EAZA Husbandry Guidelines for the Leopard *Panthera pardus* spp.

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Cover photo: Arabian leopard *Pantera pardus nimr* at BCEAW. © Jane Budd.

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Introduction

Husbandry guidelines have been developed for each EEP species and subspecies with the aim of providing guidelines to achieve optimal captive conditions for the well-being and reproduction of all animals in EEP programs.

As leopard subspecies have similar physiology and ecology and these guidelines have been compiled to include the North-Chinese leopard (*Panthera pardus japonensis*), the Sri Lankan leopard (*P. p. kotiya*), the Arabian leopard (*P. p. nimr*), the Amur leopard (*P. p. orientalis*) and the Persian leopard (*P. p. saxicolor*). All of the above sub-species, except the Arabian leopard are managed as EEP populations within European zoos. All of these subspecies are listed in the International Leopard Studbook (Walter, 2005).

With the aim of enhancing the guidelines, a general husbandry survey was conducted. The questionnaire was sent to all ISIS institutions holding *Panthera pardus* spp. within their collections. At the time of the survey (2005) there were 148 institutions recorded in ISIS. Only 25 % (39) of the institutions (listed later) answered this survey, which examined the management approaches (veterinary care, enrichment, nutrition, breeding, etc.) employed within the zoological community. Special thanks go to the facilities who answered the survey as their answers made a substantial contribution to establishing these guidelines.

Thanks go to Kevin Budd, Thierry Jardin, Jean-Marie Carenton, François Huygues, Patrick Jardin and Grégorie Breton for their advice and assistance. Thanks also go to the EAZA Felid TAG for their support, comments and corrections, and in particular Alexander Sliwa, the EAZA Felid TAG Chairperson, who wrote a letter of support encouraging all EAZA institutions housing leopards (*Panthera pardus* spp.) to participate in the husbandry survey.

These leopard husbandry guidelines were corrected and revised by Tom de Jongh, Alexander Sliwa, Martina Raffel, Michael Flügger, Paul Vercammen and Gary Batters.

Natural History and Status

Figure 1. Wild Amur leopard. © Yuri Shibnev

Taxonomic Classification

The leopard (*Panthera pardus*), along with the lion (*P. leo*), the tiger (*P. tigris*) and the jaguar (*P. onca*), falls into the relatively young felid genus *Panthera* (roaring cats). The genus is thought to have diverged from a common ancestor 2-3 million years ago.

Class: Mammalia
Order: Carnivora
Suborder: Fissipedia
Family: Felidae
Subfamily: Pantherinae
Genus: *Panthera*
Species: *Panthera leo*
Panthera tigris
Panthera onca
Panthera pardus

It is now acknowledged that the snow leopard (*P. uncia* or *Uncia uncia*), belongs to a different genus than *Panthera*.

In the literature, 29 leopard subspecies are historically described, these are listed below (scientific name, author, date and common name):

P. p. adersi, Pocock 1932 – Zanzibar Leopard. Extinct.
P. p. adusta, Pocock 1927 – Ethiopian Leopard.
P. p. antinorii, de Beauz 1923 – Eritrean Leopard.
P. p. chui, Heller 1913 – Ugandan Leopard.
P. p. ciscaucasica, Satunin 1914 – Caucasus Leopard. Synonym for *P. p. saxicolor*.
P. p. dathei, Zukowsky 1964 – Iranian Leopard.
P. p. delacouri, Pocock 1930 – Indochina Leopard.
P. p. fusca, Meyer 1794 – Indian Leopard.
P. p. ituriensis, J.A. Allen 1924 – Congo Leopard.
P. p. japonensis, Gray 1862 – Chinese Leopard.
P. p. jarvisi, Pocock 1932 – Sinai Leopard.
P. p. kotiya, Deraniyagala 1956 – Sri Lankan Leopard.
P. p. leopardus, Schreber 1777 – Senegal Leopard. Probably includes *P. p. ituriensis*.
P. p. melanotica, Günther 1775 – South African Leopard. *P. p. shortridgei* is probably a synonym.
P. p. melas, Cuvier 1809 – Javan Leopard.
P. p. millardi, Pocock 1930 – Kashmir Leopard.
P. p. nanopardus, Thomas 1904 – Somali Leopard. Probably includes *P. p. antinori*.
P. p. nimr, Hemprich and Ehrenberg 1833 – Arabian Leopard.
P. p. orientalis, Schlegel 1857 – Amur Leopard.
P. p. panthera, Schreber 1777 – Barbary Leopard.
P. p. pardus, Linnaeus 1758 – Sudan Leopard. *P. p. chui* is probably a synonym.
P. p. pernigra, Hodgson 1863 – Tibetan Leopard. Synonym includes *P. p. milladi*.
P. p. reichenowi, Cabrera 1918 – Cameroonian Leopard.
P. p. ruwenzorii, Camerano 1906 – Congolese Leopard.
P. p. saxicolor, Pocock 1927 – Persian Leopard. *P. p. ciscaucasica* is a synonym
P. p. sindica, Pocock 1930 – Iranian Leopard.

P. p. shortridgei, Pocock 1932 – Central African Leopard.
P. p. suahelica, Neumann 1900 – Eastern African Leopard.
P. p. tulliana, Valenciennes 1856 – Anatolian Leopard.

Because subspecies description is based on morphology and geographic distribution, the number of subspecies described has been the subject of controversy. There have been up to 29 subspecies of leopard described (one of them already extinct: *P. p. adersi*) however, not all of these are accepted as distinct by all authorities. Currently, scientists acknowledge nine distinct subspecies of leopard, one of which is African (*P. p. pardus*) and the remaining eight Asian (Uphyrkina *et. al*. 2001), however, this classification is still under debate (Sunquist and Sunquist, 2009).

The African populations are clearly distinct from Asian populations. Morphological analyses of the cranial characteristics of the African leopards have shown no significant variation between these populations. All African populations are therefore combined under one trinomial, *P. p. pardus,* using the principle of priority in taxonomic nomenclature (Miththapala, 1992).

Miththapala *et al* (1996) described six phylogeographic groups of leopards. Using explicit definition criteria, they recommended that the classical leopard trinomials be reclassified into only eight subspecies: *P. p. pardus* in Africa, *P. p. saxicolor* in Central Asia, *P. p. fusca* in India, *P. p. kotiya* in Sri Lanka, *P. p. melas* in Java, *P. p. orientalis* in the Russian Far East, *P. p. japonensis* in North China and *P. p. delacouri* in South China. Their limited sampling does not, however, support the distinctiveness of the East Asian subspecies (Miththapala, 1996).

Uphyrkina *et al* (2001) carried out genetic studies on 13 of 27 potential subspecies. Phylogenetic analysis of mitochondrial DNA sequences revealed diversity supporting the modern classification of nine discrete subspecies of leopard: *P. p. pardus*, *P. p. nimr*, *P. p. saxicolor*, *P. p. fusca*, *P. p. kotiya*, *P. p. delacouri*, *P. p. japonensis*, *P. p. orientalis* and *P. p. melas*.

The five leopard subspecies discussed in these guidelines are known to be genetically distinct.

North-Chinese leopard *Panthera pardus japonensis,* Gray, 1862

Range: Northern China

Figure 2. North-Chinese leopard at the Exotic Feline Breeding Compound. © Nancy Vandermey.

Although this subspecies is absent from Japan, its scientific name originally arose as a result of skins purchased in Japan. The North-Chinese leopard has a broad distribution extending from Sichuan to Southern China and north to Beijing.

These are large leopards, with a darker orange background colour than most other leopard subspecies. The rosettes are large and have orange patches within, and sometimes even a spot within the rosette.

Hunting is prohibited in China.

This subspecies is listed as Near Threatened in the IUCN Red List. Approximately 2500 individuals remain in the wild within a highly fragmented home range.

Sri Lankan leopard *Panthera pardus kotiya*, Deraniyagala, 1956

Range: Sri Lanka.

Figure 3. Sri Lankan leopard at CERZA Zoo. © Gerard Lacz.

This is the only naturally occurring large carnivore in Sri Lanka.

The Sri Lankan leopard is a small, long-tailed subspecies.

The main threats to this subspecies are poaching (for skin and bones) and habitat loss.

This subspecies is listed as Endangered on the IUCN Red List (www.iucnredlist.org). The population of the Sri Lankan leopard is estimated to number approximately 500 individuals, restricted mainly to protected areas.

Arabian leopard *Panthera pardus nimr,* Hemprich and Ehrenberg, 1833

Range: Arabian Peninsula.

Figure 4. Arabian leopard at BCEAW. © Jane Budd

The Arabian leopard is found exclusively in the mountains of the Arabian Peninsula. Its historical habitat extends along the mountains of the United Arab Emirates, Oman, Yemen, Saudi Arabia and in the north of the peninsula of Jordan.

The Arabian leopard is the largest felid of Arabia but is the smallest of the leopard subspecies. Males generally weigh 25-30kg, while females weigh 18-23kg. Its coat is pale in comparison to other leopard sub species.

Hunting is prohibited throughout the range of the Arabian leopard. The Arabian leopard is threatened by retaliatory killing in defence of livestock, habitat loss, degradation and fragmentation, and uncontrolled hunting of wild prey (Breitenmoser, 2006, Edmonds *et. al.*, 2006).

The Arabian leopard is listed as Critically Endangered in the IUCN Red List (www.iucnredlist.org). There are less than 250 mature individuals remaining in the wild, with no sub-population containing more than 50 mature individuals.

Only 50 Arabian leopards are currently housed in captivity in collections in the United Arab Emirates, Sultanate of Oman, Republic of Yemen and Kingdom of Saudi Arabia.

Amur leopard *Panthera pardus orientalis,* Schlegel, 1857

Range: Korea-Amur region.

Figure 5. Amur leopard at Rotterdam Zoo. © Rob Dolaard

Recent field studies estimate the following distribution of this subspecies: in the Russian far East, there are 25-40 individuals; in two adjacent provinces of China there are only 10 to 15 animals. It is believed there are leopards in north Korea but these estimates are unconfirmed (www.amur-leopard.org). According to the IUCN Redlist, however, the Amur leopard is now extinct in China and the Korean Peninsula (Jackson and Nowell, 2008).

The Amur leopard is particularly distinctive due to its pale coat compared with most other subspecies. It also has dark-centred rosettes which are large and widely spaced with thick, unbroken rings. The Amur leopards have extremely long hair in response to the cold climate they inhabit. Typically, males weigh between 32-48kg and females between 25-43kg (www.amur-leopard.org).

Hunting is prohibited in Russia and China. Poaching for their skins and bones continues to be a threat.

This subspecies is listed as Critically Endangered in the IUCN Red List (www.iucnredlist.org). With only 30-35 individuals remaining in the wild the Amur leopard, also known as the Far Eastern leopard, is considered to be one of the most endangered large cats in the world (www.amur-leopard.org). This population is susceptible to rapid inbreeding depression with such low numbers remaining. In mid 2008, there were approximately 300 Amur leopards housed in zoos worldwide (www.amur-leopard.org).

Persian leopard *Panthera pardus saxicolor*, Pocock, 1927

Range: Iran, Azerbaijan, Armenia, Turkmenistan, Uzbekistan, Tajikistan and north-western Afghanistan.

Figure 6. Persian leopard. © Gelsenkirchen Zoo.

The range of this subspecies extends across Afghanistan, Armenia, Azerbaijan, Georgia, Iran, Pakistan, Russia, Tajikistan, Turkey, Turkmenistan and Uzbekistan. Leopards have fared better than the other big cats (lion, tiger, cheetah), which historically occurred in the region. However, the future of the leopard in Iran is far from secure. Throughout the region, leopards generally exist as small, threatened and widely isolated populations.

The Persian leopard is said to be the largest subspecies of leopard in the world. Their long hairs resemble those of the unrelated snow leopard.

Hunting is only prohibited in Iran, Pakistan, Turkmenistan and Uzbekistan.

This subspecies is listed as Endangered in the IUCN Red List (www.iucnredlist.org). The population is estimated to number about 1300 individuals. It is severely fragmented; no sub-population is estimated to contain more than 250 mature individuals.

Conservation Status

At the species level the leopard (*Panthera pardus*) is listed as Near Threatened throughout its range, however five of the recognised nine sub-species have been assigned a threatened status. The Amur leopard *(P. p. orientalis)*, Arabian leopard (*P. p. nimr*) and Javan leopard (*P. p. melas*) are listed as Critically Endangered and the Persian *(P. p. saxicolor)* and Sri Lankan leopards *(P. p. kotiya)* are classified as Endangered.

All leopard subspecies are listed in Appendix I of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) and are therefore banned from international trade, except when the purpose of the import is non-commercial, for instance scientific or conservation reasons.

Distribution and Habitat

Leopards are still found throughout much of their historical range, although their numbers have been significantly reduced over the last hundred years due to increasing human populations. Leopard populations have also become increasingly isolated due to habitat loss and fragmentation (CBSG 2000 and Owen, 2006). Fragmentation results in isolation of populations and a lower probability of genetic exchange (Smith and McDougal, 1991). Even so, the leopard has the greatest geographic distribution of any felid (Fig. 7). Its range extends across sub-Saharan Africa, and South Asia, with scattered populations in North Africa, Arabia and the Russian Far East. Historically, it was distributed throughout Northern Africa. The leopard is found in Java but not on the islands of Sumatra and Borneo.

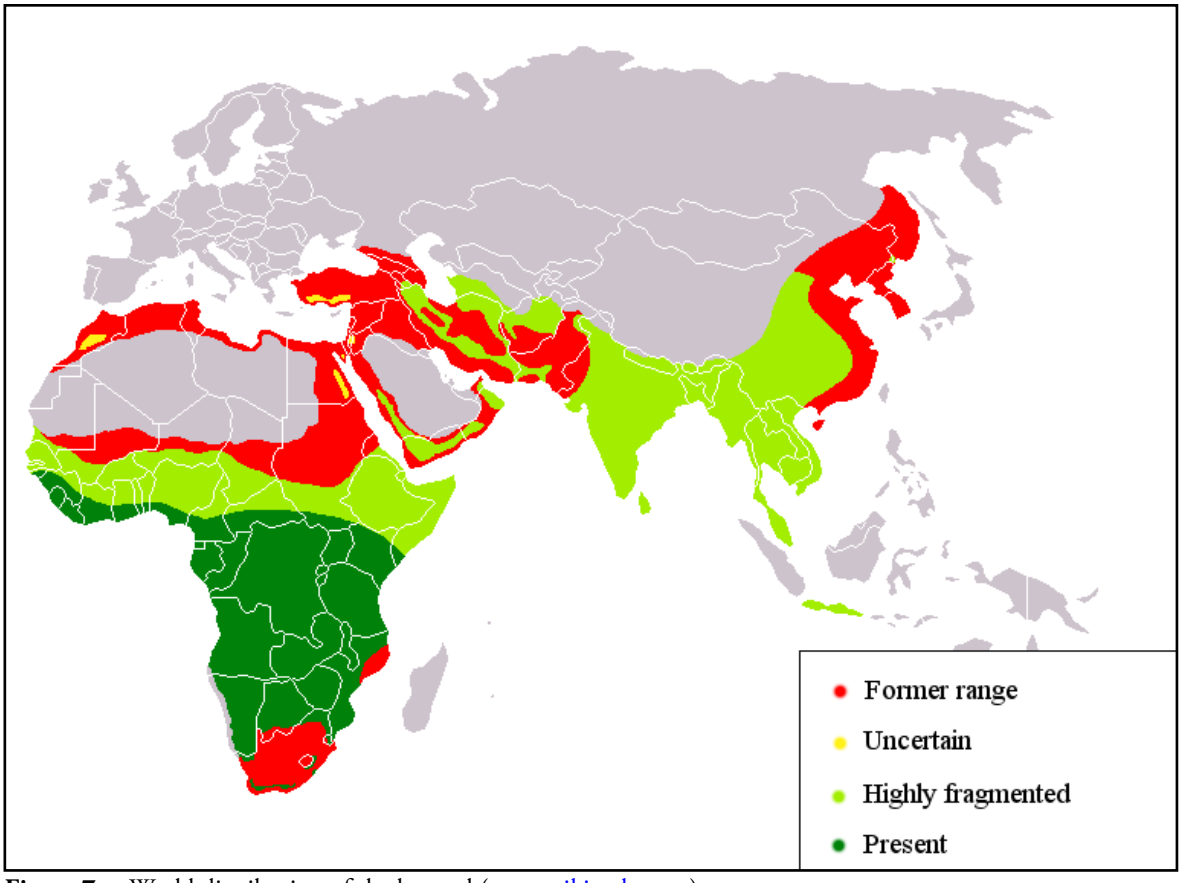

Figure 7. World distribution of the leopard (www.wikipeda.com)

The two major factors that appear to limit the distribution of leopard are the presence of competitors and the presence of humans (Skinner and Smithers, 1990) though in many parts of its range the leopard is known to coexist with other large predators. In Africa, for example, it lives alongside lions and hyenas, and in Asia it shares many habitats with tigers and wild dogs. Most of these competitors are capable of killing leopards (young and adults) and appropriating their prey, so in order to coexist, leopards need to have access to some type of escape cover.

The leopard has adapted to a broad range of habitats, however, they are thought to be absent from true deserts. It is more commonly associated with grasslands, woodlands and riverside forests, but is found in all forest types. It preferentially inhabits rocky hills and mountainous terrain from sea level to elevations of over 5,000m and can survive in areas receiving almost no rainfall (<50mm per year) to areas where the mean annual rainfall is well over 2,000mm (West Africa and tropical Asia).

Figure 8. Typical habitat of the Arabian leopard, in Wada'a, Republic of Yemen. © Jane Budd

Home range size is influenced by biomass density. It has been shown that in semi-arid regions such as Namibia, where the prey density is low, leopards occupy home ranges of up 200km^2 (Stander *et. al.* 1997). In comparison, studies conducted in areas with high prey densities (Kenya) have shown that leopards occupy much smaller home ranges of only 33km^2 for males and 14km^2 for females (Mizuntani and Jewell, 1998). The minimum individual home-range of the Sri Lankan leopard is thought to be 4-10km^2 per adult (Sri Lankan Wildlife Conservation Society).

Morphology

The leopard shows a great deal of variation in body size over its broad geographic distribution. Overall size depends very much on the subspecies and the region. The trunk is comparatively long for a cat and the legs are short. Head and body length has been reported between 125-165cm, and the tail reaches 60-110cm. Shoulder height varies from 45-80cm. The larger-bodied populations of leopard are generally found in areas isolated from competing large predators, especially from dominant big cats like lions and tigers.

There is also a wide variation in body weights between the different subspecies, ranging from 45-70kg for the African leopard, 20-30kg for the Arabian leopard and up to 90kg for Persian leopards.

Adult males are about 30% larger than the females. The male skull is much larger, longer and more angular than that of the female (Skinner and Smithers, 1991). Males also have larger teeth and a well-developed sagittal crest, which is almost absent on most females' skulls (Skinner and Smithers, 1991).

The characteristic markings of the leopard are composites of black spots commonly referred to as "rosettes" (Fig 9). Rosettes of the different subspecies vary in size, shape, thickness of margins, and whether the margins are broken into two, three, four or even five spots. The rosettes cover much of the leopards' body, including the back of the neck, shoulders, flanks, hips and the upper part of the limbs. Solid black spots of varying size cover the lower limbs, belly, throat and face. The marks along the dorsal midline may also be solid and form fairly clear lines. Under parts, from the chin to the tail, are pale in colour.

The colour and density of the fur varies according to geographic distribution and therefore also according to subspecies (Fig. 9). Some have a slightly darker coat while others have a thicker or denser coat, dependent on their environmental requirements.

Individual identification is possible based on the unique spot patterns and coat characteristics of each animal. A combination of muzzle whisker spots, forehead patterns, and those below the eye, necklace characteristics, and colours and patterns along the abdomen was found to be sufficient for reliable identification (99% accuracy) (pers. comm. A. Spalton).

Melanistic leopards (Fig. 9) have been a topic of debate for many years. Speculation started when Cuvier (1809) described the melanistic form as a separate species, *Felis melas*. Later, in 1949, Van Dooren described 19 specimens, all found in one of the wettest regions of Java. Interestingly, melanism does appear to be more common in rainy West Java than in the drier regions of the island. Melanism may serve as camouflage in the rainforest environment (Eizerik *et. al.* 2003). In 1965, Prater suggested that melanism is encouraged by high temperatures together with high humidity and low light conditions. The phenomenon is said to occur much less frequently in dry, more open territory than in heavily wooded and damp regions.

Melanistic individuals do not only appear on Java. In 1880, Günther (in a note) revealed the presence of a black variety of leopard found in the district of Albany in South-Africa. He specified that four specimens were caught from this district. The South African leopard, *Panthera pardus melanotica*, was certainly so called because the first South-African leopard described was dark or slightly dark.

It is now acknowledged that melanistic leopards are not a different species, but belong to the *Panthera pardus* family. Melanism can occur in all leopard subspecies. It is ascribed to a heritable recessive gene locus (Eizerik *et. al.* 2003), which results in an abnormally high percentage of melanin, thus eliminating all the lighter shades in the pelage. However, when the light falls on such an almost black coat from a certain angle, the normal spotted pattern remains clearly visible.

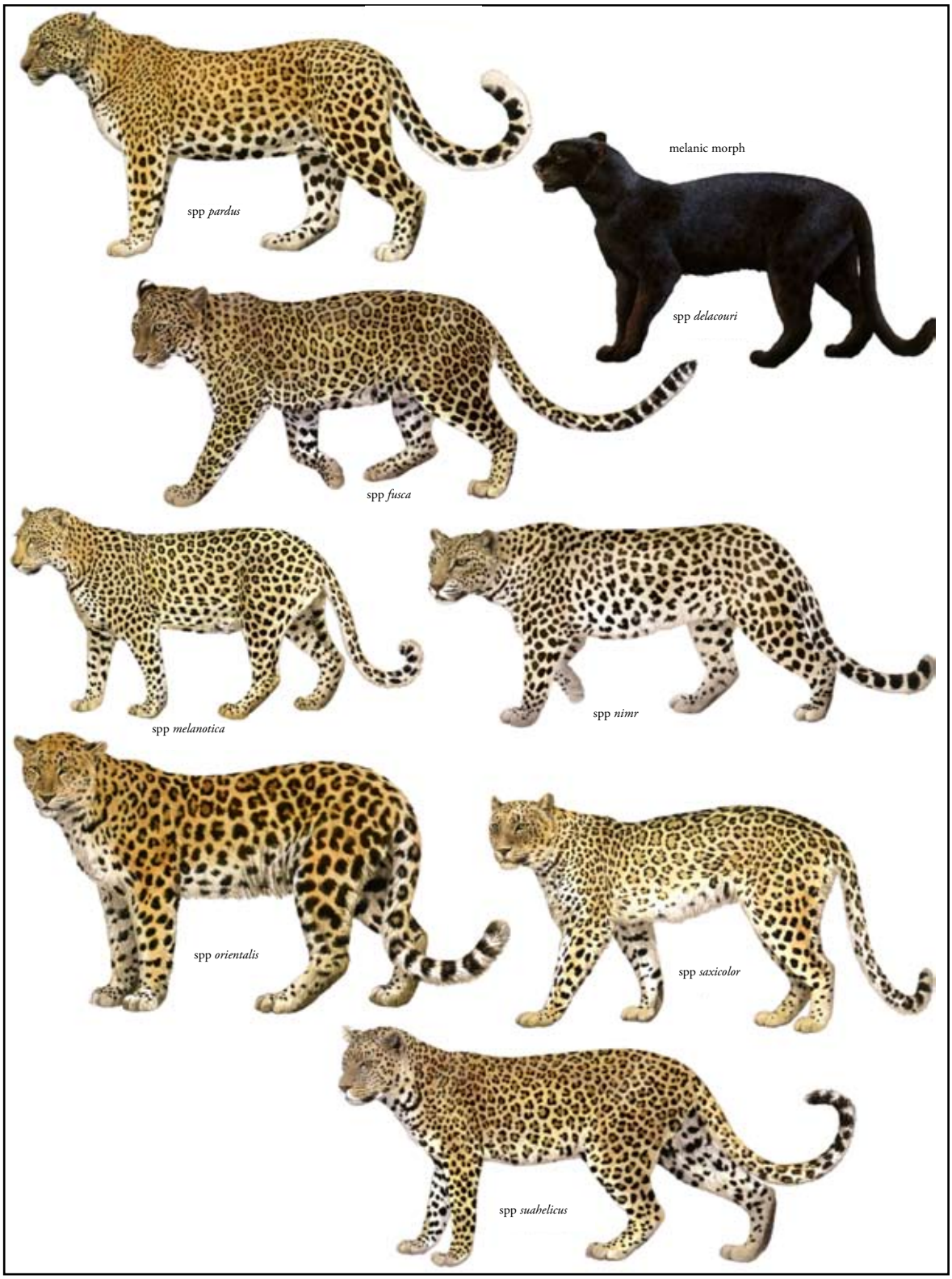

Figure 9. Illustrations of leopard subspecies in "Handbook of Mammals of the World".

