-Würsten 2008; Kachamakova & Zlatanova 2014). Intromission is signalled by a "copulatory cry" given by the female; this vocalization is typically a low, barely audible growl (Mellen 2003).

Vital signs

Vital sign	Adult lynx
Body temperature (BT)	37,8 to 39,9 °C
Heart rate (HR)	40 to 50 beats per minute*
Respiratory rate (RR)	10 per minute

Table 3. Vital signs of adult Eurasian lynx (Fowler 1986)

*the HR, RR and BT varies widely with the various anaesthetics used; basically the lynx has the same HR, RR and BT as a similar sized dog

Eurasian lynx cubs have a lower body temperature in the first weeks after birth, when they are not yet able to fully thermoregulate. In the first few weeks their body temperature can range from about 36 to 38 °C (Miller & Fowler 2015).

Longevity

In the wild, the Eurasian lynx has no natural predators. In Europe, sporadic cases of lynx being killed by wolves and wolverines have been reported, while in some cases the lynx was defeating solitary wolves in direct confrontations injuring or killing the opponent (Sidorovich 2017). However, a lynx can be fatally injured by large prey during hunting. They can also suffer from various parasites and diseases (see chapter 5: Diseases and common causes for adult and pre-adult mortality of Eurasian lynx). Mortality among juvenile lynx is high; case studies from Switzerland indicate a survival rate of 44 % in the first year and 24% in the first two years (Breitenmoser & Breitenmoser-Würsten 2008).

The majority of deaths within the lynx populations are directly related to human activity. Poaching and traffic accidents remain the main causes of mortality in Western Europe (Breitenmoser 2000; Andrén 2006; Sindičić 2016).

In the wild, the Eurasian lynx can live up to 17 years (Breitenmoser & Breitenmoser-Würsten 2008). In captivity, individuals are known to live up to 24 years (Weigl 2005).

1.5 Ecology, diet and feeding behaviour

This subchapter relates to general ecology, diet and feeding behaviour of Eurasian lynx in the wild.

The Eurasian lynx is strictly carnivorous and hunts for prey. Carcasses that are found by chance are consumed only in exceptional cases (Hernandez 2002). The intestinal tract of the Eurasian lynx is short and adapted to rapid digestion and assimilation of meat (Nowak 1999).

The main prey items in Central Europe are small ungulates, particularly roe deer (*Capreolus spp.*), chamois (*Rupicapra spp.*) and smaller red deer (*Cervus elaphus*). If these primary prey items are scarce or absent, rodents (*Rodentia*), hares (*Lepus spp.*), foxes (*Vulpes vulpes*), marmots (*Marmota spp.*) and domestic animals such as sheep (*Ovis aries*) and reindeer (*Rangifer tarandus*) (in Scandinavia) are also preyed on (Molinari 2000; Breitenmoser & Breitenmoser-Würsten 2008).

The lynx, like many felid species, is a crepuscular and nocturnal hunter and hunts mostly by sight and hearing. Because of their physical-anatomical limitations (small heart) (Honders and Kuipers 1992), lynx can only pursue prey at a sprint for short distances and depend heavily on the element of surprise (Honders and Kuipers 1992; Hernandez 2002). Therefore, after stalking their prey at close range, they attack opportunistically from the ground. If the attack fails, the hunt is suspended until the next opportunity arises. Contrary to popular belief, Eurasian lynx do not climb trees in order to pounce on their prey but merely to escape danger (Breitenmoser & Breitenmoser-Würsten 2008).

Once the prey is caught, it is killed by a compressive bite to the throat. The lynx's canines close behind the trachea and the carnassial teeth compress in order to suffocate the prey quickly. In some cases and with smaller prey animals, lynx will deliver the bite to the neck from above cutting the spinal cord (Breitenmoser & Breitenmoser-Würsten 2008). Lynx are capable of killing prey three to four times their own size. The prey is usually dragged several hundred metres before being eaten (Nowak 1999).

Lynx prey can often be recognised by the way in which it has been consumed. The lynx starts with the muscular meat of the rear haunches, feeding mostly on the fleshy parts of the kill, while leaving entrails, coat and larger bones behind. When the lynx has finished, the carcass is still intact, with head and feet attached. Often the fur is slipped forward and the head of the prey is wrapped by the inside-out hide (Breitenmoser & Breitenmoser-Würsten 2008).

Remains that are not immediately consumed are covered with soil and foliage in order to hide the kill from other predators. The lynx returns to the kill site, if undisturbed, until the prey is completely consumed. During this time frame (until complete consumption of the prey), lynx do not wander far from the cached carcass (Böer et al. 1995).

1.6 Reproduction

This subchapter details the behavioural aspects of mating and the physiological aspects of the reproductive cycle of the Eurasian lynx.

Mating

The mating season starts in January, when males roam widely within their occupied territory (while females maintain their normal movement pattern). The males actively mark their territories and leave their home ranges in search of a mate (Breitenmoser & Breitenmoser-Würsten 2008). They spray urine and rub secretions from their cheek glands on rocks, trees, stumps and other distinctive spots, most likely in order to communicate with potentially receptive females as well as to deter other male competitors (Breitenmoser & Breitenmoser-Würsten 2008). During this time, the normally silent lynx can be heard calling, which serves as expression of their desire to find a mate (Breitenmoser & Breitenmoser-Würsten 2008; Kachamakova & Zlatanova 2014).

Once each cat has experienced the other's scent via urine secretion and a female and male meet for the first time, they greet each other by sniffing and rubbing their cheeks against each other, followed by inspecting the genital region (Mellen 2003). Like other cat species, lynx are induced ovulators. During the days of contact, the male and female spend much time together, interacting by chasing one another, hunting together and staying in close proximity. Within a 24-48 hour time frame, the female comes into oestrus, serial copulations occur, which can extend over one to three days (Breitenmoser & Breitenmoser-Würsten 2008). Mating usually takes place during late afternoon to evening (Miller & Fowler 2015).

The male approaches the female, grasps her by the nape and mounts by straddling her first with the front feet and then with the hind feet. The female responds to the nape bite by adopting a lordosis posture (front quarters lowered, rear quarters elevated, and tail moved to one side). The female sometimes also treads with her hind feet. At this point, the male may also tread with his hind feet often simultaneously rubbing against the female's flanks.

The rubbing by the male may induce the female to adjust or to exaggerate her lordosis posture. The male then begins pelvic thrusting. Mounting typically lasts for one to five minutes before intromission occurs. In most instances, the male maintains a firm grasp on the female's nape throughout the mount. Intromission is readily apparent and is signalled by a 'copulatory cry' given by the female. This vocalization is typically a low, barely audible growl (Krelekamp 2004). Five to ten seconds after the female emits this vocalization, she throws the male off her back often threatening him, and then she begins to roll vigorously on her back. Rolling on the back typically lasts five to 30 seconds. Then the male and female groom their own anogenital regions (Mellen 2003). They usually mate many times before the male leaves to find another mate. Females usually copulate with only one male each season (although there is a documented instance when an already pregnant female mated again with another male), while a male lynx „visits“ many females within and adjacent to his occupied territory and home range in a polygynous mating system (Breitenmoser & Breitenmoser-Würsten 2008).

Sexual maturity

Sexual maturity in Eurasian lynx females is reached between 21 and 24 months of age (though it may rarely be as early as 12 months), while males take 30 to 36 months to mature (Breitenmoser & Breitenmoser-Würsten 2008). Females remain fecund until the age of 14, males until they are 16 to 17 years old (Nowak 1999; Nowell and Jackson 1996).

Seasonality

Mating is seasonal, induced ovulation in the early spring between the end of February and beginning of April (Breitenmoser & Breitenmoser-Würsten 2008). In some cases, a second breeding period directly after an unsuccessful attempt is possible for females that did not conceive during the primary period, as described in Breitenmoser & Breitenmoser-Würsten (2008). The interbirth interval is generally one year, but with occasional breaks (for example three years with litters, one without) (Breitenmoser & Breitenmoser-Würsten 2008).

Gestation

The gestation period lasts 67 to 74 days (Breitenmoser 2000).

Birth

Before giving birth, the female looks for a den either in a cave, in a hollow log, at the base of a tree or in dense vegetation (Breitenmoser & Breitenmoser-Würsten 2008). Litter size is typically between two and three cubs but ranges from one to five (Breitenmoser & Breitenmoser-Würsten 2008). Cubs are usually born in May or June with their eyes closed, nearly deaf, immobile and completely dependent on their mother for food and protection. Average birth weight is 250 to 300 g (Breitenmoser & Breitenmoser-Würsten 2008).

Development

Newborn cubs are completely dependent on their mother for warmth, food and protection. At this early stage, the female only leaves her cubs in order to hunt for food, and she does not venture far from the den. Males do not participate in parental care (Hernandez 2002). Between seven and 17 days of age, the cubs' eyes open, and they start to take notice of their immediate surroundings (Breitenmoser & Breitenmoser-Würsten 2008). They begin to walk between 24 and 30 days of age. At nine weeks old, the cubs follow their mother, who leads them to killed prey (Kaczensky 1991).

The female nurses her young for three to five months, and they begin to eat solid food at around two months of age (Breitenmoser & Breitenmoser-Würsten 2008). Females lead their offspring to kill sites and subsequently start to actively hunt with them teaching proper techniques. Young lynx learn to hunt through observation and practice (Breitenmoser & Breitenmoser-Würsten 2008). The cubs are weaned at four months but remain with the adult female until the following mating season, by which time they weigh between nine and 14 kilograms (around nine to 11 months of age) (Breitenmoser 2000; Breitenmoser & Breitenmoser-Würsten 2008). Young lynx siblings may remain together for some weeks after separating from their mother. By the time they reach two years of age, they are fully grown.

Dispersal

After this period together, the siblings separate and disperse in order to occupy their own territory. Male lynx tend to disperse over greater distances, which reduces the likelihood of inbreeding, while females stay closer to their mother's home range, sometimes establishing a territory in vicinity of their mother (Breitenmoser & Breitenmoser-Würsten 2008).

Mortality

The mortality rate in kittens is high, with approximately 50% dying within the first year (Breitenmoser 2000). Mortality is highest at three to four months after birth, just after the cubs emerge from the lair for longer periods of time. Survival of newborn kittens seems to be correlated with coverage and soil humidity of the lair: more covering means a dryer lair and greater survival of the cubs (Boutros 2001).

1.7 Behavioural traits

Activity

Eurasian lynx are crepuscular with the distance of their daily movement depending on availability of food resources (Podolski et al. 2012). This ranges from two to five, up to 25 kilometres (Böer et al. 1995). Using rock ledges, roots of a fallen tree or a low branch as well as hollow tree stumps, they rest mainly around midday and midnight, except during the breeding period (Breitenmoser 2000). Eurasian lynx remain active during winter- only sheltering in caves, hollow logs and trees during extremely bad weather (Hernandez 2002).

Social behaviour

The Eurasian lynx is a solitary animal. The only social units are a mother with dependent offspring (male and female) during breeding season. Eventually, after leaving their mother and living cooperatively for a short time, siblings disperse to find their own territories. On rare occasions, females have been seen hunting together when rearing offspring (Breitenmoser & Breitenmoser-Würsten 2008).

Home range

The home range size of the Eurasian lynx depends on terrain, habitat type, prey availability and potential territories of neighbouring individuals (Breitenmoser & Breitenmoser-Würsten 2008). Male core areas show overlap, while those of females are exclusive. With the exception of the overlap zones, one male and one female share the same area. On average, 86% of a female's home range is covered by a male's home range. Studies from Sweden and Russia have also concluded that males generally share their ranges with just one female and cubs. However, males seem to avoid the females' core areas, and thus appear to control a zone around females and their cubs, avoiding competition for prey and excluding other male competitors (Breitenmoser & Breitenmoser-Würsten 2008).

Lynx mark their home ranges/territories by spraying urine vertically against rocks, trees, stumps and similar natural structures, as well as fence posts or wood stacks (Breitenmoser & Breitenmoser-Würsten 2008).

2 Legislation

This chapter offers a brief overview of European legislation directly applicable to conservation and management of the Eurasian lynx within Central Europe. Prior to any action being taken, it is necessary that applicable legislation and responsible institutions or departments within the particular country in question be consulted. Additional information concerning approval of captures, permitting the use of radio telemetry equipment, as well as the use of anaesthetic agents and tranquilizer guns is provided.

2.1 Conservation

National regulations that aim to safeguard the survival of Eurasian lynx in Central Europe by imposing restrictions on hunting, trade and other potentially harmful actions are based on the status of the species within the project partner country's specific regulations and legislation. For information regarding legislation related to lynx conservation and species protection in the EU beyond this region, consult the specific national and EU legislation as appropriate.

International regulations trade are stated in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The Eurasian lynx is listed in Appendix II, which contains species that are not necessarily threatened with extinction but may become so if trade is not controlled. International trade in specimens of Appendix II species may be authorized by granting an export permit or re-export certificate (e.g. in the case of translocations and releases of specimens for reintroduction or reinforcements of small populations within the EU); no import permit is necessary). Permits or certificates may only be granted when the relevant authorities can ascertain that procedures and specifically trade are not detrimental to the survival of the species in the wild.

2.2 Health and Welfare

National regulations established to prevent the transmission and spread of infectious diseases (pertains e.g. to rabies), in order to safeguard the health and welfare of individual animals and populations, are available from the relevant national governmental bodies. Further information on comprehensive regulations can be obtained by the World Organisation for Animal Health (www.oie.int) and within the participating countries of the 3Lynx project.

International regulations concerning welfare during air transportation are outlined by the International Air Transport Association (IATA). The current regulations can be found on their website: <http://www.iata.org/publications/store/Pages/live-animals-regulations.aspx>.

2.3 General legislation

The Eurasian lynx is listed under Appendix III of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention).

This convention requires contracting parties to take appropriate and necessary legislative and administrative measures in order to ensure the protection of Eurasian lynx, as well as to regulate any exploitation in order to ensure the physical integrity of individuals and (sub) populations. This includes measures (i.a. monitoring and reporting) and regulations, such as defined closed seasons and/or other procedures regulating the exploitation, temporary or local prohibition of exploitation in order to sustain or restore satisfactory population levels and the regulation of trade in live and dead individuals.

2.4 Legal aspects concerning anaesthesia, application of drugs, capture activities

All captures in the wild must be approved by the appropriate authorities in the respective countries. The use of motor vehicles, landing with a helicopter and setting up live (box-) traps will require specific permits from local, regional and/or national authorities and possibly the hunting concession holder and landowners in general. The use of anaesthesia equipment and anaesthetic agents is regulated in EU-level and the national veterinary, animal welfare and experimentation legislation. A clear understanding of these various pieces of legislation and associated regulations is vital prior to taking any form of action. Before undertaking capture activities the respective authorities must be informed according to the legislative process in the particular country. Additionally, the use of radiotelemetry transmitters and receivers requires a permit.

Note:

Additional permits may be needed from county and/or national authorities when working in protected areas. All anaesthetic agents are prescription drugs and must be used by or on the order of a licensed veterinarian. Some of these drugs are also controlled substances, i.e. drugs that can be abused, for which specific regulations may apply. In some countries, a veterinarian has to be on site when wild animals are chemically anaesthetised; in others a special permit and prior training/certification is required for non-veterinarians.

3 Handling of free-ranging lynx (e.g for radio-collaring; physical examination; capture for reintroduction, translocation)

3.1 Biomedical Protocol for free-ranging lynx

Capture and chemical anaesthesia of free-ranging Eurasian lynx should be carried out by a team of professionals with proper training, experience and expertise in wildlife capture, veterinary anaesthesia and animal handling. Capture data should be collected according to the lynx capture form (see appendix). Individuals might be captured for a variety of reasons (e.g. radio-collaring or sampling) and should be handled with the ultimate goal in mind. Capture data must be recorded within a lynx capture form and photos must be taken.



Figure 3. Anaesthetised Eurasian lynx (darted with DAN-INJECT® dart syringe on right foreleg) - © Veterinærconsult, Norway

3.1.1 Trapping methods

Darts and delivery system

After capture in box traps set around fresh roe deer kills (in Central Europe), adult lynx and juveniles (> 8 months) are usually captured and immobilized at short distance via a blowpipe. In case of applying anaesthesia at long range (e.g. from a helicopter, often used in Scandinavia) cartridge- and CO²-powered long range dart projectors or tranquilizer guns can also be used (Arnemo & Evans 2017; Kock et al. 2013).

The following passage/chapter provides information and an overview of frequently used darts and delivery systems to anaesthetise Eurasian lynx in the field. It includes information of which dart and remote delivery system to use and the amount of drug to be administered, the distance between delivery system and target animal, as well as prior personal experience of the person undertaking the procedure.

Darts

Darts used to administer drugs come in a large variety of systems. Here we concentrate on the two most common systems which are applicable to lynx anaesthesia. Basically all darts consist of five basic parts: the needle, the syringe barrel, a separating plunger, the injection solution, and the tailpiece (for flight stability and constant velocity). The most commonly used dart-syringes in lynx are air discharged (Kock et al. 2013).

Dan-Inject® and Telinject® dart syringes

Dan-Inject® (Dan-Inject, Børkop, Denmark) represents one of the most commonly used dart projector systems that enables efficient delivery of anaesthetic agents with a blowpipe or CO₂ powered pistol and gun. An advantage are the simple, multi-use Dan-Inject® darts, which are light weight and suitable for most environmental conditions.

As dart failures can arise after repeated use [e.g. failure to hold pressure, discharge failure]. It is recommended to only use new darts for procedures with wildlife in order to maximise the success rate. Note that new darts must also be tested prior to their first use. Seamless plunger movement, correct dart pressure and needle patency constitutes the minimum checklist before any deployment. (Kock et al. 2013). An alternative to the Dan-inject products are Telinject® dart systems.



Figure 4. Dan-Inject® dart syringes fitted with high visibility luminous stabilisers (1.5, 3.0, 5.0 and 10 ml - for anaesthetisation of *L. lynx* 1.5 and 3ml are usually used) for delivery systems such as dart projectors and blowpipes (adapted from Dan-Inject®).



Figure 5. Dan-Inject® filling and maintenance kit (©Dan-Inject®)

Pneu-Dart® dart syringes

Another system used in wildlife anaesthesia is the Pneu-Dart® dart syringe (Pneu-Dart, Williamsport, PA, USA), a single-use dart system. In contrast to Dan-Inject® and Telinject® darts, this system combines dart and needle in a single unit. The explosive discharge mechanism is already inserted and ready to use with minimal preparation required. The anaesthetic drug is rapidly injected when an explosive cap within the dart detonates on impact (Kock et al. 2013). Because single-use darts are disposable, they do not require cleaning, thereby reducing the risk of accidental exposure to potent anaesthetics and reducing malfunction to a minimum. The darts are available in various volume and needle combinations. The darts have low visibility and a good accuracy which can be an advantage over longer distances.



Figure 6. Pneu-Dart® Type C dart syringe (<http://www.pneudart.com>)



Figure 7. Pneu-Dart® Type P dart syringe (<http://www.pneudart.com>)

Delivery systems

Dart guns or projectors

Remote Delivery Systems (RDS) comprise injection poles, blowpipes, dart guns and pistols. All can be used to remotely apply liquid anaesthetics or medical agents with a dart syringe system as described above.

A wide variety of different dart guns and projectors are available. Here we describe only the most commonly used for anaest. These include the Dan-Inject® CO₂ Injection Rifle Models IM, JM-SP. & JM-SP25. (Dan-Inject, Børkop, Denmark), as well as the Pneu-Dart cartridge-fired Model 389 projector. Alternatives to the CO₂ propelled system from Dan-Inject® are the Telinject GUT50 and the Pneudart X-Caliber™ (Pneu-Dart, Williamsport, PA, USA).

Dan-Inject® CO² Injection Rifle Model J.M.ST. & Dan-Inject CO₂ Injection Rifle Model J.M.SP. & J.M.SP.25

The Dan-Inject JM Standard (below left) is an extremely compact injection rifle (weighing only 2,9 kg) used for anaesthesia in a wide variety of mammalian species in the wild. It is extremely reliable and offers exceptional accuracy over darting distances from 1-40 m and can be used for maximum distances of 80 meters with an 0.5 ml capacity dart (Walzer unpublished, www.dan-inject.com). The rifle is robustly constructed, allows for the ergonomic control of CO² pressure in order to adjust effective range. The manometer and telescopic sight can be viewed simultaneously (www.dan-inject.com).



Figure 8. Comparison of Dan-Inject JM with a short and long barrel

The Dan-Inject CO₂ JM Models are available with two barrel lengths in 11mm and an additional long 13mm barrel. The short barrel is an advantage when manoeuvring within confined areas, such as a vehicle, thick brush and densely forested areas. The JM is also available in a 25 bar version (JM-SP). This enables very experienced operators to dart at extended ranges up to 80 m under optimal conditions (www.dan-inject.com). The 13mm barrel allows for the use of Pseudart darts in the JM.

Potential alternative drug applicators (rarely used in Europe)

Telinject® GUT50



Figure 9. Telinject GUT 50 (© Telinject®)

More info: <https://www.telinject.de/en/products/vario-product-line/g-u-t-50/>

Pneu-Dart® X-Caliber™



Figure 10. Pneu-Dart® X-Caliber™ (© Pneu-Dart®)

More info: <https://www.pneudart.com/products/projectors/>

Pneu-Dart® Model 389



Figure 11. Pneu-Dart® Model 389 (© Pneu-Dart®)

More info: <https://shop.pneudart.com/389BK/>

Blowpipes

Dan-Inject Blowpipe system: Blow 180

Modern blowpipe systems made from carbon fiber or reinforced plastic are available in many sizes (with longer versions enhancing accuracy and range) and are popular as a short-range limited-volume drug delivery system. These systems are commonly used for anaesthesia of captive individuals and wildlife translocation (e.g. after trapping of lynx in box traps) or rescue, with these animals initially captured in traps or snares. Advantages are the relative silence and ease of use, their light-weight, low velocity and impact energy. Disadvantages are the light-gauge needles and limited range. However, blowpipes must be seen as the RDS of choice for most applications in Eurasian lynx (Nielsen 1999).



Figure 12. Dan-Inject® Blowpipe180 (© Dan-Inject®)

Once an appropriate delivery system and projectile dart have been chosen, the equipment must be systematically assembled and checked for functionality. Ideally, a routine method for preparing equipment is established and followed; this will anticipate and avoid equipment failure by checking and rechecking the delivery system, darts and needles regularly. Additionally, repeated training sessions and practice in using blowpipes and tranquilizer rifles, filling and maintaining equipment correctly is highly recommended.

3.1.2 Anaesthetic drugs

The use of anaesthetic drugs for immobilisation of wild animals is never entirely without risk. Wildlife anaesthesia can potentially engender capture stress, injury and extremely rarely death of an individual. An experienced wildlife veterinarian is key in minimising capture related issues.

Standard protocol for the anaesthesia of free-ranging Eurasian lynx

Recommended target-sites for darting of felids are muscular parts of hindquarter of the animal, in order to allow an efficient and rapid anaesthetic induction and avoid accidentally puncture heart/thorax region, if aimed at shoulder (Kock et al. 2013).

Adult lynx (males 18-28 kg, females 14-19 kg) are darted with an initial dose of 4 mg/kg ketamine (K) and 0.06mg/kg medetomidine (M) (Painer et al. 2014). If needed this combination can be supplemented with 1-2.5 mg/kg ketamine, as antagonist 1 mg/kg atipamezole (Kreeger and Arnemo 2018). As alternative drugs, 5mg/kg tiletamine-zolazepam, 0.5 mg/kg tiletamine- zolazepam plus 0.05 mg/kg medetomidine (antagonise with 0.25 mg/kg atipamezole) or 10mg/kg ketamine plus 1.5 mg/kg xylazine can be used. The ketamine-medetomidine dose can be reduced by 50% for captive lynx (see below) (Kreeger and Arnemo 2018).

Partial reversal

If necessary medetomidine can be safely reversed 30 minutes after induction with the antagonist atipamezole at 5mg/mg medetomidine. Similarly in an emergency zolazepam can be reversed with flumazenil.

Cubs (4-5 weeks of age; mean body mass 1.5 kg) are captured by hand in their daybed, weighed and immobilized with 5mg/kg (K) and max. 0.08 mg/kg (M) (Kreeger and Arnemo 2018).

Capture by box traps

For adults captured in box traps (calm animals) and juveniles (6-12 months 9-16 kg, yearlings 12-21 kg), the dose can be reduced to max. 2,5 mg/kg ketamine (K) and 0.1 mg/kg medetomidine (M). A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject®¹) is used (Kreeger and Arnemo 2018).

Capture with foot snares

Capture at prey sites with foot snares are a safe method to capture returning lynx on kill sites. The round base of the foothold snare features a throwing mechanism that withholds a thin steel wire to capture the leg of the individual. Thereby, the throwing mechanism keeps the loop of a steel wire (6 x 9 mm) above the mechanism open, when a lynx enters the centre of the, with branches and leafes camouflaged, round base, a trigger mechanism is released and the loop wraps around the animal's leg, while a gps signal triggers an alarm, which informs the wildlife technicians or vets of a successful capture and the animal can be within 20 - 30 minutes anaesthetised for further treatment. In the bar that is adjusted to the wire, spring in the bar accelerates the pull effect of the wire, but acts also as a shock absorber when the captured lynx tries to free himself from the foot snare. Additionally it assists in the immediate prevention of paw or leg injuries as well. The setting is usually executed by installing two to four camouflaged foot snares near the lynx kill. The bars of each foot snare is anchored with steel wire to a nearby tree, or with earth studs, depending on the prevailing conditions during the capture event (Kubala et al. 2018).



Figure 13. From left to right: KORA 08 foot snares consist of a steel bar, and a split groove that acts as a throwing mechanism, however, the jaws cannot be closed to avoid injuries of the animal. Foot snares „KORA 08“ set in close proximity to a lynx kill, preferably camouflaged to allow efficient capture (© KORA, .Kubala et al. 2018)

Capture by helicopter

When capturing Eurasian lynx in the wild by helicopter, adult lynx (males 18-28 kg, females 14-19 kg) are darted with an initial dose of 4 mg medetomidine (M) + 100mg ketamine (K) (Arnemo & Evans 2017). The drugs are delivered via a 3ml dart syringe (Dan-Inject®¹) with a 1.5 x 25mm barbed needle (Dan-Inject®¹) injected via tranquilizer gun (Dan-Inject ®¹) (Arnemo & Evans 2017).

Darting inside enclosures

Inside enclosures, Painer et al. (2014) used a blowpipe and a 3 ml dart syringe equipped with a 22 gauge dart-needle (1.2x38 mm) for Eurasian lynx. An initial dose of 0.06 mg/kg Medetomidine plus 4.0 mg/kg Ketamine was used.

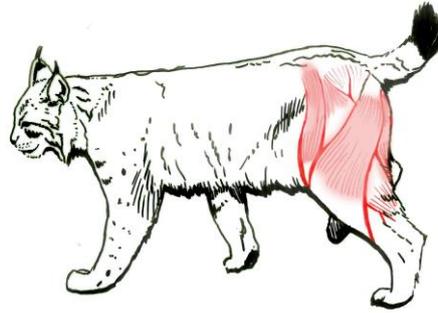


Figure 14. Dart placement sites for darting free-range lynx (Illustration: © S. Knöpfer)

3.1.3 Alternative drugs and Supplemental dose

Supplemental administration of drugs depends on the situation and whether surgical anaesthesia is required or not. Animals that are not sedated 15 minutes after delivery of the initial dose via tranquilizer gun or blow pipe are redarted with a 10mg/kg Medetomidine combination or 15mg/kg xylacin combination. If the animal is sedated but incompletely anaesthetised, application of additional drug dose is necessary:

If the animal is recumbent but incompletely anaesthetised, 0.25- 1.0 mg medetomidine should be given. If a veterinarian is not present 25 - 50 mg ketamine (K) can be given intramuscularly by hand syringe injection. If required, medetomidine (1 mg) can be used to keep the animal anaesthetised without causing prolonged recovery if antagonised (Arnemo, n.d.). In case of a prolonged procedure or signs of recovery, 0.5-1.0 mg medetomidine can be given intramuscularly to keep juvenile and adult lynx anaesthetised for additional 15-30 minutes.

If extra time is needed to complete a surgical or other painful procedures, a combination of medetomidine-ketamine should always be administered. Tiletamine-zolazepam should **not** be used for normal procedures, but can be used for procedures exceeding two hours, due to long elimination time (Arnemo & Evans 2017; Mair et al. 2014).

3.1.4 Handling of anaesthetised animals

Anaesthetised animals should be monitored and clinically examined by veterinarians (or specially trained personnel with experience in wildlife medicine and anaesthetic procedures). Possible side effects caused by anaesthesia respiratory are depression with hypoventilation and hypoxemia (inadequate amount of oxygen in the blood as appreciated via pulse oximeter or visual evaluation of mucous membranes), and thermoregulatory dysfunction (hyperthermia or hypothermia).

In the most cases this is the direct result of a drug overdose in individuals with poor body condition, aspiration of vomitus/saliva, pneumothorax or overexcitement due to stress subsequent to a misplaced dart (Arnemo & Evans 2017).

To prevent aspiration of saliva or vomitus, immobilized animals should be kept in sternal or lateral recumbency with the mouth and head low relative to the body. Ophthalmic lubricant without antibiotics (e.g. Viscotears®⁴) should be applied to the corneas to prevent drying, and eyes should always be protected from direct sunlight with a blind-fold/eye drape. Ear plugs (e.g. gauze in outer canals) should be used to reduce acoustic stress (Arnemo & Evans 2017).

Thermoregulation should be closely monitored by frequent measuring of the rectal temperature (RT). Typical and acceptable RT for lynx, according to Arnemo & Evans (2017), is between 36.0 to 39.5 °C. Individuals showing signs of hyperthermia (> 40.0 °C) should be cooled by applying a lukewarm (18-20 °C) enema or far less efficient snow (respectively water in summertime) to the axilla, groin, thoracic inlet/thorax and/or tongue. If hyperthermia does not resolve within 15 minutes, i.v. fluid therapy should be initiated with (10-15 ml/kg) of lukewarm (18 - 20° C) Ringer®-acetate. Additional, oxygen supplementation is recommended for hyperthermic animals because of increased oxygen demand.

Hypothermic individuals (RT < 37.0 C for Eurasian lynx) should be protected from wind and cold surfaces to avoid further cooling using a waterproof, insulated cover. Chemical heat packs or hot water bottles can be placed in the groin and axilla as an external heat source in the field. In case of prolonged immobilization and recovery, hypothermic animals should be warmed and body temperature (38° C) Ringer®-acetate should be administered intravascularly (Arnemo & Evans 2017).

Cardiorespiratory function must be monitored using a pulse oximeter with the sensor applied to the tongue (Arnemo & Evans 2017). Hemoglobin oxygen saturation (SpO₂) should be above 90% efficiency (but varies a lot between used equipment). Decreasing SpO₂ values are always a concern and should be acted on immediately. A decreasing trend indicates hypoxemia. Airways should be checked and if available intranasal oxygen should be given (Arnemo & Evans 2017). The colour of oral mucosa can also be used to assess blood oxygenation. A pink or red colour is normal; bluish membranes indicate profound hypoxemia. The capillary refill time (CRT) can be used to assess peripheral circulation by pressing a finger on the gums, releasing and counting the seconds it takes for the blood to return. Normal CRT is < 2 sec.

In helicopter and vehicle-supported deployments a portable oxygen cylinder (due to safety issues no transport in helicopters) or oxygen concentrator should be part of the standard field equipment. If veterinarians are present a laryngoscope, endotracheal tubes, and a ventilation bag should also be in the field kit so that in the rare case of apnea (absence of breathing), ventilation with a bag can be initiated.

Doxapram at 5-10 mg/kg i.v. can be given to stimulate respiration. The effect of doxapram is short-acting and, as a general central nervous system stimulant, it may lead to the animal waking up from anaesthesia. Trained personnel with experience in wildlife medicine and anaesthetic procedures should be able to intubate (place an endotracheal tube in the trachea) and provide assisted ventilation to an animal with apnea (Arnemo & Evans 2017). Preferably local anaesthesia is administered to the surgery site/operating field to reduce direct pain.

Note: In case of emergency, intravenous access has to be ensured, to provide fluid for kidney activity due to water loss by overexcitement. The use of additional oxygen is recommended due to impaired oxygen transfer during anaesthesia.

3.1.5 Reversal of anaesthesia

For reversal of immobilization in individuals that have received medetomidine, 5 mg of atipamezole per 1 mg of the total medetomidine dose is administered intramuscularly. Note: With the exception of an emergency such as apnea, atipamezole should not be administered prior to 20 min following induction

In an emergency (e.g. cardiac arrest, respiratory arrest), atipamezole can be given at any time, but recovery may then be rough with possible ataxia, excitation and convulsions. In cases of excessive excitation, midazolam may be given intramuscularly (suggested dose 0.1-0.2 mg/kg) (Arnemo & Evans 2017).

Recovery

Immobilized animals can usually be left to recover undisturbed at the site of capture. Potential side effects and dangers during and immediately after recovery include hypothermia (especially smaller individuals and younger cubs with a small body mass relative to body surface area or in cases of prolonged procedures), hyperthermia (e.g. due to capture, sun and/or high ambient temperatures), intraspecific aggression (males trying to mount immobilized females in estrus), inappropriate lack of fear, traffic and poaching. GPS and radio-instrumented animals should be checked the day after capture (Arnemo & Evans 2017).

3.1.6 Surgery

3.1.6.1 Analgesia and anaesthesia for surgery

See anaesthetic induction section in subchapter 3.1.1 and 3.1.2 for recommended anaesthetic drugs and doses. For post surgical analgesia 4mg/kg carprofen or 0.2mg/kg meloxicam is administered subcutaneously as soon as possible after induction of anaesthesia and ideally before surgery is initiated (Arnemo & Evans 2017).

3.1.6.2 Surgical procedures for implantation

Intraperitoneal transmitters

For surgery, the animal is placed in dorsal recumbency. An appropriate area (corresponding to the extent of surgical intervention), caudal to the umbilicus is clipped, the area washed with soap to remove the skin fat layer before applying chlorhexidine in 60% ethyl alcohol (e.g. Klorhexidin®) with a swab. Clipping of hair for surgical procedures should not be done in fall or winter in order to avoid frostbite and excessive heat loss at low ambient temperatures. Instead, an antiseptic cream can be used to rub into the fur along the midline, and the hair can then be parted to expose the skin an appropriate sized surgical cover is used to keep the surgical site sterile (Arnemo & Evans 2017).

For access to the peritoneal cavity, a ventral midline incision is made using standard surgical procedures. Incision length is tailored to suit implant dimensions. Skin and subcutis are incised, the site is blotted as necessary, linea alba is identified, lifted with forceps and gently incised (to avoid inadvertent bowel perforation). The linea incision is extended with blade or scissors. Then forceps/fingers are used to gently grasp the implant and ,drop' it into the abdominal cavity.

The weight of the implant to be placed should not exceed 2% of the body mass of the animal [note: 2% of body mass is the maximum weight of the collar and implant combined].

The radio transmitter should be tested with the receiver prior to placement, and implants need to be gas sterilized (e.g. Approlene[®]) prior to implementation. The implant should be pre-warmed prior to insertion into the peritoneal cavity. The incision is closed in two layers with monofilament absorbable sutures (PDS Eticon), using a simple interrupted pattern for the linea alba (US 0 for lynx and US 2-0 for lynx kittens, using a round needle) and an intradermal interrupted horizontal mattress pattern for the skin (US 2-0 for lynx, using a cutting needle). The skin wound can be covered with skin glue (cyanoacrylate) or a spray bandage (Arnemo & Evans 2017) though this may incite increased wound grooming (Walzer unpublished).

Temperature loggers, ECG monitors and physiological sensors

In Scandinavia, surgery to implant temperature loggers and physiological sensors is carried out as described for intraperitoneal transmitters with the incision length appropriate for the size of the logger or sensor. See Arnemo & Evans (2017) for further information on the topic. It is strongly recommended to contact experts concerning the implementation of temperature loggers and physiological sensors prior to application.

Micropchips and ear tags

A microchip should be implanted subcutaneously in the left interscapular region. The microchip should be tested with the scanner before and after implantation. Ear tags insertion sites should be inspected for signs of infection or irritation (redness, swelling or discharge) and if found, the ear tag should be removed (Arnemo & Evans 2017).

3.1.7 Radio collaring

Radio collars should be adjusted to the size, age and sex. The weight of the collar should not exceed 2% of the animal's body mass. While checking or changing collars, the neck should be examined for hair loss and any form of irritation/abrasion or other kind of injuries. Important: Collars need to be fitted with a drop-off mechanism. The minimum collar circumference for the individuals that are about to be equipped with radio-collars must be evaluated. At least one finger should easily fit between the collar and the neck of the individual (Arnemo & Evans 2017).

The transmitter (VHF) and GPS unit should be activated by removing the magnet(s) and should be tested with the receiver before the animal is released. It should be confirmed that the GPS unit is working properly before any capture is initiated (Arnemo & Evans 2017). It is strongly recommended to contact colleagues working on lynx or similar species in comparable landscapes to gain additional and up-to-date information on the performance of the various collar model available on the market.



Figure 15. Eurasian lynx equipped with radio collar (© M. Krofel)

3.1.8 Examination and sampling of free-ranging lynx

At the outset, body measurements are recorded for an anaesthetised individual in the field, (according to the lynx capture form - see appendix II: attachment III).

Blood

Blood samples can be taken from the jugular, cephalic or femoral vein by using vacuum sealed plastic tubes and multisample needles. Full blood, serum and plasma should be collected on every possible occasion.

Whole blood tubes with anti-coagulant (EDTA) are gently inverted several times and preferably stored at 4°C for transport to the lab. In remote locations it is advisable to make 2-4 blood smear slides on site. Following whole blood evaluation this sample can be used to collect a plasma sample.

Sample tubes without anticoagulant for serum biochemistry and serology should be kept at room temperature for one to two hours to ensure complete coagulation. Subsequently, serum should be separated by centrifugation (1500 g for at least 15 minutes) and transferred to 2 ml cryogenic vials. Serum for banking (serology and back-up) is stored at the lowest possible temperature until being shipped at a similar temperature (limit thaw-freezing cycles).

Blood samples should be taken from all captured individuals for ongoing and future studies regarding health, disease and stress. These samples will also be available for investigations of previously identified animals found dead (poaching/traffic accidents etc.) and submitted for necropsy.

Plasma

Collect whole blood by peripheral venipuncture using a 21 gauge butterfly needle into a EDTA tube, invert tube gently 8-10 times. Centrifuge tubes at 500 xg for 10 minutes at room temperature. Transfer the plasma to a fresh 15 ml centrifuge tube. Avoid the buffy coat (the white blood cell layer that lies between the plasma at the top and the red blood cells at the bottom). Centrifuge tubes at 2000 xg for 10 minutes at room temperature. Using a fresh pipet, transfer the top 80% of the plasma, avoiding the pellet at the bottom of the tube, to a fresh 15 ml tube. Transfer 1 ml of plasma into each screw-cap microcentrifuge tube. Sample should be processed, aliquoted and placed into the freezer as quickly as possible. Place the 1 ml aliquots into a -80°C freezer for storage (Laurent and Alexander 2015).

Whatman FTA cards

Whatman FTA® cards (<http://www.whatman.co.uk/>) consist of filter paper impregnated with a proprietary mix of chemicals which serve to lyse cells, to prevent growth of bacteria and to protect the DNA in a collected sample (Smith and Burgoyne 2004). They constitute a useful method to collect samples in remote areas without access to cooling.

Blood samples but also cheek swabs, saliva or tissue from dead individuals can be spotted on FTA cards and stored long-term at ambient temperature.

Skin - muscle - fat tissue biopsies

Skin/tissue samples are taken from the pinna (outer ear), after clipping hair and cleaning the skin with chlorhexidine alcohol, using a 4 or 6 mm sterile dermal biopsy punch. The sample is transferred to a 2 ml cryogenic vial and preserved by adding 96% ethanol. To prevent the bleeding, Arnemo & Evans (2017) recommend applying pressure to the area using a piece of gauze held in place with a clothespin.

If muscle biopsies are required, they are taken from the biceps femoris. Routine surgical preparation of the skin overlying the biopsy site is done prior to the biopsy. A short incision is made through the skin, fat, and fascia overlying the biopsy site, and the required amount of muscle tissue is excised. Muscle tissue is washed with sterile saline before placement in sterile cryovials (Arnemo & Evans 2017).



Figure 16. Location of biceps femoris in felids (adapted from anatoref.tumblr.com©)

Fat samples are taken from the same surgical site as for the biceps femoris muscle biopsy before closing the incision in two layers (muscle fascia and skin) using 2-0 absorbable sutures. If there is significant subcutaneous fat, it can also be apposed using similar sutures (Arnemo & Evans 2017).

Hair

During examination of anaesthetised individuals, hair samples should be collected with gloves and tweezers. The collected samples must be preserved in paper envelopes at room temperature to assist in the process of drying. Neglecting these details will hamper the subsequent DNA-analysis.

Faeces/Scat

Faeces may be sampled from an anaesthetised individual by inserting a sterile swab, sterile glove with digital collection or spatula to manually extract faeces for sampling from the rectum. Faecal samples are stored either cooled, if processed in the short term (e.g. for parasitic infestation) or frozen at -20°C or -80°C. If cooling is not available faecal samples can be stored at ambient temperature in a SAF solution (Marti and Escher 1990).

Important: If faecal samples are stored incorrectly, e.g. are frozen. An infestation with parasites is no longer detectable because parasites eggs are lysed. The storage temperature always depends on the purpose of sampling.

Urine

Urine is collected, if relevant for research purposes, by using a 0.6 x 40 mm needle with a 20 ml syringe. The animal is placed in dorsal recumbency and the location of the bladder is determined by palpation or ultrasound. The skin should be wiped with alcohol for disinfection and to improve contact of the ultrasound probe with the skin. The needle is inserted dorsally through the midline of the abdomen into the bladder, and urine is withdrawn using the syringe.

Urine can also be sampled by manual pressure on the bladder, expressing urine through the urethra that can be collected in a sterile container and stored at -20°C or -80°C (Arnemo & Evans 2017).

Samples should be labelled according to the method used for urine extraction, as either extracted by “cystocentesis” or “expressed bladder” - method.

For detailed information on non-invasive collection of genetic samples see chapter 4.

3.1.9 Necropsy procedures

If mortality occurs during or after capture, the carcass should be sent to a diagnostic laboratory for necropsy. If transportation of the body to the laboratory is not possible within 24-48 hours, the carcass must be frozen and shipped. As an alternative, a field necropsy *in situ* and proper tissue sampling can be carried out by a veterinarian (Arnemo & Evans 2017). For further details see chapter 7.

4 Non-invasive genetic sampling

The following subchapter focuses on the non-invasive collection of samples (chance findings) for genetic analysis (scat, urine, hair and saliva), storage and processing of the sample, as well as providing a short introduction to the analytic methods being used.

4.1 Collection of non-invasive genetic samples

In the last decades, non-invasive genetic sampling using DNA samples, such as scat, hair, urine and saliva collected without physical presence of an individual, has quickly become a state-of-the-art technique to gather valuable individual- and population-based genetic information and to monitor rare, sensitive and difficult to detect species such as the Eurasian lynx. The non-invasive approach offers a number of advantages over conventional methods used for detection including an increased probability of confirming the presence of individuals and species and minimizing the need for invasive monitoring e.g. the physical capture and tagging of individuals.

Today, non-invasive genetic sampling can be considered one of the most important tools to monitor and collect information on wildlife species that are difficult to detect by other methods. It can also aid in subsequent management planning. Furthermore, genetic sampling and analysis provide valuable information with regard to presence, estimated density, and other population parameters such as relatedness and genetic variation within a population as well as potential inbreeding scenarios or genetic bottlenecks.

Appropriate collection and storage of samples as well as handling and processing is critical for successful genetic sampling, as improper handling during collection can lead to contamination and deterioration of DNA.

4.2 Scat

Fecal DNA originates from cells sloughed from the intestinal lining and can be extracted from lynx scat samples, which can be collected at sites used by the species (e.g. paths, roads as well as prominent spots that are used for intraspecific communication) (Boitani & Powell 2012). In the subsequent process of genetic analysis, scat samples can have a higher success rate (85%) than hair samples (10%) for genetic analysis when using four microsatellite loci and a multiple-tube approach to verify individual genotypes for felids. Consequently they may offer a better choice for studies, as suggested by Ruell & Crooks (2007).

Characteristics

Size & Shape: Typical diameter of lynx scat, which is cylindrical, is approx. 2-3 cm, rarely more, with a length between 6-10 cm with blunt ends (“tufts”), often 2-3 (4) small „sausages“.

Contents: Lynx scats usually contain hair of prey animals (e.g. ungulates, leporids, rodents) but only in very small proportions as compared to wolf scat. Additionally, bone fragments of prey animals may be visible.

Plant material might be present, but in very small proportions (as in all felid species, blades of grass might be eaten to aid digestion/purging of inedible contents - (Skrbinšek 2017)).

Location: Like many smaller felid species, lynx tend to hide their scats by burying them, hiding them under heaps of foliage, soil or snow especially when defecating near their dens. This is not always the case with older, more dominant individuals, who might leave their scat beside forest roads or paths especially when travelling. Scat is often deposited near to kill sites. If suspected lynx scat is found in an exposed spot, it is more likely to be from a canid like wolf or fox. In some cases lynx will use latrines, which means they repeatedly use the same spot for defecation. Latrines are often located in rock shelters (Breitenmoser & Breitenmoser & Würsten 2008).



Figure 17. Scat of Eurasian Lynx. The blunt end and the little "tuft" in the left image are considered as the part containing the most valuable DNA for subsequent genetic sampling. (Illustration: © Igor Pičulin, photo: © Franc Kljun taken with permission from Skrbinšek (2017))

Note

It can be difficult to discriminate lynx scat from other species' defaecations, and they are most commonly confused with fox or wolf scat; additionally, scat of wild cats can be mistaken for lynx scat, even though dimensions of wild cat scat tend to be smaller and they are not usually „sausage“-shaped.

Even if there is uncertainty, the scat should be collected, labelled and photographed with an object like a pen or pocket knife for scale comparison. The image should be sent to the responsible person sampling the area, and/or the institution responsible for the genetic analysis.

Age estimation

Age estimation of scat is important in order to predict the expected DNA quality and to plan the most effective method for subsequent analysis of the sample.

Estimating the age of scats is not always easy, as appearance and odor intensity are significantly affected by weather conditions and scat content. Estimating age may be easier during winter when tracks of lynx are present.

Fresh scat will appear moist, possibly slimy with a strong, pithy odor to it (unless frozen in sub-zero weather conditions). In contrast to bear scat deposits, fresh lynx scat may quickly develop a desiccated appearance - consequently, the felid-specific odor of defecations is the best means of differentiating lynx scat.

Older scat can still look fresh, with a less intense feline smell. After a couple of days, the scat will no longer appear slimy or moist. This is especially true in dry weather conditions during summer, when scat is exposed to sunlight and wind (which accelerates the process of desiccation). However, it still possesses the distinctive odor, indicating acceptable quality for genetic analysis.

Old scats have almost no odor and are no longer moist. They are usually completely dried out but may appear damp in wet weather. These samples contain little to no valuable DNA, which disqualifies old scats for genetic sampling.

During winter, age of scat is of less concern, since fresh scat freezes; consequently genetic material will be conserved. Scats collected in wintry conditions are always acceptable for subsequent analysis.

Collection of scat sample

A sample should be collected from the surface of the scat, when possible collecting a portion without previous contact with soil. However, it is best to avoid the part most exposed to surrounding conditions, as is also the first to dry out, resulting in reduced amounts of conserved DNA. If a sample was exposed to heavy weather, the collection of drier parts not as heavily exposed to the weather is better for subsequent DNA sampling, containing more feasible DNA. If the conical tufts are present, a sample should be collected from this part, potentially providing DNA of better quality (Skrbinšek 2017). If mucus is present on the sample, it should be collected since it contains a lot of target DNA.

Storage of scat sample

After retrieval in the wild, small sample tubes, such as those used for urine or scat samples in human medicine work best for storing a scat sample. For DNA-analysis a pea-size sample of scat is sufficient.

Subsequently, the pea-sized scat sample will be preserved in the flask containing a scat conservation liquid (e.g. DET buffer (at 5-10 parts per sample), or ethanol (>95%)) (Boitani & Powell 2012). The sample should not exceed the prescribed filling level of the preservation liquid, because the DNA will not be properly conserved and may be more easily damaged. The liquid in the flask should never reach the top of the container nor spill out of it. (Skrbinšek 2017).



Figure 18. Left: Collection of a scat sample (with 50ml sample tubes (large) for reference (wolf scat) in the front); right 8ml (small sample tube) containing a scat sample (Illustration: © Igor Pičulin, taken with permission from Skrbinšek (2017))

In order to prevent contamination while collecting the sample, a device like a wooden stick or something similar is helpful for collection. This implement may be left at the collection site to indicate that a sample has been already gathered from this location. A new device must be used for each scat sampled in order to prevent cross-contamination.

Labeling the sample

Information concerning the collection of the sample should be recorded on the label of the sampling flask. If a small sample flask is used, it is possible to deposit the flask with a completed label in a resealable plastic bag or to link a number on the sample flask to a database sheet, where all information is stored separately.