Anatomy

Aside from being generally larger than all other cats, *Panthera* sp. have an incompletely ossified hyoid apparatus that allows the vocalization known as roaring, but restricts purring on exhalation.

Leopards, like all cats, have five digits on the front feet (Fig. 10) and four on the hind. The claw of the first digit on the front foot lies caudally and proximally to the palmar pad, and is used for holding prey. This digit is not visible in the spoor (Fig.11). All digits are equipped with strong, very sharp, curved claws that can be retracted at will and are a unique adaptation of the felid family for catching and securing prey. In contrast, the claws of canids play only a secondary role in catching prey (Hand *et al* 2000). The claws of the front feet are typically sharper than the claws of the hind feet. The carpal pad is thought to be used as an anti-slip device during jumping and climbing. This pad is not used during normal ambulation.

Figure 10. Right forefoot of an Arabian leopard, *Panthera pardus nimr* © Jane Budd

Figure 11. Tracks of Arabian leopard. L: Forefoot R: Hind foot. Note the rounded shape of the forefoot impression in comparison with the more pointed shape of the hind foot. © Jane Budd

Little has been published about the skeletal and muscular anatomy of leopards, therefore the domestic cat is generally used as a reference. Fig. 12 and 13 show the different muscle groups, tendon attachments and bone structure of a cat, representative of what one can expect to encounter in a leopard. Further anatomical details will not be discussed here and the reader is directed to the various texts available that outline the general anatomy of the domestic cat.

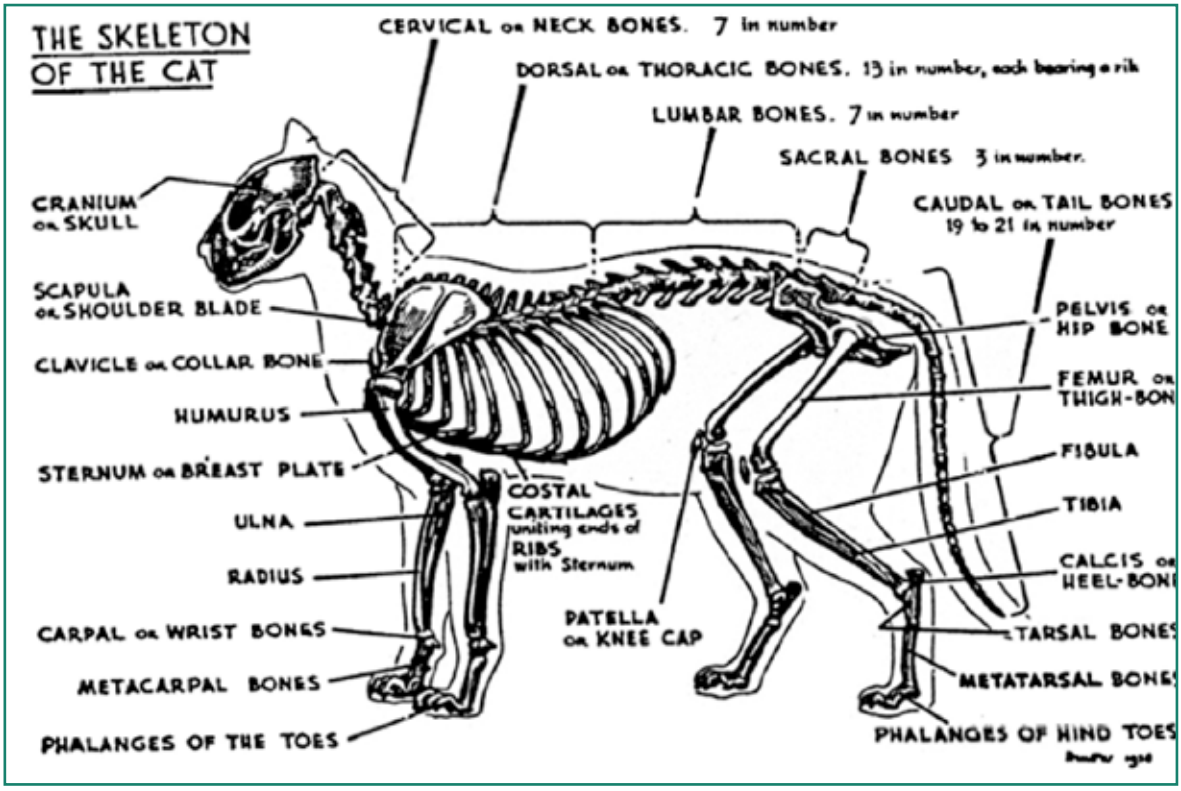

Figure 12. Illustration if the skeleton of the domestic cat.

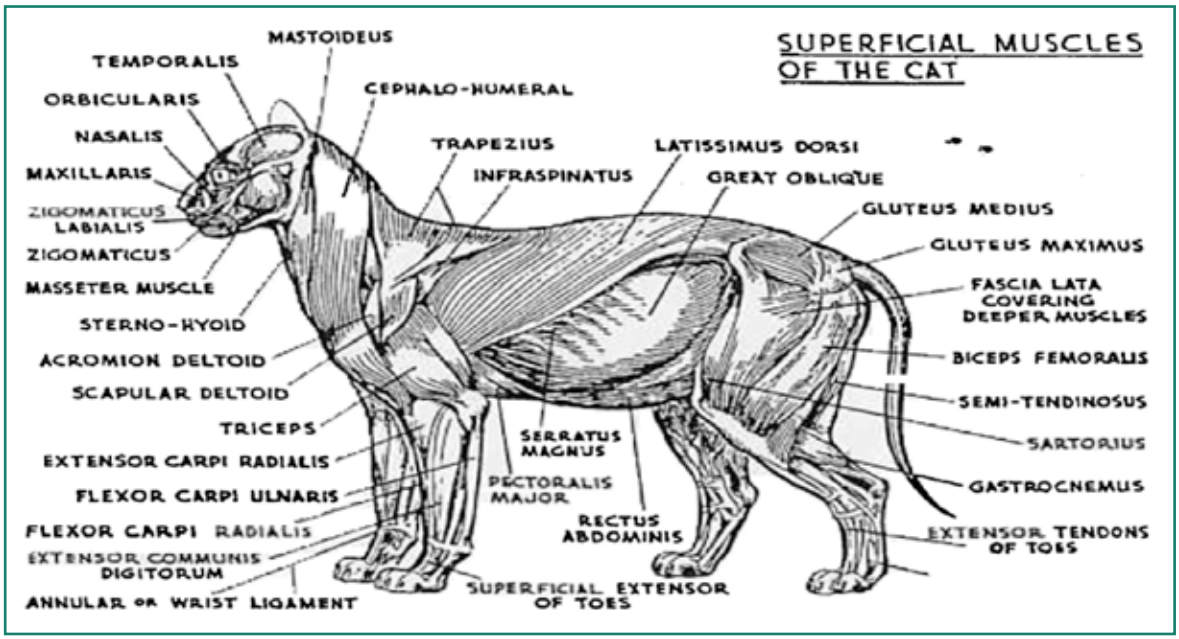

Figure 13. Superficial muscles of the cat.

The dental formula for permanent teeth is:

I	3	C	1	PM	3	M	1	x 2 = 28 or 30
I	3	C	1	PM	2/3	M	1	

The upper first premolar teeth, which are often absent in some of the smaller felids, are usually present in leopards. Felids have the same number of incisor, canine and carnassial teeth as canids, but they have fewer molars and premolars. The incisors are not specialised. Canine teeth

are large and sharp-pointed for holding while tearing and the premolars and molars are adapted for gripping and tearing prey apart (Fig. 14). Deciduous teeth are replaced by permanent teeth from 8-9 months of age.

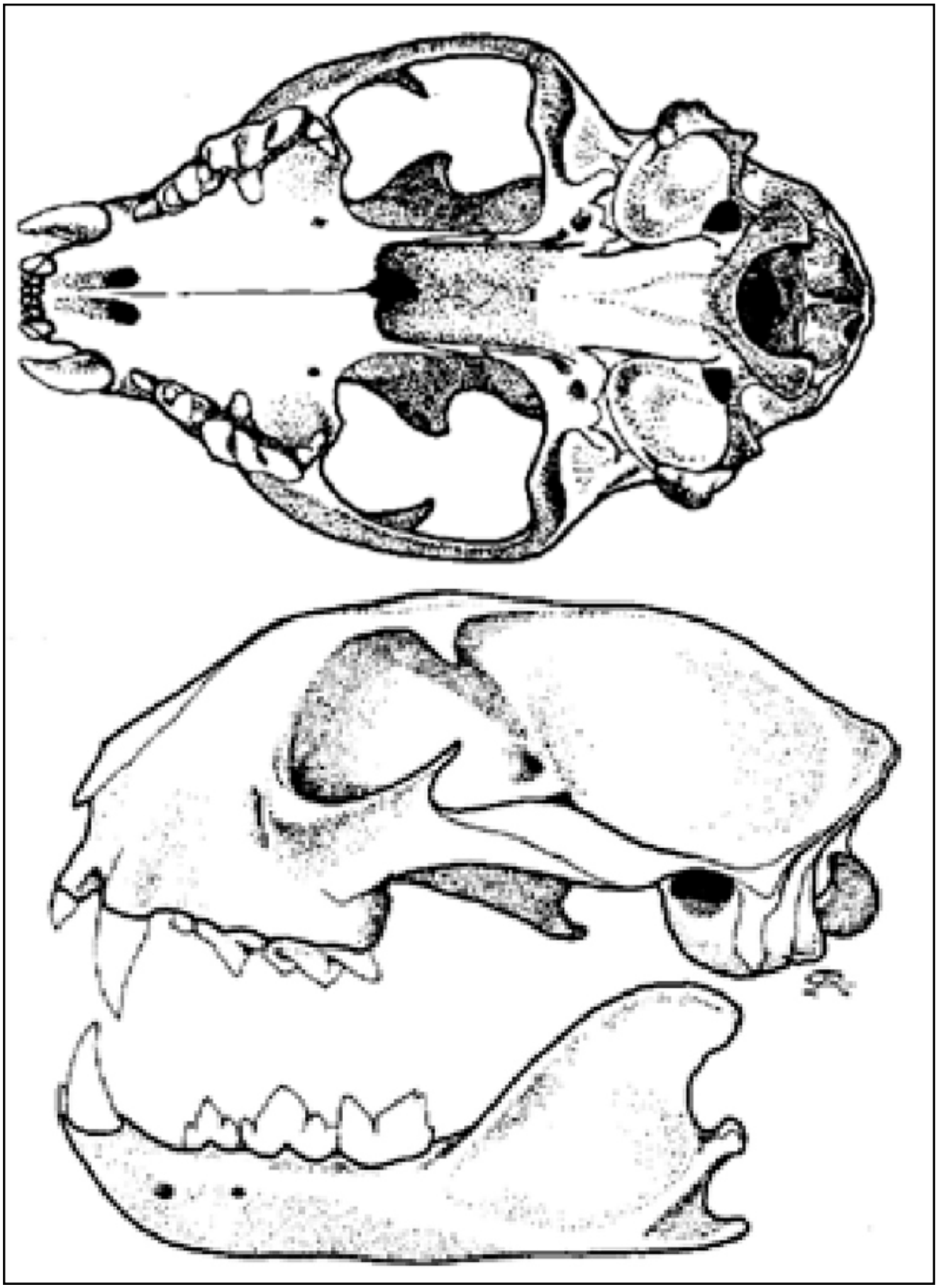

Figure 14. Skull of an African leopard, from the "The Mammals of Southern African Subregion".

Physiology

The physiology of the domestic cat is used as a model on which to base the physiology of Felidae. Further physiological details will not be discussed here; the reader is directed to the various texts available that outline the general physiology of the domestic cat.

Of interest is that a study of the blood type of 26 different felid species revealed that blood type A is by far the most common type in wild felids. Leopards also fall into the A-type blood group (Griot-Wenk and Giger, 1999). The significance of blood typing is unknown in wild species; however it is possible to assume that blood transfusion reactions and isoerythrolysis in neonates are unlikely to occur within the same group of felid species (Griot-Wenk and Giger, 1999).

Longevity

Leopards can live to 8-10 years in the wild (Shoemaker, 1983); however, in captivity they are known survive to over 20 years of age. The oldest recorded Arabian leopard in captivity survived to 25 years of age.

Behaviour

Radio-tracking studies enable a better understanding of leopard behaviour in the wild (Owen, 2006, Mizutani and Jewell, 1998 and Bertram, 1982). Leopards are solitary animals, and other than a female and her young or a courting pair, they seldom associate with one another.

The land tenure system of this felid is broadly similar to that of many other large cats. Adult males typically occupy large areas that overlap the home areas of two or more adult females. Female ranges are usually smaller than those of males (Mizutani and Jewell, 1998, Bailey 1993). Mizutani and Jewell (1998) suggest that the maximum home range size of a male leopard is determined by the size of the resident female home ranges rather than by prey density. The home range size of a leopard and the distance that a leopard travels in a night is influenced by a combination of its energy requirements, its reproductive requirements, local prey distribution and density, and intraspecific relations (Mizutani and Jewell, 1998).

In arid areas or other sites of particularly low primary productivity, the home range sizes of leopards are much larger and range/territory overlap for same sex animals is more common (Mizutani and Jewell, 1998).

Communication occurs through expression and posturing, scent marking, vocalization and olfaction. The primary means of social interaction among leopards appears to be via olfactory signals left in urine, anal sac secretions and faeces but vision and auditory senses are also very important. While scent may not carry as far as a call, it is more persistent, and leaves far more information about an individual's sex, residential status, reproductive condition and individual identity. Urine and anal secretions are sprayed onto shrubs and into scraped patches of earth (pug marks). The coughing, sawing or rasping vocalization of the leopard may also function to bring animals together for mating or to space out individuals, depending upon their sex and their reproductive and social status.

Diet and Feeding Behaviour

With the exception of African lions, all large cats are predominantly solitary hunters and live a co-operative but isolated existence with others of their species (Mizutani and Jewell, 1998). Leopards do most of their hunting on the ground, but can climb extremely well when required. They are primarily visual hunters, often locating prey from vantage points such as trees, rock piles and dune ridges. Prey is also sometimes located by sound as evidenced by their upright, forward facing ears that have 20 different muscles controlling movement. Leopards are quick to investigate distress calls.

Leopards are opportunistic hunters, killing vulnerable animals as they are encountered. In general, the leopard's diet reflects the prey available in the area. Typically listed as constituting part of the diet of a leopard are rodents, rabbits, hares, several species of deer and antelope, duiker, pigs, zebras, jackals, foxes, porcupines, pangolins and monkeys. Birds, reptiles, amphibians, invertebrates and grass are eaten as well. In areas where leopards live near humans, their diet can include dogs, cats and domestic stock. Like other felids, individual leopards may develop preferences and focus on one particular type of prey.

In areas where other large predators (lion, tiger) are absent, leopards do not appear to alter their diet to include more or larger prey, suggesting that their selection of prey is not markedly constrained by the presence of competitors.

Where cover is sparse or other predators are numerous, leopards take their kill into a tree (Fig. 15) before they start to feed or drag and carry the carcass a considerable distance to find a secluded spot.

Figure 15. An Africa leopard positioning its kill in the branches of a tree. © www.lax-a.is

Depending on the size of the carcass, a leopard may consume its kill over a protracted period.

Reproduction and Development

Reproduction is the foundation on which a species survives or becomes extinct (Wildt and Wemmer, 1999). Given the secretive behaviour of large felids, it is not surprising that the majority of reproductive data comes from captive animals.

Observations of leopards in the wild suggest that females scent-mark more frequently during the peak mating period. Zoo records show that reproductive cycles in females are non-seasonal and polyoestrus (de Haas van Dorsser, 2006, de Haas van Dorsser *et. al* 2007) although behavioural indicators of oestrus may be suppressed during less favourable breeding periods. Amur leopards have been observed to breed in June-July and give birth in September-October (Shibnev, 1989) and Santiapillai *et. al*. (1982) suggest that breeding in Sri Lanka takes place in the dry season (May-July).

Females usually display oestrus behaviour for up to seven days. The onset of oestrus is associated with an increase in friendliness, head/body rubbing, rolling and vocalizing a few days prior to oestrus itself.

The courting behaviour of leopards is different to lions where the male initiates copulation and prevents the females from moving away. Owen (2006) observed that it is the female leopard that initiates copulation and she follows the male through his territory. Mating is initiated by the female walking back and forth in front of the male (Owen, 2006 and Laman and Knott, 1996), sometimes brushing against him or waving her tail in his face. She presents her perineum by crouching on her sternum in front of the male with her tail averted to one side (de Haas van Dorsser 2006, Laman and Knott 1996). When mounting, the male stands over the recumbent female, places his front paws along her sides, and tucks his hind feet behind hers (Fig. 16). Mating is accompanied by loud vocalisation from the male and he commonly grips the nape of the female at ejaculation. Following ejaculation, the female throws the male off her back, often threatening him and then begins to roll vigorously on her back.

Figure 16. Leopards mating.

Mating occurs frequently over an average period of 2-3 days in the wild (Owen 2006 and Bailey, 1993). Laman and Knott (1997) observed a mating pair of leopards over a period of 1½ hours (n=13). They observed that mounting lasted an average of 3.0 seconds and the average interval between copulations was 6.5 minutes (n=7). Owen (2006) reports an average copulation duration of 9 seconds (n=19) with an average inter-copulatory interval of 11 minutes.

As with all felids, ovulation is copulation induced. Mating results in one of three outcomes: 1) failure to ovulate which results in a return to oestrus after 12-21 days (de Haas van Dorsser, 2006), 2) failure of embryo implantation (pregnancy) which results in a period of pseudo pregnancy under the influence of progesterone or 3) pregnancy. In pseudo-pregnancy, the corpus luteum does not regress until 35-42 days after ovulation, at which point the female returns to oestrus (de Haas van Dorsser 2006, Brown *et. al.* 1996 and Schmidt *et. al.* 1993).

Following a gestation period, ranging from 90 to 105 days (depending on subspecies), the female gives birth to one to three cubs. Of the five subspecies discussed, only the Arabian leopard has been studied in any detail in captivity and is recorded as undergoing a 97-day pregnancy period (de Haas van Dorsser *et. al.*, 2007), counted from the second day of oestrus. Females use secluded caves, thickets, hollow trees, abandoned burrows and rock piles as birth dens. For the first few days after the cubs are born, the mother spends all her time at the den, resting, nursing and looking after her young. However, she must hunt and so, leaves the cubs unattended. The highest rate of cub mortality occurs during the first months of life, and in the wild the young commonly fall prey to lions, tigers and hyenas (Skinner and Smithers, 1990). The female may move the cubs to different dens every two to five days.

The family begins to travel together when the cubs are two to three months old (Fig. 17). At this point, the cubs are generally starting to eat meat. Captive observations of Arabian leopards show that mother-reared cubs start to experiment with taking meat with the dam from 6-8 weeks of age. Cubs initially learn to hunt by playing and pouncing on leaves, sticks, siblings and their mother.

Figure 17. Arabian leopard female with cub at BCEAW © Kevin Budd

At twelve to eighteen months old, young leopards are generally independent of their mother, but the time of dispersal varies with the sex of the animal, local vacancies, resource availability and the reproductive status of the mother (Owen, 2006, Le Roux & Skinner, 1989, and Martin & de Meulenaar, 1988). Dispersal may be delayed in areas where prey is abundant, especially if the adjacent habitat is occupied by a resident leopard. In general, males seem to become independent at an earlier age than their sisters. Mother-daughter relationships may also be extended by the tendency of daughters to settle near their mother.

According to Bailey (1993), only 41% to 50% of young African leopards survive beyond their first birthday. Bailey also estimates sub-adult survival (1½ to 3½ years old) to be as low as 68%, a statistic which is probably related to still naive hunting skills.

Assuming that their young survive to independence, female leopards would appear to be able to produce a new litter every fifteen months to two years. In her study of African leopards in Karongwe, Owen (2006) recorded that the average inter-birth interval was 14 months.

Eaton (1977) suggests that sexual maturity is attained by 24-35 months while Bailey (1993) observed that females typically have their first litter at three years. In captivity, faecal hormone studies have confirmed that the male and female Arabian leopard enters puberty by two years of age (de Haas van Dorsser, 2007). Behavioural observation of captive Arabian leopards indicates that overt behavioural oestrus does not commonly occur before two to three years of age in females with males beginning to respond from three to four years of age.

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Holding and Exhibit Facilities

Figure 18. Leopard enclosure at Burger's Zoo. © Paul Vercammen

Enclosure Design

Modern exhibits have moved away from cramped, barred enclosures and are now large naturalistic fenced or glassed displays, which require detailed planning.

Many institutions have old enclosures in need of renovation of their outdoor and/or indoor enclosures (Fig. 19). It should be noted that indoor accommodation requires special attention, as the time spent indoors can be longer than the time spent outdoors.

Figure 19. Leopard enclosure at Sana'a Zoo in Yemen. © Jane Budd

Easy maintenance of the enclosures is worth considering when planning enclosures. Well-constructed enclosures are easier to maintain and are subsequently more likely to be responsibly managed (Rosenthal and Xanten, 1996).

The design and layout of the enclosure also depends upon its specific purpose, the local climate and resources available. Remember that this is where the leopard spends 24 hours of the day and the enclosure should therefore provide; 1) a choice of den space, 2) climbing poles and solid resting surfaces that are off the ground, 3) sand or gravel for defecating/urinating, 4) behavioural stimulation and 5) sunlight. Providing a quiet retreat towards the rear of the enclosure is advised so that the animals have the opportunity to isolate themselves from the public.

There are no set criteria defining how leopards should be housed, however, due to the agility of this species, secure containment is an essential aspect to consider when designing a new enclosure. Dimensions and minimum standards for large cat enclosures vary from country to country. Most government standards are generally accepted to be too small.

Providing complexity in the environment is important. There is a negative relationship between time spent pacing and enclosure complexity. If possible, enrichment should already be considered at the design phase so that large rocks, logs and substrates can be included in the enclosure and even easily replaced if suitable access for heavy duty equipment is provided in the initial design.

Irrespective of the purpose of the enclosure, the design should avoid situations in which the animal cannot be monitored, or suitably accessed for treatment or immobilisation.

A security guardrail should be spaced far enough away from the enclosure fencing to prevent visitors reaching the enclosure; a minimum of 1.5 metres from the enclosure should be adequate. The guardrail should be installed in accordance with safety standards recommended in each country.

The enclosure foundation should be deep enough to provide structural strength and should take into consideration ground type and hardness. The enclosure skeleton must be strong enough to prevent escape and must also be able to withstand damage caused by storms, snow, falling trees, etc.

Metallic nets provide excellent structural security and are visually attractive but are very expensive (Fig. 20 and 21). A more traditional type of construction, with a frame of steel pipe or wooden poles and traditional fencing is acceptable. Wooden poles offer the advantage of being easily replaced when damaged; however, they are an ideal substrate for pests and also weaken as they age creating a potentially dangerous situation. The use of glass should be carefully considered. Whilst it is aesthetically pleasing, it can be expensive, requires high maintenance and is susceptible to cracking if unstable. Careful evaluation regarding strength requirements should be carried out.

Figure 20. A new design for leopards' outdoor enclosure at Burger's Zoo. © Officium Design Engineering.

A number of fencing types are available. Selection is influenced by the type of animal being housed, the available budget and maintenance requirements of the fencing selected. Galvanised chain-link wire fences are most commonly used for leopard/big cat enclosures. Fences in open top enclosures should be at least 3.5m high. The wire mesh should have a minimum diameter of 3.5mm and should be rust and weather resistant.

The ability to transfer animals safely from one enclosure to another or between holding areas is extremely important. Movements between enclosures should be able to be carried out with as little stress as possible whilst still providing absolute safety for both animal and keeper (Rosenthal and Xanten, 1996). Guillotine (care with long tails) or sliding doors are most practical for felids, they are inexpensive to build/maintain and also optimally utilise all usable enclosure space. All doors should be clearly identified to avoid accidental opening of the wrong door.

Substrate and Vegetation

Exhibits often now include vegetation and soil to provide a more natural environment. Sand and gravel based substrates are preferred but may become contaminated with microorganisms and parasites over time and should be periodically replaced.