The following information for the collected sample should be recorded:

- Species: the name of the species from which the sample was collected (often not obvious)
- Date: when was the sample collected
- Name: finder/collector of the sample - this makes it possible to get additional information and feedback (e.g. concerning location and circumstances of finding) from the collector
- Location: where the sample was found; GPS coordinates should be written down; if that is not possible, then record the name of the spot, where the sample was found, together with information on the particular area/region; this allows a more precise mapping of the finding.
- Age: it is important to make an estimate of the approximate age of the scat, as this affects DNA quality of the sample
- Note: any additional information considered useful (concerning the circumstances of collection) should be written down.

species <input type="checkbox"/> bear <input type="checkbox"/> wolf <input type="checkbox"/> lynx	
name/surname	
date	location
GPS coordinates and/or geographic name	
SCAT	
scat age (circle) 0 1 2 3 4 5 days	
size (diameter of the „sausage“, cm):	
URINE / SNOW TRACKS	
non-marking (ground): Y N	blood in urine: Y N
object of marking?	
num. of animals in group	track size (cm)
note	
[cm] 1 2 3 4 5 6 7	



Figure 19. Label on flasks for urine and scat samples; for smaller sample flasks (8 ml) labeling with information about the collection is written directly on a plastic bag into which the sample flask is deposited (Illustration: © Igor Pičulin, taken with permission from Skrbinšek (2017)).

Prevent double sampling

After collecting a sample, the scat should be marked in order to prevent double sampling, but not removed, buried or covered in order to not influence a potential increase in male-male aggression (C. Walzer, pers. comm.).

Storage

After initial collection, the samples should be kept in a cool and dry place (Boitani & Powell - 2012) to increase DNA yield. Currently, a two-step approach with soaking of the collected sample into ethanol and then transfer and desiccation onto silica gel within a week of collecting is used for complete drying of the sample (Roeder et al. 2004). Samples can then be stored at room temperature or 4°C until analysis. While silica-preserved samples yield a lower DNA concentration, the two-step method yield significantly more DNA (Roeder et al. 2004) Furthermore, samples should be utilised for extraction and analysis within three to four weeks of collection in order to increase amplification success. However, the handling of storage of the samples depends individually on each laboratory.

Note:

Data organization (e.g. labelling of flasks) may be facilitated by a bar code system using peel-off labels to link physical samples to information (described above) on data sheets (Boitani & Powell 2012). Methods for extracting and storing DNA are developing quickly; consequently frequent review of papers and forensic genetic literature on the topic is recommended in order to remain abreast of current practices.

4.3 Hair

Hair samples may be collected systematically via a snag or rubbing device, and for lynx the device is often baited (e.g. cat nip, castor or valerian oil). When the lynx rubs or marks the site of the collecting device (often placed in dens or daybeds), hair is collected opportunistically (Boitani & Powell 2012). Hair samples provide another good and non-invasive source of DNA.

Location of opportunisticly identified hair (samples):

Most opportunistic hair samples are found at spots where lynx rub prior to marking with urine for intraspecific communication and where they are resting/rearing their offspring (dens and daybeds). It is important to examine nearby objects like the bark of trees, broken branches and rocks as well as to scan the surroundings for structures that induce or attract an individual to rub and consequently leave hair. If hair samples can be found, the characteristic smell of feline urine indicates a high probability that the sample comes from a lynx (Skrbinšek 2017). When snow tracking, chances to find hair are increased as tracks may lead directly to spots where individuals mark or rest, e.g. under a fallen tree, in a rock shelter etc.

Collection & Storage

Collected samples are stored in a dry place, e.g. paper envelopes that are placed in sealed plastic containers containing silica gel or bags of desiccant drying agents. Desiccant is critical, in order to dry out the sample and prepare it for DNA preservation. The paper envelope assists in the drying process and protects the sample from environmental influences (moisture, direct sunlight etc.). Several collected hair samples in separate envelopes can be stored in the same plastic container with desiccant, as long as the samples are appropriately labeled.

The follicle, the bulbous basal part of a hair, contains the most reliable source of DNA; although extraction from the tip of hair is also possible (which potentially allows the extraction of mtDNA but no genotyping). Hair follicles can be hard to detect without a magnifying glass; consequently it makes sense to collect as much hair as possible. Intact hair shafts are far superior to those that are broken or incomplete. Kemp hair provides by far the most DNA for extraction, but thin, short undercoat hair can provide valuable DNA, too and should be also collected.

If at a certain site, hair samples can be found in several locations, hair should be collected from each of these places in separate envelopes, in order to prevent the mixing of different genotypes.

Ideally, hair samples are collected with gloves and tweezers that have been heated (e.g. using a lighter) or sterilized during the ongoing process of hair collection in the field (depending on the primers used, in many laboratories a potential contamination with human DNA while collecting samples in the field is no problem, since the used primers do not respond to human DNA).

After collection, it is vital to cap the sample tube tightly, in order to protect the sample from moisture, which can damage the sample.

Labelling the sample

The recorded parameters are similar to those recorded for scat samples, with the following additional parameters:

Age of the hair sample: An estimation of how old the deposited hair sample was, when collected. There is no objective way or method to estimate the age directly from the hair, but circumstances like the collection of hair samples during snow tracking can suggest an approximate time frame based on weather records (e.g. the latest snowfall). It is also worth ascertaining when the site was last checked for hair, as this may help to narrow down the time frame for the current sample.

Location of hair sample: On which structure/location the particular hair sample was collected. What type of object (e.g. dead tree, den, daybed, rock shelter etc.)

species	<input type="checkbox"/> bear	<input type="checkbox"/> wolf	<input type="checkbox"/> lynx
name/surname			
date		location	
GPS coordinates and/or geographic name			
H A I R			
age (estimate)	0-3	4-7	8-14 >14 days
hair collected from:			
<input type="checkbox"/> hair trap			
<input type="checkbox"/> other (description):			
I F T H E R E A R E S N O W T R A C K S			
number of animals		track size (cm)	
notes			
cm 1 2 3 4 5 6 7			

Figure 20. Example for labelling a collected hair sample (including the most important parameters) (Illustration: © Igor Pičulin, taken with permission from Skrbinšek (2017))

IMPORTANT: When exposed to air, desiccant will absorb moisture and become ineffective. Sampling kits to collect hair samples are vacuum-sealed, and, if the seal is not broken, provide an unlimited shelf life. On the other hand, if a kit is already opened, it should be used within days or be discarded, because the desiccant will accumulate moisture, and the storage capabilities will be impaired, which can damage a hair sample and makes it unusable for subsequent DNA analysis.

Note:

The amount of DNA left in a single hair or fragments is usually very small. Hairs collected with follicles provide higher quality DNA extracts than scats, which have more agents that inhibit and prevent amplification. However, a single hair in comparison to scats usually yields much less DNA. For the purposes of individual identification one must realize that hair snagging devices usually contain hair samples from more than one individual, which is suboptimal, because it can create false new genotypic individuals (Boitani & Powell 2012).

4.4 Urine

Another viable source of DNA for genetic sampling is urine, which can be collected with swabs taken from the ground or directly from snow that has been kept frozen until DNA extraction. Since lynx use urine to mark their territory, urine may be abundant when snow tracking a resident individual. This is especially true for males, who mark more frequently than females, especially during the breeding period in February/March.

Before collection

Urine is most easily collected during snow tracking sessions in winter. Prior to collection, the tracked individual should be confirmed as a lynx (see identification of paw prints below). Data concerning the circumstances of the collection should be recorded (following the instructions provided for scat samples -> see above).

Collecting urine samples

The desired sample is the „yellow snow“, which will be placed in a 50 ml sample flask. Before collection, a careful removal of surrounding „white snow“ around the „yellow snow“ is recommended to make the collection easier and to facilitate collection from some of the deeper parts of deposited urine. Enough urine-saturated snow to fill the sample flask is collected, and the flask is then tightly sealed.

When following a lynx track during mating season, findings of multiple urine deposits from the same individual are possible. Since not all samples will be successfully genotyped, it is preferable to collect several samples along the followed track. Skrbinšek (2017) recommends the collection of samples of the first two urine deposits from the same individual in separate small sample flasks. When additional urine samples are found on the same snow track that appear to be from the same individual, they should be collected in a single large 50ml sample flask. However, if there is any doubt that the samples is from the same individual, then individual sample flasks should be used for each sample.

Additional Information for labelling the sample:

The recorded parameters are similar to the ones recorded for scat and hair samples, except for the following additions:

Track size:	size of paw prints (to draw conclusions about age and sex)
Marking/Non-marking behaviour:	deposited urine directly on the ground; or a prominent marking spot was used. If marking, which structure was marked (e.g. stump, rock...)
Signs of blood in the urine:	sometimes visible after mating, very valuable source of DNA

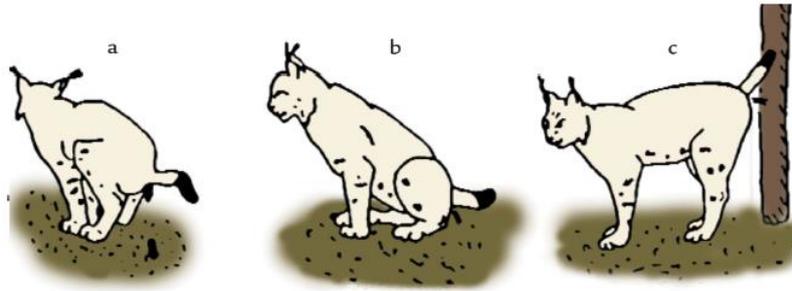


Figure 21: Defecation (a), urination (b) and marking (c). Defecation and urination will be typically on the ground producing a larger quantity of deposited urine; marking behaviour induces usually drops of urine sprayed against a vertical object like prominent trees, rocks or stumps (illustration taken with permission from Hucht-Ciorga 1988)

4.5 Saliva

Should prey freshly killed by lynx be found, saliva samples from a bite wound constitute a potentially useful source of DNA. Studies (e.g. Harms et al. 2015) indicate a rapid decline in DNA yield over time.

Rapid deterioration of DNA over time and potential for contamination with scavenger DNA can make use of saliva difficult.

Identification of lynx prey

Lynx usually ambush and kill prey with a bite to the throat and subsequent suffocation. Occasionally, lynx will kill prey by biting the neck from above, severing the spinal cord.

Consequently, lynx prey often have injuries in the larynx area with the wounds being typically clean, deeply punctuated by the sharp canines but without the large lacerations surrounding them that are more characteristic of wolf kills. In some cases, there are no immediately visible bite wounds, with puncture sites only found on closer examination after skinning.



Figure 22. Killing bites by a lynx, in nature (left) barely visible punctures in the back of the neck that are more prominent and visible after necropsy (right, indicated by arrows). Saliva samples would ideally be taken by rubbing forensic swabs around and between the wounds of the left photo (photos: M. Krofel© taken with permission from Skrbinšek 2017)

Collection

The collection of saliva samples should be undertaken with special saliva oral swabs (e.g. SalivaBio Oral Swabs © Salimetrics) applied to the most obvious and deepest bite wounds of the prey animal, because, the individual will have spent the most time in this area, producing and depositing larger amounts of saliva in the process. The portion of the carcass that seems most recently eaten from may also provide useful material. Saliva is collected using two to three swabs on chewed and gnawed on parts of the prey. In order to enhance the success rate of genotyping during subsequent DNA analysis.



Figure 23. SalivaBio Oral Swab for Saliva Sampling (© Salimetrics)

After identifying the area containing a viable amount of lynx' saliva, rubbing with the tip of the swab over the entire area. It is important to avoid areas containing significant haemorrhage in order to avoid the rapid saturation of the swab with blood rather than saliva. Samples within a single area have to be collected by using the same swab.

Note:

Felids often lick the neck after an initial kill - if a kill is fresh, there is an increased chance to collect DNA of reasonable quality for subsequent analysis (Skrbinšek 2017).

Forensic swabs used for sample collection are stored in sealed bags and have desiccant integrated into the storage tube to prevent the entry of moisture. After collection of the saliva samples, the swab has to be returned to the tube, sealed tightly and secured within a plastic bag. If a sealed bag containing swabs and desiccant is opened but not used within a couple of days, the desiccant collects moisture and making it unsuitable for storage, and the bag should be thrown away.



Figure 24. Forensic swab (© Sarstedt)

Labelling the sample

The recorded parameters are similar to those recorded when collecting scat, hair or urine samples, except for the following additions:

- Prey species: description of the sampled prey species
- Solitary / Multiple (family) lynx: appearance of kill site, e.g. were cubs present at the kill site (usually only possible to identify in situations with snow cover)
- Sample origin (which body part): which part of the prey animal the sample was taken from (when finding a fresh kill, it is very important to take a saliva sample from the neck wound)
- Degree of decay: informs conclusions about the chance of successful genotype determination from the collected sample

species <input type="checkbox"/> bear <input type="checkbox"/> wolf <input type="checkbox"/> lynx	date:
name/surname:	location:
GPS coordinates and/or geographic name:	
prey species:	% consumed:
prey description:	
single predator <input type="checkbox"/> family <input type="checkbox"/> if family, how many?	
sampled from: neck <input type="checkbox"/> rump <input type="checkbox"/> shoulder <input type="checkbox"/> other:	
carcass decomposition (circle):	0 1 2 3 4 5
(0 - fresh; 3 - bloated t.; 5 - liquification of tissue, mummification...)	
notes:	

Figure 25: Example for labelling a sampling containing lynx saliva (Illustration: © Igor Pičulin, taken with permission from Skrbinšek (2017))

Storage

Special attention should be paid to ensure that the swab tube is sealed tightly and properly. In order to conserve the swab for a longer period, the connection between swab handle and tube can be sealed with duct tape or other material in order to ensure an airtight fit.

5 Diseases and common causes for adult and pre-adult mortality within the genus lynx in captivity and in the wild

Felids are susceptible to many infectious and noninfectious diseases. This chapter briefly outlines conditions commonly associated with Eurasian lynx in the wild as well as for newborn and adult individuals in captivity; most common causes for adult and pre-adult mortality are described as well.

5.1 Common problems, disease diagnostics and prophylaxis in newborn individuals and cubs held in captivity

Condition	Characteristics/Signs	Treatment
Diarrhoea	Diarrhoea can dehydrate a newborn lynx very rapidly and result in death. In most cases, diarrhoea is related to over-feeding or a feeding formula that is too concentrated; sometimes it is caused by a bacterial or parasitical infection, usually due to a lack of hygiene.	<p>Mild diarrhoea responds well to a more diluted formula with mineral water or electrolyte solution at a 1:1 ratio until diarrhoea stops. In more serious cases, feeding formula has to be taken completely off and only electrolytes (a 5-10% glucose solution) given - using the same amount and feeding schedule as with formula until diarrhoea stops (Mellen and Wildt 2003). If the neonate is hypothermic no oral medication or fluid should be applied.</p> <p>In these cases, a veterinary intervention is needed to start an intravenous or subcutaneous treatment. Gradually reintroduce formula, beginning with extremely dilute formula (1:10-1:5, slowly returning to its original strength as stool consistency solidifies. Consult a veterinarian if diarrhoea persists. Bene-bac® by PetAg®, a gel that stabilizes the natural gut flora of kittens, is very effective (Andrews 1998). Antibiotics are not recommended, particularly for treating diarrhoea, as they often upset the cubs growth of a normal bacterial gut flora, they can even worsen the situation (Gunn-Moore 2006).</p>
Constipation	A cub whose faeces are hardened and has difficulties defecating or has not defecated in 36 hours is considered constipated (Mellen and Wildt 2003).	Normally, a few doses of liquid paraffin (Hodernal®, Emuliquen®) in approximate doses of 0.5 ml per feeding for 2-3 days solves the problem (Gunn-Moore 2006). Another possibility is to give the cub a few drops of corn syrup added to each bottle for 2-3 feedings (Mellen and Wildt 2003). If the cub does not defecate after applying the explained treatment, a mild warm soapy enema may be applied. If these steps do not resolve the constipation, then veterinary help should be sought.

Condition	Characteristics/Signs	Treatment
Bloat	Bloat is swelling of the abdomen caused by gas in the intestines or the peritoneal cavity of the cub.	Applying electrolyte therapy as for diarrhoea using Nutrical®; At a rate of 4 cc daily divided by the number of feedings or Simethicone (Mellen and Wildt 2003).
Aspiration	Aspiration can occur when the feeding formula is not well accepted or when the cub has difficulty swallowing and liquid enters the airways. Reasons may include unfamiliarity with the bottle or excessive volume of formula being offered.	Apply the following technique to clear the airways: hold the cub belly down on the palm of your right hand, placing its head between the index and middle fingers. Place the palm of your left hand on the cub's back, holding the upper part of its head with the fingers of this hand. Bend forward with your legs flexed and swing the cub downward between your legs gently but firmly so that it can expel the fluid from its airways.
Hypothermia	Cubs are unable to regulate their own temperature, which can drop from optimal to a critically low or high temperature in just a few hours. Risk is highest in the first few post-natal weeks, when body temperature ranges between 35-37 °C, the shivering reflex is not yet present and there is not much subcutaneous fat. A cub with a temperature of 38 °C has a blood pulse between 200 and 250 bpm. However, if its temperature drops below 34 °C, the heartbeat will drop to 40-50 bpm. This may cause a malfunction of the respiratory system and ultimately lead to cardiac arrest (Gunn-Moore 2006, 2006a).	It is vital to re-stabilize the cub's temperature gradually. The cub may be placed in a warmed isolette or incubator. It should be done in a timeframe lasting between 1 to 4 hours, depending on the severity of hypothermia. A rapid increase in temperature may cause cardiovascular collapse and death of the animal.

Table 4. Common problems, disease diagnostics and prophylaxis in newborn individuals and cubs held in captivity

5.2 Viral diseases in captivity and in the wild

Name	Epizootiology	Cases	Signs	Diagnosis	Treatment	Captivity	Wild
Feline panleucopenia or parvovirus (FPV) also known as Feline infectious enteritis (FIE)	Highly contagious virus that is shed in all secretions and excretions. It is shed in faeces for up to six weeks after recovery. Illness last five to seven days; usually fatal. Mortality is highest in felids <five months of age.	In a study of Naidenko et al. (2018) on seprevalence in wild felids, none of the tested wild Eurasian lynx were positive FPV-tested. Ryser-Degiorgis (2009) tested one wild individual positive for FPV antibodies. Wasieri et al. (2009) showed infections in a captive held individual in a wildlife park, potentially caused by contact with feral domestic cats. In 2019, an individual captured in Romania, held in quarantine for reintroduction in the LIFE Lynx project in Slovenia tested positive for FPV, without showing clinical signs (M. Krofel, pers. comm.). FPV can pose a threat to lynx held in captivity, as well as to individuals in the wild exposed to temporarily quarantined individuals reintroduced for population reinforcement. Vaccination of (temporarily) captive held individuals is therefore recommended.	Can be subclinical; acute cases show fever, depression, anorexia and dehydration, vomiting and diarrhoea may be present; death.	Presumptive diagnosis is based on clinical presentation; confirmation by demonstrating FPV antigen in faeces; Test kits for canine parvovirus antigen may detect FPV antigen during the acute phase.	Virus is resistant to inactivation and can survive >1 year in a suitable environment; Virus is inactivated by 6 % household bleach (sodium hydrochlorite); Prevention relies on vaccination, and use of a product containing an inactivated or killed virus is recommended (Miller & Fowler 2015).	X	X
Rabies	Rabies is caused by a lyssavirus that affects the central nervous system. Bites of infected animals (carnivores or bats as vectors), contact of saliva with mucous membranes or open wounds. Aerosol in an enclosed environment, fatal disease within two to seven days of illness (Miller & Fowler 2015).	Rarely have cases of rabies been reported in Eurasian lynx (Matjuschkin 1978; Stahl & Vandel 1999; Ryser-Degiorgis 2009).	Salivation, abnormal behaviour (aggression), neurologic signs (paresis, seizures).	Recommend euthanasia and shipment of head to a qualified laboratory for FA or VI Serology used to monitor response to vaccination (Miller & Fowler 2015).	Immunization with Purevax® Rabies ad us. vet. or Nobivac® Rabies ad us. vet. from the age of 12 weeks to prevent mortality from infection recommended;. Lyssaviruses are not stable; inactivated by common disinfectants (www.vetpharm.uzh.ch).	X	X

Table 5. Viral diseases in captivity and in the wild

Name	Epizootiology	Cases	Signs	Diagnosis	Treatment	Captivity	Wild
Orthopoxvirus (OPV)	Orthopoxviruses are a group of large, complex, double-stranded DNA viruses that replicate in the cytoplasm of the host cell. Most orthopoxvirus infections are zoonotic, with humans serving as accidental hosts (Munoz 2014).	In 2011, orthopoxvirus (OPV) DNA was detected in tissues (lung, kidney, spleen) of 24 (9%) of 263 wild Eurasian lynx (<i>Lynx lynx</i>) from Sweden. The prevalence was higher among individuals from regions with dense, rather than rural human population (Tryland et al. 2011). According to Tryland et al. (2011), lynx are probably exposed to OPV through predation on small mammal reservoir species. They concluded that “OPV is widely distributed in Sweden and may pose a threat to humans”, so far no detection of OPV in other lynx populations is known of.	Orthopoxvirus infections may be localized to the skin or disseminated. The initial site of infection may be the skin, a mucosal surface, or the respiratory tract. The virus then spreads through regional lymphatics to cause viraemia and involvement of the reticuloendothelial system with secondary viraemia. The typical pock skin lesions result from direct viral infection of the skin (Munoz 2014).	Diagnostic laboratory testing for orthopoxvirus infections can include polymerase chain reaction, viral culture, and electron microscopy of rash lesion material, as well as serologic testing of serum (Peterson and Damon 2015).	Vaccinia virus continues to be used as a vaccine. Vaccinia Immune Globulin Intravenous (VIGIV) is licensed for the treatment of certain complications of vaccinia vaccine administration (Peterson and Damon 2015). No antiviral drugs are currently licensed for use in the treatment of orthopoxvirus or other poxviral illnesses. Antibodies directed against a member of the orthopoxviruses can provide cross-protection against other poxvirus species. Although no specific antiviral treatment is available, certain compounds, such as cidofovir and ribavirin, have in vitro activity against all pox viruses. Drugs active against herpes virus, particularly acyclovir, are not active against pox viruses (Munoz 2014).		X
Feline leukemia virus (FeLV)	FeLV is a retrovirus of domestic cats; FeLV in nondomestic felids is rare	Individuals infected with FeLV occurred in captive <i>Lynx rufus</i> (Sleeman 2001) and an outbreak occurred in the wild in <i>Lynx pardinus</i> in 2006/7 (Geret et al. 2011)	infection indicated by pale gums, yellow color in mouth and whites of eyes, enlarged lymph nodes, bladder, skin, or upper respiratory infections	Early infections: diagnose by blood testing (ELISA); IFA blood testing detects progressive phase of the infection	Presently, there is no cure for FeLV infection	X	X

Name	Epizootiology	Cases	Signs	Diagnosis	Treatment	Captivity	Wild
Feline immunodeficiency virus (FIV)	FIV is a lentivirus that affects mainly domestic cats	FIV strain was detected in <i>Lynx rufus</i> (Lagana et al. 2013)	initial stage (acute phase) is accompanied by mild symptoms such as lethargy, anorexia, fever and lymphadenopathy; followed by the asymptomatic stage with no noticeable symptoms, followed by final stage, where the individual is extremely susceptible to secondary diseases which induce death	blood testing for FIV antibodies; testing identifies those individuals, that carry the FIV antibody but does not detect the actual virus; individuals tested positive might be tested later negative due to seroreversion	Treatment via Lymphocyte T-Cell Immunomodulator (LTCl); LTCl is a potent regulator of CD-4 lymphocyte production and function (Beardsley et al. 1983. It has been shown to increase lymphocyte numbers and Interleukin 2 production (Beardsley et al. 2007).		X
Feline herpes / rhinotracheitis virus (FHV-1/FVR)	FVR is caused by the feline herpesvirus 1 (FHV-1)	Low prevalences of minimally positive titres were found in <i>Lynx pardinus</i> (Roelke et al. 2008)	Upper respiratory infection of the nose and throat; uncontrollable sneezing; watery or pus containing nasal discharge; loss of sense of smell; eyelid spasms resulting in closure of the eye blepharospasm); conjunctivitis; keratitis	Diagnosis of FVR by corneal ulceration; Definitive diagnosis can be done by direct immunofluorescence or virus isolation	No specific antiviral famciclovir is effective at treating this infection in cats. Conjunctivitis and corneal ulcers are treated with topical antibiotics for secondary bacterial infection.		X
Feline calicivirus (FCV)	FCV is caused by a virus strain of the family Caliciviridae, causing respiratory infection in cats	In a study by Meli et al. (2009), antibodies to FCV were detected in 29 of 74 (39.2%) tested free ranging <i>Lynx pardinus</i>	Acute upper respiratory infection (URI); gingivitis and stomatitis; limping syndrome (in young individuals); rare: Virulent systemic FCV infection (vsFCV)	Presence of typical signs of URI; sample of pus by ocular; virus detection by PCR	FCV infections frequently complicated by secondary bacterial infections; supportive treatment with antibiotics; severe cases need intravenous fluid therapy, nutritional support		X
Feline corona virus (FCoV)	FCoV is a enveloped single-stranded RNA virus	Antibodies to feline coronavirus (FCoV) were detected in 19/74 (25.7%) tested free ranging <i>Lynx pardinus</i> (Meli et al. 2009)	Fever, lethargy, inappetence, vomiting, diarrhea, dehydration, icterus, tachypnea, uveitis, neurologic signs, abdominal distention due to ascites.	Virus detection via electron microscopy or PCR from diarrheic feces aid diagnosis of FCoV enteritis	Treatment largely supportive and includes fluid and nutritional support. Disease is progressive and ultimately fatal.		X

5.3 Rare or incorrectly diagnosed viral diseases in captivity and in the wild

Name	Epizootiology	Cases	Signs	Diagnosis	Treatment	Captivity	Wild
Bluetongue virus (BTV)	Bluetongue virus; orbivirus (with at least 24 serotypes described)	Recent emergence of BTV8 in northwest Europe with over-wintering and seasonal pattern of disease - in 2007 one case of BTV in Eurasian lynx in a zoo in Belgium (Jauniaux et al. 2008)	Hemorrhage and ulceration, oral cavity and teats, epiphora and periocular inflammation, transient but severe corneal edema, limb edema - in Eurasian lynx anemia, emaciation, enlarged and gelatinous lymph nodes, pneumonia, sub-cutaneous hematomas, petechial hemorrhages, lung congestion with edema (Jauniaux et al. 2008)	History & clinical signs, histopathologic examination, PCR test & serologic examination	Vector control, vaccination with BTVPUR ALSap 8, resp. 4 ad us. vet.; The vaccine contains inactivated serotype 8 or 4 bluetongue virus. It gives the animal an active and specifically anti-serotype 8 Bluetongue virus immunity (www.vetpharm.uzh.ch).	X	
Borna disease (BDV)	Order: Mononegavirales Family: Bornaviridae (enveloped single-strand RNA virus) - Multiple genotypes	In Sweden, one individual in the wild showed abnormal behaviour and was shot. PCR analysis showed the presence of Borna disease virus infection in the brain. To our knowledge, this is the first confirmed case of Borna disease in a large felid. Host spectrum of BDV is very broad and includes horses, sheep, cattle, cats, rabbits, and ostriches, rarely wild mammals such as lynx (Degiorgis et al. 2000).	BDV infection causes severe neurologic syndrome called Borna disease (BD), manifested as nonsuppurative encephalomyelitis with a predilection for the limbic system, the basal ganglia, and the brain stem. Symptoms vary but may include excited or depressed behaviour, ataxia, ocular disorders and abnormal posture and movement (Miller & Fowler 2015).	PCR, VI, IFA of cell culture, IHC; Western blot crop biopsy and histopathology. Antibody test not commercially available. When present, gross lesions consist of dilated proventriculus and cardiac enlargement. Histopathology of proventricular dilatation disease is characterised by nonsuppurative inflammation in the central, peripheral and autonomic nervous systems (Miller & Fowler 2015).	No proven zoonotic potential. Care limited to supportive with easily digestible high calorie food, NSAIDs. Screen for viral infection and shedding via PCR. Maintenance of an ABV free collection preferred but is rarely currently feasible. Testing and separation is difficult based on inter-mittent shedding and latency of virus. No recommended commercially available vaccine. Appropriate disinfection uninvestigated (Miller & Fowler 2015).		X

Table 6. Rare or incorrectly diagnosed viral diseases in captivity and in the wild

5.4 Bacterial diseases of Eurasian lynx in captivity and in the wild

Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Salmonellosis	Salmonellosis can be acquired from contaminated raw meat fed to captive held individuals. The symptoms range from mild diarrhoea to severe gastroenteritis with generalised infection, causing dehydration. Some infected animals appear unaffected (Krelekamp 2004).	One case of <i>Salmonella arizonae</i> was reported in a young captive Eurasian lynx (Macri et al. 1997)		The treatment apart of replacing fluids and electrolytes, consists of administering broad-spectrum antibiotics such as Synulox® ad us. vet. (active agent Amoxicillin and Clavulan acid) (Miller & Fowler 2015). To lessen the risk of salmonella infection, it has been suggested that the intestines are removed from the carcasses before offering poultry to captive individuals (Blomqvist et al. 1999). In general, the offering of poultry should be avoided and rabbits or similar naturally accepted prey items should be offered instead to avoid salmonella infection.	X	X
Tuberculosis	Tuberculosis (TBC) is usually transmitted from feeding infected meat with wild birds acting as potential vectors for <i>Mycobacterium bovis</i> , the bacteria that causes TBC. The disease can take several months or even years to develop. The bacterium is also transmitted in aerosols and other secretions. Symptoms include localized lymph node swelling, acute respiratory distress, weakness and chronic emaciation/strong weight loss.	So far there have been no known cases in Eurasian Lynx, but TB has been reported in free-living Iberian Lynx (Briones et al. 2000; Pérez et al. 2013).	Antemortem testing: multimodal approach and cautious interpretation. Clinical and necropsy findings; detection of <i>M. bovis</i> by acid-fast and immune-histochemical stains, PCR, and culture (“gold standard”); tuberculin skin test and antigen-stimulation tests (Miller & Fowler 2015).	TBC is rarely treated (Miller & Fowler 2015). In captivity prevention consists of deep freezing whole or partial prey items prior to feeding them (Mellen 2003). With the general expansion of TB in Europe this could become a major concern in the future.	X	X

Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Pseudo-tuberculosis (<i>Yersinia pseudotuberculosis</i>)	<p>Occurred in the wild as chronic form with multifocal necrotic foci in inner organs; in a rescue station facility, an acute form with diarrhoea, anorexia and apathetic behaviour followed by septicaemia was observed (Ryser-Degiorgis and Robert 2006);</p> <p>Outer Symptoms: skin injuries, lymph nodes enlarged, abscesses in lymph nodes open with infectious, yellow-green pus; abscess in internal organs, mostly lungs/liver</p>	Observed in individuals from the alpine population from Switzerland, both in the wild and in rescue station facilities (Ryser-Degiorgis and Robert 2006).	Diagnosis by PCR	Regular blood testing to establish unsuspecting virus free individuals (applies for individuals held in captivity)	X	X
Feline infectious anaemia (FIA)	<p>Feline infectious anaemia (FIA) is a feline retrovirus caused by a variety of infectious agents, most commonly <i>Mycoplasma haemofelis</i> (Mhf), which is transmitted by blood sucking insects (cat fleas) (Blomqvist et al. 1999).</p> <p>Signs include lethargy, decreased appetite. Physical examination findings are non-specific and can include anaemia, such as mucous membrane pallor, tachypnea and tachycardia, pyrexia, occasionally splenomegaly and jaundice. The anaemia can be severe and fatal in some cases. Coinfection often occurs with other infectious agents including feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV).</p>	In a study of Willi et al. (2007), prevalence for feline hemoplasma infections in wild Eurasian lynx from Switzerland 44% tested via real-time PCR for <i>M. haemofelis</i> positive.	Most common findings from complete blood counts from cats showing a Mhf infection are a macrocytic, hypochromic regenerative anaemia. Reticulocytes and Howell-Jolly bodies can be identified on cytologic examination. Mhf infection can be diagnosed by identification of organisms on a blood smear. However, examination of a single blood smear is less than 50% sensitive, as the animal's immune response causes organisms to disappear from the blood stream for several days often to reappear a few days later (Hagiwara 2009). The gold standard for diagnosis of Mhf infection is PCR, which detects the 16S RNA gene.	Treatment consists of blood transfusion (in cases of severe anaemia) and antibiotics for at least two weeks (e.g. Doxycycline) (Miller & Fowler 2015). Only cats who are anaemic and have clinical signs and laboratory results consistent with haemoplasmosis should be treated, as the drug does not reliably eliminate the organism (Sykes 2010). Enrofloxacin is also an effective treatment but should be considered as secondary choice due to the risk of acute retinal damage in cats (Tasker et al. 2004). In view of the known risk factors that exist for FIA infection, it is wise to take measures to prevent flea infestation (flea treatment see p. 61) in captivity. Treatment with antimicrobials may result in false negatives on PCR; so collecting before beginning therapy is preferable (Miller & Fowler 2015).		X

Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Anthrax	<p>Anthrax is caused by a bacterium called <i>Bacillus anthracis</i> that forms spores and may remain dormant in soil for years (Miller and Fowler 2015). The disease is usually associated with feeding of contaminated carcasses (Blomqvist et al. 1999).</p> <p>Animals infected with the bacterium show signs of septicaemia, fever, depression and weakness. Acute death, with blood draining from body cavities is possible (Miller & Fowler 2015).</p>	<p>Grigoryan (2002) described a case, where a silver puma, a serval and a black lynx died of anthrax in an Armenian zoo after being fed contaminated carcasses.</p>	<p>Staining smears of peripheral blood, postmortem lesions.</p>	<p>The disease can be treated with antibiotics (Penicillin: streptomycin). However, a diagnosis is rarely made in time for antibiotics to be effective (Miller and Fowler 2015). Prevention consists of deep-freezing whole or partial prey items prior to feeding them to individuals.</p>	X	
Borreliosis	<p><i>Borrelia burgdorferi</i> is the agent causing borreliosis or Lyme disease.</p> <p>Although domestic cats do produce specific antibodies, it is unclear whether they develop clinical symptoms.</p>	<p>Antibodies to <i>B. burgdorferi</i> were demonstrated in one of two investigated free-ranging lynx from France (Ryser-Degiorgis 2009). A recent study on borreliacidal effect of carnivore's serum complement indicated that wolf and lynx probably are competent reservoir for <i>Borrelia spp.</i> (Ryser-Degiorgis 2009).</p>	<p>Serology is the main way of confirming a clinical impression of Lyme disease</p>	<p>Standard treatment for disease caused by <i>B. burgdorferi</i> infection in captive held individuals is doxycycline at 10 mg/kg orally every 24 hours for 30 days. Longer courses of treatment may be necessary in some cases, particularly those with nephropathy (capcvet.org).</p>		X

Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Helicobacter sp.	<p>Histological: Macroscopic lesions in the stomach or liver. Helical, coiled, corkscrew-like organisms closely resembling <i>Helicobacter spp.</i> in gastric mucosa</p> <p>Gastritis, vomiting, and diarrhea have been associated with <i>Helicobacter</i> infection, although a direct causal relationship has not been identified (Mirzaeian et al. 2013).</p> <p>Mucosal inflammation, glandular degeneration, and lymphoid follicle hyperplasia accompany some infections (Blois, n.d.).</p>	<p>Mörner et al. 2009 detected <i>Helicobacter spp.</i> in the stomach by PCR analysis in 17 (68%) of the lynx tested in the study. PCR fragments, amplified from the tested lynx were sequenced and compared with those of known <i>Helicobacter</i> species, which resulted in a closely relatedness to <i>H. heilmannii</i>.</p>	<p>Upper GI endoscopy or exploratory laparotomy. Surface mucus from a large area of the stomach can be obtained by taking brush samples via endoscopy. If organisms are present, they are readily identified under 100× oil-immersion magnification. Because brush cytology samples a large area of the stomach, the sensitivity of this test is high. Gastric biopsies should be obtained from multiple areas in the stomach, because organism distribution can be patchy. Routine H&E staining is usually sufficient to identify organisms, although special silver stains may be required if the organisms have a glandular location.</p>	<p>Recommended treatment regimens include amoxicillin or tetracycline, metronidazole, bismuth subsalicylate, and a proton pump inhibitor (eg, omeprazole) or H2-receptor blocker (eg, famotidine) for 2-3 wks. Other treatment combinations of omeprazole and azithromycin or clarithromycin have been described (Blois, n.d.).</p>		X
Conjunctivitis/ Chlamydiosis (<i>Chlamydia felis</i>)	<p>Clinical signs typically include a marked hyperemia of the nictitating membrane, prominent chemosis, blepharospasm, and ocular discharge. Often start unilaterally but become bilateral after a few days. Chlamydiosis in cats can additionally be associated with fever, infection of the upper respiratory tract, pneumonia, reproductive disorders (Gruffydd-Jones et al. 2009).</p>	<p>A free-ranging adult Eurasian lynx (<i>Lynx lynx</i>) captured in Switzerland presented with a severe purulent unilateral conjunctivitis.</p>	<p><i>Chlamydia felis</i> was detected in conjunctival swabs by real-time quantitative PCR (Marti et al. 2019)</p>	<p>Systemic treatment with oxytetracycline and ketoprofen led to complete recovery.</p>		X

Table 7. Bacterial diseases of Eurasian lynx in captivity and in the wild

Other common bacterial infections encountered in Eurasian lynx in the wild and in captivity

According to Ryser-Degiorgis (2009), most bacterial induced mortalities are caused, apart from those already mentioned above, by commonly occurring bacteria strains from infected wounds. Pulpitis following canine teeth injuries, associated with alveolar periostitis; osteomyelitis and/or septicemia in lynx orphans kept in rescue station facilities; as well as dental abscess in carnassial tooth associated with *Arcanobacter pyogenes* infection. Furthermore, purulent bronchopneumonia due to *Streptococcus spp.*, *Pasteurella sp.* and occasionally associated with pyothorax and/or pericarditis; purulent cystitis and pyelonephritis due to an ascending urinary tract infection with haemolytic *Escherichia coli*.

5.5 Parasitic diseases: Endoparasites

Faecal samples from injured or dead individuals should be screened for endoparasites. In cases of diagnosed endoparasitism in captive lynx, faecal samples have to be screened more frequently and appropriate therapy be applied. If a persistent parasitic problem exists, the specific animal as well as the surrounding environment (e.g. timely cleaning and removal of faeces to reduce the risk of reinfection) has to be considered (Blomqvist et al. 1999)

Phylum	Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Nematodes	<i>Gastro-intestinal parasites</i>						
	<i>Toxocaridae (Toxascaris, Toxocara spp.)</i>	<p>The most common nematodes (roundworms) found in lynx faeces are ascarids such as <i>Toxascaris</i> and <i>Toxocara cati</i>, both of which have an indirect life cycle, most Eurasian lynx carry them.</p> <p>Ova of <i>Toxascaris</i> and <i>Toxocara cati</i> are very resistant and difficult to eliminate from the environment. Re-infection readily occurs by contaminated food, by ingestion of secondary hosts (rodents) and can also be passed to cubs in mother's milk.</p> <p>Heavy infestation can cause diarrhoea, vomiting, weight loss, poor hair coat (Miller and Fowler 2015) and in severe cases, death due to intestinal obstruction (Krelekamp 2004).</p>	<p>In Sweden, according to Ryser-Degiorgis (2009), 71% of more than 200 individuals were infested most commonly with ascarids such as <i>Toxocara cati</i>.</p> <p>Investigations from Switzerland and other northeastern European countries reveal similar results with infestation rates reaching from 63% and 93%, respectively (RySource?). The prevalence of <i>T. cati</i> is with 26% of tested lynx individuals reported as rather low in Białowieża, Poland (Szczęsna et al. 2008).</p>	Scat contains ova of <i>Toxascaris</i> and <i>Toxocara cati</i>	Captive held animals particularly should have regular parasitic egg counts carried out on faeces (Mellen and Wildt 2003). Because elimination is essentially impossible once infection has occurred, periodic treatment with anthelmintics (e.g. Zantel® ad us. vet.) is necessary. A variety of effective compounds is available and can be administered in food (Miller & Fowler 2015). It is good practice to vary the type of anthelmintic (e.g. Fenbendazol®, Praziquantel®) employed (Blomqvist et al. 1999).	X	X

Phylum	Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Nematodes	<i>Trichinella spp.</i>	<p>Trichinella spp. are small parasitic nematode worms that infest the intestines of various mammals and whose larvae move through the bloodstream, becoming encysted in muscles. In Europe, Trichinella is common in foxes that are considered as an important infection source for other wildlife.</p> <p>Lynx harbour Trichinella without developing a disease condition.</p>	<p>Prevalence in Eurasian lynx is usually high, reaching from 30-50% (Ryser-Degiorgis 2009).</p> <p>In Finland, prevalence varies from 5-70%, depending on the geographical region (Oksanen et al. 1998; Oivanen et al. 2002), and in Sweden, it is generally very low with 5% prevalence (Pozio et al. 2004). Trichinella species identified in Eurasian lynx are <i>T. pseudospiralis</i> in Sweden (Pozio et al. 2004), <i>T. nativa</i> in Finland (Oivanen et al. 2002) and Estonia (Järvis et al., 2001), and <i>T. britovi</i> in Switzerland (Frey et al. 2008).</p>	Blood tests. An increase in the number of eosinophils or existing antibodies (several weeks after infection) Muscle biopsy to look for trichinella larvae.	Anti-parasitic medication with anthelmintics such as albendazole (Albenza) or mebendazole can be effective in eliminating the intestinal worms and larvae of captive held individuals		X
	<i>Capillaria sp.</i> , <i>Uncinaria sp.</i>	<p>Capillaria sp. are extremely thin, filamentous worms measuring 15-25mm long (males) and 35-80mm long (females).</p> <p>Uncinaria sp. hookworms range in size from 10 to 20 mm by 0.4 to 0.5 mm.</p> <p>Clinical signs of infection include: weight loss, diarrhoea, regurgitation, anaemia and oral necrotic plaques (wikivet.net).</p>	<p>In a study on endoparasite infestation of Eurasian lynx in Finland, only eggs were detected for <i>Capillaria sp.</i> and <i>Uncinaria sp.</i> nematodes, and only adults were detected for <i>Mesocestoides sp.</i> cestodes (Deksne et al. 2012). <i>Capillaria sp.</i> was found in the bronchi and trachea of 33% lynx from Latvia and in less than 2% of lynx from Sweden (Bagrade et al., 2003; Ryser-Degiorgis and Robert 2006)</p>	Faecal flotation, to identify the typical barrel-shaped eggs. Eggs laid within the gastrointestinal epithelium are only released into the lumen of the digestive tract when the epithelium sloughs. Severe clinical signs may be associated with negative or low faecal egg counts.	For captive individuals Fenbendazole, Mebendazole, Pyrantel Pamoate and Ivermectin have been used and efficacy of therapy should be checked through repeat faecal flotation tests (wikivet.net).	X	

Phylum	Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Nematodes	<i>C. feliscati</i>	Adult female worms are ~ 5 cm long; males are half that size. In cats with a heavy infection, symptoms can include frequent urination, painful urination, bloody urine, straining to urinate. Infected cats are usually over 8 months of age.	<i>C. feliscati</i> were a.o. observed in the urinary bladder of lynx from Estonia (Bagrađe et al. 2003) and captive held lynx in Poland (Filip and Demiaszkiewicz 2017).	Faecal flotation - finding eggs in the urine. Adult worms can be seen and removed if the bladder is surgically opened.	No approved treatment is available, an oral dose of 0.1 mg/lb. of Ivermectin has been suggested.	X	X
Flatworms	<i>Mesocestoides sp.</i>	Causing mild gastrointestinal symptoms: nausea, diarrhea, abdominal discomfort, vomiting.	<i>Mesocestoides sp.</i> was a.o. observed in free-ranging Eurasian lynx in a study in Finland (Deksne et al. 2013)	Adult mesocestoides intermittently shed proglottids, not eggs. ; M. adult tapeworm infections will not be detected during routine ova and parasite screening procedures such as faecal flotation.	Therapy with mebendazole, after recurrence of the initial episodic clinical signs postoperatively. Daily use of mebendazole for intermittent periods of up to 3 months to reduce gastrointestinal signs in captive held individuals (Barsanti et al. 1979)		X
	<i>Spirometra janickii</i>	Tapeworms in the genus <i>Spirometra</i> (Cestoda: <i>Diphyllobothriidae</i>) are mainly reside in the small intestines of cats and dogs. Clinical signs vary from unthriftiness, malaise, irritability, capricious appetite, and shaggy coat to colic and mild diarrhea; rarely, intussusception or blockage of the intestine, emaciation, and seizures are seen	<i>Spirometra janickii</i> was identified as the most common parasite in Eurasian lynx in Poland (Szczęsna et al. 2008).	Faecal flotation and identifying proglottid segments in faeces	Can be treated with praziquantel at 7.5 mg/kg, PO, for 2 consecutive days.[10] <i>Spirometra</i> species infections in cats can also be treated with a single dose of praziquantel at 30 mg/kg, SC, IM, or PO. Mebendazole at 11 mg/kg (Peregrine, n.d. Veterinary Manual)		X

Phylum	Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Lungworms	Lungworms						
	<i>Aelurostrongylus</i> sp.	A moderately common lungworm in felids. Felids may be subclinically infected and display no clinical signs, but heavy infections cause coughing or increased respiratory rate.	In Bialowieza Forest, Poland <i>A. abstrusus</i> was first recorded and subsequently identified in 17% of the investigated samples (Szczęsna et al. 2006). The species was also found in Eurasian lynx in Switzerland and in Iberian Lynx (Schmidt-Posthaus et al. 2002, Rodriguez and Carbonell 1998)	Faecal flotation - to count and see helminthic eggs	1-5 day: Fenbendazole 4.8g (Panacure®PetPaste 187.5mg in 1g) -at a dose of 20mg/kg, p.o. once every 24 hours. On 6th and 20th day: Ivermectin (Kepromec®10mg/ml) at a dose of 0.4mg/kg s.c On the 30th day: Advocate cat spot on, and it is recommended this treatment to be monthly.		X
Tapeworms	<i>Taenia</i> spp.	Cestodes are transmitted by inter-mediary or paratenic hosts, are less commonly found in lynx (Ryser-Degiorgis 2009).	Mostly found in Estonia Latvia, where all investigated lynx harboured <i>T. psifomes</i> (Bagrađe et al. 2003; Valdmann et al. 2004). Found in Poland as well (Kołodziej-Sobocińska et al. 2018).	Faecal flotation - to count and see helminthic eggs	Anthelmintic Therapy: Albendazole or Praziquantel.		X
	<i>Diphyllobothrium</i> sp.	D. species are large (~2m) and can have a mechanical effect on the host, many infections are asymptomatic. Diarrhea, discomfort, fatigue, constipation, pernicious anemia	Found in free-ranging lynx from Estonia and Poland (Valdmann et al 2004; Szczesna et al. 2008; Kołodziej-Sobocińska et al. 2018)	Faecal flotation - to count and see helminthic eggs, respectively identifying proglottid segments in faeces	Anthelmintic Therapy: Albendazole or Praziquantel.		X

Phylum	Name	Characteristics/Signs	Cases	Diagnosis	Treatment	Captivity	Wild
Protozoans	<i>Isospora sp.</i> , <i>Cystoisospora sp.</i>	Protozoans (<i>Isospora sp.</i> , <i>Cystoisospora sp.</i>), transmitted by intermediary or paratenic hosts are less commonly found in lynx (Ryser-Degiorgis 2009).	<i>Isospora sp.</i> was found in free- ranging Eurasian lynx from Finland (Deksne et al. 2013) and <i>Cystoisospora sp. in</i> <i>captive individuals in Poland</i> (Filip and Demiaszkiewicz 2017)	Isosporosis is more often diagnosed by histology than by fecal floatation only.	Sulfadimethoxine given at 50 mg/kg orally once a day for 10to14dayswilleliminateocyst excretioninmostdogsandcats(1 04, 191). The combination of ormetoprim (11 mg/kg) andsulfadimethoxine (55 mg/kg) given orally for up to 23 days hasbeen used effectivel		X
Blood parasites	<i>Aconoidasida</i> (<i>Cytauxzoon</i> <i>felis</i>)	Cytauxzoon felis is an apicomplexan protozoal parasite that causes severe, and often fatal disease, in cats. Organisms parasitize erythrocytes and schizonts are found in macrophages in blood and tissue.	Cytauxzoon felis infection has been demonstrated in various non-domestic felids including the Eurasian lynx, with a prevalence of 26% in Switzerland (Meli et al. 2006)	In wild felids, <i>C. felis</i> infection is usually subclinical with a fatal progression of the disease been reported in bobcats (<i>L. rufus</i>) under experimental and natural conditions only (Ryser-Degiorgis 2009).	The organism can be recognized in blood smears as 1-2 um small, ring or safety pin-shaped bodies in red blood cells, but can be readily missed with low levels of parasitemia		X
	<i>Alphaproteo-</i> <i>bacteria</i> (<i>Anaplasma</i> <i>phagocytophila</i>)	The presence of <i>Anaplasma</i> <i>phagocytophila</i> , which causes tickborne fever in domestic ruminants has been reported in a number of domestic and wildlife species.	Seroprevalences lower than 10% have been demonstrated in Eurasian lynx in absence of clinical signs (Ryser-Degiorgis et al. 2009).	Anaplasmosis is diagnosed by culture, histopathology, PCR, or serology.	An effective treatment for feline anaplasmosis is doxycycline administered orally at a dosage of 5 mg/kg once a day for 14-28 days.		X

Table 8. Parasitic diseases: Endoparasites

Other nematodes and trematodes found in Lynx

Other nematodes found in Eurasian lynx, include *Diphyllobothrium latum*, *Ancrylostoma tubaeforme*, *Eucoleus aerophilus*, *Metastrongylus sp.*, *Nematodirus sp.* and *Alaria alata* not been reported previously in Eurasian lynx (Szczena et al. 2008).