Figure 21. Elevated view of the outdoor, closed roof enclosure at Burger's Zoo. © Officium Design Engineering.

Vegetation should be chosen carefully to avoid the use of potentially toxic plant species and should aim to provide shade and shelter. It is important too, to provide vegetation that allows the leopards to climb. This encourages natural behaviour and provides behavioural enrichment options.

Trees and large shrubs should not be placed too close to the perimeter fence and branches growing towards the fence should be regularly pruned back to avoid potential escape.

Climbing plants could be used in the fence, but their growth should be closely monitored to ensure that the function and integrity of the fence is not affected.

Watering points are very important; the enclosure could be further enhanced with a stream and/or waterfall.

In the areas of the enclosures that have non-dirt floors (dens, feeding cages, etc.) the most common material used is concrete, which should be skimmed but should also have a nonslip texture. Importantly, any surface should be easily cleaned, rapid drying and nonporous to prevent the accumulation of organic debris and bacteria. The slope of the floor should promote rapid drainage away from the enclosure (Rosenthal and Xanten, 1996). Disinfectants and detergents should be selected according to effectiveness and toxicity levels and should only be used as advised by the manufacturer.

Outdoor Facilities

Closed-top Enclosure

The ground surface areas of these enclosures are often smaller than open park designs but they do provide the opportunity to use furnishings and additional structures to increase the overall usable space, both vertically and horizontally. The focus in these enclosures should be on quality rather than quantity of space. The type of furnishing used should be carefully chosen to avoid injury to the leopard whilst offering complexity and opportunities for locomotor activity and exercise (Maple and Perkins, 1996). Choices include trunks, branches and vegetation which provide behavioural stimulation and increased activity. Stumps and poles provide places for the leopard to scratch thereby helping to prevent ingrown claws. The use of high platforms is also important, not only to provide areas for the leopard to survey its surroundings but also to provide additional enrichment within the enclosure.

The minimum ground surface area should be 150-200m^2 for a pair with an additional 50% of floor space for each supplementary animal in the enclosure. a minimum of 100m^2 is advised for a single animal. The enclosure should be a minimum of 10 metres in depth. The recommended height is 5-6 metres.

Enclosures at the BCEAW in Sharjah have been designed with internal angles of greater than 90º to avoid animals becoming trapped during fighting (Fig. 22). The enclosures themselves contain basic furnishings but lack vegetative enrichment. Better use of horizontal space could also be made in these enclosures.

Figure 22. Leopard enclosure at BCEAW. © Kevin Budd

Open-top Enclosures

The surface area of open park exhibits are generally markedly bigger than that of the closed-roof enclosure as elevated surface area is not as readily available in this type of enclosure. A minimum of 600m^2 is advised for a pair, 100m^2 should be added for each supplementary animal.

The fence should be constructed with a minimum of a 1m overhang at an angle of 45-90° to the fence, along the inside edge (Fig. 23). This should be well tensioned to prevent injury to the animal and be firmly anchored along the entire perimeter. The use of a concrete foundation for the posts is advised. The use of electrical cables along the inside is strongly advised.

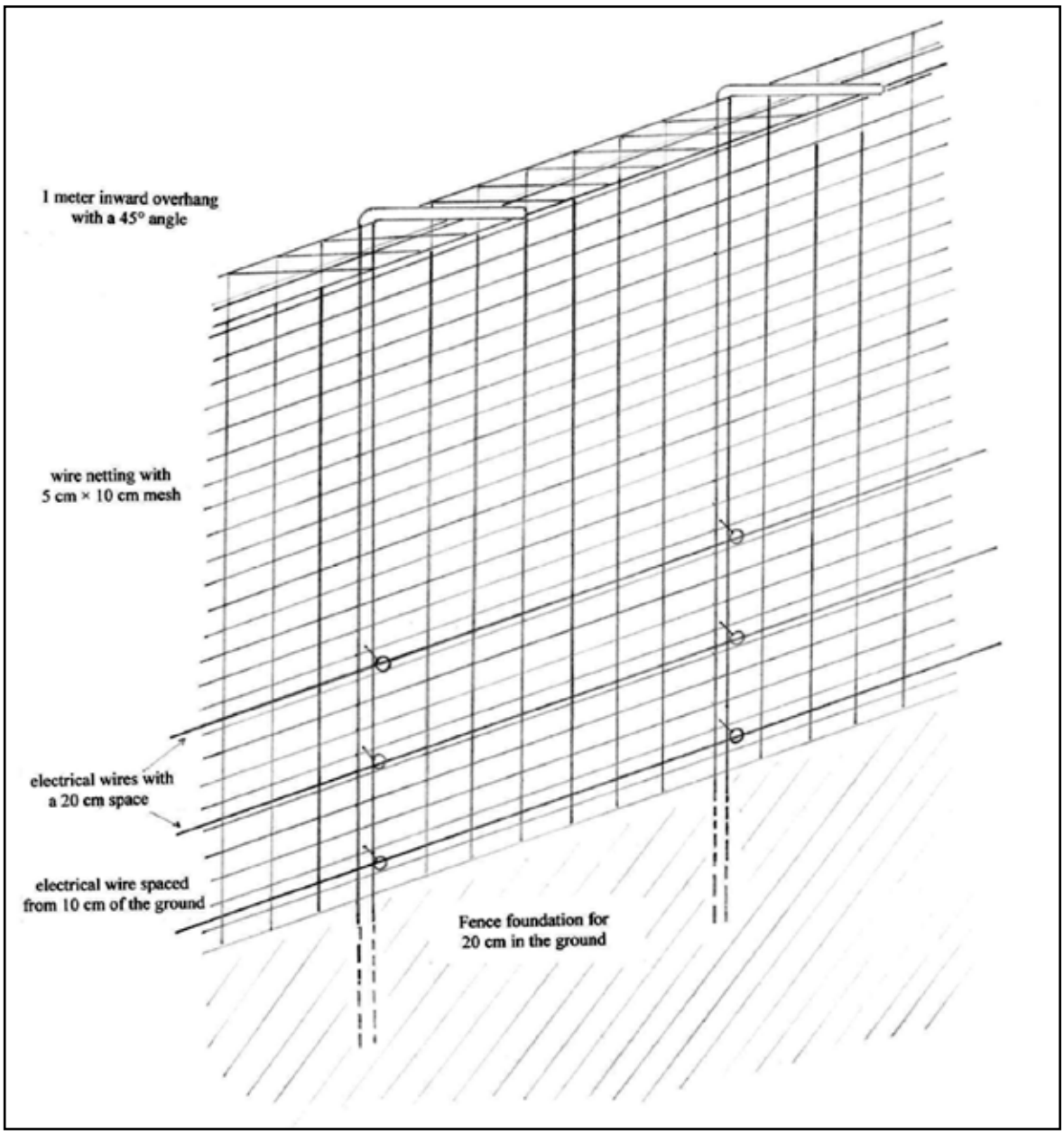

Figure 23. Fence diagram of an open park enclosure type.

Furnishings such as plants, loose ground, quiet zones, trunks and trees should be used. High platforms could be used but require careful planning to prevent escape. Tree stumps, rocks, poles or combinations of the three could be used to make platforms. Logs and trunks could be used

to construct posts which allow the leopards to scratch and claw. Trees and plants should provide shelter and shade and are also often used by the leopards for scent marking. Big trees could serve as raised resting platforms.

Indoor and Off-exhibit Facilities

A service or off exhibit area should be included in the design of every enclosure where power points, light switches and taps, etc. are safely located. Access to the enclosure should be gained through this area and not directly from the outside, thereby providing a double security system against escape from an enclosure. Keeper access doors should be large enough for keepers to gain entry without having to stoop or crawl into the area (Rosenthal and Xanten, 1996). Doors should also be large enough to permit passage of crates, exhibit materials such as branches, stumps, etc. All doors including shift doors must have the ability to be locked and electrical switches should be clearly marked to prevent the accidental opening of motored doors. Viewing windows in the doors are important to allow keepers to look into the enclosure before entering, thus preventing animal escapes, but should have mesh or bars preventing the animal from getting its paws through.

Off exhibit animal holding areas (Fig. 24) are useful as treatment areas and provide seclusion for stressed or sick animals, they are also useful as holding areas during cleaning or even as introduction areas away from public view.

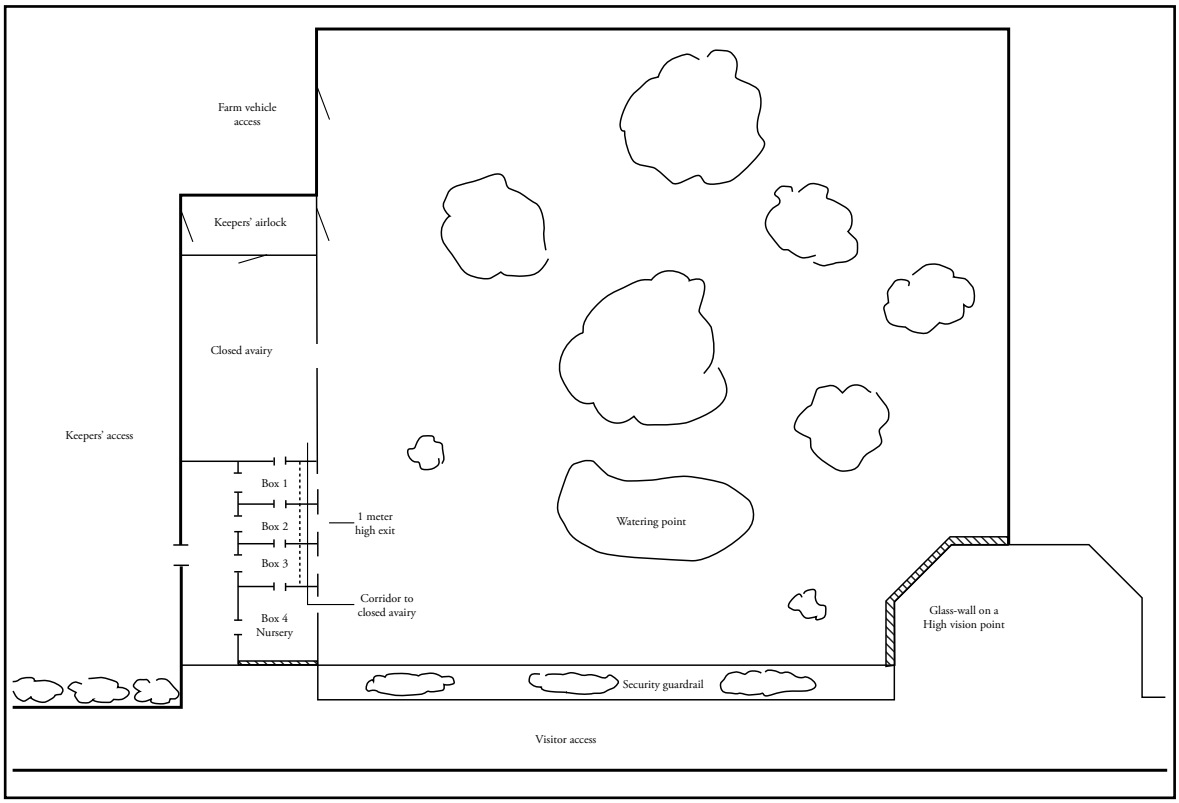

Figure 24. Enclosure design, with service area.

Provision should be made for the keepers to be able to feed the animals remotely, without having to deal with the animals directly. This can be done by using a shoot and trap door to deposit food directly into the enclosure or by including a separate feeding cage into which the food is placed before the animal is allowed access. Most importantly the indoor accommodation

must be easily accessible for the keepers so that it can be cleaned on a regular basis. A sliding door on the animal side should be used to close the leopard in or out of the den.

The minimum size recommended for overnight quarters is 25m^2 for each animal. The interior should include various raised sleeping areas which permit the leopard to select a preferred sleeping surface and should also include a sand box for toileting. The leopard should always be easily visible. An observation window could be added in the door.

A ventilation system can be used; however this ventilation system should be maintained separately from the exhibit and public areas. There should be 8-10 complete air changes per hour with a minimum 15-40% intake of fresh air. The use of separate ventilation systems reduces potential disease transmission from/to the public and also reduces odour in the visitor areas. Adequate ventilation also ensures drying of exhibit and work areas, limiting the growth of mildew and algae (Rosenthal and Xanten, 1996).

Temperature extremes should not exceed those of the native habitat of origin. Where applicable, it is important to provide good insulation against extreme cold and heat. A heated floor or radiator could be used for winter in some cold countries. It should be noted that tropical subspecies have more need for heating than those from colder climates.

Breeding den

Stability in both the social (solitary for leopards) and physical environment of the new mother is crucial at parturition (Baker *et al* 1996). The breeding den should aim to offer security and should be warm, dry and dark. It is recommended to provide a nesting shelf approximately 1 meter above the ground as well as the opportunity to nest on the floor. Nesting boxes with a peripheral lip to contain the young cubs are commonly used and are advised for any raised nesting shelves.

An observation window allows access to monitor the activity of the mother and her cubs but should be used with caution at all times, if at all during the first days. A closed circuit television (CCTV) camera system is a worthwhile investment and allows monitoring of the new family from a distance, thus reducing the likelihood of inadvertently disturbing the nesting environment. In colder climates, a heating floor is particularly useful for maintaining an optimum temperature in the den for mother and cubs.

Also of importance in the sleeping dens, but particularly in breeding dens where cubs are reared is that the floor surface of all potential nesting areas should be non-slip to promote correct locomotor development as the cubs become ambulatory.

Sanitation

Cleaning protocols vary among zoological institutions. Generally accepted procedures include removal of faecal material, waste products, and enrichment items. Water features that do not incorporate filtration systems should be drained and cleaned on a schedule or as needed. Night house operations and exhibits that utilize hard surface floors in the keeper work areas, shift corridors, individual holding units, and enrichment items should include daily removal of faecal material. It is good practice to implement a regular cleaning and sanitizing schedule incorporating

detergents (examples include lotionized soap and degreasing dish detergents) and disinfecting agents (examples include quaternary ammonium disinfectants, phenols, chlorhexidine and diluted bleach). After any chemical application, surfaces should be rinsed with a high-pressure water stream. Access should not be provided until wet areas have dried to prevent injury. Many institutions place disinfecting footbaths at exhibit and night house entry points, especially in areas in which keepers service a number of exhibits containing diverse species. Steam cleaning of hard surface areas is recommended annually, where practical.

Pest Control

An effective and safe method of controlling insects and rodents is mandated, and accurate records should be kept to reflect supervised monthly licensed pest control inspections and service. Rodent control can be achieved using snap traps, glue boards, and other non-chemical systems. Poison baits should be used only when there is no possibility of felid access to the bait or to treated rodents resulting in secondary poisoning. Bait traps are highly effective, but must be kept dry and should be refilled at regular intervals. Insect control may include electronic insect killers, growth inhibitors, pest strips, and natural or synthetic pyrethrins. All chemicals should be veterinarian approved prior to use.

Safety and Escape Policies

The escape of big felids is a serious and potentially dangerous event. Leopards are wild animals and dangerous. No matter how well designed facilities are, it is important to respond immediately in a calm and professional manner in order to protect zoo staff and visiting public (where applicable), and to return the leopard safely to its exhibit. An emergency protocol should be in place to provide action guidelines should a leopard escape from its exhibit into a service or public area or into another animal exhibit.

You must attempt to confine the leopard. If possible confine the animal into a service area with access for it to return to its enclosure. Given the opportunity, the leopard will usually choose to return to its cage.

If you have confined the animal, be sure that the surrounding area is secured. If this means closing a building or any area, do it. Do not try to return the escaped leopard by yourself. Monitor the animal's location until help arrives.

If you cannot confine the animal, summon help as quickly as possible, but stay in the area to monitor the animal's location and keep unauthorised people out of the area.

Do not excite the animal and keep your distance.

Several institutions have an alarm system, which can be activated as soon as possible in an emergency situation.

Chemical immobilisation equipment should be transferred to sites where the leopard is as soon as possible. The person in charge should determine if the leopard needs be immobilized and will assess the number of people needed to assist in capturing the escaped leopard. In most cases, the fewer people, the better. Only trained staff will use immobilization equipment.

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Feeding and Nutrition

Figure 25. Feeding of meat on the bone maintains dental health. © Jane Budd

Introduction

Provision of food and water is a fundamental component of animal husbandry and welfare. It is well recognised that diet is closely associated with many disease processes (Hand *et. al.* 2000). Correct nutrition and care throughout life maximises health, performance, longevity and disease prevention (Hand *et. al.* 2000). The evolution of felids as true carnivores has resulted in unique nutritional and metabolic adaptations to foods that are composed strictly of animal tissue. Felids have far more specific nutritional requirements than other carnivores, which are facultative carnivores or omnivorous.

The diet should be balanced and should contain the required energy and protein components as well as mineral and vitamin supplementation. The age, sex, reproductive status and general health of each animal should always be considered when designing a nutrition program. Feeding below or above the optimum nutrient requirement for a particular life stage can have a negative impact on physiologic performance and health (Hand *et. al.* 2000).

An American nutritional study showed that diet has a positive impact on the reproductive health of male felids (Swanson *et. al.* 1994). This study compared supplemented versus non-supplemented diets in eight different felid species. Differences in semen quantity (volume, sperm/ejaculate) and quality (motility, sperm morphology) were examined and showed that ejaculate volume is reduced in the non-supplemented diets.

Hygiene during food preparation and storage is extremely important. Meat based products are susceptible to bacterial growth and spoilage (Allen *et. al.* 1996, Dierenfeld *et. al.* 1994). Frozen products should be thawed under refrigeration so that the temperature at the thawed surface is kept low enough to prevent bacterial growth whilst the remainder of the product thaws (Allen *et. al.* 1996). Personnel handling the products should be adequately trained in hygienic food handling practices.

Feeding times vary with different animal management techniques; most of the zoological parks questioned fed their leopards in the evening or late in the afternoon. It is worth coordinating feeding times with delivery of thawed meat-based products to minimise spoilage.

Nutrient Requirements of Felids

The nutritional requirement of the domestic cat has been studied in detail, and currently remains the only model for establishing the dietary composition parameters of non-domestic cats (Morris, 2002).

Felids are strict carnivores, they show no tendency toward omnivory (Morris 2002, Allen *et. al.* 1996) and they have unique metabolic adaptations to a diet high in proteins. They require high levels of most essential amino acids as they cannot be synthesised in sufficient quantities by conversion of other amino acids. Felids also have a limited ability to conserve nitrogen when intake is low (Allen *et. al.* 1996). They are especially sensitive to arginine deficiency, which causes rapid increases in blood ammonia concentrations and ammonia toxicity (Morris 2002, Hand *et. al.* 2000).

Felids have high requirements for dietary taurine compared with most other mammal species. Deficiency can lead to progressive retinal degeneration, and decreased reproductive performance in females and has been associated with dilated cardiomyopathy (Morris, 2002, Allen *et. al.* 1996). Taurine is well supplied in animal tissue, particularly where whole carcasses are consumed.

Also essential to felids is dietary niacin, vitamin D and vitamin A. Unlike other mammalian species, cats do not convert plant pro-vitamin A compounds (beta-carotene) to retinol but rather rely on preformed vitamin A from animal tissue in the diet. This vitamin occurs predominantly in the viscera of prey, particularly the liver. Interesting, but not surprising, is the observation that cats have a higher tolerance than other species for high levels of vitamin A (Morris, 2002). Neurological conditions, hair loss and ill thrift result from vitamin A deficiencies.

Requirements for all of the essential compounds can be satisfied by consumption of whole prey (Dierenfeld *et. al.* 2002, Morris, 2002, Hand *et. al.* 2000 and Allen *et. al.* 1996). Where only muscle meat is fed, supplementation is required to prevent deficiencies.

Nutrient Composition of Zoo Diets

The nutrient composition of vertebrate carcasses is similar with regard to most major nutrients. Protein concentrations are reasonably high, calcium and phosphorus concentrations are generally adequate and in the correct ratios (Allen *et. al.* 1996). Water and fat content of prey will vary.

In the wild, carnivores eat all (or nearly all) of the prey captured, including bones, fat, viscera etc. (Allen *et. al.* 1996). The nutritional composition of muscle meats is quite different from whole prey (Table 1 and 2).

Table 1: Nutrient composition of selected vertebrates (dry matter basis) (Allen *et. al.* 1996, Spitz *et. al.* 2003 and USDA Online Database).

Species	Mass (g)	% Dry matter	% Crude protein	% Fat	% Ash	% Ca	% P
Rat							
Adult	280	34	59.7	23.6	15.7	4.0	1.8
Juvenile	5.9	14	77.1	7.1	15.7		
Mouse							
Adult	27.6	31.5	58.3	23.9	11.0	3.4	1.8
Juvenile	1.6	16.7	74.9	12.6	12.6		
Rabbit	per 100g	28.6	67.2	15.4	5	3.26	2.15
Quail	per 100g	34.6	67.6	29.7	10.8	3.82	
Guinea pig	per 100g	31.3	51.04	46.1	9.2	3.02	
Chicken							
Adult	per 100g	33.5	56.7	26.9	9.5	1.94	1.4
Chick	34.3	33.0	67.9	16.8	8.2	1.7	0.9

Table 2: Nutrient composition of selected muscle meats (dry matter basis) (Zootrition v2.6)

Food /100g	Form	% Dry matter	% Crude protein	% Fat	% Ash	% Ca	% P
Horse meat	Raw	27.37	40.52	56.27	1.9	0.02	0.36
Beef	Raw	42.7	40.52	56.27	1.9	0.02	0.36
Pork	Raw	50.17	69.9	69.9	1.44	0.04	0.31
Chicken	Raw	34.1	54.69	44.28	2.32	0.03	0.43

The use of muscle meats as the sole diet is not suitable and results in pathological bone conditions due to dietary imbalances of calcium, phosphorus and vitamin D (Allen *et. al.* 1996). Alternating between whole prey, muscle meat and prepared diet is recommended to compensate for their individual disadvantages. Commercially prepared felid diets provide all the necessary minerals and vitamins, while whole prey and meat on the bone reduces the incidence of oral disease and allows the expression of species-appropriate behaviours.

Care should be taken when feeding bone. Shattered pieces of bone or bones with sharp edges could penetrate the oesophagus or digestive tract and potentially result in the death of the animal.

Discussed below are various types of meat commonly available for feeding. Each food source has advantages and disadvantages that should be considered:

- Commercially prepared feline diets require little or no preparation and are assumed to be nutritionally balanced (Dierenfeld *et. al.* 2002) as they already contain all the necessary vitamins, minerals and trace elements. Feeding an exclusively soft diet is, however, not recommended due to the high incidence of associated oral disease. These diets also take very little time to consume and are therefore not fulfilling any behavioural/psychological needs.
- Commercially produced chicken maintains good oral heath, requires little preparation and is comparatively inexpensive (de Haas van Dorsser *et. al.* 2001), but there are no viscera or feathers provided with the carcass. Provision of balanced vitamin and mineral supplements are essential, as are novel behavioural enrichment techniques in presenting this carcass.
- Large felids are known to eat most of their prey (Allen *et. al.* 1996). In accommodating this natural behaviour, captive leopards are often fed whole carcasses such as chickens, quails, rabbits and guinea fowls. Provided most of the soft tissue and some bones (or other calcified tissue) is consumed, whole prey generally satisfies protein and essential trace element requirements (Dierenfeld *et. al.*2002 and Allen *et. al.* 1996).

  The greatest benefit of feeding whole prey is the encouragement of natural feeding behaviour. Plucking the fur/feathers from the carcass requires time and effort and so provides an ideal behavioural enrichment tool. Good dental hygiene is also maintained with whole carcass diets and the ingestion of the fur, feathers and skin of whole prey helps to maintain gastro-intestinal health by stimulating the passage of ingesta through the gut (de Haas van Dorsser *et. al.* 2001).

  A major disadvantage with feeding whole carcasses is expense. In-house production of feeding animals is labour intensive and requires specialised management and accommodation to ensure that these animals will not be a source of parasites or disease.
  The feeding of wild birds and animals is not advised as they can carry a variety of

transmissible diseases. It is rarely possible to subject the carcasses to adequate inspection prior to feeding.

- Muscle meat is a good source of protein, B vitamins and some minerals but it is low in calcium, manganese and fat-soluble vitamins and requires suitable supplementation to ensure that it provide s a complete balanced meal. As with commercial diets, a diet of muscle meat alone is considered too soft to maintain adequate dental hygiene and should therefore be alternated with whole carcass meals and/or meat on the bone.
- Live prey diets follow the same principal as a whole carcass diet plan, but the prey source is fed alive. There are welfare considerations that govern the use of live prey for behavioural enrichment and this technique is therefore seldom used.