5.6 Parasitic diseases: Ectoparasites

Name	Characteristics	Cases	Diagnosis / Signs	Treatment	Captivity	Wild
Ear mites (<i>Otodectes cynotis</i>)	Otodectes cynotis is a non-burrowing mite commonly causing otitis externa in domestic cats.	Otodectic or ear mange has been reported in free-ranging Eurasian lynx from Sweden (Degiorgis et al., 2001) and Switzerland (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005c). It seems to be very common in lynx from Switzerland (Ryser-Degiorgis 2009).	Ear mites can be seen macroscopically in the external ear (Krelekamp 2004). According to a report on occurring otacariasis in free ranging lynx presented by Degiorgis et al. (2000), histologically, hyperkeratosis and acanthosis was present and the epithelial surface was overlain by hyperkeratotic and parakeratotic crusts of mites, mite detritus and cerumen. In the subcutis was a slight to moderate infiltration of lymphocytes and macrophages found. The ceruminous glands were hypertrophic and hyperplastic and an hyperplasia of the sebaceous glands was visible. The found lesions seemed to correlate with the degree of infestation (Degiorgis et al. 2001).	Affected animals and those in contact with them should be treated regularly with an ear preparation (commonly used is ivermectin and selamectin as drop preparations) to kill the mites (Mellen and Wildt 2003). Furthermore, the ears of a captive held individual can be regularly checked and cleaned, if necessary, but this requires anaesthesia.	X	X
Fleas and Louse Flies	Fleas are wingless insects, 1.5 to 3.3 mm) long, that are agile, with a proboscis, or stylet, adapted to feeding by piercing the skin and sucking their host's blood through their epipharynx; winged louse flies, dark brown in colour, flat shaped, leathery in appearance.	Louse flies and fleas occasionally found on free-ranging Eurasian lynx (Ryser-Degiorgis 2009).	Fleas can be found in the hair coat over the entire body (Miller and Fowler 2015). Infestation is difficult to detect unless there are obvious clinical signs such as excessive scratching, loss of hair or poor coat condition. Heavy flea infestations may cause anaemia in young lynx (Blomqvist et al. 1999).	Flea shampoos (e.g. pyrethrins) can be used as treatment for captive individuals but must not be employed on individuals about to be reintroduced in the wild (Miller & Fowler 2015). Flea control is a matter of prevention, with a wide array of products existing - commonly used are products that contain Etofenprox or Pyriproxyfen (Rust 2011).	X	X

Name	Characteristics	Cases	Diagnosis / Signs	Treatment	Captivity	Wild
Ticks	-	-	Ixodid ticks are regularly found on free-ranging Eurasian lynx (Ryser-Degiorgis 2009).	Removal of tick, eventually application of antibiotic cream		X
Mange	Mange is a skin disease caused by mites that are highly contagious (Muller et al. 1989).	Sarcoptic and Notoedric mange mites occasionally affect Eurasian lynx (Mellen & Wildt 2003; Ryser-Degiorgis et al. 2002).	Infestation is difficult to detect unless there are obvious clinical signs such as excessive scratching, loss of hair or poor coat condition (Blomqvist et al. 1999). Diagnosis is attempted with skin scrapings from multiple areas, which are then examined for mites and mite eggs microscopically. Additionally, if available, a serologic test may be useful in diagnosis. Lesions consist of an extensive encrusting dermatitis that cover the entire body, but usually more prominent on the head, ears, feet and tail. Typical macroscopical changes are thick crusts with deep fissures. Lymph nodes are generally enlarged. Towards the end stage of the disease, individuals are cachectic and often harbour a large amount of Ascarids in the intestine (Ryser-Degiorgis 2009).	With Simparica® 5 mg ad us. vet. tabs containing the active agent Sarolaner. Sarolaner is an acaricide and insecticide from the isoxazoline family. Sarolaner blocks GABA- and glutamate-controlled chloride channels in the central nervous system of insects and mites and kills them. Fleas, mites and ticks must attach to the host and start feeding to be exposed to the drug (www.vetpharm.uzh.ch).		X
Sarcoptic Mange	Sarcoptic Mange is caused by <i>Sarcoptes scabiei</i> . Sarcoptic mange is the most frequent infectious disease in Eurasian lynx, reaching up to 22% of non-hunted dead lynx in Sweden (Ryser-Degiorgis et al. 2005).	In Eurasian lynx, sarcoptic mange has been reported in captivity in China (Jeu and Xiang 1982), as well as in the wild in Norway and Sweden associated with an outbreak of sarcoptic mange in red fox (Holt and Berg 1990; Mörner 1992).	See Diagnosis/Signs - Mange (above)	Simparica® can also be used for treating Sarcoptic mange (see above - treatment mange)	X	X

Name	Characteristics	Cases	Diagnosis / Signs	Treatment	Captivity	Wild
Notoedric Mange	<p>Notoedric Mange is caused by <i>Notoedres cati</i>.</p> <p>Known to be a sporadic disease in domestic cats and presumed to reflect contact of Eurasian lynx with domestic cats; also a mixed infection with <i>N. cati</i> and <i>S. scabiei</i> has been documented.</p>	<p>Very occasionally observed among captive lynx in zoos, but has been rarely reported in free-ranging lynx (Dobias 1981). In 1999, in Switzerland, there were two lynx found dead infested with <i>N. cati</i>. Other cases to be known of occurred in Norway, Sweden and Germany, in association with outbreaks of sarcoptic mange in red foxes, which are considered as the main source of infection for lynx (Ryser-Degiorgis et al. 2002).</p>	See Diagnosis/Signs - Mange (above)	<p>Treatments with Avermectins (Ivermectin, Selamectin (e.g. Revolution®) or Moxidectin (Advantage Multi®) and/or acaricidal shampoos for local treatment (e.g. Amitraz®) and lime-sulphur baths for captive held individuals are usually effective, and in-contact animals should also be treated to prevent further spread. This treatment is not allowed for individuals about to be reintroduced in the wild (Mellen & Wildt 2003).</p>	X	X

Table 9. Parasitic diseases: Ectoparasites

General antiparasitic solutions for felids

As a broad spectrum antiparasitic for cats, Broadline® spot-on Felids - a topical solution for direct skin application that contains the insecticidal and acaricidal active substances fipronil (adulticide) and (S) -methoprene (ovicidal and larvicidal), the endocytogen eprinomectin and the cestozide praziquantel is recommended. This combination provides efficacy against fleas, ticks, gastrointestinal nematodes, tapeworms, and against urinary bladder worms (www.vetpharm.uzh.ch).

5.7 Protozoal diseases in captivity and in the wild

Name	Epizootiology	Cases	Signs	Diagnosis	Treatment	Captivity	Wild
Toxoplasmosis (<i>Toxoplasma gondii</i>)	Toxoplasma gondii is a coccidian parasite primarily affecting felids, in which infection usually does not lead to disease symptoms. The causative agent is the protozoan parasite <i>Toxoplasma gondii</i> , for which cats are the main host.	In Fennoscandia, seroprevalence in lynx reaches 70-75% and is significantly higher in subadult and adult lynx than in juveniles. Prevalence of infection appears to be highest in southern regions of Sweden, which are more densely populated by humans, possibly due to the presence of domestic cats shedding oocysts to the environment, to climatic differences (i.e. to survival of oocysts) and/or to variations in prey availability, since prevalence of infection greatly vary between different prey species. Information on toxoplasmosis in Eurasian lynx is still being collected, and so far only the Karelian lynx population in Finland has been confirmed to host <i>T. gondii</i> commonly. There has been no evidence of contribution to an environmental oocyst burden or any of the investigated individuals dying from the infection (Jokelainen et al. 2013. Seropositivity has also been reported in captive lynx (Sedlák and Bártová 2006).	Rarely the disease does cause clinical signs of diarrhoea in cats. Only during the initial infection do cats excrete large numbers of eggs (oocysts) of the pathogen. Most cats then develop a lifelong immunity. No clinical disease has been reported in Eurasian lynx so far, but sporadic cases have been reported in young bobcats (<i>Lynx rufus</i>).	Serum samples have to be tested for <i>T. gondii</i> -specific antibodies by direct agglutination test.	Clindamycin or Tetraseptin (Active antibiotic agent: tetracylin) <u>Clindamycin:</u> Dosage: 10 - 12 mg / kg depending on the clinic p.o. or parenterally twice a day - duration of treatment: 4 weeks <u>Tetraseptin:</u> Dosage: 4 drops of forte (20 mg) / kg body weight, mixed directly with the feed 3 times a day or with liquid (tea, water). The best absorption of Tetraseptin is achieved when administered 1 hour before feeding. The usual duration of use is about 3-10 days. It should be continued for 1-3 days after the symptoms have resolved. Tetraseptin acts against both extracellular and intracellular bacteria by inhibiting the protein synthesis of the bacteria on the ribosomes (www.vetpharm.uzh.ch).	X	X

Table 10. Protozoal diseases in captivity and in the wild

5.8 Non-infectious diseases

Other non-infectious diseases include the development of congenital heart diseases and pelvic malfunctions as well as changes in variation in teeth number, teeth and skull disorders, these anomalies and malformations are believed to be caused by genetic bottlenecks and inbreeding scenarios prevalent in the Swiss-Alpine population as well as the Dinaric population with other populations showing similar cases possible with low prevalent heterozygosity (Morend et al. 2019, Mihaylov et al. 2018, Gomercic et al. 2008).

6 Forensic Investigations

6.1 Introduction

Pathological and forensic work executed by specially trained and skilled veterinarians form an important part in the entire process of investigation of an supposed to be illegally killed/of unknown causes, prematurely deceased Eurasian lynx individual. Subsequently, it is the responsibility of legislative and legal bodies (e.g. police and justice) to collate all available forms of found evidence from the forensic investigation and pass subsequent judgement and allow legal measures to be taken (Brownlie and Munro 2016).

Forensic veterinary necropsies play a pivotal role when illegal killing of a protected wildlife species is suspected. Accordingly, a forensic investigation is launched, based on suspicions and relevant findings, in order to draw exact conclusions concerning the cause(s) of death. In depth investigations include parasitological, bacteriological/virological, toxicological as well as histological examinations, which assist and allow precise statements on the circumstances and time of death of a found individual. In forensic investigations, the veterinary pathologist is required to act in an unbiased manner as an objective and independent assessor. The variety of motivations and weaponry involved (e.g. gun-related poaching, the use of snares, poison) in these crimes necessitates a comprehensive and in-depth approach and extensive knowledge of forensic necropsy procedures as well as (wound) ballistics.

The role of the pathologist is to document, interpret and explain the pathological findings to the legal investigators and ultimately to the court, in order to allow for adequate legal prosecution. It is vital that investigation and examination of the found carcass is meticulous, that detailed records and protocols are kept and a proper chain of custody (for each item of evidence within the ongoing investigation) is maintained. Complete, clear and comprehensive documentation, ensure and allow reexamination for subsequent steps in the process of investigation and prosecution in cases of illegal killing of strictly protected species, respectively where without objection non-natural circumstances of death (e.g. traffic accidents) can be determined.

Preliminary considerations

The following steps are usually helpful preliminary to an investigation (taken with permission from Beiglböck and Walzer 2019):

- Identify the respective national and regional legal and veterinary authorities
- Identify potential stakeholders (hunting agencies, conservation organisations, NGOs)
- Identify an appropriate first responding veterinary facility (and the facilities for ancillary investigations)
- Familiarize yourself with your national/regional legal aspects regarding wildlife and wildlife crimes
- Establish a working relationship with all parties and institutions potentially involved in the persecution of wildlife crimes
- Discuss their respective requirements and needs in a forensic investigation
- Discuss a preliminary working- and communication plan and the respective protocols in case of the occurrence of a suspected wildlife crime case
- Discuss the potential costs of a forensic investigation and the meeting of costs

6.2 Getting started – at the scene

Depending on the case and the circumstances of retrieval, the cadaver of the deceased individual and accompanying findings (e.g. cartridge cases, foot prints at the scene..) are considered as items of evidence. A (potential) crime scene with all the associated material (e.g. ballistic material) included and associated with the present “scene” are part of the ongoing investigation (Brownlie & Munro 2016).

Meticulous records on scene and later in the laboratory during necropsy are critical to an indisputable line and continuity of evidence.

6.3 Prior to necropsy

Radiography

Prior to dissection, the lynx carcass has to be observed radiographically, in order to visualize metal residues from projectiles and other radio-opaque fragments. Even without externally apparent wounds, radiographic imaging can provide important information concerning the cause of death. Bone fractures and dislocations are identifiable *in situ* before potentially causing artifactual misalignment of bone fragments by dissection. In most cases, digital radiography is sufficient for the localization of metal-containing structures in the body of an individual, additional computer tomography (if available) might be helpful.

Radiographic images should be always taken from at least two perpendicular views to allow a three-dimensional view of the individual being examined.

As a rule: The search for metal containing bullet fragments has to be always supported by radiography of the carcass!!

6.4 Evidence management

Proper evidence management ensures the maintenance of continuity of evidence, providing a chain of custody for involved personnel (e.g. veterinarians/forensic investigators, wildlife ecologists, forester and hunters as well as legal representatives from the police etc.).

The whole process, from retrieval of the dead individual in the field up to the necropsy executed in the lab, requires secure custodianship of the evidence adhering to procedures and standard reporting forms and protocols (see Appendix II: forms, protocols and lists).

Appropriate handling, storage and inventorying of material evidence (this includes proper recording, labelling and inventorying of each item during the procedure, noting whereabouts, responsible persons etc.) ensures further use in a wildlife crime investigation.

Photography

The different steps, from initial discovery of the body until necropsy, including the different steps of dissection are documented with serial photographs (followed by including labels and scales within each photograph consistently to allow continuous identification of the recordings later on and a comparison of size and measure) throughout the process. All findings, including incidental or nonsignificant changes have to be recorded. These can subsequently be used within a pathology report to underline findings and assumptions, factors responsible for the cause of death of the examined individual.

First of all, a photograph of the crime scene, allowing an overview, is taken as well as a photo of the bagged carcass. During the recording, label and scale have to be always placed close to the area of particular interest. Without moving the label, a closeup shot of the particular finding should be taken, thereby ensuring that the area of interest aligns with centre of the view-finder and that the label is also included (Brownlie and Munro 2016). Then, photographs of the body to be examined are taken, before the dissection begins, from as many different angles as appropriate. It might offer and reveal additional information concerning the circum-stances of death, any wounds or alterations, the position of retained projectiles, fragments or gunshot residues within or under the skin, and the tracts or dislodged bone fragments caused by the shot.

Note: Each image taken of the investigation has to be always properly stamped and labelled to allow a clear assignment to the respective case and animal body (also information clarifying whether the photo is taken from the left or right side of the individual has to be recorded - e.g. by placing a left/right tag at the object being recorded - see figure 33 and 34).

Evidence and image identification (findings/photographs/samples) by labelling / barcoding
For proper evidence identification, it is most important to use permanent indelible and freezer-proof labels for all pieces, parts and photos evaluated and photographed during the examination.

Additional evidence, such as material removed from or found with the carcass (e.g. bullet fragments, buckshot, pellets) must be carefully collected, labelled and stored appropriately. A record of where all means of evidences (biological and non-biological) have been stored, how they are labelled, when they were disposed etc. have to be maintained in event logs and saved appropriately (e.g. in a cloud or hard drive).

Photos

Photographs during investigation have to be taken in a continuous numbered series. Subsequently, a complete set of unaltered photographs should then be stored securely and traceably, and this complete set needs to be available to investigators, all involved legal bodies without any alterations or subsequent deletions.

Labels

Labels need to be included in the entire photo documentation, a complete copy of all photographs must be retained securely in a cloud or hardcopy folder as well. Thereby, the images can be securely stored locally or in a central storage facility and can be retrieved and viewed at any time by responsible persons and authorities as needed, even after disposal of the animal body.

Note: It is important, at the outset, that a correct time and date stamp in the photographic and/or (eventually used) barcoding device is adjusted (also regarding daylight saving time).

Sample collection

For information on collection of non-invasive samples see Chapter 4.

Secure storage

Samples must be documented, properly packed, labelled and transported, with attention throughout to both ensure the quality and integrity of the sample material and maintain the chain of custody (Cooper et al. 2009).

It is extremely important, depending on ongoing and planned analyses, to check with the responsible laboratory about the exact storage modalities. Otherwise, incorrectly stored, damaged or destroyed samples might hamper or disqualify further analysis schemes entirely. Samples deriving from the parent cadaver should also be labelled carefully and retained securely during movement for analytic purposes (e.g. for histology) in order to track its whereabouts at any time (Brownlie and Munro 2016).

Method	+	-
Chilled at +4°C	<ul style="list-style-type: none">• Good preservation for few days	<ul style="list-style-type: none">• Autolysis continues slowly
Frozen at -20°C	<ul style="list-style-type: none">• Almost indefinite storage• Pathogens usually not killed (thus can still be analysed)	<ul style="list-style-type: none">• Artefacts• histological examination usually not possible anymore• Pathogens usually not killed (thus sample may be hazardous)
Formalin	<ul style="list-style-type: none">• Indefinite storage• Pathogens usually killed (thus sample is safe to handle)	<ul style="list-style-type: none">• Affects appearance of organs• Genetic studies difficult• Pathogens usually killed (thus cannot be analysed)
Ethanol/Methanol	<ul style="list-style-type: none">• Almost indefinite storage• Pathogens usually killed (thus sample is safe to handle)	<ul style="list-style-type: none">• Affects appearance of organs• Genetic studies difficult• Pathogens usually killed (thus cannot be analysed)

Figure 26. Pros and Cons of usual sample storing methods (Beiglböck and Walzer 2019)

Note: Deep-freezing a carcass for conservation purposes disqualifies the tissue for further histological examinations that represent an pivotal part of the necropsy process!

6.5 Standard operating protocols

For all mentioned steps, written standard operating protocols should be used (see appendix II). In this way, a consistent approach is assured. Existing protocols should be reviewed regularly and revised as needed to ensure they are relevant, feasible and thoroughly followed (Brownlie & Munro 2016).

6.6 Necropsy

Necropsy examinations consists of macroscopic and histological examination in order to allow for conclusions regarding the circumstances that caused mortality.

6.7 Ballistics

The interpretation of shooting wounds by the veterinary pathologist requires some knowledge of firearms and the ammunition used in wildlife crimes. This subchapter shall present the respective background knowledge in more detail (for more information see Beiglböck and Walzer 2019, respectively PAW 2014).

There are several types of firearms used in wildlife crimes, mostly rifles and shotguns. To a lesser extent, handguns and air- or gas-powered guns, although they produce significantly less energy and hence are deadly only in small animals and at short distances.

Most firearms except shotguns have rifled barrels, meaning that spiral grooves have been cut into the bore of the barrel. The purpose of this is to force the bullet into a longitudinal spin for better stabilisation and accuracy during flight. Rifling typically leaves toolmarks on a fired bullet that may be characteristic for an individual weapon. The toolmarks may be investigated in a special forensic laboratory and serve as important evidence and thus bullets (and its fragments) recovered from a carcass should be handled with great care and kept as evidence (Beiglböck and Walzer 2019).

6.8 Ammunition

For the veterinary forensic examiner, classification of ammunition according to the different behaviour of the bullets in the body is of importance to assess tissue damage:

Stable shape bullets

These projectiles maintain their general shape in the target and retain their mass. They are mostly solid or fully metal-jacketed projectiles and are mostly used for hunting very large animals because they penetrate deep into the body.

Deforming bullets

They deform their shape in the target (e.g. “mushrooming”) and lose only a small percentage of their mass, thereby ensuring high energy- transfer to the tissue.

Depending on type and bullet composition, average viable shooting distance of stable shape and deforming bullets are approx. 100 - 200m.



Figure 27. The shape of a deforming bullet before (right) and after hitting a body (left). Note the typical “mushroom shape” ensuring high energy-transfer in the body (Beiglböck and Walzer 2019).

Frangible bullets

This design is intended to completely fragment in the target, again ensuring high energy-transfer.



Figure 28. A frangible bullet and its small fragments that will be found along the wound path in the carcass (taken with permission from Beiglböck and Walzer 2019).

Shotgun shells

These consist of a (mostly) plastic casing and, beginning from the bottom, a primer, propellant, wadding and shot. The shot vary in size, ranging from pellets with a diameter of <2mm (often referred to as “birdshot”) to larger pellets (<9 mm, often referred to as “buckshot”). Pellets used to be composed solely of lead, but due to environmental concerns, today are increasingly made of other metals as steel or tungsten.

Labelling of the shotgun ammunition may vary between countries. In Europe, the most common terminology to describe the shells is a non-metric calibre specification, the most common being 12, 16 and 20 (with a respective bore diameter of app. 18,5, 16,8 and 15,7 mm), the length of the shell (in mm) and the size of pellets (in mm).



Figure 29. Cross section of two shotgun shells and their main components loaded with small pellets (“birdshot”) and, respectively, large pellets (“buckshot”) (taken with permission from Beiglböck and Walzer 2019).

Slugs

Shotgun shells may also carry slugs, in German often referred to as “Brenneke”. These are commonly made of solid lead. They have a weight typically between 20g and 30g and rapidly lose velocity when fired and thus are also used only at short distances (<40m).



Figure 30. Cross section of a shotgun shell containing a slug (at top).

Depending on type and bullet composition, average viable shooting distance for shotgun shells (loaded with pellets or slugs) is approx. <40m.

Despite the information about the type of projectile collected through retrieved bullet fragments, an exact assignment to a specific weapon is only rarely possible.

Air- or gas-powered weapons

These come in various shapes, the most common type being the diabolo-style pellet that has a “wasp-waist”. They have only limited wounding capacity due to their small weight and rather low velocity. Nevertheless, when fired at closer distance on a small animal, they may inflict serious wounds.

The average viable shooting distance for air- or gas-powered gun pellets is approx. <100m.



Figure 31. Various shapes of projectiles for air- or gas-powered weapons.

6.8.1 Wound ballistics and wound capacity

Gunshots

The wounding capacity of a gunshot projectile is mainly dependant on the energy it transfers to the body tissue and its behaviour in the animal body (wound ballistics).

Tissue damage occurs through:

- Laceration of tissue and complete organs that are directly hit by the bullet, its residues and fragments on their trajectory through the body. Fluid-filled organs like the heart may completely rupture due to the non-compressibility of fluids (Beiglböck and Walzer 2019).
- The cavitation effect

Deforming or frangible bullets begin to deform or to fragment as soon as they enter the body, thereby transferring much of their impact energy to the tissue. Especially with rifle bullets used for hunting the cavitation effect is prominent. A pulsating, temporary wound cavity develops along the pathway due to the forces the rapidly decelerating bullet exerts on the surrounding tissue. This compression and contraction process cause damages in tissue and organs not in the direct path of the bullet or its fragments (Beiglböck and Walzer 2019).

Stable shape bullets also cause a temporary wound cavity by tumbling. However, this type of bullet penetrates deeper into the body before they begin to tumble due to the rapid deceleration. However, a temporary wound cavity may be absent when a thin part of the body is hit with the bullet travelling straight through the animal (Beiglböck and Walzer 2019).

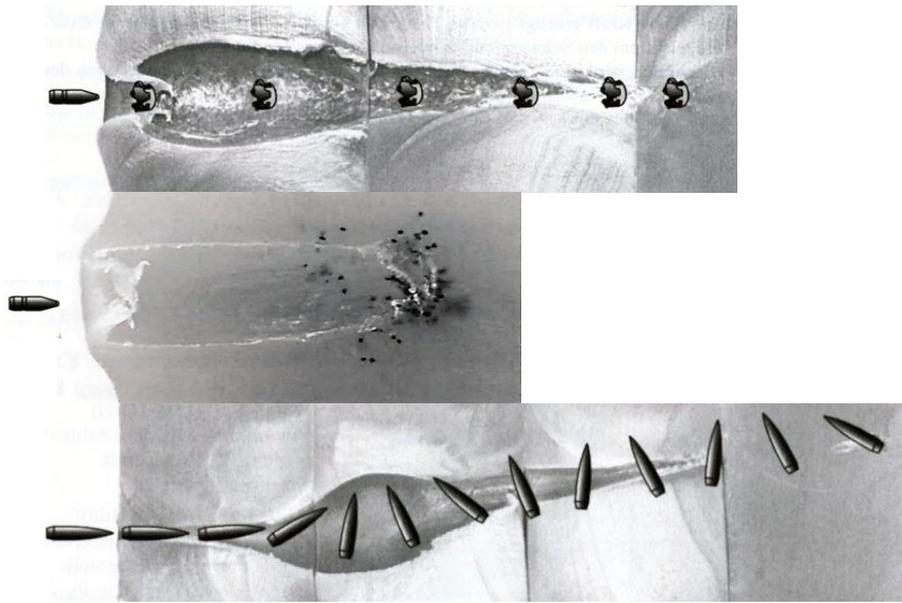


Figure 32. Illustration of the temporary wound cavities formed by different projectiles. From Top to bottom: Deforming bullet, frangible bullet, stable shape bullet.