Within the institutions that answered the survey, a wide variety of diets are fed including rabbits, goat, rats, chicken, hamsters, deer, mutton, horse, beef, dromedary camel, donkey, eggs, guinea pigs, pigeon and kangaroo. The diets most commonly used are muscle meat of beef and horse (with bone), rabbits or chicken (Fig. 26).

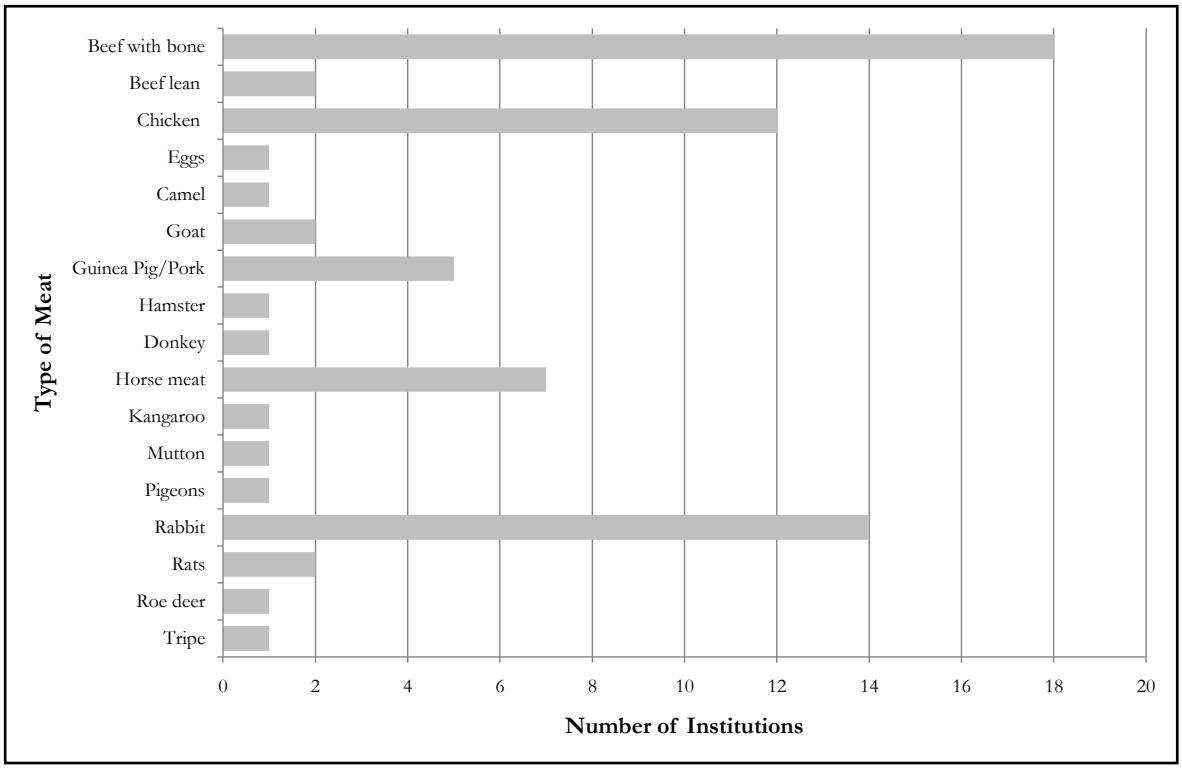

Figure 26. Types of meat used in different EAZA institutions.

A wide variety of vitamins and mineral supplement formulations are also used in the different institutions across North-America, Asia, the Arabian Peninsula and Europe.

Calculating Dietary Requirements

As discussed, it is important that a diet contains all the necessary nutrients to avoid mineral and vitamin deficiencies. The amount of energy provided also has to be taken into account to maintain normal physiological processes. The basal metabolic requirement (BMR) is the energy required for all resting activities (such as respiration, circulation, kidney function, etc.), which can be calculated as:

BMR (kcal/day) = 70 (BWkg)$^{0.75}$ (Hand *et. al.* 2000)
Expressed in kJ, the BMR can be calculated as 293 (BWkg)$^{0.75}$ (Hand *et. al.* 2000)

For a 40 kg leopard, the BMR = 70 (40)$^{0.75}$ =1113 kcal/day (4460 kJ/day).
For a 90 kg leopard, the BMR = 70 (90)$^{0.75}$ = 2045 kcal/day (8562 kJ/day).

Remember that the total energy requirement for any individual is more than just the BMR. Many factors determine the total energy requirement, including body mass, age and hormonal status and subspecies (Table 3). Young leopards need more energy than an adult, and a female in lactation needs more energy than an inactive female. It is also important to monitor the body condition of each animal as individual requirements are often very different. Enclosure size can also play a role in dietary requirements as this may influence the activity levels of the animals concerned. The energy requirements in Table 3 should be used as a guideline and not as absolute requirements.

Table 3: Calculating energy requirements, accounting for normal activity or production (Hand *et. al.* 2000)

Activity or Status	Energy Requirements
Inactive adult	1.4 x BMR
Active Adult	1.6 x BMR
Obese prone	1.0 x BMR
Weight loss	0.8 x BMR
Weight gain	1.2 x 1.4 BMR (at ideal weight)
Gestation	1.6 x BMR in early pregnancy, increasing to 2 x BMR at parturition.
Lactation	2 - 4 x BMR
Growth	2.5 x BMR

Additional food used for enrichment, training programs and/or treats should be included in calculations when assessing the total diet. To stimulate natural feeding behaviours, the diet should encourage methods of consumption similar to those employed in the wild. Behavioural enrichment techniques are discussed separately (see page 41).

The keepers play an important and essential role in the management of portion size and frequency of feeding. They know each individual animal and are best able to judge the condition of each leopard.

Frequency of Feeding

In the wild, leopards are frequently faced with periods of fasting. In simulating natural events, many zoos routinely fast their large carnivores one day a week. This practice is also thought to be beneficial for weight management in inactive animals although there is no scientific evidence to support this (Allen *et. al.* 1996). Based on the survey results (see Appendix 8); some institutes also fast their leopards two or more days a week. These fast days should not be on two consecutive days. The number of fast days depends on the activity level of the animal and the status. Pregnant or lactating females and juveniles should not be fasted.

Of the 38 institutions that answered the survey, 40% include one fast day per week, 23% include two fast days per week and 17% include three fast days per week. 8.5% of institutions have no fast days and 11.5% change the number of fast days during the year; with one fast day in winter and two fast days in summer (Fig. 27).

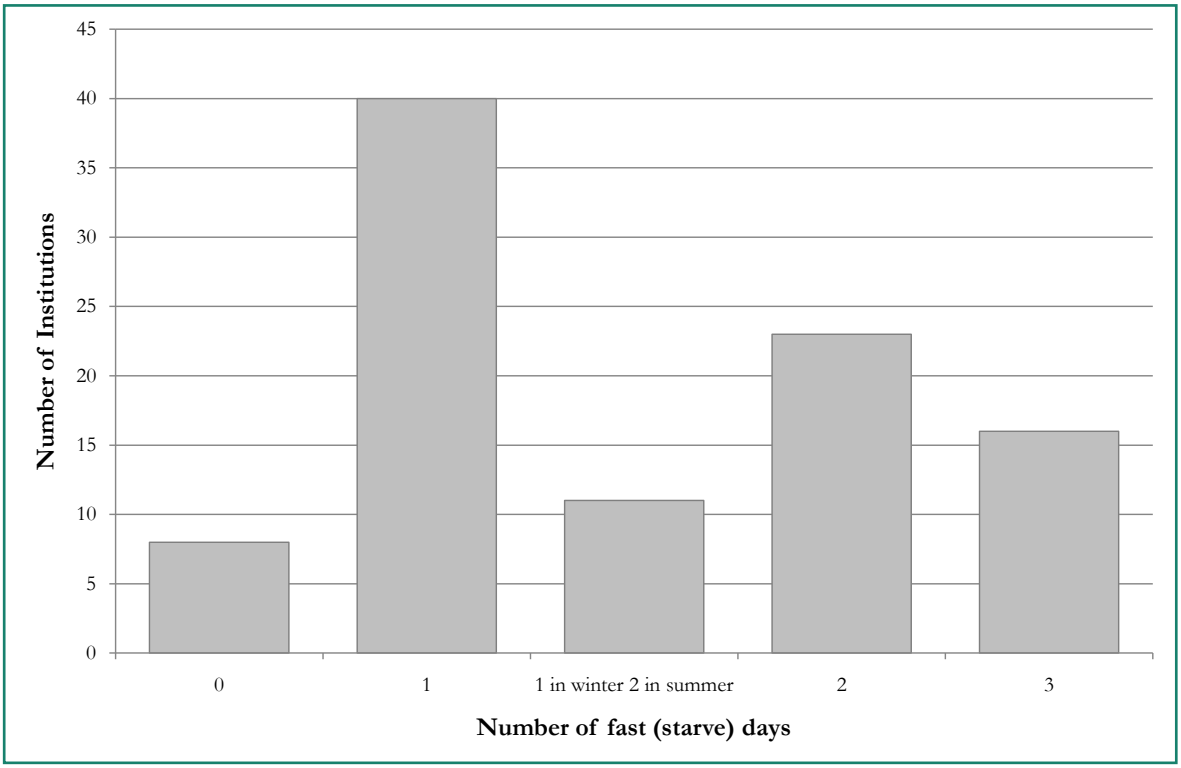

Figure 27. Comparison of the number of fast days at EAZA Institutes

Water Requirements

Clean drinking water should be available at all times. Water should be provided in containers that cannot be easily overturned or emptied and containers should be cleaned daily. The use of automatic watering devices is often avoided with large cats due to the potential for the cats to damage them. Watering devices that pose no safety hazard to the animals should be selected. Adequate water pressure is important as is the ability for the keeper to access taps etc. from the service area rather than from within the enclosure (page 29).

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Behaviour and Enrichment

Figure 28. Leopard exploring a hessian bag stuffed with rabbit bedding. © Jane Budd

Social structure

For naturally solitary species, the safest group structure is single animals, however many zoological institutes prefer to hold multiple individuals for aesthetic reasons or due to space constraints. Larger female groups may be safely maintained, such groups are best established while the individuals are sub-adults.

A group of males has been successfully established at the Singapore Zoological Garden. This zoological institution holds four males and one female, which are divided into a breeding pair and a group of three related males. Holding all male groups, however, is generally not advised due to the high risk of injury or death during territorial squabbles and competitive challenge at feeding times.

Changing group structure

Because of the potential for serious or fatal injuries, all introductions should be well planned and closely monitored. It is generally accepted that younger, immature individuals are easier to introduce than older adults, however, this is not always the case.

Leopards that are relaxed and familiar with the keeper staff and their environment can be more predictable during introductions. Newly acquired animals should be given adequate time to settle into their new environment, become familiar with and learn to trust the normal daily routine.

It is strongly recommended that the animals to be introduced have some form of "remote" introduction prior to physical introduction. Remote contact can be made through a connecting fence. Aggression may be displayed initially but should settle as the animals become familiar with one another. Introductions should not be attempted until the pair has accepted, or at least tolerates one another.

It is also useful to allow the two animals to scent a "no-man's land" where available or to have alternating access to indoor/outdoor enclosures. This allows the animals to become familiar with each other's scent over time and introduces each animal to the idea that there is another of their species in the vicinity.

Utilising the oestrus period of the female for introduction will help to reduce some of the initial aggression. The attention seeking behaviour displayed by a female in oestrus may sometimes be startling to an inexperienced mate/companion but is usually well tolerated.

The newly introduced animals should be supervised at all times initially. It may be suitable to introduce the animals for short periods each day, gradually increasing the time spent together. Only once the pair is relaxed together should they be left unsupervised.

Preparation for introductions should include ensuring immediate access to some form of distraction that can be used to separate the pair in the event that a serious fight does break out. Large dustbin lids that can be banged together loudly or a strong jet of water may create enough distraction to allow separation. Ensure a quiet environment and limit outside distractions. Know the availability of the veterinarian and the location of transport carriers in the event injury from

fighting is life threatening. If the area is large enough, it is recommended that introductions take place off-exhibit. Once the pair has been introduced (off-exhibit), the process of introducing the pair to the exhibit can begin.

Before considering an introduction between two leopards, ensure that the enclosure provides adequate sleeping platforms, tree limbs, cargo nets, etc. to allow multi-level use of the enclosure for both animals. The enclosure should also provide visual barriers and areas of retreat for both animals.

Some facilities leave their cats together 24 hours a day once the pair is comfortable with one another. Others continue to separate pairs at night, when feeding or only introduce pairs when the female is in oestrus.

Behavioural enrichment

Captive environments are considerably less complex than wild environments and confined animals consequently express boredom in response to an environment that fails to meet their stimulatory needs (Carlstead. 1996). Carlstead (1996) also states that it is difficult to define the stimulatory needs of animals as these vary markedly between species and even between individuals. Carlstead (1996) further states that to maintain wild-type behaviour in captivity it is essential to adapt environmental conditions to the animal rather than expecting the animal to adapt to the environment.

The goal of behavioural enrichment is to provide stimuli that promote appropriate behavioural and mental activities to encourage natural behaviours in captive animals and reduce the incidence of stereotypic, self destructive or abnormal behaviours. These behaviours can include inactivity, over-excitability, pacing, over-grooming, and head swinging.

The development of an enrichment program should be based on the natural biology of the species, considering such things as social structure, habitat use, diet, primary senses, activity cycles etc. Evaluation of abnormal behaviours will also help to decide what approaches could be implemented. Various enrichment studies have been carried out; they are a good source of information and provide novel ideas that can be implemented easily.

Social stimulation

Leopards are solitary animals except during breeding. However, companionship in captivity can be beneficial as it permits natural behaviour such as play, competition, co-operative behaviours and even occasional aggression. As discussed previously, introductions should be carried out with caution.

Olfactory cues such as animal scent, perfume, aromatic oils, herbs, spices and food placed around the outdoor enclosure provide investigative opportunities and often also encourage scent marking to re-establish a perceived territorial intrusion. Bedding, faeces or fur from prey animals and even faeces or urine from other leopards will also stimulate investigation.

Interactions between the keeper and the animal can be beneficial to animal well being by providing a positive and secure relationship and environment. Mellen (1998) and Poole (1998)

report improved reproductive success and decreased pacing in small felids where a rewarding relationship has been established with the keeper.

Physical environment

The use of trees, unevenness, observation points, and various levels with perches provides opportunity for climbing, leaping and jumping. Swinging perches can simulate the naturalness of trees. Investigative behaviour can be further stimulated by randomly moving the furniture in the enclosure.

Visual barriers can be created with the use of vegetation, hollow logs, solid logs, rock piles etc. Visual barriers are useful in providing this naturally secretive species with privacy and may also decrease food competition and aggression. Trees (check for escape potential) and logs provide a rubbing surface and scratching post to sharpen nails and even shade areas to rest in.

Novel substrates such as mulch, soil, leaf litter and cut grass provide unusual textures for investigation and can be easily placed in the enclosures randomly.

Non-natural toys are commercially available and are generally well received. They can be used in off-exhibit enclosures if a natural exhibit is a priority for display.

Some examples of physical environmental enrichment techniques that could be considered include:

- Trees/logs that are hollow or have holes drilled into them for hiding treats
- Trees/logs/poles for scratching or rubbing or climbing
- Unstable (but not unsafe) poles that simulate tree branches
- Natural substrates to stimulate normal scooting/marking behaviours
- Novel substrates such as freshly cut grass, mulch, leaf litter, soil, moss for investigation, rubbing, rolling
- Substrates such as prey bedding for investigation
- Holes placed in high rocks/poles for hiding food to encourage climbing
- Rotation of exhibit furniture
- Vines or ropes
- Spices, herbs, perfumes
- Faeces of other leopards or other species (care with parasite transmission)

Feeding enrichment

Numerous feeding methods are used in zoological institutions; all are based on stimulation of the animal in its search for food. Carnivores naturally use large amounts of energy foraging for food and efforts should therefore be made to allow them to work for their food.

Novel presentation of food can be achieved by hiding the food throughout the enclosure in woodpiles, under rocks, in high trees/perches or even inside a cardboard box (remove metal staples). The woodpile method has been observed to encourage further investigation even after all the food has been found.

If random feeding schedules, multiple feeding times and unpredictable approaches can be implemented, these will help to reduce stereotypic behaviours and increase foraging (Shepherdson *et. al.* 1993).

The use of meat sticks, treat boxes, lures, cow tails and horse tails can also be used to encourage hunting behaviours such as stalking, chasing, grabbing, pulling, jumping and climbing (Fig 29 and 30). Whole carcasses encourage natural behaviours because the carcass needs to be plucked or the fur pulled before it can be consumed. The action of crushing bone and tearing flesh during consumption has the added benefit of helping to maintain healthy teeth and gums.

Figure 29. Sequence of an Arabian leopard retrieving food from a feeding pole. © Kevin Budd.

Some examples of feeding enrichment techniques that could be considered include:

- Carcass foods with fur/feathers and organs: chicken, rabbits, mice, rats, guinea pigs, quail, lizards, small ungulates.
- Scattered or hidden food items

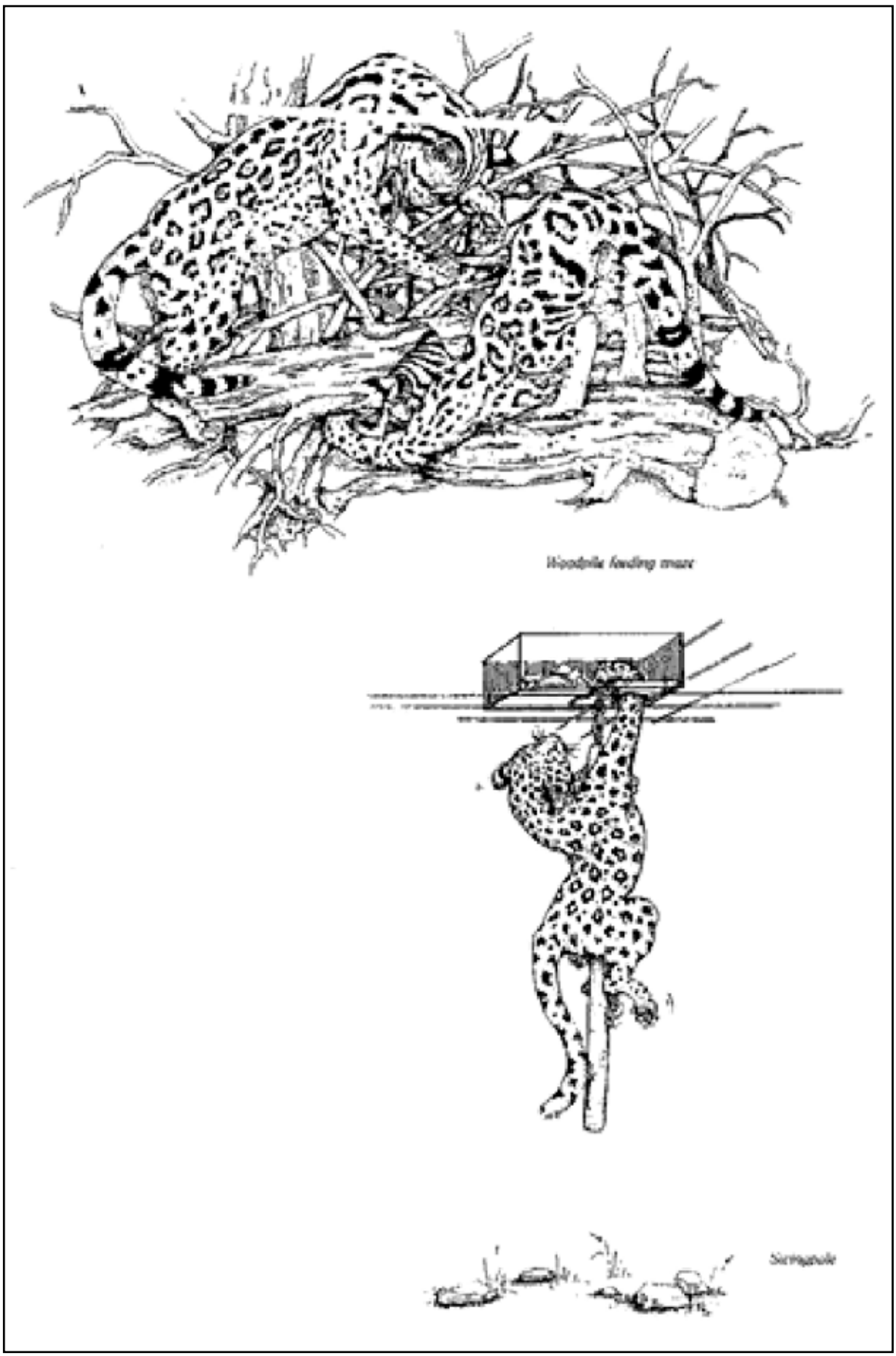

Figure 30. Two methods of feeding enrichments, the woodpile method (top) and the swingpole method (bottom).

- Bones: horse tail, cow tail, ribs, meat on the bone (as part of or additional to daily ration)
- Hides from rabbit, ungulates or other prey species
- Varied/random feeding schedules and routines to reduce predictability
- Woodpile for hiding food or blood trail
- Feeding poles that encourage exercise to get the food
- Melons, pumpkins etc. that provide novel textures, meat pieces could be hidden inside
- Hiding food inside cardboard boxes or short cardboard tubes
- Pig ears, hooves, rawhide bones

The use of feeding boxes has proved useful with Amur Tigers *(Panthera tigris altaica)* and in Sri Lankan leopards *(Panthera pardus kotiya)*. The meat is hidden in the boxes, which are closed by a sliding door with a strong magnet. When the magnet is switched on, the animal cannot open the door. Each magnet is switched off during two 15 minute periods, semi-randomly. This happens without associated noise and the sliding door does not move or open by itself. To get the meat, the cats have to investigate the feeding boxes regularly. This method of feeding has demonstrated a decrease of permanently frustrated appetitive foraging behaviour and stereotypic behaviour (Fig. 31).

Figure 31. Feeding boxes used for tigers and leopards. (Source: Effect of Feeding Boxes on the Behaviour of stereotyping Amur Tigers *(Panthera tigris altaica)* in Zurich Zoo, Zurich, Switzerland, Jenny S. and Schmidt H.)

Novel Enrichment

Artificial and novel objects can also encourage natural behaviours through the expression of investigation and manipulation. Cardboard boxes are commonly used at the BCEAW, Sharjah and many of the felid (and canid) species rip the boxes apart as if it were prey.

It is important to realise though, that while the provision of novelty will stimulate natural behaviours, novel items should also be randomly removed for a period of time to maintain interest in the item when it is returned. Behavioural enrichment requires some degree of randomness to stimulate interest and novelty.

Some examples of novel enrichment techniques that could be considered include:

- Hessian bags filled with hay/faeces or other novel smells
- Tires
- Carpet tubes, paper towel rolls
- Boomer balls, Kong toys
- Paper bags

- Pine cone, palm fronds, tree branches
- Cardboard boxes
- Blood balls or icicle with meat pieces
- Spices, herbs, perfumes
- Bungi ropes with dangling toy/food

Safety Considerations

Behavioural enrichment items should be chosen with care to avoid accidental ingestion which can result in serious medical complications. Ropes and chains should be used with extreme care and should be hung in such a way that feet, legs or heads cannot get entangled in them.

Responses to enrichment objects should be closely monitored at all times as these can vary markedly between animals. Timely intervention of unsuitable behaviour/response to an object can avoid serious accidents.

Investigate which plants are safe to use in enclosures as some plants are toxic if ingested and some animals will eat the plants.

Faeces from other animals should be checked for parasites on a regular basis to prevent parasite transmission between species.