Shock waves

Shock waves from the impacting bullet will travel through the whole body via blood vessels, nerves and other tissue and may seriously impair body function, causing a “neuronal shock”, which can lead to death of the shot individual (Beiglböck and Walzer 2019).

Shotgun pellets

The shot charge from shotguns radiates from the muzzle in a cone-like shot distribution pattern. The wounding capacity of shotgun shots is highly dependent on the firing distance.

At close range (<5 m) it will have destructive effects due to high energy transfer to the tissue, but as the distance gets longer, the pellets not only disperse but lose their energy very quickly. Thus, pellets will not even penetrate a thicker hide at longer ranges (>50m). When entering the body, pellets from a shotgun rapidly lose their energy and only rarely deform or disintegrate (unless they hit a bone), they produce no temporary wound cavity (Beiglböck and Walzer 2019). The likelihood of finding shotgun pellets or fragments in the carcass is generally high due to the operation mode of a shotgun, as the penetration of the pellet at normal firing distances does not develop sufficient velocity to fully penetrate the animal body.

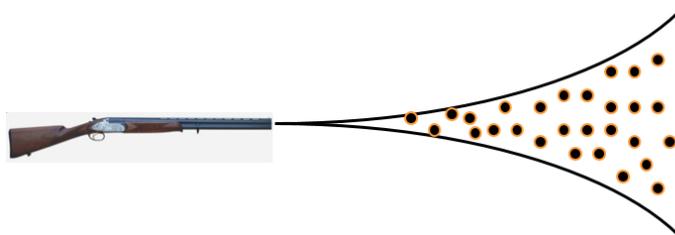


Figure 33. Scheme of a shotgun pattern after firing (taken with permission from Beiglböck and Walzer 2019).

Surprisingly still, the exact mechanism of killing is not fully understood and is mostly referred to as a neuronal shock (“shock waves” - see above) due to the body being hit by multiple pellets on various locations of the body simultaneously.

Shotgun slugs

As with shotgun pellets, slugs fired at close range from a shotgun have devastating effects due to their shear mass. At farther distances, however, they rapidly decelerate thus losing energy. Since they are mostly made of lead, they very often deform and disintegrate in the body but produce no temporary wound cavity. When hitting a body with sufficient energy, the wounds will resemble a (massive) blunt trauma (Beiglböck and Walzer 2019).

The determination or assignment to a certain type of rifle/shotgun, respectively bullet/projectile, material and caliber is significantly dependent on the work of a ballistic-technical examination experienced and accomplished wildlife forensic scientist.

7 Common anthropogenic causes for adult and pre-adult mortality in Eurasian lynx

7.1 Shooting

Shooting appears to be the most common poaching method for Eurasian lynx. Thus, 22% of examined lynx in Switzerland harboured lead pellets from non-fatal shooting attempts, indicating that poaching is much more common than it appears from the “official” recorded deaths known to be caused by illegal shooting (Ryser-Degiorgis 2009). The verified findings of several animals of the Bavarian-Bohemian-Austrian (BBA) Eurasian lynx population killed illegally by shooting, as well as the numerous individuals in this population simply “disappearing” after being already identified (by systematic monitoring via camera trapping) as having territories established, indicate the dark figure of illegally killed individuals has to be assumed as much higher (Engleder, T.; Wölfl, M., S.; Minarikova, T.; Belotti, E. pers. comm.).

Shooting at lynx

In forensic investigations, the question arises often as to whether there are still fragments of the projectile or even the entire projectile can be detected in order to elucidate and verify a supposed shooting attempt on a dead found individual. Projectiles from modern cartridges deliver, depending on the shooting distance, a projectile at a speed of approx. 600 m/s - 900 m/s, regardless of the projectile type. Due to the low target resistance of the lynx body in broad-based shelling (body width about 12cm-14cm), it is unlikely, that the entire projectile remains to be found. The low target resistance of the lynx body is usually not sufficient to stop the bullet in the animal. Even a deformation (mushrooming) and/or splinter delivery of the projectile is not necessarily given at such a low target resistance (see chapter 6.7).



Figure 34. Remaining projectile tip stuck in the spinal musculature in the processus transversus region of the 5./6. Lumbar vertebra in a female Eurasian lynx (© Pathology - FIWI Vienna)

Bullet fragments

Remaining projectile fragments can be very small depending on the type of projectile being used and they can be clearly located away from the bullet channel in the carcass. Thus, it is easily possible that tiny bullet remnants and fragments are overlooked by qualified, but in forensic investigations, inexperienced veterinarians.

Consequently, individuals showing corresponding injuries have to be subjected to an exact pathological, forensic, anatomical and ballistic-technical examination. **An x-ray of the carcass must be always carried out in order to find residues of projectiles macroscopically prior to necropsy.**

All further investigations are depending on the initial inspection of an experienced wildlife forensic veterinarian. Particular attention has also to be paid to non-metallic bullet components such as e.g. plastics (projectile tips).

If bullet residues are found, their composition is usually not very significant, whereby the de-termination of the material group (lead-free/lead-containing/plastic) of the used projectile is a minor problem. After determining the texture, it is possible to draw conclusions about the intended mode of operation of the projectile through the previously precisely determined distribution of the bullet residues within the animal body. Moulding of the entering and exit bullet-hole and the determination of the bullet channel can give further indications for the determination of the type of projectile and the calibre being used (see chapter 6.8).



Figure 35 Bullet fragments found in a female lynx allegedly been killed by “collision with a train” (© Pathology Unit - FIWI Vienna)

7.1.1 Pathological findings in firearm victims

Handling of suspected firearm victims

Handling of the carcass should be reduced to a minimum both at the crime site and during external examination to avoid the loss of any evidence (e.g. a bullet fragment falling out while located near an exit wound). After removal from the body bag used for transportation, the bag should be examined closely for the remaining presence of any evidence/bullet residues, that has exited the body during transportation.

Necropsy of suspected firearm victims

Finding bullet parts or shots in a carcass does not necessarily mean that this relates to the death of the animal! Animals may suffer non-fatal shooting injuries (especially pellets) that do not interfere with any vital function. Especially when shotgun pellets are found in the hide or muscles, it is of paramount importance to determine if these are freshly inflicted wounds or if they stem from an older shooting event, or even if the animal was shot at after death.

Look out for any signs of bleeding, wound healing processes like the formation of fibrous tissue etc.! Further, despite various attempts by forensic experiments, determination of the exact shooting range is almost impossible. Especially with shotguns, due to the dispersal of the pellets after firing, the pattern, the amount and hence the density of the pellets in a carcass have been used to derive the probable shooting distance. However, since almost all shotguns comprise two barrels (that are normally both loaded), it cannot be excluded that an animal has been shot twice or more times in a single shooting incident. Thus, the pattern of the pellets in the carcass are of no practical use when determining the shooting distance!

When shooting is suspected in wildlife crime cases, it can generally be assumed that the victims were shot at larger distances. Thus, evidence like gunsmoke stains or soot marks (e.g. stains left on the haircoat/skin from the ignition process of a bullet fired at very close distance) or scorching of the skin or hair from the superheated gases leaving the muzzle, will normally be absent.

7.2 Injuries caused by traffic accidents/vehicle collisions

The rapid increase of transport infrastructure development like roads and railroads, negatively affect wildlife both in indirect ways - loss of connectivity, landscape degradation and habitat fragmentation, as well as directly by increased animal mortality caused by direct collisions.

In Switzerland, traffic accidents account for 20-50% of mortality causes of lynx found by chance, with subadult lynx dying significantly more often, explained by inexperience and increased movement for dispersal over long distances (Ryser-Degiorgis 2009).

In forensic investigations of individuals injured or killed by suspected vehicle collisions some aspects have to be obeyed:

Not all animals die in fatal head-on collisions and stricken animals may even survive if they received only slight injuries (e.g. in “near-misses”). Especially larger animals, involved in vehicle collisions may cover some distance from the road until they succumb to their injuries. Internal bleedings from ruptured organs or blood vessels may continue for a while until the animal dies from the subsequent loss of blood. Consequently, victims of vehicle collisions may be found distant from nearby roads (Beiglböck and Walzer 2019).

7.2.1 Pathological findings due to collisions with vehicles

Multiple bleeding in various tissues will be evident, injuries to body areas that are remote from the direct impact area may be present due to projection of the animal, coup-contrecoupe effects due to the rapid acceleration/deceleration process in collisions etc. Multiple abrasions of the skin and dirt embedded in the fur are often found in collision-victims (Beiglböck and Walzer 2019).

Larger collision-victims such as Eurasian lynx with a higher body mass, more muscle tissue may show more subtle pathological changes like bleedings in the hide and musculature when hit not frontally but in a peripheral region. **External examination may reveal no obvious wounds, but check the exterior for vehicle paint chips.** Nevertheless, because of the forces involved in collisions, death can occur due to rupture of inner organs and subsequent internal bleeding (Beiglböck and Walzer 2019).

7.3 Snaring/Trapping

Snares

Snares are simple devices to catch mammals through anchored cables. They trap an animal around its neck, body or feet. Animals caught in snares alive are at risk of starvation and dehydration or hyper-/hypothermia if trapped for a longer period. They may struggle vigorously leading to progressively tightening of the wire and thereby cutting deep into the tissue with typical lesions, detected easily during necropsy.



Figure 36. A typical snare to catch certain species of wildlife.

Snares and traps might be used intentionally to catch/kill endangered wildlife. Wire snare techniques are a forensic difficult to detect, illegal method for killing large carnivores such as Eurasian lynx. For instance, the "Lynx Snare" made from highly flexible twisted and looped steel wire equipped with a mechanism blocking a return caused by liberation efforts of the individual trapped therein leads to an inevitable strangulation when the snare is located around the neck.

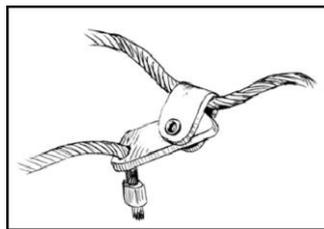


Figure 37. Detail Steel wire blocking mechanism - Lynx Hair Snare (Source: alcesjournal.org)

Foothold traps/spring traps

Different types and sizes of traps exist, ranging from cage traps to catch animals alive to body-gripping traps (e.g. conibear traps; spring-traps), which are designed to quickly kill an animal (Beiglböck and Walzer 2019).

Foothold traps, such as the type Conibear 330 as well as the still available "swan neck" kill "smaller" felids, such as Eurasian lynx instantly. Since felids like to "fish" injuries like fractures on legs, paws and in the area of the metacarpals are symptomatic.



Figure 38. Different types of traps (from left to right): Cage trap for trapping animals alive, conibear trap and spring trap, both designed for fatal trapping (Beiglböck and Walzer (2019)).

Body gripping traps kill the target animals (e.g. foxes, martens) quickly by tightly shutting jaws around the body, especially the neck, of an animal. However, non-target animals are at high risk by this type of traps, even if they are used legally. Especially if placed incorrectly many non-target species, triggering the release mechanism, get injured. Ranging from superficial wounds to severe fractures and even amputation of the leg. The degree of the injuries is highly dependent on type and size of the trap (Beiglböck and Walzer 2019).

Note: Snaring techniques used for live capture and monitoring schemes can also have significant injury potential and can cause fractures! Special attention during application is advised!

7.3.1 Pathological findings in snaring/trapping victims

In case of a lynx hair snare, the entire process of suffocation takes only a few seconds and leaves hardly any visible external injuries on the animal body. The strangulated individual appears externally intact/unharmed with often barely or hardly recognizable abrasions on guard hair and kempy wool (in the area of the noose closure). Especially for individuals in winter coat, forensic evidence of strangulation is considerably more difficult. During the forensic examination, meticulous attention to visible, conspicuous, subcutaneous haemorrhage has to be paid. Sometimes, animals caught alive are killed by shooting, thus radiography should always be applied in such cases.

Detected injuries, seized and documented positions of the bone fragments may provide an indication of the applied snare or trap type, as long as the injuries and evidence are fresh and are not too severely decayed by maggot infestation. The fracture edges and position of the fragments of a possible multi-fragment fracture (3 to 6 fragments) or fragmentary fracture (more than 6 fragments) can provide further valuable information on the trap technique and type being used.

7.4 Poisoning

In some parts of Europe, poisoning is still a matter of concern and a threat for wildlife species. However, not all poisoning events are intentional or malicious. Poisoning may occur, e.g., by the improper use of otherwise legal rodenticides (“secondary poisoning”). Another issue of non-intentional, secondary poisoning is the uptake of dead animals or their bowels that were shot with lead-containing ammunition. Lead is a chronic toxic substance that may cause disease and death in predators that feed on these animals or their remains containing lead particles (Beiglböck and Walzer 2019). T

Malicious poisoning mostly aims directly at “unwanted” raptors and carnivores by dispersing different types of baits (e.g. carcasses, meat, eggs) prepared with illegal, highly toxic substances like carbofuran. This kind of wildlife crime does not only threaten the targeted animals but also poses a risk for companion animals and humans that come into contact with the prepared baits. Rarely, poisoning will apply directly to Eurasian lynx, due to the fact that carrion is normally neglected, while hunting actively for prey. Due, to their behavioural pattern of returning to their killed prey item until its entire consumption, a preparation of the prey item with poison is possible and can induce “secondary” poisoning, tough.

While chronic lead poisoning and other unintentional secondary poisoning events are typically not very conspicuous, the findings at a crime scene of a malicious poisoning incident are often suspicious.

These may include (but are not confirmative of intentional poisoning) (taken with permission from Beiglböck and Walzer 2019):

- Baits (pieces of meat, whole animals, eggs, etc.) near the carcass
- Unusually coloured items/soil
- Dead insects on or near carcass
- Vomitus
- Ante mortem spasms
- Disturbed grass/soil (due to mortal distress of victim)

Care must be taken when processing the crime site and all participants should done their PPE (see p. 127 equipment forensic sampling).

Confirmation of poisoning in wildlife can only be achieved by necropsy of the carcass and subsequent toxicological investigation since findings at necropsy are seldom typical for a certain toxic substance. Lesions found at necropsy that may raise suspicion of intentional or unintentional poisoning:

- Poor body condition
- Generalized anaemia
- Ulcerations of gastrointestinal tract
- Abnormal content in gastrointestinal tract (e.g. discoloration)
- Bloody content in gastrointestinal tract
- Non-clotted blood in body cavities without obvious injuries
- Pulmonary edema
- Enlargement of organs
- Rapid onset of rigor mortis
- Histopathology: Inclusion bodies in cell-nuclei of kidney (indicative of lead intoxication)

Cases of lynx poisoning

In Switzerland, poisoning has been observed several times; cases of poisoning are also known from Bulgaria, Macedonia and Serbia: single individuals and family groups were poisoned by applying poisonous substances as bait to their kills. Ryser-Degiorgis et al. (2005) identified poisons such as alpha-chloralose and cyanide. In France, secondary poisoning with bromadiolone was recorded (Ryser-Degiorgis 2009).

8 Websites, contacts and further relevant information on forensic investigations, necropsy procedures and animal health relevant information

Below, contact details on institutions and knowledgeable persons within Austria, the Czech Republic, Germany, Italy and Slovenia are provided (please see. These include group of people and institutions, that have to be contacted in case of dead found lynx, where mortality is suspected to be caused by anthropogenic influence . Contacting forensic experts is of great importance, in order to allow an efficient investigation and precise analysis of the exact causes and circumstances leading to the non-natural death of the found individual.

Animal Health, Forensic Investigations and Welfare

Austria:

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Italy / Switzerland :

Marie-Pierre Ryser-Degiorgis, Prof. Dr. med. vet., Dipl. ECZM (WPH); Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Länggassstr. 122, CH-3001 Bern, Switzerland.

Slovenia / Croatia:

Magda Sindičić, PhD, DVM; Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, Zagreb, Croatia, Email. magda.sindicic@vef.hr; Webpage: <http://www.vef.unizg.hr/>

8.1 Useful literature addressing wildlife crimes and forensic investigations

A useful guide is the Handbook on Standard Operating Procedures (SOP) in Forensic Investigations of Suspected Illegal Killing of Wildlife (Beiglböck and Walzer 2019), some parts of this chapter were abbreviated and taken from this publication. It presents basics, techniques and methods available, not just for veterinarians and wildlife pathologists, also for wildlife crime investigators and practical recommendations to fight illegal killing of wildlife. It explains the use of forensic and other specialist techniques in the investigation of wildlife crime in every detail. Another useful publication is PAW (2014) It includes additional information on specimen identification, poisoning and pesticide analysis, forensic veterinary pathology, taxidermy, DNA technology and other lab-based procedures.

For information on the topic concerning conservation and enforcement, the authors recommend: the society for wildlife forensic science (<https://www.wildlifeforensicscience.org/>), the trace network (<https://www.tracenet.org/>) , as well as the wildlife trade monitoring network TRAFFIC , providing information on illegal trade of wildlife, (<https://www.traffic.org/>).

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Xylazine (e.g. Xylapan ad us. vet.®, Vetoquinol AG, Bern, Switzerland)

Tiletamine (e.g. *Zoletil*®, *Tilest*, ®Telazol® (only available as combined compound - ingredients:
50 mg/ml Tiletamin; 50 mg/ml Zolazepam), Pharmavista, E-Mediat AG, Schönbühl, Switzerland)

Zolazepam (e.g. *Zoletil*®, *Tilest*, ®Telazol® (only available as combined compound - ingredients:
50 mg/ml Tiletamin; 50 mg/ml Zolazepam), Pharmavista, E-Mediat AG, Schönbühl, Switzerland)

Flumazenil (e.g. Anexate®, Lanexat®, Mazicon®, Romazicon®; e.g. General Injectables and Vaccines,
Inc., VA, United States)

Appendix I: Husbandry Guidelines

A1 General handling of captive held individuals (injured/orphaned individuals for physical examination, e.g prior to reintroduction / translocation or as part of long-term captive holding plan)

This chapter discusses aspects related to the handling of injured or orphaned lynx in captivity as well as those quarantined prior to reintroduction into the wild. It also covers individuals affected by disease or disability that prevents their reintroduction into the wild. Species-appropriate husbandry and therapy guidelines are provided in compliance with relevant conservation laws. Finally, handling and husbandry of Eurasian lynx following translocation and/or reintroduction is considered, and ways to facilitate these procedures are suggested.

Captive held lynx are still wild animals and should be treated as such. Daily interaction should only include visual examination by staff, and contact should be kept to an absolute minimum to prevent conditioning of the species to direct human presence (Krelekamp 2004).

A1.1 Behavioural conditioning – Transportation box

The training of lynx should be limited to acclimating them to their crate (prior to transportation) (Blomqvist et al. 1999). Lynx can be acclimated to their crate by giving them regular access to the crate (at least 3 weeks prior to release) (Mellen 2003). They can be trained to enter the crate voluntarily by feeding them inside the container during this time (Blomqvist et al. 1999).

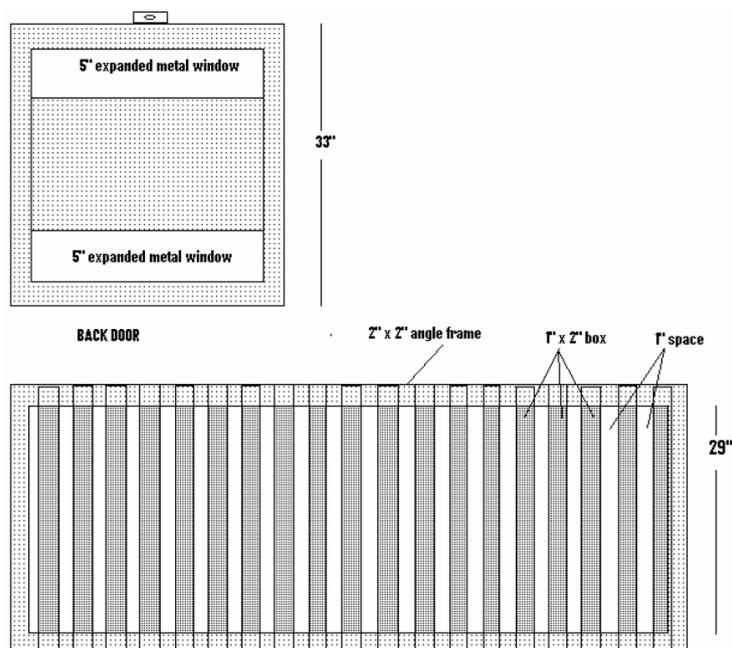


Figure 39. Box trap scheme example (Deem et al. 2005)



Figure 40. A box trap in Østfold (Norway) waiting for a lynx to pass through (taken with permission from ©Mengülluoğlu 2013)

Boxes constructed from solid wood or ones with wood floor coverings are recommended in order to reduce potential claw damage. Odden et al. (2007) found these superior to metal mesh boxes historically used in Central Europe for lynx research.

A1.2 Capture and restraint

Previously, capture and restraint for felids was accomplished by netting. These methods required expert technique and entailed risk of trauma to animals and/or people. More recently, behavioural training has been successfully used to facilitate capture. Behaviours for lynx that are particularly helpful include shifting into transport crates (by placing the crate in the enclosure thereby engendering familiarity with the structure); other behaviours can be disregarded for individuals about to be reintroduced into the wild, because it is important to avoid habituation to human handling (Fowler 2011).

A1.3 Transportation

Welfare and health of the individual to be transported and/or translocated is the primary consideration and can only be assured if the individual is contained safely in an appropriately designed animal transportation crate or box that minimizes stress and discomfort during the procedure.

All relevant paperwork should be prepared in advance of the transport. Documents consisting of a biomedical protocol, clinical report, husbandry notes and biography of the particular individual to be transported should accompany the lynx, along with a veterinary health certificate that contains a complete record of the individual's health status, which is required by the receiving institution.

Depending on the origin and destination, transport of lynx between countries may require export health certificates signed by a veterinarian acting on behalf of the appropriate government agency (Krelekamp 2004). Additionally, for the transport of strictly protected species such as the Eurasian lynx, CITES licences are required.

Lynx should, if possible, be acclimated to their transport box before relocation, in order to reduce stress and discomfort. The person(s) responsible for the transport should make every effort possible to facilitate this outcome. (see chapter A1.1 Behavioural conditioning - Transportation box).

Only healthy individuals should be transported, unless the movement is necessary to enable treatment or therapy. Ideally, the animal's health would be evaluated prior to transport via physical examination and blood collection under anaesthesia. However, this is not always practical or possible. In a majority of cases, examination will be limited to visual parameters, followed by discussion with people involved in the animal's previous capture (for wild individuals planned for reintroduction) or those involved in veterinary care and husbandry of the individual (in the case of animals already held in captivity). The animal's medical record and results of previous physical examinations are also evaluated. It is very important that the health of the animal(s) concerned is assessed and any indicated treatment administered prior to transport. If anaesthesia is necessary for crating, the animal must be allowed adequate time to recover fully before being transported (Blomqvist et al. 1999).

During transportation

In case of shipment via aircraft, the transport box should meet International Air Transport Association (IATA) recommended criteria and guidelines (see chapter A2.1.2). Dimensions of the transport box/container must allow the lynx to stand in a natural posture with its head held in a natural position. Recommendations for the design of transport boxes for felids in Blomqvist et al. (1999).

Furthermore, the width must allow the animal to easily turn around, stretch and lie down comfortably. Thus, a transport box should have dimensions as described in 1.4 Biology - measurements (Krelekamp 2004).

Transportation box design

The design should include an access port for a pole syringe, and the floor of the box should be slatted or perforated over a leak-proof droppings tray in such a manner that all faeces fall onto the tray. The floor should be leak-proof and covered by absorbent material to prevent excreta from escaping. This should also provide comfort and ensure that the animal is not prone to direct contact with its own excrement for the duration of the transport. Straw might be used for this purpose, but if international shipments are involved, it should be confirmed that plant material is accepted by the receiving country (Mellen 2003).

The transport box should be clearly labelled with the words „Live animal“ plus additional labels required by specific regulations if needed (Blomqvist et al. 1999).

Temperature & Ventilation

During transport an ambient temperature within the appropriate range (+15 to +25 °C) must be maintained and temperature extremes avoided, as stressed and excited individuals overheat very easily in confined spaces. Cooling should be achieved by fresh air (e.g. fans) and not water. During delays when ambient temperature is projected to exceed 25 °C, drinking water should be provided (Blomqvist et al. 1999). Proper ventilation is crucial, consequently free flow of air via a sufficient number of holes and ventilation slots on at least two opposing sides must be guaranteed (Blomqvist et al. 1999).

Acoustic stress

Lynx tend to become aggressive and/or stressed when exposed to excessive external noise and activity. During the transport, crates should be located away from any sources of noise (e.g. people, loud equipment other sources of potential sonic stress).

Light management

Light intensity and incidence during transport should be kept to a minimum. Ventilation slots on the transportation box should be covered with a material such as burlap that filters light but allows sufficient air circulation (Mellen 2003).

Food and water supply

During short journeys (under 12 hours) it is not necessary to provide food and water. Assuming that the animal has been fed well prior to transport, food need not to be given for 48 to 72 hours. Water should be provided for transport times over 12 hours though. Provision of food and water during transport requires placement of food and water containers within the transport box.

If feeding is required, canned cat food may be sufficient. Water containers should be positioned at the front of the box and fixed off the floor to prevent the device from soiling. If animals are unattended on long journeys, written instructions concerning feeding and watering should be attached to the outside of the transportation box (Blomqvist et al. 1999).

Accompanying veterinarian

Usually it is unnecessary for a veterinarian to accompany the transport of an animal unless it has been anaesthetised for the journey, which is rarely required. It is desirable that an experienced and trained person familiar with the species accompanies a transport, which allows for close monitoring and reassurance of the animal's well being (Blomqvist et al. 1999).

Arrival

Upon arrival, veterinary staff and/or wildlife ecologists should perform a visual examination of the animal as soon as possible.

A1.4 Quarantine

Lynx should be quarantined for at least 30 days after transport and prior to release into the wild. Quarantine should be carried out in a separate enclosure, which is physically isolated from other facilities holding felids or other wild animals. Strict hygiene precautions must be observed within the quarantine area. Beyond basic testing (CBC, serum chemistry panel, serum banking and a physical exam), serology testing for feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP), feline leukaemia virus (FeLV), toxoplasmosis and tuberculosis should be completed before the animal is released and is allowed to make contact with conspecifics. The animal should have three negative faecal exam results and should be treated for external parasites (fleas, ticks, ear mites etc.). If the exams prove positive for parasite infestation, appropriate therapy has to be instituted and faecal samples have to be screened more frequently. If persistent parasite problems exist, the individual as well as the environment have to be taken into consideration to address the problem (see chapter 5 for information for testing schemes and treatment of diseases).

During quarantine, the enclosure should be provided with a den box, where the individual can hide. Noise and proximity to other large carnivores, as well as other stressors should be avoided.

If the above tests are negative and the veterinarian in charge is convinced that an individual is healthy, it should be vaccinated against e.g. FVR, FCV. Subsequently, sufficient time must be allowed for a protective antibody response to develop to the dispensed vaccines before release from quarantine (Blomqvist et al. 1999).

When releasing into the wild, care should be taken to allow the reintroduced individual to exit the transportation box in his own time.

A1.5 Safety

During transport and prior to release into the wild, keepers and animal managers should limit direct (hands-on) contact to a absolute minimum (Krelekamp 2004). They must avoid entering the lynx enclosure when the animal is present.

Interaction between lynx and caretaker/keeper/animal manager should only be possible through a mesh screen or through fencing (protected situation). Enclosures must be covered or the barriers made in such a way that lynx cannot jump out. For further details on safe enclosure designs see below (Appendix 2.1.1).

A2 Husbandry guidelines (management of Eurasian lynx in captivity)

This chapter gives a general introduction to design and infrastructure/furnishing that have been developed to provide an appropriate enclosure for Eurasian lynx.

A2.1 Enclosure

Lynx are a temperate species that generally live and hunt on the ground. Therefore, they are usually kept in outdoor enclosures, but need some form of shelter and retreat, as place to rest and to protect themselves from extreme weather conditions.

A2.1.1 Fencing

Most enclosure's fencing or boundaries consist of wire mesh, glass and/or gunite (cement and aggregate mixed with water). If wire mesh is used as fencing material, it should be imbedded into concrete base footings that prevent animals from digging through. Gauge of wire mesh should be small enough that a lynx cannot reach through and strike a keeper. Climbing skills of the species must be taken into account; enclosures must therefore be covered and/or barriers must be made high enough, with the top portion facing inward in the direction of the enclosure, to prevent the lynx from climbing out. Additionally, electric fencing should be attached on the inside along the top to further deter escape (see figure 41).

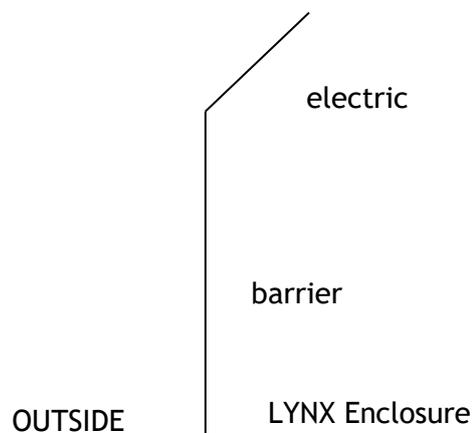


Figure 41. Barrier/fencing of a non-covered enclosure for Eurasian lynx

To reduce stress and any visual distraction, the fencing/barriers of the enclosure used for quarantine purposes prior to release/reintroduction must be almost fully covered, leaving only a few spy-holes for visual observation of the individual. In a non-covered enclosure, trees and plants must be so arranged that a lynx cannot use them as aid to climb out.

Enclosures should have a secondary holding area in order to safely separate lynx from their primary enclosure to allow for cleaning, feeding and medical procedures. Alternatively, the primary enclosure may be divided into separate compartments (Krelekamp 2004).



Figure 42. Enclosure for lynx cubs prior to re-introduction into the wild (author's photo)

A2.1.2 Dimensions

For a quarantine or pre-release enclosure in a captive environment, it can be difficult to recreate the substantial size of a normal lynx home range. According to Krelekamp (2004) minimum space is: 4 x 2 x 2,5m per individual (l x w x h), subsequently the spatial density accounts to 20m³ per cat. As a terrestrial species, floor space should be planned with complexity and usability prioritized. Felids should also have access to at least 75% of the enclosure's vertical space. Studies have shown that the greater the height of the enclosure, the lower the levels of faecal corticoids (hormones, whose production is induced by stress) (Mellen 2003).

A2.1.3 Substrate

Flooring should be made of materials like cement or concrete, which can be easily cleaned. An additional provision of layers of straw, wood shavings or sand is recommended (Mellen 2003).

A2.1.4 Furnishing

Complexity and usability of an enclosure is very important. Lynx that are housed in an enclosure that provides more structure and complexity spend less time pacing than those housed in enclosures that are more sparsely furnished (Mellen & Shepherdson 1997). Enclosure complexity is defined by, amongst other factors, the number of physical barriers and structures that an individual could use to hide and rest in seminatural circumstances.

Every enclosure must be equipped with at least one den box for every lynx housed in the particular enclosure structure. Animals that are continuously housed outdoors should be provided with a den that protects them from inclement weather and extreme temperatures (Mellen 2003).

Bedding materials for dens include straw, wood shavings or wood wool. Presence of bedding material sometimes induces felids to urinate or defecate in the nest box. If individuals persist in using the den box as latrine, a second den box should be provided. Lynx will typically use one box as a latrine and the other one as a den (Mellen 2003).

Additional efforts should be made to allow lynx to utilize vertical components within an enclosure by providing aerial pathways in the form of logs and similar structures. Lynx seem to prefer perching platforms at elevated heights within the enclosure in order to rest, hide and peer out. Wooden as well as plastic materials make good platforms or shelves. Lynx also require logs in order to sharpen their claws; rotting logs will stimulate clawing activity (Mellen 2003).

A2.1.5 Maintenance

Substrates should be spot-cleaned (faeces removed) and raked; urine-stained flooring should be disinfected weekly. Food containers and water bowls should be cleaned and disinfected daily. Perches and shelves that individuals use to climb should also be kept free of faeces and urine, but it is not necessary to clean them daily. Since scent-marking behaviour is important in lynx, layers of straw, wood shavings or sand present on the floor must be changed and floors and walls disinfected after the quarantine period ends in order to provide a scent-free and clean environment for subsequent individuals to be housed in the enclosure.

Footbaths should be used prior to entering and after exiting the enclosure. The footbaths should be filled with a disinfectant and used by all persons as they enter and leave the enclosure to prevent the spread of fungi, virus and bacteria.

Disinfectants most commonly used in zoo operations usually have a broad spectrum of microbicidal activities, such as o-phenylphenol salts, especially sodium o-phenylphenol. Equally popular for routine cleaning and sanitizing operations in zoos are quaternary ammonium compounds and chlorine bleach (sodium hypochlorite) (Heuschele 1995).

A2.1.6 Temperature

Eurasian lynx can tolerate a wide range of temperatures with in situ temperatures ranging from -25° C to +25° C. However, higher temperatures lead to exhaustion and overheating. Consequently, lynx that are kept in non-covered enclosures exposed to sunlight must be offered sufficient shade and, in case of extreme weather conditions, must be provided with shelter.

A2.2 Feeding

This chapter describes a basic dietary plan for captive Eurasian lynx individuals, including supplementary requirements, non-nutritional aspects of feeding, feeding methods and water provision.

A2.2.1 Basic diet

Lynx are obligate carnivores that rely on ingestion of other animals for food. In a captive setting it is rarely possible to provide natural prey species (e.g. such as ungulates or smaller mammals like hares); therefore a thorough understanding of the nutritional requirements of felids is essential in order to formulate an artificial diet.

Because felids lack much of the metabolic flexibility of facultative carnivores or omnivores, they have more specific and demanding nutritional requirements than canids or ursids. In general, non-domestic cat species share similar nutritional requirements with their domestic counterpart (Blomqvist et al. 1999). This can inform dietary planning for lynx.

Two different types of diets can be fed to captive lynx (abbreviated after Krelekamp 2004):

A dry extruded diet

If only the nutritional aspects of diets are considered, dry extruded diets are recommended for small felid species, as they can be made nutritionally complete. Commercially, a few extruded diets with high palatability are available.

High-moisture, ground diet

A formula for a nutritionally supplemented ground meat diet can be considered, but is just a poor substitute for a provided carcass, especially for individuals about to be reintroduced in the wild (Mellen 2003).

Whole or partial carcasses

For a diet consisting primarily of meat, it is also possible to feed a variety of commercially available whole animals (e.g. rabbits, rats or guinea pigs), gutted carcasses (e.g. rabbits) and carcass parts (e.g. beef) as the major part of the diet. Birds (e.g. poultry) should only be used if a free-range source is available, otherwise contamination with hormones and antibiotic residues, which are often present in commercial poultry farms, presents a risk to the captive individual. Day old chicks are easy and convenient to use for food, but Krelekamp (2004) mentions they are too rich in vitamin A. The feeding of wild birds and animals such as rabbits should be avoided, as they can carry a variety of transmissible diseases, and it is often not possible to inspect the carcass prior to feeding.

A2.2.2 Methods of feeding

Daily feeding of lynx is recommended with fast days once or twice per week (where bones can be offered as supplement and to provide dietary enrichment). In order to reduce habituation and association of food with people, food should be provided while the individuals are not directly present (e.g. while withdrawn to the den). When feeding whole or partial prey items, these should be deep frozen (-30 to -18° C) to kill any parasites prior to presentation (Crissey et al. 2001). They should then be thawed under refrigeration in a clean area and subsequently delivered to the enclosure in insulated containers in order to reduce the risk of exposure to potentially harmful microbes (Mellen 2003). It is also important to ensure that carcasses are not fed whilst still frozen at the centre, as this may lead to gastric problems (Krelekamp 2004). Once a carcass has thawed, the whole carcass should be offered at or around ambient temperature.

A2.2.3 Water

Fresh, clean, potable water should be made available at all times in containers that cannot easily be overturned or emptied. Felids are known to routinely defecate in water bowls (a behaviour that is difficult to discourage). Therefore, water bowls should be cleaned and disinfected daily. Elevating water bowls 15 to 30 cm above the ground might discourage this behaviour.

Appendix II: Forms, Lists and Protocols

Attachment I Lynx Capture Form

Lynx Capture Form

Recorded by:	Institution/Phone:
Animal ID:	
Date:	Time:
Administrative district: City:	Coordinates: /
Location of capture: <input type="checkbox"/> Forest <input type="checkbox"/> Meadow <input type="checkbox"/> driveway Other:	
Purpose of capture / cause (accident etc.)	
Body condition (general appearance) please check: poor (emaciated, very underweight) good (thin/lean) excellent (ideal) <u>Bleeding:</u> Location _____ <u>Breathing:</u> Normal Labored Choking Noise _____ Comments _____ <u>Neuro:</u> Convulsions Head tilt Comments _____ <u>Right Eye:</u> Dilated Blood Swelling Other Lesions Palpebral reflex: Normal/Slow/None <u>Left Eye:</u> Dilated Blood Swelling Lesions Palpebral reflex: Normal/Slow/None <u>Nares/Nose:</u> Blood Discharge Sounds _____ <u>Mouth:</u> Blood Lesions Teeth _____ Colour of gums _____ <u>Ears:</u> Blood Discharge Parasites Comments _____ <u>Dehydration:</u> Skin turgor: _____seconds <u>Abdomen:</u> Normal Distended Painful Wrinkled Trauma Comments: _____ <u>Legs/Feets:</u> Normal Swelling Fracture Paralysis Cold Hot Comments: _____	
other (injuries, scars, ectoparasites):	
sex: <input type="checkbox"/> female <input type="checkbox"/> male	interspace anus-genital: _____mm
age: estimate:	<input type="checkbox"/> degree of tooth abrasion:
coat pattern: <input type="checkbox"/> large spots <input type="checkbox"/> small spots <input type="checkbox"/> rosettes <input type="checkbox"/> plain	

Body dimensions

weight:	height at the withers:
body length (with tail):	body length (head to torso without tail):
neck girth:	tail length (tip to anus):
hind foot length:	ear length: right: left:
shoulder height:	ear tuft length: right: left:
head length ¹ :	head girth ² :
chest length ³ :	chest girth ⁴ :
microchip: <input type="checkbox"/> no <input type="checkbox"/> yes, number:	

Dimensions paws: (without claws from the back of the main bale to the tip of the longest toe bale)

	length	width	comment
front right:			
front left:			
back right:			
back left:			

Condition dentition: _____

Length canines:

upper left:		upper right:	
bottom left:		bottom right:	

Interval between canines (from tip to tip): maxilla _____

mandible _____

Sampling:

blood storage: _____

hair deriving from (belly, coarse hair, guard hair etc.):
_____ storage: _____

Scat consistency: _____ parasites: _____

storage:

Genetic Sampling: oral mucosa muscle tissue blood none

¹ from Prosthion (rostral end of the interincisive suture) to Akrokranium (the most caudal median point)*

² Zygomatic width: greatest width between the zygomatic arches*

*(Gomercic et al. 2010)

³ from the tip of the nose to the sacrococcygeal joint, following the animal's contours, with the lynx in a physiological recumbent position*¹

⁴ from the upper abdomen; measurement around the sternum*¹(Marti and Ryser-Degiorgis 2018)

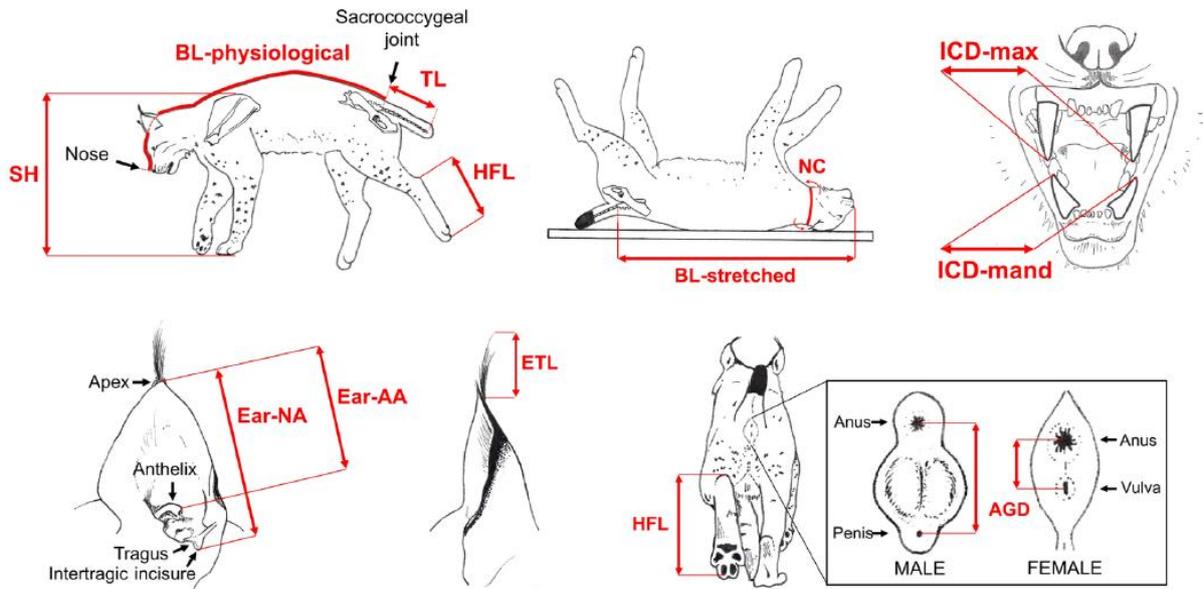


Figure 1. Physical parameters measured on Eurasian lynx (*Lynx lynx*): BL-physiological = Body length measured with the animal placed in a physiological position, SH = Shoulder height, TL = Tail length, HFL = Hind foot length, BL-stretched = Body length measured in a stretched position, NC = Neck circumference, ICD-max = Inter-canine distance of the maxillary canine teeth, ICD-mand = Inter-canine distance of the mandibular canine teeth, Ear-NA = Ear length measured from the bottom of the lateral ear notch (intertragic incisure) to the apex of the pinna, Ear-AA = Ear length measured from the bottom of the fold behind the anthelix to the apex of the pinna, ETL = Ear tuft length, AGD = Anogenital distance (Marti and Ryser-Degiorgis 2018).

Vital signs – heart rate, respiration, rectal temperature (RT), O² saturation, capillary refilling time (CRT):

1. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
2. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
3. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
4. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
5. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
6. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
7. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
8. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
9. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____
10. time	_____	beats/min	_____	resp./min	_____	RT	_____	°C	O ² sat.	_____	CRT	_____

Anaesthesia:

Time	Drug	Dose(mg or ml)	Method	Location
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

Time animal immobilized _____ Time animal recovered _____

Radio-collar: Equipped Yes No Frequency _____
 Radio Signal Checked Yes No

Disposition:

Examination Relocation Treatment and Release Tagging
 Transported to: _____

Closure: Released Transported Euthanized

Documentation: photos video x-ray other: _____

- overview pictures
- photos animal, dorsal / ventral – different perspectives with background
- externally visible injuries in overview and close-up
- close-up of teeth/dentition in case of dental injuries
- vertical overview and close-up of the entire animal for identification (individual photographed from left and right, legs with inside and outside parts from left and right, back from dorsal, abdomen and chest from ventral, shoulder from left and right, hip with thigh from left and right right and flank from left and right)

Attachment II: Mortality Event Form

 	Lynx Mortality Event Form		
	Recorder's name:		
	Institution:		
INITIAL REPORT			
Name of reporter:	Date:		
Affiliation:	Telephone:		
Address:			
Email:			
Location of event:	GPS coordinates:		
Type of landscape, land-use and/or facility:			
Number sick	Number dead	Age(newborn/juvenile/adult)	Sex
First detection of sick/dead animals (date):			
Carcass condition: Fresh <input type="checkbox"/> Bloated <input type="checkbox"/> Early decomposition <input type="checkbox"/> Advanced decomposition <input type="checkbox"/> Complete decomposed <input type="checkbox"/> Skeletonization <input type="checkbox"/>			
Clinical signs of sick/injured animal (describe behaviour):			
External signs on dead animals:			
Environmental factors: (Record conditions such as storms, precipitation, temperature changes, or other climatic factors that may have contributed to encountered condition)			

Other relevant history (population movement and dynamic, on-going human activity in the area, recent unusual event):

FIELD INVESTIGATION			
Name(s) of investigator:		Date:	
Affiliation:		Telephone:	
Address:			
Email:			
Location of event:		GPS coordinates:	
Type of landscape, land-use and/or facility:			
Number sick	Number dead	Age (newborn/juvenile/adult)	Sex (M/F)
Report of diseases in livestock or humans in the region:			
First occurrence of sick/dead animals (estimate from records, from local reports, or from the last field activities in the area with no detection?):			
Clinical signs of dead animals (physical appearance):			
Carcass condition: Fresh <input type="checkbox"/> Bloated <input type="checkbox"/> Early decomposition <input type="checkbox"/> Advanced decomposition <input type="checkbox"/> Completely decomposed <input type="checkbox"/> Skeletonization <input type="checkbox"/>			
External signs on dead animals:			

Additional information
Environmental factors: (Record conditions such as storms, precipitation, temperature changes, or other natural or anthropogenic changes that may contribute to stress.)
Other relevant history and observations (population movement and dynamic, on-going human activity in the area, recent unusual event):
Other Comments and Preliminary suspicions

Pictures of sick and dead animals

Attachment III: To Do List Necropsy

All veterinary pathological facilities will have their distinct protocols according to their needs and practical experience and, although undoubtedly adhering to best-practise standards, these protocols may vary slightly between institutions. We therefore refrain from presenting a necropsy protocol-template but rather point out some issues that should be obeyed during the forensic necropsy process (Beiglböck and Walzer 2019).