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Reproduction and Development

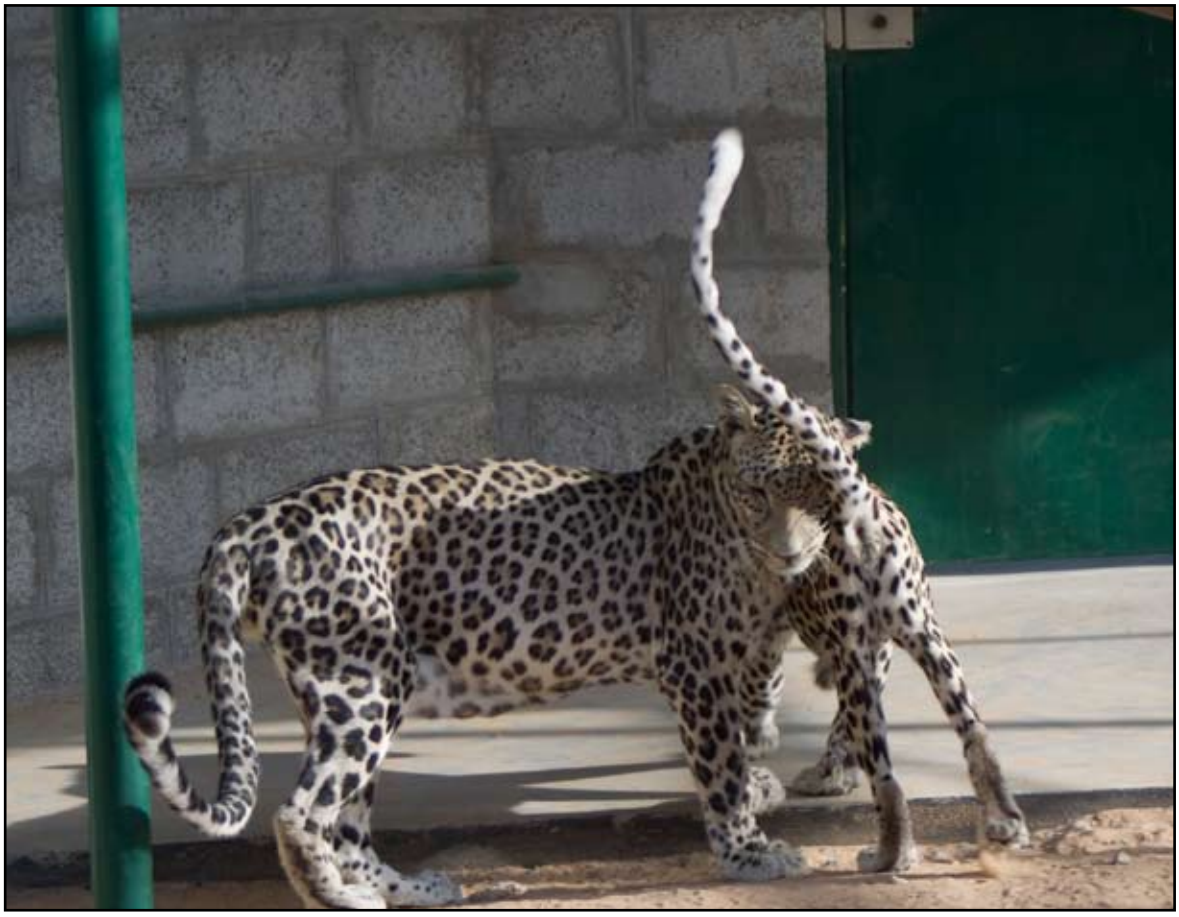

Figure 32. Typical soliciting behaviour of a female Arabian leopard in oestrus. © Jane Budd

Breeding

It is well known that the leopard is an extremely adaptable animal and has been shown to breed well in captivity. Leopards have little mate preference and few health concerns when given adequate husbandry (de Haas van Dorsser *et. al.* 2001).

It is strongly recommended that pairs are only introduced when the female is in oestrus (see Changing Social Structure, page 42). The pair should have a common enclosure wall (mesh screen) and the male should be rotated onto the female's territory so that behavioural responses indicating oestrus can be observed.

As discussed previously, ovulation is copulation induced. Mating results in one of three outcomes: 1) failure to ovulate which results in a return to oestrus after 12-21 days (de Haas van Dorsser, 2006), 2) failure of embryo implantation (pregnancy) which results in a period of pseudo pregnancy under the influence of progesterone and a return to oestrus after 35-42 days or 3) pregnancy. Ideally, the male should be left with the female for the duration of two oestrus cycles after copulation has been observed to ensure that a return to oestrus following pseudopregnancy is not missed. In the absence of copulation after this length of time, the female can be presumed pregnant.

Although leopards are known to be non-seasonal poly-oestrus breeders there are situations in captivity where breeding seasons are regulated based on various management factors such as environment, season etc. The Arabian leopard at the BCEAW, Sharjah is such an example, where leopards are only introduced for breeding in late summer and autumn so that young are born during winter. This approach avoids undue physiological stress on pregnant/lactating females and neonates during the extreme summer temperatures. Female Arabian leopards continue to show oestrus behaviour throughout the year, however, semen evaluation of male Arabian leopards at the BCEAW has shown that there is a reduction in semen quality and volume during the extreme UAE summer months.

As previously discussed (page 19), it is recognised that females of most leopard subspecies reach sexual maturity when approximately 24 to 36 months old (Eaton 1977). A study on the reproductive physiology of Arabian leopards has shown that females begin to undergo hormonal changes from the age of two years (de Haas van Dorsser *et. al.* 2007). The earliest age at which behavioural oestrus has been recorded in captive Arabian leopards is 22 months. Under captive conditions a female could potentially give birth to a litter every 12 to 14 months. It is generally advised to allow the cubs to remain with the dam until they are at an age where they would naturally begin to disperse (12-18 months).

Mating

As observed in wild leopards, oestrus duration in captive leopards is one to two weeks.

As discussed in the Natural History and Status chapter (page 19), most of the mating cues are initiated by the female in oestrus and include increased vocalisation, increased scent marking, cheek rubbing etc (Fig. 32). In the captive situation, the male has been observed to show increased interest in the female, following her closely, approaching her and sniffing her anogenital region. Both males and females show increased urine marking.

The breeding pair will mate frequently during the night and occasionally during the day. Less shy animals have been observed copulating frequently throughout daylight hours as well (Fig. 33). The mating period can last as long as 7 days, reaching a peak between 3 to 5 days. Compare this period with observations of wild African leopards that only mate for 2-3 days (Owen, 2006 and Bailey, 1993). The inter-oestrus period lasts between 12-21 days in Arabian leopards. Owen (2006) reported an inter-oestrus interval of 22 days (range 10-48) for wild African leopards.

Figure 33. Leopards in the normal mating position. © Jane Budd.

Pregnancy and birth

Pregnancy lasts for 95-105 days and is generally calculated from the first or last day of mating respectively. An approximate date for the birth of cubs can be calculated based on when mating was observed. The gestation period of Persian leopards is recorded as 93-98 days (Weston, 1991) and for Arabian leopards it is 92-97 days (de Haas van Dorsser *et. al.* 2007).

It is also possible for observant keepers to monitor the progress of the female through the pregnancy. Physical changes generally only become apparent during the last trimester of pregnancy although, in smaller leopards or primiparous females, pregnancy can be more visually obvious. Changes in the physique of a pregnant female include abdominal distension during the last trimester and the development of mammary glands along the ventral abdomen during the last week of pregnancy.

Nesting behaviour normally begins within a few days of partus and includes restlessness, aggression towards keepers and increased seclusion or secrecy. Indicators that birth is imminent can include a loss of appetite as well as restlessness, calling and seclusion or secrecy.

Females entering the last trimester of pregnancy (3-4 weeks prior to parturition) should be given free access to a secluded enclosure or exhibit and should be allowed isolation with free access to at least one cubbing den, preferably two. They should not be relocated to new and unfamiliar enclosures.

Changes in husbandry procedures or the physical environment should also be planned and implemented well in advance of the impending birth so that disruptions at, and following, parturition are kept to a minimum. It is well known that females are less than normally tolerant of stressors (Baker *et. al.* 1996) postpartum and are prone to exhibit excessive carrying, neglect and cannibalism if not offered a higher level of privacy at this time. It is advised to 1) avoid changing husbandry routines, 2) increase seclusion of the new mother, 3) avoid personnel changes and 4) restrict access of nonessential personnel in the weeks immediately before and following the birth of young cubs.

The birth den should not be cleaned until the cubs are leaving the den of their own accord. The rest of the enclosure should receive only minimal cleaning. Routine protocol followed at the BCEAW, Sharjah is to avoid entering the enclosure during the first two weeks if at all possible, except to refill water bowls. Routine cleaning thereafter until the cubs are beginning to wean includes cleaning and changing the water and removing faeces/bones from the enclosure only.

Cubbing facilities for leopards often have straw or grass hay added for bedding. If bedding is used, the substrate should be carefully selected so as not to cause stricture around the feet or legs of the cubs. The floor of the den should also be suitably textured for traction to prevent splaying of the limbs as the cubs begin to ambulate. Closed nesting boxes can be added to open/large cubbing dens in order to offer the female a dark, quiet enclosure.

The use of CCTV monitoring systems (see page 30) allows remote monitoring of the progress of a female and her cubs. CCTV monitoring systems also provide the opportunity to monitor the female during partus to ensure there are no complications during or after the birth of the cubs (Fig. 34). Constant monitoring during partus also allows prompt intervention when required.

Figure 34. CCTV still of a female Arabian leopard and her cub.

Dystocia (difficulty giving birth) is not commonly reported in leopards. Of 14 litters of Arabian leopards born at the BCEAW, none presented with dystocia. Cubs can be born 30-40 minutes apart or up to 4-5 hours apart. Active, regular contractions should result in the birth of a cub within one hour and the placenta is expelled shortly after the cub is born. Active contractions for more than an hour with no cub born, requires medical intervention. The female will usually rest between cubs, using this time to clean and nurse cubs already born.

Litter size is usually one to three cubs, weighing less than a kilogram (depending on subspecies). The average weight of newborn Arabian leopard cubs is 360g.

As discussed previously (see page 38), diets for females should be increased in the last month of gestation with an equivalent increase post-partum to accommodate for increased energy requirements during lactation.

Development and care of young

Examination of the dam and her young is important to ensure the ongoing health of both and is useful for the early detection of illness (Read and Meier, 1996). Any examination should be carried out with extreme care to minimise the risk of rejection (Meier, 2003). Limit personnel to the regular keeper and the examining veterinarian, wear clean latex gloves covered in scent from the bedding. Examine the young out of sight and hearing of the dam and carry out the examination as quickly as possible. Examining the young at feeding time when the dam is busy with her food provides a useful distraction. Before allowing the mother access to the young again, the cubs should be gently rubbed with scent from the bedding to remove any odours.

Check vital signs, weight, hydration, and congenital abnormalities. Remember to check the umbilicus for infection. Some facilities test the suckling reflex, however, this is really only of interest if the cub is still newborn (1-2 days). Ideally, examinations should be postponed for the first two weeks if the female and young appear normal. Thereafter, cubs can be weighed weekly or two weekly, depending on the reaction of the mother following each handling (Fig. 35). Weighing should be carried out at the same time and on the same day each week.

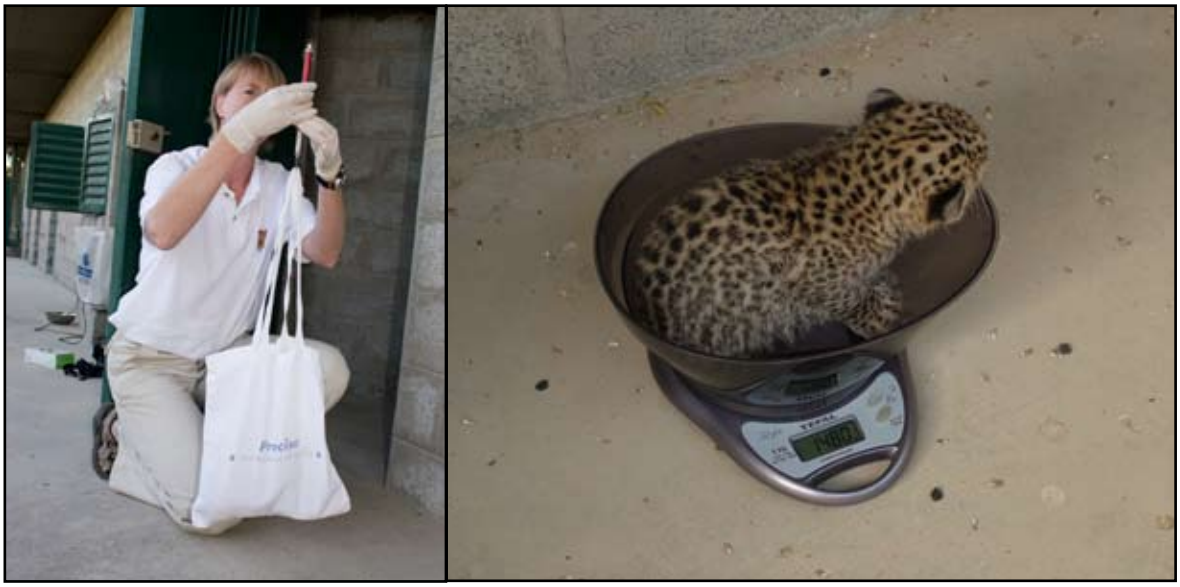

Figure 35. Monitoring weight gain gives a good indication of whether the cub is receiving adequate nutrition. © Kevin Budd

Cubs are born blind and begin to open their eyes at 8-10 days. They also start to attempt ambulation from this age and begin to explore their immediate surroundings inside the den. They generally only start leaving the den at 4-5 weeks of age, once they are able to walk well. Based on observations of Arabian leopards at the BCEAW, Sharjah, the cubs begin to investigate meat from 6-8 weeks old but are not completely weaned until approximately five months of age.

Deciduous (first) teeth begin to erupt at three weeks with incisors appearing first. Molars are usually the last teeth to appear. Deciduous teeth are replaced by permanent teeth from 8-9 months of age. It is usually the adult canine teeth that are first noticed.

Hand-rearing

Most felid management plans prefer that young are parent-reared. However, there are times (maternal neglect, cub ill-thrift, maternal illness or death, etc.) where hand rearing cannot be avoided. It is not uncommon for a young primiparous female to abandon her first litter. Experienced females may also abandon or inadvertently kill their young if stressed at the time of birth or during the first few weeks following birth. It is important, therefore, that the husbandry conditions under which the pregnant/nursing females are housed provide adequate security and few disruptions to ensure a stress free nursing environment.

The decision to hand-rear should be carefully considered and should not be attempted unless absolutely necessary. The costs and benefits to the species and the genetic contribution of the individual to the captive population should also be carefully evaluated. It is well documented that mother reared cubs are more likely to successfully reproduce and rear young than their hand-reared counterparts (Baker *et. al.* 1996). Conversely, animals that are used for educational purposes are often hand-reared so that they are accustomed to the close proximity of people and conditioned to the captive environment (Read and Meier, 1996).

Success in hand rearing requires preparation. Ideally, impending births are expected/planned and potential problems have been anticipated and prepared for. Thawed serum from an adult leopard can be used sub-cutaneously or orally in hand-reared neonates to facilitate passive transfer of antibodies

A veterinarian should provide First Aid, particularly if the neonate is unstable or injured. Most commonly, abandoned neonates suffer from low glucose (hypoglycaemia) and low body temperature (hypothermia), both of which are potentially fatal if left untreated.

Initial isotonic fluid therapy should be administered via sub-cutaneous, intravenous or intra-peritoneal routes. Ensure the fluids are warmed prior to administration. Glucose should also be provided to correct hypoglycaemia and meet the demands of the central nervous system (Read and Meier, 1996) as the animals' body temperature returns to normal. Subcutaneous fluids are easily administered but remember that peripheral circulation in cold individuals will be reduced which means that fluid uptake is limited. Local vasodilation in response to warmed subcutaneous fluids may also worsen shock conditions. Oral rehydration should not be attempted until the body temperature approaches normal (36°C in the first days, up to 37.2°C in the first 2-4 weeks).

Hypothermia should always be corrected slowly. The use of electric blankets, heat lamps etc. should be approached with extreme care. Burns are easily caused in a hypothermic animal as the ability of the body to redistribute heat is reduced (Read and Meier, 1996; Meier, 2003). Attempt to maintain a temperature gradient within the neonate's confined space to allow selection of the most comfortable resting place.

Rearing protocols are followed once the neonate is stable. There are numerous protocols available (e.g. the AAZK Handrearing Protocol). Record keeping is important. Initial records should include signalment (common and scientific names, sex, age, birth location etc.), history, and physical examination findings. A rearing plan should be drawn up (formula type and concentration, feeding frequency, rate of weight gain, weaning plan etc.) and the cubs' progress should be recorded on a feeding chart. The choice of formula type and feeding frequency is based on the nutritional requirement and condition of the neonate and also on maternal milk analyses where known.

Weight (and vital signs until stable) should be recorded daily at the same time for an accurate indication of weight gain or loss. The feeding chart should include fluid and food intake, urination, defecation and behaviour of the cub.

A daily weight gain of 50-100g can be expected, but will vary. It is useful to know the normal growth curves of mother-reared cubs with which to compare the progress of the hand-reared cub (Fig. 36). Deviations from the expected growth rate can give an early indication that medical intervention is required. As discussed, cubs should be weighed at the same time each day and their body weights recorded. Aim to ensure that there is at least some weight gain daily, no weight gain or weight loss should be further investigated by a veterinarian.

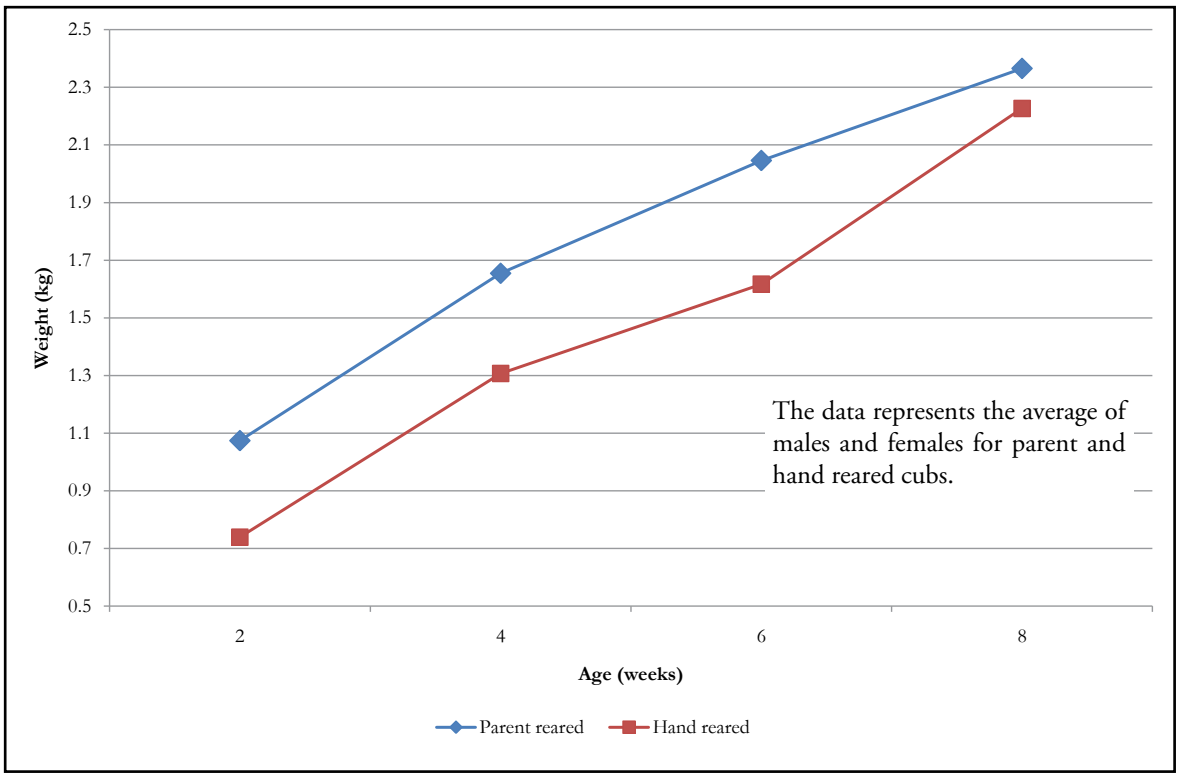

Figure 36. Comparison of the growth rate of parent reared and hand reared Arabian leopard cubs at BCEAW.

It is important to use sterilized formulas and feeding equipment. Stock formula can be prepared and stored for 24 hours and bottles filled from the stock formula. Any formula that is not consumed at a feed should always be discarded. All equipment should be thoroughly washed with detergent to remove any residue and rinsed well before sterilisation.

Normally, cubs are fed 12-18% of total body weight in each 24hour period, depending on the progress and growth rate of the cub. The maximum capacity of the stomach is 5-8% of total body weight, no more than this volume should be fed in a single feed. Gastrointestinal tract stress can be avoided if the cub is given appropriate and consistent volumes. Animals should not be fed as much as they will take as this results in overfeeding and diarrhoea. Some references suggest that satiation can be achieved with a top up of cooled boiled water from the bottle if desired.

Frequency of feeding depends on the age of the neonate and on the developmental progress of each individual. During the first week, cubs are generally fed every 2-3 hours. During the second and third weeks, overnight feeds can be reduced and the neonate fed every 3 hours during the day, extending to every four hours by three weeks of age. At this point the neonate should be receiving five meals per day. By 4-5 weeks of age, the neonate should be able to be fed four times daily, monitor satiation of the cub and adjust the feeding schedule accordingly. The neonate can also now be taught to lap (if desired) and solids can be gradually introduced into the diet. The age of complete weaning varies between facilities and depends on the care giver and the progress of the cub. Weaning is usually complete by 10-12 weeks of age.

The first bottle-feeding should only be attempted if the neonate is stable and an oral electrolyte solution should be used. If a strong suckle response is present, a dilute milk formula of 25% formula and 75% electrolyte can be offered at the next feed. The strength of the formula can be gradually increased over the next 24-36 hours.

Figure 37. Hand-reared neonates should be held in a sternal position with the head and neck extended during feeding. © Kevin Budd

Bottle-feeding can be difficult to initiate, however, with careful teat selection the suckling response is easily stimulated. Formula should never be forced into the neonate's mouth by squeezing the bottle/teat. Once the cub has accepted the teat and the formula, the flow rate can be adjusted to achieve a slow, controlled feeding that reduces the risk of aspiration of milk during feeding. The bottle and nipple type used vary according to the size and age of the cub and preference of the care giver. Most *Panthera* spp. respond well to short, soft standard human infant teats (Hedberg, 2002) and speciality teats for premature babies. Once a cub has accepted a teat type, the same brand should be used throughout the nursing period if possible.

Feeding posture in neonates is equally important and should always aim at having the cub in its natural feeding position, which is sternal (on its stomach) with the head and neck extended towards the mothers' udder (Fig. 37). Feeding in this position also helps to avoid accidental inhalation of milk. Cubs will often knead at the mothers' udder to stimulate milk let-down, providing a towel or surface against which the cub can knead will help facilitate this action.

For the first several weeks, neonatal carnivores require ano-genital stimulation to urinate and defaecate. A warm, moist cloth or cotton ball can be used to stimulate the ano-genital region before or after each feed. Care should be taken to avoid abrasion. Most young will defecate by themselves by 6 weeks, if not sooner. Self-controlled urination will occur sooner but varies between individuals.

It is important to consider the natural requirements of the animal and to provide an environment that allows "normal" development. Historically, neonates were reared in sterile environments, providing for physical needs but ignoring behavioural and psychological needs which often resulted in maladjusted individuals (Read and Meier, 1996).

Some authors consider that hand-reared animals should be exposed to animals of the same or similar species (Read and Meier, 1996 and Schoemaker, 1997). If there is just one cub to rear, a domestic kitten can be carefully introduced at 3 - 4 weeks of age (Schoemaker, 1997). This companionship, even if only for a few hours a day, provides valuable play experience, socialization and encourages normal developmental skills (Read and Meier 1996 and Schoemaker, 1997). Disease risk should be carefully evaluated before introducing a companion.

Figure 38. Arabian leopard cub playing with its toys. © Kevin Budd

It is essential for young felines to exercise. Adequate space and time are needed to allow investigation and development of climbing/stalking/

pouncing skills. Enrichment "toys" can be provided to encourage stalking and pouncing (Fig. 38). All natural behaviours should be encouraged, although the cubs should not be encouraged to play with their caregiver. Biting and clawing the caregiver should particularly be discouraged.

Formula

A wide variety of different milk formulas are available for use in feeding felid neonates. Some of them are listed in detail in Appendix 5. The choice of formula is based on factors such as similarity to maternal milk composition, product availability and individual tolerance/ acceptance.

The milk of *Panthera* spp. is low in carbohydrates (see Appendix 5.1). Formulas high in carbohydrates are therefore often not well tolerated and tend to cause diarrhoea (Hedberg, 2002). The inability of leopard cubs to digest lactose can be assisted with the addition of a lactase enzyme to the formula.

It is also important to note the taurine content of the formula selected for rearing. Taurine is an essential amino acid for all felids; deficiency can cause serious changes in heart function and retinopathy (Howard *et. al.* 1987, Hedburg 2002). If the formula does not contain taurine it should be added prior to feeding.