- Adopt your necropsy protocol according to the needs and requirements of a forensic investigation
- Take photographs of all steps during necropsy, beginning with reception of carcass (packaging, earmarks etc.). Use tags and rulers for best documentation and start with overview photos before going into detail. Take photographs also of non-significant findings (e.g. an organ without any alteration),
- Consult CSI form and photos of crime scene for full background history of case
- Recover and retain all potential pieces of evidence like snares, traps and others still attached to the carcass. Handle them with care and use gloves (Fingerprints).
- Recover and retain all markings of the carcass like rings in birds, microchips, telemetric collars
- Record storage conditions of carcass prior to necropsy
- Record body-measurements and -weight as well as those of relevant organs
- Perform a FULL, THOROUGH and EXHAUSTIVE NECROPSY & HISTOPATHOLOGY, even if the cause of death seems obvious
- Bear in mind that autolysis may be prominent in dead wildlife. Do not confound the alterations caused by this process with those of other origin.
- Pay attention to signs of (see text):
 - Shooting
 - Snaring
 - Poisoning
 - Blunt trauma indicative of vehicle collision, wind turbine/power line collision etc.
 - Electrocution (in birds)
- In shooting incidents, recover and retain at least the larger bullet fragments. Use plastic forceps, padded forceps or gloved fingers for recovery
- Select best samples for ancillary investigations and fill out sampling log
- Initiate ancillary investigations deemed necessary
- Try to establish a post mortem interval
- Determine cause, mechanism and manner of death

Attachment IV: Necropsy Protocol Form

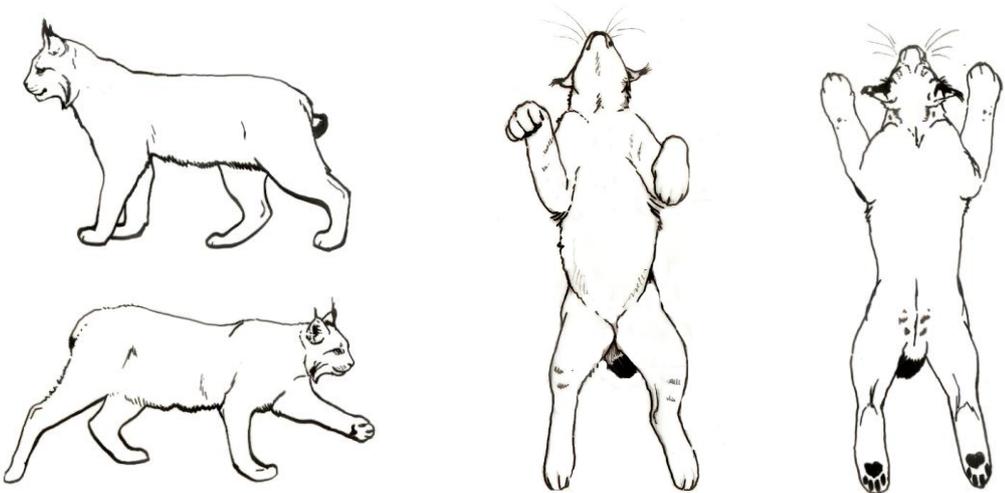
 	 Lynx Necropsy Protocol Form
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NECROPSY EVENT INFORMATION				
Event ID:				
Location of event:				
Location of Necropsy:				
Necropsy Date and Time:				
Storage (prior to necropsy):	Ambient <input type="checkbox"/>	Refrigerated <input type="checkbox"/>	Iced <input type="checkbox"/>	Frozen <input type="checkbox"/>
Multiple Animal Deaths?:	Yes	No	Number of Animals (if yes):	

ANIMAL INFORMATION				
Animal ID (Event ID-A0001...):				
Location of Death		GPS Location:		
Date of Death:		Time of Death:		
Carcass Recovery Date & Time:				
Carcass condition		Fresh <input type="checkbox"/> Bloated <input type="checkbox"/> Early decomposition <input type="checkbox"/> Advanced decomposition <input type="checkbox"/> Complete decomposition <input type="checkbox"/>		
Sex:	Male <input type="checkbox"/>	Female <input type="checkbox"/>	Undetermined <input type="checkbox"/>	
Age:	Neonate (days)	Juvenile weeks/months)	Adult	Geriatric

SUSPECTED HISTORY					
Manne r of Death:	Predation <input type="checkbox"/>	Disease <input type="checkbox"/>	Human Interaction <input type="checkbox"/>	Accidental <input type="checkbox"/>	Unknown <input type="checkbox"/>
Type of Human Interaction (check any that apply)					
Euthanasia <input type="checkbox"/>	Problem animal control <input type="checkbox"/>	Poaching <input type="checkbox"/>	Gunshot <input type="checkbox"/>		
Snared <input type="checkbox"/>	Poisoned <input type="checkbox"/>	Vehicular Trauma <input type="checkbox"/>	Other <input type="checkbox"/>		
<p>Signs/structures in the environment (<i>signs of struggle, signs of weather events such as lightning or flood, signs of convulsion/paddling, other gear/debris/evidence/(infra-) structures/ found near animal, or evidence supporting vehicular trauma, poisoning etc.</i>)</p>					

GROSS NECROPSY EXAMINATION
<p>Use the spaces below to record the size, shape, colour, consistency/texture of the organs examined and the location, number and distribution of any abnormal findings.</p> <p>Take a photograph of all abnormal findings</p> <p>If the examined system is normal record NGL (no gross lesions). If not found record NF.</p>

EXTERNAL EXAM
<p>Proceed to external examination</p> <p>Collect oral and rectal swabs</p> <p><input type="checkbox"/> Take a photograph</p>
<p>Signs on carcass: (<i>briefly summarize clinical signs, presence of wounds, broken bones, external parasites, tumor, including structural alterations caused by humans to head/appendages, pelt/fur, or body</i>):</p>

<p>Illustrations: © S. Knöpfer</p>

Length (from the tip of the nose to the sacrococcygeal joint (without tail) (in cm):	Weight (kg):
Nutritional Condition (<i>circle the appropriate description</i>):	
Subcutaneous fat:	Very fat Normal Little to No fat
Muscle mass: Ideal <input type="checkbox"/> Underweight/Lean <input type="checkbox"/> Thin <input type="checkbox"/> Very underweight <input type="checkbox"/> Emaciated <input type="checkbox"/>	
Nostrils (<i>e.g. discharge</i>):	
Ears (<i>e.g. discharge, wounds</i>):	
Eyes (<i>e.g. discharge, cornea clear or cloudy</i>):	
Mouth (<i>tongue, teeth condition, ulcers, other lesions</i>):	
Skin/Hair Coat/Nails (<i>colour, condition</i>): Adequate <input type="checkbox"/> Lapsed <input type="checkbox"/> Borderline <input type="checkbox"/> Poor <input type="checkbox"/> Terrible <input type="checkbox"/>	
Wounds/Scars (<i>location, length, depth, presence of bruising/bleeding around wound</i>):	
External parasites (<i>location, type, number or estimate of number</i>):	
Anus/perineum:	

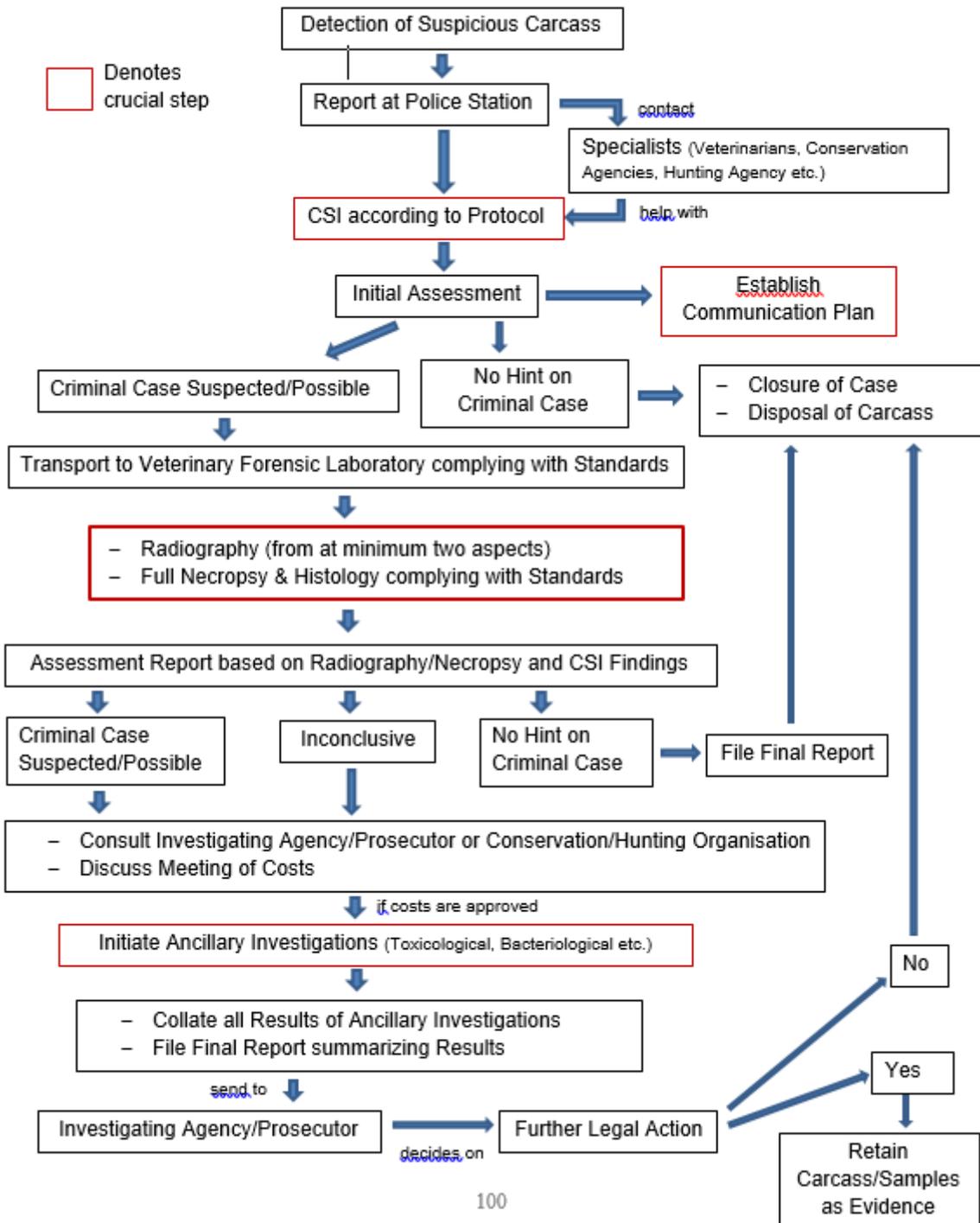
BODY SYSTEMS	
<p><i>Reflect fore leg and rear leg. Disarticulate hip</i> <i>Reflect skin</i> <i>Sample eye, skin, muscle, sciatic nerve, bone marrow</i></p> <p>Photographs Yes <input type="checkbox"/> No <input type="checkbox"/></p>	
<p>Musculoskeletal System (Examine bone and muscle for fractures, dislocations, arthritis, joint infection, muscle atrophy, trauma):</p>	
<p><i>Then, open abdomen, thorax</i></p> <p>Photographs Yes <input type="checkbox"/> No <input type="checkbox"/></p>	
<p>Body Cavities (examine thorax, pericardium, abdomen for fluids, trauma, other abnormalities):</p>	
<p><i>Remove and examine lung, liver; sample thyroid, heart, tongue, oesophagus, trachea</i></p> <p>Photographs Yes <input type="checkbox"/> No <input type="checkbox"/></p>	
<p>Circulatory System (<i>heart, valves, vessels</i>):</p>	
<p>Respiratory System (<i>Larynx, trachea, bronchi, lungs (externally and after cut)</i>):</p>	
<p>Lymphatic System (<i>spleen, tonsils, lymph nodes - tracheobronchial, mesenteric, popliteal, axial, etc.</i>)</p>	
<p><i>Remove and examine GI tract & liver</i> <i>Sample liver, gallbladder, stomach, pancreas,</i> <i>Open and examine intestine</i> <i>Sample small intestine, large intestine</i></p>	

<i>Sample spleen</i> Photographs Yes <input type="checkbox"/> No <input type="checkbox"/>	
Gastrointestinal system (<i>oesophagus, stomach(s), intestine, faeces, pancreas, liver, gallbladder; please also report the amount and type of food in the stomach(s), and the presence of any abnormal material</i>): 	
<i>Examine and Sample kidney, adrenals, ureters, bladder, urethra</i> Photographs Yes <input type="checkbox"/> No <input type="checkbox"/>	
Urinary System (<i>kidneys, internal and external urinary tract</i>): 	
Examine and Sample gonads Photographs Yes <input type="checkbox"/> No <input type="checkbox"/>	
Reproductive System (<i>ovary, testicles, uterus; mention the presence of fetus</i>): 	
<i>Remove head and skin off, remove skull and examine brain</i> <i>Sample brain and pituitary gland</i> Photographs Yes <input type="checkbox"/> No <input type="checkbox"/>	
Nervous System (<i>brain, spinal cord, peripheral nerves, eyes</i>): 	

PRELIMINARY DIAGNOSTIC:

Attachment V: Flowchart Wildlife Crime Investigation Scheme

FLOWCHART INVESTIGATION SCHEME



(taken with permission from Beiglböck and Walzer (2020))

Sample Collection checklist

- Rectal swab in RNAlater
- Rectal swab in VTM (if available)

- Oropharyngeal swab in RNAlater
- Oropharyngeal swab in VTM (if available)

- Blood in plain tube by cardiac puncture (or puncture/section of vena cava)

If a centrifuge is available, collect the blood in a plain tube and spin down to separate serum and red blood cells:

- Serum in plain cryovial
- Red cells in RNA later
- Red cells in VTM (if available)

If a centrifuge is not available:

- Full blood in RNA later
- Full blood in VTM (if available)

Blood spot on filter paper

- Brain in RNAlater
- Brain in VTM (if available)

- Lung in RNAlater
- Lung in VTM (if available)

- Liver in RNAlater
- Liver in VTM (if available)

- Kidney in RNAlater
- Kidney in VTM (if available)

- Urine in RNAlater
- Urine in VTM (if available)

- Faeces in RNA later
- Faeces in VTM (if available)
- Faeces in plain cryovial

- Spleen in RNA later
- Spleen in VTM (if available)

skin biopsy for genetic analysis in 95% alcohol

Note: samples listed above should be stored in the freezer (except skin snip and Dried Blood Spot)

Additionally:

- collect a sample of each observed lesion into a formalin container (1 part tissue for 10 parts formalin)

Attachment VI: Tissue examination and collection checklist

Tissue	Histo	Cyto	Photo	Storage temperature (-20° / - 70°)	Tissue	Histo	Cyto	Photo	Storage temperature (-20° / - 70°)
GENERAL - external					ABDOMEN				
Oral cavity & teeth					Diaphragm				
Tonsils					Stomach				
Skin and nails					Small intestines				
Subcutis					Large intestines				
Skeletal muscle					Pancreas				
Peripheral nerves					Spleen				
Mammary gland					Liver & gall bladder				
Umbilicus					Lymph nodes				
					Aorta & vessels				
BONES & JOINTS					Kidneys				
Bone marrow (femur)					Ureters				
Bones					Urinary bladder				
Hips					Urethra				
Stifles					Adrenal glands				
Tarsi					Ovaries				
Elbows (Interphalangeal Joints)					Oviduct/Uterus				
Shoulders					Vagina/vulva				
Carpi					Testes				
Atlantooccipital					Accessory sex gland				
					Penis / Prepuce				
CAVITIES					HEAD				
Thoracic cavity					Eyes				
Abdominal cavity					Ears & bullae				
					Skull / nasal cavity				
PLUCK					Brain / Meninges				
Tongue					Pituitary gland				
Thyroids/parathyroids									
Oesophagus					SPINE				
Trachea					Vertebral column				
Lungs					Spinal cord				
Heart/Pericardial sac									
Aorta & other vessels									
Thymus & lymph nodes									

Cyto = cytology slide prepared (e.g. tissue impression)

PHOTO = photograph taken

-20/-70 = frozen tissue temperature: list storage temp as -20, -70 or other temp., if applicable

Attachment VII: Skeletal Lesions Form

Encircle/Record injuries or abnormalities to lynx skeleton. Describe in necropsy protocol form and/or in comment section below

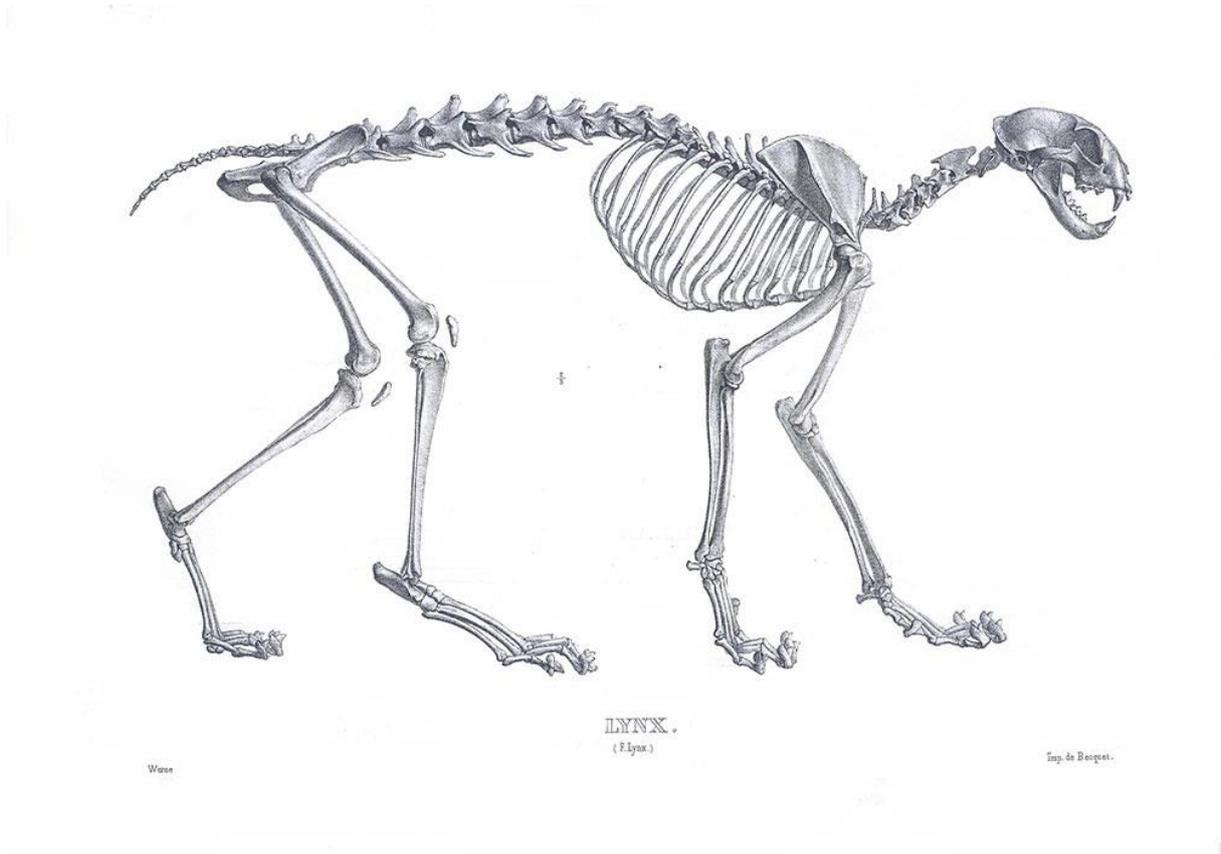


Figure 43: Skeletal Anatomy of Eurasian Lynx - (Source: https://www.thewildlifemuseum.org/media/photo/LR_dB_Lynx_Skeleton.jpg)

Comments:

Attachment VIII: Fixed Tissue List

Fixed Tissue List for Histopathology in wildlife crime scene investigation

Institution:

Case #:

Animal ID:

Lab Submission:

Date:

Veterinarian:

1. Preserve the tissues in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin.
2. Samples should be no more than 0.5cm to 1.0 cm thick and 3x4 cm (length and width) to fix properly. The exceptions are brain, spinal cord, and eye. The ratio of tissue to formalin is 1:10 in wide mouth containers.
3. Tissues collected should be based on case information, medical history, and necropsy findings including lesions, wounds, and evidence of injury or disease.

Salivary gland

Oral/pharyngeal mucosa and tonsil

Tongue - cross section near tip including both mucosal surfaces.

Lung - sections from several lobes including a major bronchus

Trachea

Thyroid/parathyroid

Lymph nodes - cervical, mediastinal, bronchial, mesenteric and lumbar; cut transversely.

Thymus

Heart - sections from both sides including valves

Liver - sections from 3 different areas including gall bladder

Spleen - cross sections including capsule.

GI Tract - 3 cm long sections of:

Oesophagus

Stomach - multiple sections from all regions of the lining

Intestines - multiple sections from different areas

Omentum - 3 cm square

Pancreas - sections from two areas

Adrenal - entire gland with transverse incision.

Kidney - cortex and medulla from each kidney

Urinary bladder, ureters, urethra - cross section of bladder and 2 cm sections of ureter and urethra.

Reproductive tract - uterus and ovaries with longitudinal cuts into lumens of uterine horns; both testes (transversely cut) with epididymis; entire prostate transversely cut.

Eye

Brain - cut longitudinally along midline.

Spinal cord - sections from cervical, thoracic and lumbar cord.

Diaphragm and Skeletal muscle - cross section of thigh muscles

Opened rib or longitudinally sectioned femur - marrow must be exposed for proper fixation

Skin - full thickness of abdominal skin, lip, and ear pinna.

Neonates: umbilical stump - include surrounding tissues

Lists

General Equipment:

- Good packs for carrying equipment
- Writing utensils
- Clipboard with a clear piece of plastic to keep rain off
- Protective plastic sheets to keep rain off documents
- Notebook
- Other recording devices (recorder, computer, smart phone etc)
- Camera
- Spare batteries and chargers for all electronics
- GPS unit and maps
- Packing equipment: cooler box, cardboard box, ziploc bags, packing tape

Supplies for Animal Capture Activities (derived from Sanchez et al. 2016):

Adapt as appropriate to field conditions and perceived risk:

PPE (Personal Protective Equipment)

- Nitrile (recommended) gloves
- Leather or Kevlar gloves
- Face-mask
- Sharps-container
- Closed-toed shoes

Monitoring

- Thermometer
- Stethoscope
- Stopwatch or other timing device
- Pulse oximeter with probes
- Penlight
- Scale and Measuring Tape
- Warm-water bottles (to prevent hypothermia)
- Buckets or water bottles (to prevent hyperthermia)

Immobilization equipment

- Dart equipment (rifle/pistol/blowpipe, CO2 or powder cartridges, and dart protectors)
- Drugs (sedatives, tranquilizers, anaesthetic drugs, reversals or antagonists)
- Calculator to use for calculating drug dosages
- Darts and dart needles
- Nets
- Pole-syringe
- Cargo-net / Tarp
- Blindfold
- Ear-plugs
- Carrying bags
- Syringes and needles
- Towels/Snare-pole

Emergency

- Emergency medications (doxapram/ atropine/epinephrine/diazepam)
- IV catheters
- Fluids (NaCl, lactated Ringer's, Dextrose)
- IV administration set
- Antibiotics, disinfectants
- Tongue swabs
- Vet wrap and tape
- Flashlight
- Minor surgery (and suture) pack
- Euthanasia solution
- Alcohol (to treat hyperthermia)
- Tissue glue or super-glue
- Blanket/towel (to help treat hypothermia)
- Cold pack/hot pack Laryngoscope, tracheal tubes, ambu bag

Recovery and release

- Crates/containers in which to place animal during recovery
- Binoculars

Necropsy (see chapter 8)

- Necropsy protocol
- Necropsy Form
- Anaesthesia and/or euthanasia equipment
- Metal ruler and measuring tape
- Spring scale
- Knives and steel (knife sharpener)
- String
- Manila labels
- Scalpel handle (# 4) and disposable blades (#24) or disposable scalpels
- Forceps - various
- Scissors - various
- strong scissors (to clip bones)
- Sharps disposal unit
- Cutting board

Sample Collection Equipment for genetic sampling and forensic sampling:

(see chapter 5 & 7)

- Sample Log Form
- Anaesthesia and/or euthanasia equipment
- Permanent marking pen
- Pencils
- Labels
- Syringes - 5, 10, 20 ml
- Needles - various gauges
- Serum collection tubes
- Sterile plastic bottles - 90 ml (for formalin fixed organ samples)
- Sterile cryovials - 2 and 5 ml
- 50ml falcon tubes
- Sterile plastic bags (Whirl-pak® bags)
- various size zip-lock bags
- One litre plastic containers filled with 10% neutral buffered formalin (x 3)
- 100 ml of 70- 90% ethanol
- 2ml vials with Viral transport medium (VTM, or equivalent)
- 2ml vials with RNA later solution (or equivalent lysis buffer)
- Sterile polyester swabs
- Capillary tubes
- Glass microscope slides, coverslip and slide storage box
- 12 volt portable centrifuge
- Staining kit
- Faecal flotation vials and solution
- Cooler and ice packs or dry ice
- Liquid-nitrogen dry shipper or dewar
- Sharps disposal unit
- EDTA (purple top) blood collection tube
- Serum (red top) collection tube
- FTA cards
- Tube holder
- Sample storage box
- Disposable transfer pipette
- Gauze swabs

Basic procedures for personnel working in the field with wild animals (taken from Sanchez et al. 2016)

- Coordinators should provide all personnel a “Useful Contacts” list with address and numbers of local medical and emergency response services.
- Researchers working with wild mammals should consider pre-exposure rabies vaccination.
- Researchers and their assistants should also consider vaccination against tetanus in those situations where exposure to this pathogen is possible.
- Individuals who are exposed to potential vectors of rabies (e.g. animals with neurological signs) should immediately report the exposure to medical authorities and the supervisor.
- All animal tissues, fluids, and excrement should be handled so that the potential for human contact is minimized.
- Staff should thoroughly wash and/or sanitize hands and any other contaminated skin surfaces with a germicidal skin cleanser immediately after handling wild animals or their samples.
- All personnel handling wild animals should practice good hygiene and avoid rubbing their eyes after animal handling.
- Appropriate planning and specific precautions (trained staff, equipment and tools in good working condition, PPE, etc.) should be taken in order to prevent injuries from bites, scratches and skin punctures from wild animals. Even minor wounds or scrapes may become infected and can potentially result in disease transmission.
- If an injury occurs, clean the wound with a disinfectant and immediately contact a coordinator/supervisor.
- Where there is a risk from aerosolized pathogens from saliva, faeces or urine, protective gear such as gloves, eye protection, respiratory protection (masks, face-shields or respirators), foot protection and protective clothing should be used as necessary. Researchers should always wear gloves and facemask when handling sick or dead animals.
- Personnel performing post-mortem examinations in the field should wear at least a plastic apron, gloves and facemask or goggles.
- After any post-mortem examination is performed, staff should wash and disinfect hands and any other contaminated skin surface.
- All contaminated equipment should be cleaned and disinfected immediately after use while still wearing the appropriate PPE. Disposable used equipment must be adequately disposed of on site (i.e. buried, burnt, etc.).
- All drug containers, needles, scalpel blades, suture needles and other sharp instruments should be used and disposed of in a manner that prevents accidental human injury.
- Physical restraint of wild animals should be kept as brief as possible.
- Care should be exercised when using equipment such as nets, gloves, rabies-pole, etc. to capture wild animals.
- Staff should be familiar with dart equipment, sites of injection and drugs when chemical restraint is elected.
- Anaesthesia monitoring equipment and emergency drugs must be available and staff should be familiar with their use.
- Staff will make sure each animal is fully recovered from anaesthesia prior to release.