Formulas that are low in protein are known to cause hair-loss. Hedburg (2002) reports hair-loss in snow leopard cubs that were fed *Esbilac*. BCEAW, Sharjah experienced hair-loss in Arabian leopard cubs fed *Cimicat*. In these cases, the addition of a portion of strained-poultry-meat baby food to the milk formula adds extra calories without increasing the volume and should correct the hair-loss problem (Hedburg, 2002). If this technique is considered, it is advised to introduce the meat source gradually over a week. The meat source can be added from 12 days of age (Hedburg, 2002).

KMR feline milk substitute (see Appendix 5.3) from Borden/Pet-Ag (www.petag.com), has been widely used in the recent past. It is marketed for hand rearing of domestic kittens. Reformulation of this brand in 1993 resulted in the addition of butterfat for improved mix-ability but the addition of the butterfat caused digestive problems in some exotic species. Lactobezoar was reported in leopards (Hedburg, 2002). A bezoar is a compacted mass in the stomach formed by ingested material that does not pass into the intestine (Blood *et. al.* 2002).

The upgraded *Zoologic* (Borden/Pet-Ag) range contains seven milk formulas that can be mix-and-matched to meet the appropriate requirements of the specific neonate. This range of milk formulations is currently recognised as the gold standard range for hand rearing exotic neonates. Their website provides detailed guidelines for formula selection and preparation (www.petag.com). Milk Matrix 33/40 and Milk Matrix 42/25 are similar formulations to *Esbilac* and *KMR* but are without the butterfat. The product numbers indicate the minimum crude protein/fat percentages in the formula. Most species require a combination of formulas from this range to more closely resemble the composition of maternal milk.

Esbilac canine milk substitute (see Appendix 5.2) from Borden/Pet-Ag (www.petag.com) has also been one of the more commonly used formulas for rearing felids in the recent past. Care should be taken with the use of this formula as it is designed for the canine neonate. As mentioned

above, it is low in protein and contains no taurine and therefore requires supplementation to provide a balanced diet. It is available in liquid and powder form. Esbilac has been successfully used for Amur and North Chinese leopards that were hand-reared at the Feline Conservation Center in Rosamond, California.

Milkodog (see Appendix 5.5) was successfully used to rear two female Sri Lankan leopards at the CERZA Zoo. This formulation is not recommended for use in felids. A complementary diet, *Sofcanis* (see Appendix 6.10), was added to ensure this formula met all mineral, amino acid, trace element and vitamin needs. *Sofcanis* is a dietary supplement that can be used during growth, pregnancy, lactation.

Below are two examples of hand rearing protocols used for hand-rearing leopard cubs. Weight curves are included (Fig. 39 and 40).

Hand-rearing at the CERZA Zoo, Hermival les Vaux, France:

To distinguish the two females, during the hand-rearing, one was clipped. Cubs have to be bottle-fed. A tablespoon (=15ml) of *Milkodog* mixed with 2 tablespoon of hot water (50 to 60°C). The table of growth is in the Appendix 3 and *Milkodog* composition is shown in Appendix 5.5. Quantities of meat and milk and administration are indicated in Table 4.

Days 1-2:	Feed every 2 hours (day and nights).
Days 3-4:	Feed every 2 hours during the day and every 3 hours at night.
Days 5-27:	Feed every 2 hours during the day and every 4 hours at night.
Day 28-70:	Feed every 3 hours from 08h00 to 23h00.
Day 70 to weaning:	Feed every 4 hours, between 08h00 to 23h00.

Table 4: Quantities of milk and meat ingested and mode of provision during the first 16 weeks of a hand-reared leopard. (Source : Plan d'élevage artificiel pour des panthères du Sri Lanka. CERZA)

Week	Milk per day (ml)	Meat per day (g)	Provision
1	232	None	Baby's bottle
2	288	None	Baby's bottle
3	374	None	Baby's bottle
4	430	None	Baby's bottle
5	540	None	Baby's bottle
6	545	10	Baby's bottle
7	495	48 (twice daily)	Baby's bottle
8	502	56 (twice daily)	Baby's bottle and start slowly to mix milk with meat
9	661	121 (twice daily)	Baby's bottle and start slowly to mix milk with meat
10	648	145 (twice daily)	Baby's bottle and mix milk with meat
11	525	195 (twice daily)	Baby's bottle and mix milk with meat
12	411	278 (twice daily)	Baby's bottle and mix milk with meat
13	437	307 (twice daily)	mix milk with meat
14	425	400 (twice daily)	mix milk with meat
15	342	450 (twice daily)	mix milk with meat
16	150	450 (twice daily)	mix milk with meat

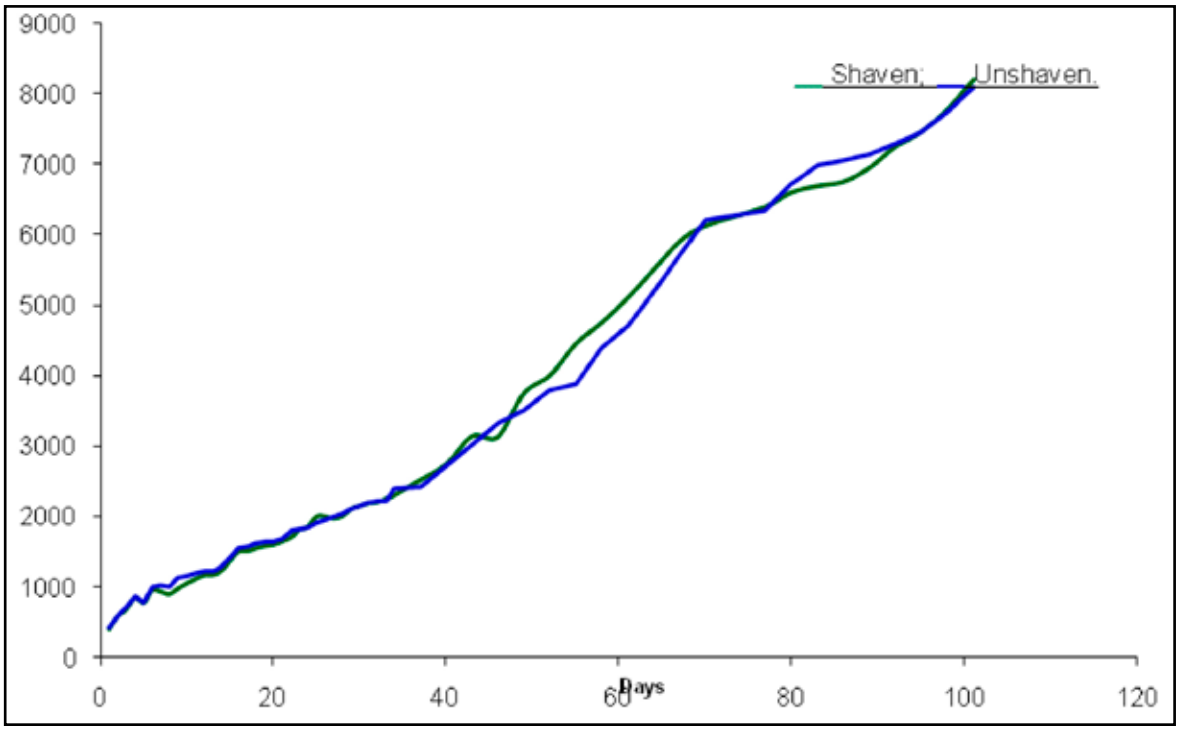

Figure 39. Weight curves obtained in leopards hand reared at the CERZA Zoo.

Hand-rearing at the Feline Conservation Center, Rosamond, California, U.S.A

Formula used: Esbilac liquid (12oz cans), Pedialite and Lactaid drops, ratio at 50/50. Two drops Lactaid per 8 oz formula (see Appendix 5.2 for lactaid composition).

Days 1-7: Feed every 3 hours, 6-7 times a day with ½ to ¾ oz of *Esbilac*.
Days 8-14: Feed every 4 hours, 5 times a day with 1 to 2oz. Neo-calglucon brand liquid calcium supplement and poly-visol liquid vitamins added to formula.
Days 14-28: Start solids. Beech-nut brand baby food (turkey or chicken).
Days 28: Continue adding solids and feed every 4 hours, 4 to 5 times a day.

Observations: Umbilical cord falls off at 5-7 days,
Eyes open at 8-10 days,
Starts teething at 3 weeks,
Up on all fours at 3 weeks and walking well at 4 weeks.

Control of reproduction

There are many different reasons for using contraceptive techniques within a captive population. The most important of which is as a method of establishing genetically variable populations within the constraints of the limited captive habitat.

Contraception may be permanent or temporary. For example, where there is not enough available space to house an expanding population, temporary contraceptive techniques could be utilised until new holding facilities become available. Thus allowing an established pair to continue to be housed together for aesthetic value or to accommodate space constraints.

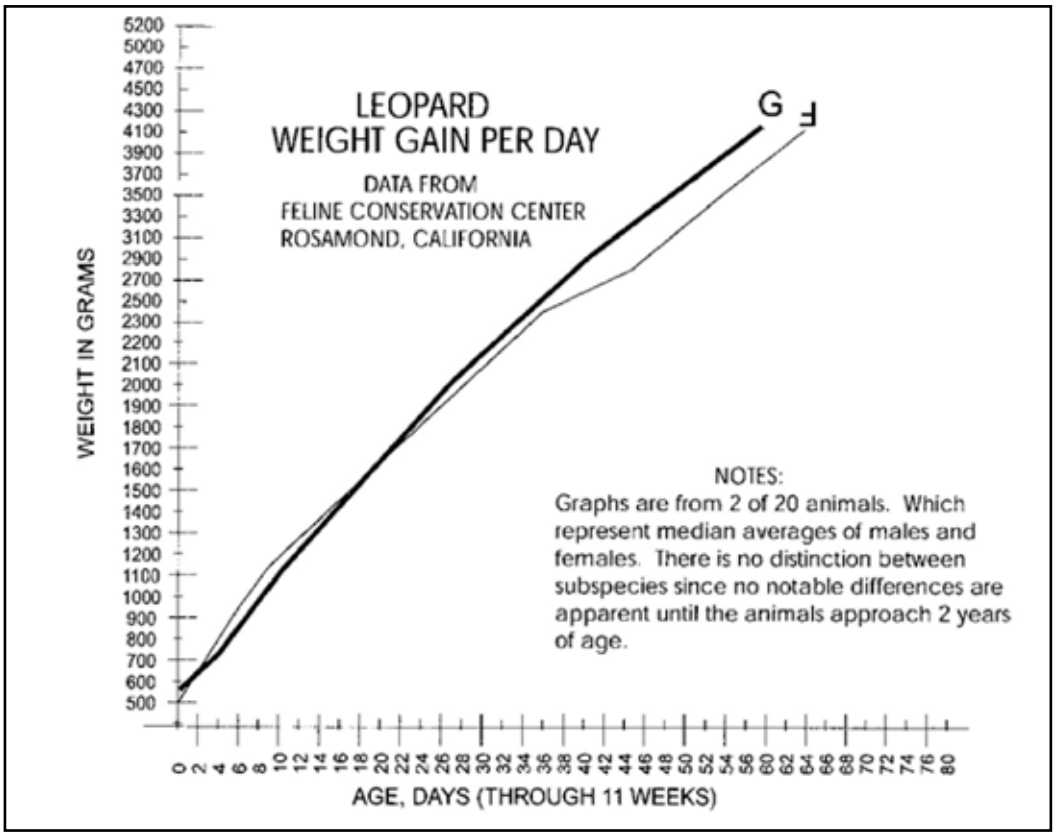

Figure 40. Weight curves obtained in leopards hand reared at the Feline Conservation Centre.

The need to avoid inbreeding, selectively prevent breeding of over-represented individuals or even reduce fighting in large male groups may result in the use of permanent or temporary contraceptive approaches, dependent on the specific situation.

Management of disease processes such as recurring infection or neoplasia in individual animals may also require the use of permanent contraceptive techniques. Many zoological institutions now realise the importance of maintaining pure subspecies. Permanent contraceptive techniques can be used to prevent the introduction of unwanted hybrids into the population.

Permanent Contraceptive techniques for females

Permanent contraceptive techniques have the advantage of being 100% effective, but there is also the drawback of removing the animal from the captive gene pool (Lewis, 2000). These techniques are generally used when the animal is of no genetic interest such as with hybrids or offspring from closely related pairings. Animals with congenital deformities may also be subjected to permanent contraception.

Ovariohysterectomy

This is the best technique for the long-term health of an animal and is commonly used in animals with abnormalities of the reproductive tract. This technique involves an abdominal laparotomy to surgically remove the ovaries and uterus under anaesthesia. Ovaries are the source of endogenous steroids that can promote mammary cancer and endometrial hyperplasia in non-breeding females. The procedure does carry some risks including peri-operative complications, post-operative infection and post-surgical interference with surgical wounds. Anaesthesia is also always a risk.

Ovariectomy

Only the ovaries are removed, the uterus is left in place. As the operation can be carried out by laparoscopy, the surgical procedure is simpler than with ovariohysterectomy and hence the potential for post-surgical complications is reduced. Although the source of endogenous steroids is removed, infections and even neoplasia of the uterus may still occur after recovery.

Tubal ligation and salpingectomy

Tubal ligation involves tying the Fallopian tubes and salpingectomy removal of part of the Fallopian tubes. These procedures can also be carried out using laparoscopic techniques. The patency of the tubes following clamp removal cannot be guaranteed. The risk of the animal developing reproductive tract cancer, endometrial hyperplasia and uterine infections is probably the same as for intact, non-breeding cats, but less than for cats receiving long-term progestagen contraception. Normal reproductive behaviour is maintained but fertilisation of the oocyte is not possible. This technique is not commonly used.

Permanent contraceptive techniques for males

Castration

This is a very simple operation in all cats. This method involves surgical removal of the testes through a testicular incision thereby totally eliminating the production of gonadal sex hormones, which are essential for the development and maintenance of secondary sexual characteristics. Whilst castrated animals tend to exhibit less territorial aggression, non-sexual aggression, such as is seen with food competition, is unaffected.

Vasectomy

The removal of a section of the vas deferens is also a simple surgical procedure in leopards. With this procedure there is no loss of secondary sexual characteristics, libido or normal sexual behaviour. Males may remain fertile for a limited period post-surgically.

Ovulation, but no pregnancy, will be induced in females copulating with vasectomised males. The risk of reproductive tract diseases in non-breeding intact females, in the group or pair, is not diminished. Other drawbacks of this technique include the occurrence of pseudo pregnancies and persistently cycling females.

Temporary contraceptive techniques for females

Physical separation of the male and the female during oestrus is rarely practical. This method can increase the risk of reproductive tract disease in females if used as a long term strategy. Moreover, this approach does not encourage/allow normal social behaviour. Artificial techniques in females have so far been based on the use of the progestagens. When these are withdrawn, most animals will return to normal cycle activity and hence fertility - provided they were reproductively normal before contraception was initiated.

Progestagens are available as long-acting injections or very long acting implants. Because oral progestagens have many undesirable side effects, their use is not advised. Temporary contraceptive techniques for males are not currently available.

Injectable progestagens

Injectable preparations such as Melengestrol acetate (MGA) and proligesterone (P) are probably easier to manage for most collections. Single injections produce 2-6 months contraception. In general proligesterone produces fewer side effects.

Progestagen implants are available which contain either medroxyprogesterone acetate (MPA), levonorgestrol or more commonly, MGA. Placed intramuscularly (recommended) or subcutaneously by simple surgery, MGA implant provides a constant, continuous release of small quantities over a specific period of time (Lewis, 2000). This is the simplest technique for medium term reversible contraception in females but does have various side effects associated with it such as hair loss and endometrial problems.

Immunocontraception with Porcine Zona Pellucida Vaccination (PZP)

The type of vaccines available differs with the source of PZP (native or recombinant) and the adjuvant used to stimulate an immune response. The efficacy of this method in preventing pregnancy is not yet known. Most felids continue to have oestrus cycles and show behavioural oestrus after vaccination.

GnRH agonists/antagonists

GnRH agonists/antagonists are available in injectable or implantable forms. When adequately dosed, this method should be effective in suppressing oestrus. Health risks are not well known, but secondary sex characteristics and reproductive behaviour may regress after long-term treatment

When the resumption of breeding is required, an implant can be removed and a return to cycle can be expected within weeks provided that fertility was normal before implantation.

The frequency and the severity of undesirable side effects produced by progestagens are still only partially defined, but ongoing studies in the U.S.A., under the auspices of the American Zoo and Aquarium Association Contraceptive Advisory Group, have gone some way to clarifying the situation. Recent studies have found that felids continually exposed to MGA implants may develop severe endometrial hyperplasia and have higher risk of mammary gland and uterine cancers. Cats should not receive implants for continuous periods exceeding 2-3 years. Implants should not be used as permanent contraception.

If young are not desired, permanent contraceptive techniques are recommended. In the case of individuals that may be bred in the future, the safest contraceptive measure is to separate the pair when the female is in oestrus.

Temporary contraceptive techniques for males

There is no reliable temporary artificial contraceptive available for use in males. Testosterone administration disrupts spermatogenesis in the male but the long-term efficacy and safety of this method have not been assessed.

Evaluation of fertility

In general, leopards breed readily in captivity. It is important to distinguish between physiological infertility and infertility caused by inappropriate management techniques. This is especially important in the case of genetically valuable animals.

Techniques available for females are direct examination of the reproductive tract, assessment of circulating hormone levels and monitoring the hormonal response to exogenous drugs active on reproductive function.

The ovaries and uterus can be examined directly by laparoscopy under general anaesthesia, although ultrasound can also provide useful information.

The circulating hormone levels change during a normal reproductive cycle. Monitoring serum hormone levels requires blood samples taken at frequent intervals. This means that frequent anaesthesia would be required, which is ethically questionable, physiologically stressful and potentially dangerous for the animal.

Faecal steroid analysis (oestrogen, progesterone and in some cases cortisol) allows non-invasive, estimation of the status of the animal (pubertal status, ovarian follicular growth, ovulation, the function of the corpus luteum and causes of reproductive cycle abnormalities/failure). There is a biological relationship between changes in faecal steroid metabolite concentrations and physiological factors known to affect ovarian activity.

The administration of exogenous hormone such as gonadotrophin and gonadotrophin releasing hormone stimulate the development of ovarian follicles and ovulation. Blood sampling for hormone analysis allows the investigator to confirm that the ovaries are responsive and that ovulation has occurred.

Techniques available for males are direct examination, assessment of circulating hormone and semen analysis.

Direct examination of the reproductive tract includes evaluation of external genitalia, and involves examining testicular volume, tonicity and integrity. Ultrasound evaluation can also give useful information.

The assessment of hormone levels such as testosterone and luteinizing hormone could be measured from blood and/or serial faecal samples.

The simplest and most common technique used to discover if a male is fertile or not is semen analysis. Semen is generally collected by electro-ejaculation under general anaesthesia. The semen is assessed by the measurement of ejaculate volume and pH, sperm concentration, percentage of motile sperm and those with progressive motility and structural morphology.

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Medical Management

Figure 41. Leopard Procedure at BCEAW. © Kevin Budd

Introduction

All captive wildlife is handled at some point for capture, restraint or anaesthesia. All facilities should therefore be able to perform shifting and crating without the use of anaesthesia but should also be able to perform anaesthesia safely. Holding areas that can be used for confinement allow better access for observation (food intake, collect urine/faecal samples etc.), anaesthesia (or drug administration) or transfer into a transport box.

Anaesthesia

Anaesthetising any animal is a complicated and potentially dangerous procedure and should only be undertaken by experienced veterinarians.

With the appropriate equipment and planning, safe and effective anaesthesia is possible, however, it is never absolutely risk free and should therefore never be undertaken lightly. Anaesthesia is achieved by the administration of one or a combination of drugs (Cullen 2006); which should only be administered under the direct supervision of a trained veterinarian.

The aims of anaesthesia are to provide immobility of the animal for safe handling, to prevent awareness of pain and provide sufficient muscle relaxation to permit surgery.

Pre-anaesthetic considerations

Leopards should be fasted for 12-24 hours, and water should be removed at least 1-2 hours before immobilisation. Fasting reduces the incidence of vomiting or regurgitation during the procedure. Anaesthesia should be avoided where possible in pregnant, diseased, or dehydrated animals and should be administered with particular care in young and old animals.

Anaesthesia should be carefully planned and all staff briefed prior to commencement of any anaesthetic procedure. It is important that anaesthetic procedures are scheduled during quieter periods of the day with minimal personnel present during induction. If anaesthesia has to be induced outdoors, weather should be considered. Always separate the animal to be anaesthetised from its cage mates.

Stress or excitement increases anaesthetic risks by potentially increasing the induction dose required and can also cause injury during induction (Bush, 1996). Aim to minimise stress as far as possible for any manipulative procedure. Emergency drugs must always be at hand in case of complications.

Anaesthetic monitoring

It is important to monitor anaesthesia for early detection of complications. Common complications include respiratory depression, a drop in blood pressure (hypovolaemia), a drop in circulating oxygen (hypoxia), cardiac depression (bradycardia), and seizures. The use of an anaesthesia record chart provides a quick reference for the progress of the patient. Parameters should be taken at 5 minute intervals during the entire procedure.

An intravenous catheter should be inserted to allow rapid delivery of emergency drugs, additional anaesthetic drugs or supportive fluid therapy during anaesthesia. The use of a pulse oximeter will allow the anaesthetist to monitor oxygen saturation of the blood and a Doppler blood flow detector can be used to monitor the pulse and blood pressure (indirect measurement). Heart rates and lung sounds can be monitored using a stethoscope. It is important to palpate the peripheral pulse (the femoral artery is readily palpated) at the same time as listening to the heart rate to ensure the heart is still capable of producing a normal peripheral pulse.

It is recommended to take repeated rectal temperatures during the procedure as anaesthesia leads to loss of temperature control. Hypo or hyperthermia should be avoided and should be corrected as soon as the core body temperature becomes abnormal. Use heating pads judiciously as severe burn wounds can be caused.

Many anaesthetic agents reduce the blink reflex and cause drying of the cornea. Suitable ophthalmic lubricant should be applied to maintain corneal hydration and protective eye covers should be used to protect the eyes from harsh light.

More sophisticated equipment is needed to monitor respiratory carbon dioxide, blood gases and ECG.

Post-anaesthetic considerations

The rate at which the leopard recovers will depend on the anaesthetic drug combination used, the amount of drug used, the duration of anaesthesia and the age and health status of the animal. The leopard should be recovered in a warm, dimly lit, quiet box or cage. Care should be taken to ensure there are no shelves, water or sharp objects on which it can injure itself. Never allow a conscious leopard in with an anaesthetised or recovering leopard.

The drugs are eliminated gradually and the leopard may be drowsy and unable to walk in a co-ordinated fashion for several hours. Only once the animal is no longer ataxic can it be allowed access to water and food. Most leopards will be thirsty on recovery though some will not eat until the following day.

Drug Administration techniques

Administration of anaesthetic drugs can be accomplished using a squeeze/crush cage that allows the use of a hand syringe, or remotely via blow pipes and dart guns (rifle or pistol).

The blow pipe is the most basic of all remote drug delivery systems. If used correctly, a trained operator can propel a lightweight dart (3mL) up to 10m (Bush, 1996). Rifle or pistol projectors allow the administration of larger volumes and longer distances can also be achieved if needed. The use of a blow pipe causes less impact trauma than a dart gun due to the light weight and low velocity delivery of the blow pipe darts. All remote delivery systems require training on non-living targets in order to ensure accuracy. Injection sites commonly used are the large muscle masses of the proximal (upper) hind limb and forelimb.

The darts of remote delivery systems (Fig. 42) consist of a plastic body into which the drug is placed (at the head-piece) and a tail-piece into which air is placed through a one-way-valve. The

air compresses the plunger. At impact, a silicone seal on the tip of the needle is displaced which exposes a port in the side of the needle. Air pressure pushes the plunger forward and the drug is expelled through the needle port. (Telinject/Daninject)

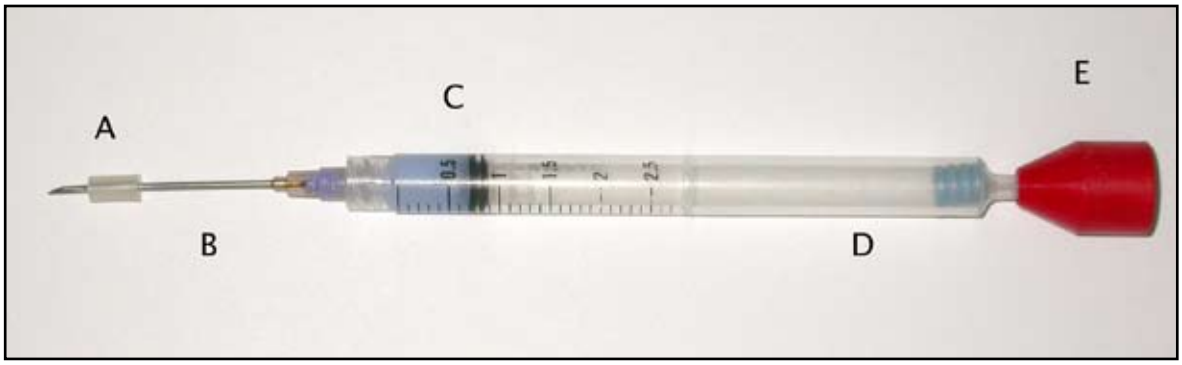

Figure 42. Telinject remote delivery system, A = silicone seal, B = needle with side port, C = head-piece, D = tail-piece, E = flight © Jane Budd

Immobilisation/induction drugs

An ideal anaesthetic drug should; 1) have a large therapeutic index, 2) be concentrated, 3) have a rapid induction/recovery time, 4) have adequate duration of action, 5) be compatible with other drugs, 6) have good sedative, muscle relaxant and analgesic properties, 7) have minimal side effects, 8) be reversible (Wenker, 1998 and Cullen, 2006).

Drugs commonly considered for use in felid anaesthesia include dissociative agents (Ketamine), alpha2-adrenergic agonists (Xylazine, Medetomidine) and benzodiazapines (Diazepam, Midazolam). The concurrent use of opioids is often advocated to reduce the overall amount of anaesthetic drug required to induce anaesthesia. All of the drugs or drug combinations available for use during anaesthesia have advantages and disadvantages that should be taken into consideration. It is important to follow a protocol with which the anaesthetist is familiar.

Dissociative agents (e.g. Ketamine) are rapid-acting drugs that produce altered consciousness. This drug class has a wide margin of safety and is one of the most commonly used chemical restraint/anaesthesia drugs. It also has good analgesic properties (Plumb, 2005). Ketamine is particularly effective in carnivores (Wenker, 1998) and is commonly used in combination with other agents. Note that normal muscle tone and blink, pharyngeal and laryngeal reflexes are maintained with this class of drugs. It is therefore recommended to use these drugs in combination with agents (such as the alpha2-adrenergic agonists) that produce suitable muscle relaxation. Side effects include muscle rigidity, excessive salivation, convulsions, vocalisation and hyper- or hypotension. There are no known antagonists.

At optimum doses, the first effects are seen in 2-5 minutes following intramuscular injection. *Ketamine* is short acting; the duration of effect in cats is usually no more than 60 minutes but can be longer at higher doses.

The alpha2-adrenergic agonists: (Xylazine, Medetomidine) are potent central nervous system depressants with sedative, muscle relaxant and analgesic properties. They are not commonly used as sole anaesthetic agents but they are often used in combination with other suitable drugs to produce the required effect. Their effect is dose dependent with drug responses ranging

from sedation to anaesthesia. At high doses, the alpha-2 adrenergic drugs cause respiratory depression and hypotension and thermoregulatory mechanisms are disrupted (causing hyper- or hypothermia). Recovery from high doses is prolonged and difficult. Antagonists are available for this class of drugs.

Xylazine has traditionally been the most widely used of this class of drugs. Its onset of action is usually seen 10-15 minutes after intramuscular injection. Analgesic (pain relieving) effects may only last for 15-30 minutes, but sedation lasts up to 1-2 hours. In cats, vomiting is a common side effect. Xylazine affects thermoregulation and may cause hyperthermia or hypothermia. Xylazine should not be used in animals that are in poor condition or suffer from depressed respiration, cardiac function disturbance or impairment of hepatic or renal function (Plumb, 2005).

Because it may have a stimulatory effect on the smooth muscle of the uterus, Xylazine should be used with caution (if at all) in pregnant animals. It is known to cause abortion in ruminants.

The specific alpha2-antagonist available for the reversal of Xylazine is yohimbine; Atipamezole can also be used. If Xylazine is used in combination with a cyclohexamine (Ketamine), the xylazine effects should not be reversed before the animal has metabolised the cyclohexamine.

Medetomidine (Domitor) is newer than Xylazine and is one of the most potent alpha2-adrenergic agonists currently available. It is also the most specific alpha2-adrenoceptor agonist, giving longer and more reliable sedation/analgesia (Plumb, 2005). Side effects include peripheral vaso-constriction, bradycardia, respiratory depression, hypothermia and vomiting. The duration of effect is dose dependent.

Atipamezole is available for reversal of medetomidine. It is a competitive inhibitor of medetomidine, and acts by displacing medetomidine from its receptor site. Recovery is usually rapid (5-10 minutes) following IM administration of the antagonist.

Tiletamine HCl/Zolazepam HCl (Telazol) is a combination of an anaesthetic agent (chemically related to ketamine) combined with a tranquillising agent (a benzodiazepine). This formulation is generally avoided for use in large exotic cats as it may cause seizures (Plumb, 2005). It is similar to ketamine/diazepam but is reported to have a duration of effect that is approximately 3 times as long as that of ketamine (Plumb. 2005).

At optimum doses, the onset of action should be within 1-7 minutes following intramuscular injection. Surgical anaesthesia in dogs lasts approximately 27 minutes, recovery can take up to 4 hours (Plumb, 2005). Side effects include increased heart rate (tachycardia), and cardiac output, respiratory depression, excessive salivation, vomiting, prolonged recovery. Reversing agents are available for Zolazepam; however they are extremely expensive and are therefore very seldom used.

Neuroleptic agents (nitrous oxide) and benzodiazepines do not produce immobilisation when used alone but are sometimes used as synergists with opiods or cyclohexamines to reduce the dose of the immobilising drug thus giving a smoother, more rapid induction (and recovery) and negating some of the undesirable side effects.

Gaseous anaesthesia is utilised for most surgical procedures or when it is necessary to prolong an anaesthetic beyond the effective period of the induction/immobilisation drugs administered. Maintenance of anaesthesia using gaseous anaesthetic agents (mixed with oxygen and delivered through an endotracheal tube) is the safest option.

Various inhalation anaesthetic agents are available such as halothane, isofluorane, sevofluorane and methoxyfluorane; each with advantages and disadvantages associated with their use. Common complications seen with inhalation anaesthesia are dose related and include respiratory depression, hypoxia, apnoea, bradycardia and seizure.

Intubation in leopards is more difficult than in other carnivore species because the larynx is located further caudally, towards the thoracic inlet.

Table 5: Commonly used injectable anaesthetic protocols for exotic cats

Dose (IM)	Comments	Reversal
Medetomidine and Ketamine		
30-40µg/kg medetomidine 2-4mg/kg ketamine	Wide safety margin Vomiting sometimes seen following administration of medetomidine	Lowest possible dose of ketamine reduces risk of seizure Reversal of medetomidine with atipamezole at 5x initial medetomidine dose (equivalent volume). Given IM
Xylazine and Ketamine		
0.5 – 1mg/kg xylazine 7-8mg/kg ketamine	Higher dose of ketamine may result in seizures. Prophylactic diazepam (5-10mg) can be administered once immobilised Xylazine can induce vomiting	Reversal of xylazine with Yohimbine (0.15mg/kg). Atipamezole can also be used.
Zolazepam/tiletamine		
3-5mg/kg	Not commonly the induction combination of choice. Better combinations are available. Prolonged recovery May cause seizures	Reversing agent expensive and not commonly used

General Health

This chapter will aim to highlight some of the more common disease conditions occurring in felids. It is by no means comprehensive. Information in this section should not be used as an alternative to consulting a veterinarian; it is included as a guideline for the reader to further understand some of the conditions occurring.

Viral Diseases

Feline infectious enteritis (Panleukopaenia) is an exceptionally contagious viral disease of cats caused by parvovirus. Due to widespread vaccination, it is a disease that is not commonly seen in domestic cats in modern times (Merck, 2005), however, infection rates in unvaccinated populations are still high.

The virus is extremely resilient and can survive for up to one year in suitable environments. Their extreme resilience means that infection is easily spread over long distances.

Transmission of the virus occurs:
- By direct contact with an ill animal, or a sub-clinical or symptom-free carrier
- By indirect contact via contaminated personnel, material, litter, dirty dishes or food.
- By contact with contaminated faeces, urine, and other secretions.
- Trans-placental infection usually causes abortion, resorption of the foetus or stillbirth. Live kittens often have cerebellar hypoplasia, in-coordination and tremor.

Most infections are sub-clinical. Animals that become infected are usually less than a year old and have naïve immune systems.

Clinical signs are severe and include marked depression, loss of appetite, fever and vomiting. Haemorrhagic diarrhoea may develop after several days. The number of circulating white blood cells is severely depressed.

Veterinary treatment requires supportive fluid therapy and intensive nursing care in isolation. Plasma or whole blood transfusion may be required in hypoproteinaemic or anaemic patients. Broad-spectrum bactericidal antibiotic therapy is indicated (Irwin, 2006). Nephrotoxic drugs should be avoided until the hydration status of the patient has been restored. Anti-emetic therapy may permit earlier enteral nutrition with soft, easily digested food (Irwin, 2006). Analgesia may also be recommended.

Feline Respiratory Disease Complex includes feline viral rhinotracheitis (FVR), a herpes virus and feline calicivirus (FCV). Other primary agents implicated in this disease complex are *Chlamydophyla felis, Bordetella bronchiseptica, Mycoplasma* sp. Bacteria such as staphylococci, streptococci, *Pasteurella* and coliforms are generally secondary invaders (Irwin, 2006).

Numerous infections with FVR are described in leopards however; infections by FCV are less common. Transmission can be direct, through contact between individuals, aerosols and infected animal secretions, or indirect (through husbandry materials or humans). FCV is shed continuously, while FVR is released intermittently when a latent infection becomes active. It is often impossible to differentiate FVR from FCV infection (Merck, 2005).

Young individuals, particularly newborns, and the weak or immunologically compromised are more susceptible to infection and often present with more rigorous signs of disease. Symptoms typically seen with infection include fever, frequent sneezing, conjunctivitis (with ocular discharge) and rhinitis with serous nasal discharge initially becoming mucopurulent and copious (Merck, 2005). Weight loss is also commonly seen. In severe cases pneumonia, ulcerative stomatitis (mouth ulcers) and ulcerative keratitis (ulcers on ocular conjunctiva) may occur.

Treatment is symptomatic and supportive and concurrent use of broad-spectrum antibiotics is useful to prevent secondary infection. If available, nebulisation will help in the removal of mucous secretions.

Vaccines are now available. It is important to avoid the use of modified live vaccines (MLV), killed vaccines only should be considered.

Feline Infectious Peritonitis is caused by a coronavirus and has been reported in most large felids, including leopards, clouded leopards, snow leopards and especially cheetah (Merck, 2005). It is currently thought that FIP is caused by a simple mutation of the feline enteric coronavirus (Irwin, 2006). Whilst many felids may be infected with the virus, very few develop clinical disease. This is predominantly a disease of young cats.

Virus transmission occurs by contact with an infected cat or its excreta. Transmission is known to be high in crowded situations (Irwin, 2006).

Two forms of FIP are recognised, a dry and a wet form. Clinical signs seen with the dry (non-effusive) form are generally associated with granuloma formation and include ocular signs, neurological signs, pneumonia, lymphadenopathy etc. This form is also associated with little antibody response. The wet form, in contrast, is associated with pronounced antibody response and rapidly developing clinical signs include icterus, fever, anaemia, ascites (distension of the abdomen) and sometimes, pleural fluid accumulation.

Treatment of this fatal disease is supportive with particular focus on nutrition and prompt treatment of secondary infections. Current therapeutic approaches include support of the immune system with the use of interferon (Irwin, 2006).

Vaccines are available in some parts of the world (USA and Europe) but have a low reported efficacy (Irwin, 2006). They are also not available in the killed form and are therefore not currently recommended for use in exotic felids.

Rabies is a lyssavirus from the *Rhabdovirus* family. It causes an acute viral encephalomyelitis that can affect any mammal but is mainly of importance in carnivores and bats. Rabies occurs worldwide although there are a few countries that are free of the disease.

Rabies is transmitted mainly by introduction of virus-laden saliva into the tissues, usually via the bite of an infected animal. The virus moves to the spinal cord and then the brain via peripheral nerves and then from the brain to salivary glands, again via peripheral nerves (Merck, 2005).

The incubation period of rabies is variable and can be prolonged (Merck, 2005). Clinical signs of infection include sudden behavioural changes and unexplained progressive paralysis. Behavioural changes include anorexia, apprehension or nervousness, irritability, uncharacteristic aggressiveness, wild animals may lose their fear of humans, nocturnal animals been seen wandering aimlessly during daylight. Progression of the disease occurs rapidly, particularly after the onset of paralysis. Death is inevitable.

The two most common forms of the disease are the furious form and the paralytic form (Merck, 2005). The furious form is the most well known form where the animal becomes irritable and aggressive at the slightest provocation followed by incoordination and seizures and death as the disease progresses (Merck 2005). The paralytic form presents with profuse salivation due to paralysis of the through and masseter muscles. These animals are usually not aggressive. Progression of the disease is the same with paralysis, coma and death occurring within a few hours.

Diagnosis of rabies is difficult clinically. If rabies is suspected, definitive diagnosis should be carried out by a qualified laboratory in accordance with standardised protocols for rabies testing. Most countries have established standard protocols to follow in cases where rabies may be suspected. The tissue sample required for diagnosis of rabies is fresh brain tissue.

Rabies is a notifiable disease and is also a zoonosis (can be transmitted to humans). Pre-exposure immunisation is strongly recommended for all high-risk groups such as veterinary staff and animal keepers (Merck, 2005).

Canine Distemper Virus (CDV) is a paramyxovirus closely related to the measles and rinderpest viruses.

Fatalities have been caused by this pathogen in leopard subspecies in recent years. When felids are at high risk, near an outbreak of disease, it would be prudent to vaccinate against CDV. Where vaccination is considered necessary, the selection of a CDV vaccine must be made extremely carefully. Only inactivated, recombinant or subunit vaccines should be used. They may not be commercially available.

Papillomavirus: This virus has been reported in domestic and non-domestic cats. It is rare to find this virus in leopards; although it has been reported in Florida panthers, lions, clouded leopards and snow leopards. It presents with proliferative lesions in the skin and oral cavity. In a non-domestic cat, a number of papillomata have undergone malignant transformation to squamous cell carcinoma. Papillomata should be removed using surgical excision, laser surgery or cryosurgery. Care should be taken that virus (in exhaust plumes or tissue remnants) does not seed the adjacent tissue. A recombinant papillomavirus vaccine is under development.

Avian Influenza H5N1: This virus is thought to be more pathogenic for felids than other influenza viruses. Clinical signs are high fever, respiratory distress and unexpected death. The post mortem findings usually include pulmonary consolidation and multifocal haemorrhage in several organs, including lung, heart, thymus, stomach, intestine, liver and lymph nodes. The infection is caused by the ingestion of infected chicken.

Bacterial diseases

Bacteria cause a number of different diseases in exotic cats. Infection can largely be avoided through optimum husbandry protocols. Treatment for most bacterial diseases includes appropriate antibiotic therapy combined with supportive therapy (fluid therapy and optimum dietary support). Bacterial culture and sensitivity profiles should be run where possible to identify the bacteria responsible and the antibiotic to which the bacterium is sensitive.

Infections with *Staphylococcus* and *Streptococcus* species are most commonly seen. These bacteria cause a wide variety of conditions ranging from localised infections in the form of simple abscesses to widespread systemic infection causing potentially fatal septicaemia or pneumonia.

Other bacteria causing respiratory disease include *Pasteurella* sp., *Klebsiella, Mycoplasma* sp. and *Pneumococci*. Pasteurelosis is very common in all species; bite wounds from an exhibit mate commonly cause infection in cats.

Contaminated food is also a common route of bacterial infection, including *Salmonella* species, which can cause severe enteritis. Wild rodents and birds also play a role in spreading Salmonella. Symptoms include lethargy, inappetance and abdominal pain. Infections vary from mild diarrhoea to severe gastroenteritis and may even progress to generalised septicaemia. Some infected animals may show no clinical signs. The disease can be transmitted to humans.

Other bacteria commonly introduced through contaminated food sources are Listeria (which can cause abortion and septicaemia), and anthrax (*Bacillus anthracis*). Anthrax is uncommon but generally fatal.

Escherishia coli also commonly causes enteritis (in all species) and is of particular concern in young cats as it may progress to pneumonia or septicaemia.

Mycobacterium species may cause tuberculosis which is a chronic, debilitating disease with progressive lesions in the lungs, liver and bone. This disease is no longer very common, however, an outbreak can be detrimental in captive populations.

Ectoparasites (external parasites)

Fleas (*Ctenocephalides* sp., *Pulex* sp., *Echinphaga gallinacea*) are a common ectoparasite of all species, as are lice, mites and ticks.

Fleas are found over the whole body but are commonly seen on the head and neck, around the tail base and on the ventral abdomen. They can cause intense itchiness, which may result in self-inflicted excoriation (superficial traumatic abrasions). Flea numbers can rapidly increase if not controlled. A heavy flea burden can cause anaemia in young cats due to blood loss. Treatment includes lufenuron, fipronil and pyrethrins.

The flea population lives and breeds in the enclosure, usually in the dens/bedding where the environment is ideal for replication (dark and warm). This is also often the place where leopards spend a large portion of their time. With frequent cleaning, the parasite burden can be kept to a minimum; however, to reduce an existing flea population it may be necessary to spray the den and cage corners, woodwork, crevices, etc. with insecticide. The treated cage must be kept free of leopards for 24 hours post-spraying to prevent ingestion of insecticide.

Mange mites, like *Sarcoptes scabiei* (Fig. 43), *Demodex* spp. and *Notoedres cati*, occasionally affect non-domestic cats. Signs of severe irritation, itching and hair loss are seen. By examining skin scrapes it is often possible to identity the type of mite involved. Frequent treatment with acaricidal shampoos is usually effective in domestic cats but is not practical in leopards, as application will usually require sedation. Treatment for mite infection can be achieved with repetitive administration of systemic ivomectin or milbemycin oxime. Dermal applications include selamectin (Meuller, 2007). Such treatment should only be carried out under veterinary supervision. In-contact animals should also be treated.

Figure 43. *Sarcoptes scabiei.*

Ear mites, like *Otodectes cynotis* and *Otobius megnini*, can cause severe irritation and discomfort, and may result in chronic bacterial infection with subsequent ruptured ear drums. In domestic cats, affected animals and those in contact with them are treated regularly with a prescribed ear preparation. Applying ear ointment to a conscious leopard twice daily is not possible without sedation. Alternative systemic preparations that are effective against otodectes include ivomectin and selamectin and generally require only a single treatment (Meuller, 2007).

Lice (uncommon) in large numbers can consume a large amount of blood and ticks can carry disease from one felid to another. Products containing fipronil or pyrethrins are usually effective in controlling ticks/lice

Where external parasites are problematic, it is advised to de-flea, de-louse and de-tick leopards at least twice a year using a topical spot on preparation or spray. If the enclosure is infested, environmental treatment is essential. Rigorous pest control in and around leopard enclosures will help to reduce the potential for introducing infectious disease, which is often carried by pests.

All products used should be used under the guidance of a veterinarian to prevent accidental poisoning or adverse reactions to the product selected.

Endoparasites (internal parasites)

Common endoparasites to which felids are susceptible include roundworms (*Toxascaris* and *Toxocara* sp.), hookworms (*Uncinaria* sp.), whipworms (*Trichuris* sp.), tapeworms (*Dipylidium, Taenia* and *Echinococcus*) and protozoa (*Eimeria, Isospora* sp., Toxoplasmosis and *Giardia* sp.).

Toxascaris eggs have been commonly identified in Arabian leopard faeces. The distinguishing features of the Toxascaris egg are the thick wall, smooth outer surface and almost spherical, yellow-brown contents. Through its development the worm embryo undergoes multiple divisions before it can be recognised as an L1 stage larva (Fig. 44).

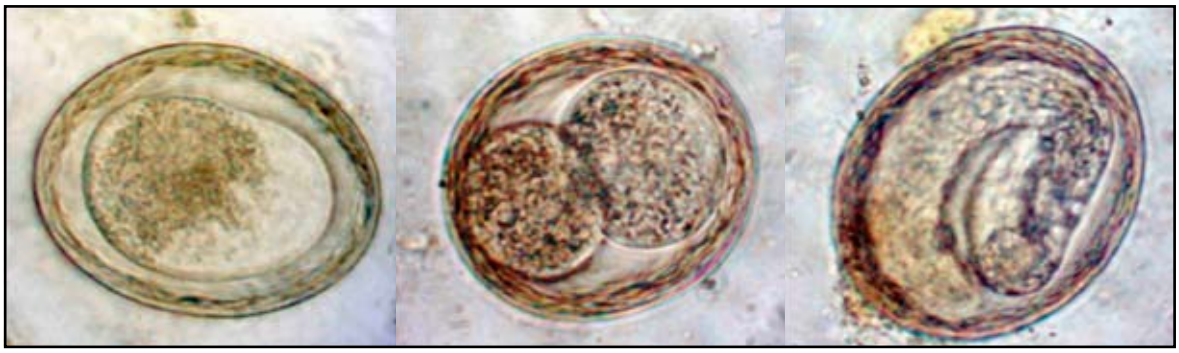

Figure 44. *Toxascaris* eggs; L1 (far right).

Endoparasite transmission is generally by the faecal-oral route. Large numbers of worms can cause low-grade chronic intestinal inflammation leading to poor growth and metabolism, poor hair coat and the presence of potbelly in infected cubs. Heavy infestation of roundworms can lead to diarrhoea, vomiting, loss of appetite, emaciation and even death if left untreated.

All leopard faeces should be examined at least biannually, more often if repeated infection occurs or where a known problem exists. Tests that should be performed include faecal flotation, faecal wet prep and examination of a stained faecal smear. The presence of parasite eggs in faeces indicates that mature parasites are present. The number of eggs counted per gram of faeces gives an indication of the severity of infection. Concentration techniques may be required for the detection of low parasite numbers or eggs heavier than the flotation fluid used.

A range of drugs are available that are effective against various worm species. Care should be taken in selecting the correct product for the parasite targeted. Animals with positive faecal exam results should be re-examined 1 - 2 weeks following treatment to check the effectiveness of treatment.

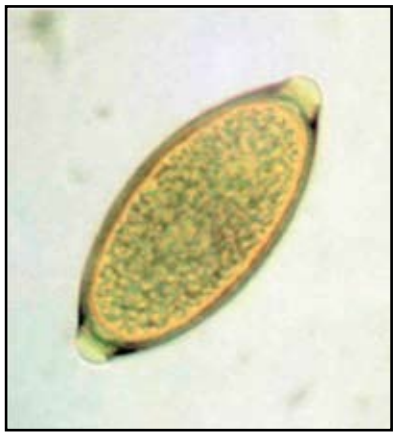

Figure 45. A *Trichuris* egg

Ascarids and whipworm eggs (Fig. 45) are very robust and can survive for years in an enclosure, potentially leading to re-infection in treated cats.

Coccidiosis (*Isospora* sp.) can produce diarrhoea and dysentery. Giardia sp. can also cause severe and chronic diarrhoea leading to malabsorption and weight loss. Toxoplasmosis is usually a symptomless infection of the intestinal lining, but generalised disease does occur occasionally in captive felids. Similarly, the non-pathogenic sexual phase of *Sarcocystis* sp. does occur in the intestines of cats.

Table 6: Common internal and external parasites and their treatments. (Taken from: Lewis J. C. M. 1996. Veterinary considerations, Management guidelines for exotic cats)

The dosages given are guidelines only and are only one of the many effective protocols available.

Parasites	Drug	Dose
Tapeworms		
(Cestodes)	Praziquantel	5mg/kg orally.
	Niclosamide	150mg/kg orally.
	Mebendazole	15mg/kg daily for 3-5 days.
Taenia spp	Fenbendazole	20mg/kg daily for 5 days or 100mg/kg once
Flukes		
(Trematodes)	Mebendazole	15mg/kg daily for 3-5 days.
Roundworms		
(Nematodes)	Fenbendazole	20mg/kg/day for 5 days or 100mg/kg once.
Ascarids:	Mebendazole	15mg/kg daily for 3-5 days.
	Ivermectin	0,2mg/kg.
	Piperazine	80-100mg/kg Repeat after 2-4 weeks.
Hookworms,	Fenbendazole	20mg/kg/day for 5 days or 100mg/kg once.
Whipworms,	Mebendazole	15mg/kg daily for 3-5 days.
Stomach worms:	Ivermectin	0,2mg/kg.

Parasites	Drug	Dose
Protozoa		
Toxoplasma:	Suphadiazine + Pyrimethamine	10mg/kg 0,5-1mg/kg.
Coccidia:	Sulphadimidine or other sulphonamides (as sulphadimethoxine)	50mg/kg orally
Ectoparasites		
Mites: Mange and ear mites	Ivermectin Lime/sulphur	0,2-0,3mg/kg orally or by s/c injection. Wash every 7 days.
Fleas	Fipronil Lufenuron	In spray Orally

Neonate Specifics

Cubs have an immature drug metabolism and like all felids, they lack certain enzymes for the breakdown of drugs (de Haas van Dorsser *et. al.* 2001). It is therefore necessary to take extra care when choosing drugs for treatment.

- Enrofloxacin may have an adverse effect on the normal development of cartilage and joints in cubs.
- Tetracyclines in cubs may stain the teeth.
- Non-steroidal anti-inflammatory drugs such as carprofen, ketofen and tolfedine are potentially nephrotoxic and should therefore be used with care and according to manufacturers recommendations.
- Flunixin should not be used in felids.
- Paracetamol in felids causes irreversible liver damage.

Diarrhoea is a common problem. Most diarrhoea is caused by overfeeding or rapid changes in milk constituents during hand rearing. Care should be taken to provide the mother with a stress free environment to allow optimum lactation. If the diarrhoea is not malodorous and is only too soft in consistency, supportive therapy only may be required. If the cubs are anorexic and depressed antibiotic treatment is required. Diarrhoea that is malodorous usually indicates bacterial infection and also requires antibiotic treatment. If left untreated, the cubs may quickly become dehydrated and die. Some viruses also cause diarrhoea and it is necessary to maintain good hygiene when dealing with such cubs.

Cubs are susceptible to cat "flu" (Feline Respiratory Disease Complex) which is an upper respiratory tract disease. This presents with ocular and nasal discharge, sneezing, corneal lesions and anorexia. The cubs are usually depressed and sometimes the eyelids are stuck together with dried discharge. This condition is best treated with a course of antibiotics to prevent secondary complications such as pneumonia. The cubs need warmth, rest and quiet. The cubs often take a few weeks to recover. It is recommended to vaccinate the dam against these viruses.

Weakness and poor growth is seen with certain blood parasites such as *Haemobartonella felis*. Fleas and other parasites from the mother or other felids transmit these tiny organisms. Adult leopards do not usually show clinical signs of infection but may be carriers of this blood parasite. Young cubs are susceptible to infection and may become very ill. They are generally successfully

treated with oral doxycycline for three weeks. The blood parasites can be demonstrated on blood film and a blood smear sent off to the laboratory will confirm infection.

Coccidiosis usually only causes disease at one to three months of age and signs may arise in association with stress or other diseases. Signs of infections include watery, mucoid, or bloody diarrhoea, dehydration and secondary bacterial infections. A combination of soluble and enteric sulfonamides is effective against the schizogonic cycle.

Weanling Specifics

The first year of life involves a great deal of skeletal growth and closure of growth plates. Abnormalities in this period will lead to lifelong deformities. It is essential that young leopards have access to sunlight, a calcium/phosphorus balanced diet and sufficient exercise. Cubs also need certain dietary amino acids, for the correct development of the central nervous system.

After weaning, young individuals fed diets composed primarily of meat may develop rickets because of the high levels of phosphorous and low amount of calcium in the diet. Vitamin D deficiency may complicate the problem. Prepared carnivore diets have helped eliminate this problem; however, if a diet rich in meat is offered, calcium supplementation should be provided.

Geriatric Specifics

Older leopards are drug frailer than young individuals. Care should be taken in selecting therapeutic drugs and immobilisation/ anaesthesia should be avoided where possible. Blood samples should be taken for assessment of kidney and liver function should the animal require immobilisation. Older leopards often take longer to recover from anaesthesia.

Kidney disease is not rare in old felids. The first signs are increased frequency of urination, production of dilute urine, increased frequency of drinking and halitosis (bad breath). Dietary correction will help to support this disease. This condition is lethal and irreversible.

Dental disease is common in old felids too. Teeth are worn by daily use and may become non-functional due to excessive wear (Fig. 46). There are potential sites of infections when the root canals are open. Tooth root abscesses present with anorexia, depression, halitosis and salivation. The leopard will need to be anaesthetised in order to treat the problem.

Figure 46. The lower canine teeth have been worn through chronic chewing on a fence © Jane Budd

Poisoning

Many poisoning substances can cause animal diseases. Cats are more sensitive to a wide variety of toxic substances than other carnivores. Any disinfectants used in felid enclosures should be thoroughly rinsed away and, if possible, those containing phenols should be avoided.

Many poisonings are caused by the incautious use of therapeutic drugs, for example:

- Aminoglycoside antibiotics such as streptomycin and gentamycin can cause damage to kidneys and the inner ear.
- Anthelminthics such as piperazine cause ataxia and depression in overdose.
- Organo-phosphorus compounds such as malathion, dichlorvos and diazinon are used against ectoparasites, but in excess can cause muscle spasm, paralysis, convulsions, coma and even death.
- Phenol-based topical are contra-indicated and even chlorhexidine can cause localised toxicity if used for cleaning ears.
- The careless use of chemicals to control vermin can also lead to poisoning in cats. Chlorinated hydrocarbon insecticides can not only be ingested or inhaled, but also be absorbed across the skin. These chemicals can cause over-excitability, ataxia, convulsion and death.
- Feeding meat from carcasses of animals destroyed with barbiturates can lead to prolonged anaesthesia and even death.

Miscellaneous conditions

Injury is the most common problem in captive felids, but a wide variety of miscellaneous problems are also seen in the digestive, respiratory, nervous and other systems.

Dental diseases such as gingivitis, gum infections, fractured canines and carnassial teeth and periodontal abscesses are not uncommon in felids. Where the vital pulp of any tooth has been exposed by a fracture, expert advice should be sought to effect root canal treatment or extraction. Periodontal disease is commonly found in older leopards, particularly when they are fed a predominantly soft diet. The accumulation of dental calculus damages and inflames the gum and allows the tooth root to become infected. These infections can lead to systemic bacterial infections and widespread amyloidosis.

Overgrowth of claws will occur readily, particularly in enclosures where inadequate scratch posts are provided, and can cause lameness and ulceration of the foot. An ingrown toenail is often not detected until the animal is obviously lame (Fig. 47).

Gastro-intestinal upsets such as diarrhoea and vomiting can be caused by the infectious diseases already mentioned. This upset could also be due to contaminated feed, decomposed food or a sudden change in diet.

A wide variety of tumours occurs, including those of the liver, mammary glands, uterus, ovaries and skin. Oral tumours are not uncommon in older leopards.

Figure 47. Ingrown claws can cause lameness and ulceration © Jane Budd

Hair loss and hairballs can together pose problems. The former is difficult to determine and can be due to nutritional deficiencies, persistent licking due to boredom or hormonal disturbances. The fungal infections known as ringworm may also cause loss or breaking of hair, together with dry scabbiness of the skin. Ringworm can affect humans.

The latter, hairball, is due to an accumulation of hair in the stomach particularly in periods of heavy coat shedding have been implicated in loss of appetite and weight loss. Treatment with liquid paraffin in the food or water is usually effective.

Congenital problems could be seen such as ocular and palpebral coloboma and hip dysplasia, umbilical hernias, cleft palate, cataracts and heart defects. Some of these conditions may be caused by drug or live vaccine administration during pregnancy (e.g. griseofulvin, ivermectin).

Preventative medicine

A preventive medicine programme aims to prevent disease and maintain optimum health status. A rigorous preventative medicine program is more important than the diagnosis and treatment of disease as it arises. Animals in captivity are generally under some degree of stress and are therefore considered more likely to develop disease.

Some zoological institutions provide annual or routine physical examination under anaesthesia. Not every zoo agrees with this approach, but it does allow complete annual health evaluation, provides a normal data set for each healthy animal and may aid in early disease detection. Tests that can be carried out include blood collection for complete blood count (CBC), packed

cell volume (PCV), serum biochemistry, heartworm antigen testing (where relevant), selected serology testing for feline infectious peritonitis (FIP), feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV), toxoplasmosis, serum banking, rectal culture for salmonella and urinalysis. In addition, prophylactic dental scaling and polishing could be done at the time of the exam, if necessary.

All animals should have a faecal exam at least every six months, or more frequently where needed. Two follow-up exams should be carried at weekly intervals 1-2 weeks post-therapy where a parasite burden is present. Routine monthly treatment for heartworm prevention (Ivermectin) should be performed year-round in endemic regions. Common internal and external parasites and their treatments are listed in Table 6 (page 77).

Vaccination

Whilst there are no vaccines specifically approved for use in non-domestic felids, it is known that the large cat species are susceptible to a number of viral diseases of domestic cats and dogs. It is generally recommended to vaccinate all leopards against feline rhinotracheitis, feline panleukopaenia, feline calicivirus, and FeLV. Most species of large felids are also known to be susceptible to canine distemper virus.

Generally, it is recommended to use killed vaccines rather than modified live vaccines (MLV). MLV generally induce stronger and more persistent antibody responses, however, these vaccines have not been approved specifically for use in this species and may cause the disease that is aimed at preventing. Equally, the efficacy of killed vaccines is unknown.

Figure 48. Vaccines can be administered by subcutaneous injection in young or anaesthetised leopards © Kevin Budd

Vaccines can be administered by subcutaneous hand injection in an anaesthetised or juvenile leopard or by intramuscular injection using a blowpipe in the conscious animal.

A wide variety of vaccine schedules are used in captive exotic felids and opinions differ as to initial vaccine schedules. Vaccines given at 8, 12 and 16 weeks and then boosted annually should provide adequate protection in the majority of cases.

It may be necessary to vaccinate at more frequent intervals (biannually) in cases where inadequate immune response is initiated.

Vaccine reactions do occur (mild malaise, temporary loss of appetite, local swelling, lameness, etc.) and all animals should be observed following

vaccination. Anaphylactic reactions to vaccines are not common but can cause death if not promptly recognised and treated.

Vaccination requirements for rabies vary between countries and are not required at all in many countries that are free of rabies. It should be noted that killed rabies vaccines only should be used in exotic species.

Quarantine

The purpose of quarantine is for detection of those animals that are incubating disease. A simple, standardised protocol for the management of newly arrived felids should be established in all zoological institutions. Precautions must also be taken to minimise the risk of exposure of the quarantine staff to zoonotic diseases.

It is important to remember that although the animal may have been considered free of transmissible diseases at the previous facility, exposure to disease during transport may have occurred (Hinshaw *et. al.* 1996). Previously undetected carriers of disease may also develop clinical disease due to the stress of transport.

Detailed quarantine guidelines have been published by the American Association of Zoos and Aquaria and the American Association of Zoo Veterinarians and are available for download at www.aazv.org.

The quarantine/isolation facility should ideally be separated from the main collection and it is advisable that wastes should also be separately disposed of where possible or burnt. Where an isolated building is not available, close physical contact between resident animals and the quarantined animals should be prevented. Ideally, staff caring for the new arrivals should have no contact with the resident felids. If this is not possible, staff should carry out their routine duties with the resident animals before dealing with those in isolation. Particular care should be taken to use a different set of boots and overalls in the isolation area. Equipment used to feed and water quarantined animals and to clean their enclosures must be used for these animals only. All quarantine equipment should be regularly disinfected with an appropriate disinfectant. Antiseptic foot baths and hand-washes should be available for use on leaving the quarantine enclosure.

It is generally advised that the new arrival should be held in quarantine for a minimum period of 30 days or until treatment for parasites is considered successful (whichever is longer). The length of the quarantine period is also influenced by the incubation periods of the diseases of concern.

Basic procedures that should be performed on admission of all quarantined animals are full physical examination, dental examination, body weight, identification marking, and collection of a blood sample for infectious disease status investigation. Infectious diseases of felids to check for include feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV), feline viral rhinotracheitis, calicivirus, panleukopaenia, coronavirus/feline infectious peritonitis, canine distemper and toxoplasmosis (zoonosis). For herpes (viral rhinotracheitis), calicivirus and toxoplasmosis a carrier state is possible.

It is also recommended to carry out haematological (CBC and PCV) and biochemistry analysis. Comparing blood sample values with the normal range for a species can give a good indication of the health status of the animal. (see Appendix 4). Blood can be easily taken from the jugular, cephalic, saphenous and lateral tail veins. Whole blood for serum and blood in EDTA should be stored at 4°C and analysed within 24 hours. Serum or plasma should be banked and stored in liquid nitrogen, an ultra-freezer (-70°C) or a -20°C freezer that is not self-defrosting. Blood smears are best made and air dried as soon as possible after collection.

Urinalysis should be carried out if possible.

The animal should be treated for ecto- and endoparasites. Faecal samples should be negative for parasites on three consecutive tests 1-2 days apart. Particular emphasis should be placed on eliminating any potential infection with *Echinococcus* spp. tapeworms. Even if the presence of these cestodes is not confirmed, it is advisable to treat new arrivals with praziquantel to kill any adult worms that may be present. Infection of the environment is also a serious hazard but should be prevented through careful quarantine protocols.

Necessary vaccinations are usually administered during quarantine; however, it is important to remember that modified live vaccines may be extremely dangerous when applied to wild animals. It is preferred to use killed vaccines where ever possible.

Post Mortem

Post mortem examination should always be carried out regardless of whether the cause of death has been determined at the time of death. The European Felid TAG necropsy protocol is presented in Appendix 1.

The carcass should be maintained at 4°C and examined as soon as possible after death. Once the carcass starts to decompose a great deal of information is lost. Alternatively, the carcass can be frozen at -20°C. This will preserve the carcass for years but again, a large amount of data can be lost in freezing.

Using a systematic approach to the post mortem, comparative data is collected from each animal and allows abnormalities to be noted and trends to be detected. A specimen post mortem report is attached in the Appendix 1 for noting all findings.

The carcass should be weighed (in kilograms) and measured for morphological studies. Photographs of the right and left lateral sides, the dorsum, the ventrum, the head face-on and the lateral tail should be made.

The coat should be checked for lesions, abscesses, areas of alopecia or the presence of ectoparasites (including fleas, lice, ticks and mites). Any ectoparasites should be collected in an Eppendorf tube in 70% ethanol with a drop of glycerol for identification.

Samples for Research

Collection of biological materials from live animals

Only when the leopard is completely anaesthetised, can the collection begin. The biological materials collected could be blood samples, semen samples, hairs and skin.

The site for blood collection is the cephalic or saphenous veins. The sample should be collected into sterile vacuum tubes with anti-coagulants such as EDTA or ACD. Samples can be stored with the anti-coagulant only, refrigerated and shipped as soon as possible or mixed with an equal volume of TES buffer "Easy Blood", in which case it can be stored for longer periods and kept at room temperature. The preparation of TES buffer "Easy Blood" is detailed in Appendix 2.

For serum samples, the blood should be placed in a centrifuge and spun down. Serum can be stored frozen in the freezer at -20°C or preferably in liquid nitrogen to be kept ultrafrozen at -70°C.

The volume and type of sample should be commensurate with the veterinary purpose with only a small proportion being used for the research purpose.

Simultaneously, semen samples could be collected using electro-ejaculation. Prior to every operation, the prepucial sheath and the penis are cleaned to avoid contamination of the ejaculate. After the ejaculation procedure, the total volume is measured and maintained at body temperature. One or two drops of the sample can be mounted for microscopic evaluation of its quality. This quality evaluation involves assessing percent of sperm motility and rating forward progressive motility.

Three to five microlitres of the ejaculate could be fixed in 100 microlitres of 0.3% glutaraldehydein for analysing morphology.

Tissue biopsy samples are generally collected from the inner thigh region. A 5 × 5cm area of the skin should be surgically prepared by shaving the area and cleaning with betadine solution and later with alcohol to remove excess oils and betadine. A 1 cm^2 piece of skin is surgically excised and stored in suitable transport medium. Cryopreservation is also available for storage of tissue samples.

Collection of biological materials from dead animals

Tissue (such as skeletal muscle, liver, or kidney) can be collected and stored frozen in ethanol or in preservation buffers such as "Easy Blood". If samples are stored in ethanol or "Easy Blood", it is helpful to cut slits in the tissue to allow for better penetration of the fluid. It is important to minimise cross-contamination of samples by using different blades or thoroughly cleaning blades between samples. In case of dead animals with uncertain identification, the entire head can be collected and placed in ethanol for shipment.

For each individual death, a systematic necropsy protocol should be followed. As well as providing data for various ongoing studies (morphological, genetic or others), the necropsy aides in the detection of disease and provides valuable data for collection management.

The European Felid TAG necropsy protocol is attached in Appendix 1. The different tissues that could be collected are listed in section 4 of this appendix.

Important care during the collection of samples:

Be sure that all samples are properly labelled with the case number and the animal identification. All samples should be accompanied by contact information (including phone number and mail address) for personnel in charge of the samples.

To assure the integrity of the samples, gloves should be worn when collecting the samples. Materials should be new, sealed in original wrappers.

References

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Kahn C.M. (Ed). 2005. *Merck Veterinary Manual* Ninth Edition. Merck and Co. Inc., Whitehouse Station, New Jersey, U.S.A.

Meuller, R.H. 2007. *Sarcoptes, Demodex and Otodectes: Treatment Options*.

Plumb D.C. 2005. *Plumb's Veterinary Drug Handbook*. Blackwell Publishing, Iowa, U.S.A.

Tenover, F. C., and J. V. Hirschmann. 1990. *Interpretation of Gram stains and other common microbiologic slide preparations*. The UpJohn Company, Kalamazoo, Michigan.

Shipping and Transportation

Figure 49. Moving leopards at the BCEAW. © Kevin Budd

General welfare

Only animals in good health should be transported. Animals in advanced stages of pregnancy or animals that have recently given birth should not be shipped. Cubs should not be transported until they are fully weaned.

Sedation for transportation is generally not advised unless the animal is severely distressed and likely to cause itself harm during transportation. Sedation should only be carried out under the supervision of an authorized qualified veterinarian. Animals that have been sedated should not travel unsupervised in case of complications arising from the sedation administered.

Each animal should be transported in a separate container in order to prevent stress and aggressive behaviours. Providing crate training and acclimating the animals to be shipped with their transport box will also help to reduce transport stress. Several ways in which animals can be acclimated to their container include leaving the open crate in the enclosure for the animal to investigate, feeding in the open crate and gradually adding front and rear doors once the animal is used to using the crate for feeding.

Animals that have become sick or that have been injured during transport should receive veterinary treatment as soon as possible. A record of any such occurrences should be kept. Any rest periods prescribed by a veterinarian should be complied with.

To avoid cross-infection, and for health and hygiene reasons, human contact with animals should be avoided, and they should not be housed near foodstuffs or in places to which unauthorised persons have access.

Transport Container

The International Air Transport Association (IATA) provides detailed guidelines with regard to container and shipping requirements. The section below summarises some of the more important points to consider when constructing a transport container.

The container (Fig. 43) should be constructed in such a way as to ensure that it is strong enough to house the animal securely and to withstand the handling involved during transport. The frame should be of solid wood or metal bolted/screwed together and should include a spacer bar of 2.5cm for air circulation. The container should be lined with sheet metal or suitable mesh that will not cause damage to the animal. The roof should be solid with ventilation openings. The container should be related to the size of the animal (allowing the animal to stand and sit erect and lie naturally) and should also reflect the welfare and ventilation requirements of the animal. Ventilation openings should provide through ventilation at all levels.

The front should be of mesh of suitable strength, or of metal bars, and so designed and constructed that the animals are not able to come into contact with persons handling the container. Equally, the container should be secured to prevent unauthorised or accidental access to the animal. A burlap or similar cover can be fitted to the front but should be fitted in such a manner that it may be removed if necessary.

Avoid the use of toxic or skin irritant paint or wood preservative.

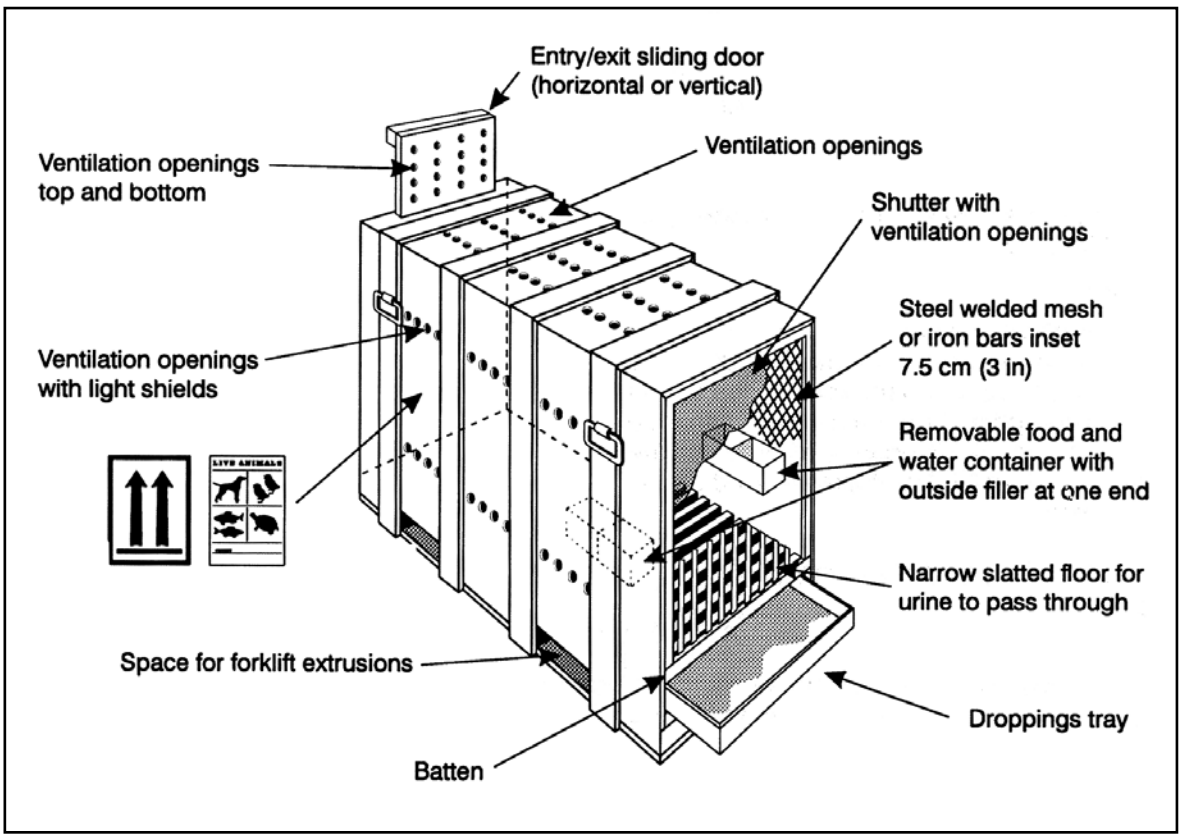


Figure 50. IATA Live Animal Regulations, Container No. 73 for large felids.

Container dimensions should allow the animal to lie down easily and comfortably but should not allow the leopard to turn around. A 10cm (4 inches) clearance space should be provided around the animal in the normal standing position. The height and length of the container should permit the leopard to stand up with the head carried normally. The dimensions will vary according to the status of the animal (cubs, adults etc.).

The floor of the container should be slatted, without the possibility of the animal's feet being trapped between the slats. The floor can be positioned over a removable, waterproof tray with sufficient absorbent material in the tray to catch urine. A catchment tray is not required if absorbent material is used as bedding for the animal. International regulations with regard to importing plant material may limit the choice of bedding that can be used and should be checked prior to transportation.

Feed and water containers should be fitted to the front of the container and should be fixed off the floor. Safe external access must be provided for filling in emergency.

Suitable lifting handles or gripper bars should be provided and, when the container is heavily loaded hooks for crane slings and facilities for handling by fork-lift should also be fitted. Spacer bars of adequate size (2.5cm) should be fitted to all walls, roof and base of the container, to ensure that there is a free flow of air to the animals in the event of stacking or close stowing of cargo.

Labelling and Documentation

Durable and waterproof labels like "LIVE ANIMALS- DO NOT TIP" or/and "THIS WAY UP" should be provided on all sides and the top. The consignor's and consignees' name, address and telephone number must be included. Detailed information should accompany the animal including the number of animals, scientific name and common names used in the exporting and importing countries, the temperature range required, the required diet, feeding and watering instructions, the details of any sedation given, and the official stamp of carrier showing date of his receipt of consignment.

Reference

IATA. 1997. General container requirements. *Live Animals Regulations*. International Air Transport Association, Montreal, Geneva.

Captive Population Management

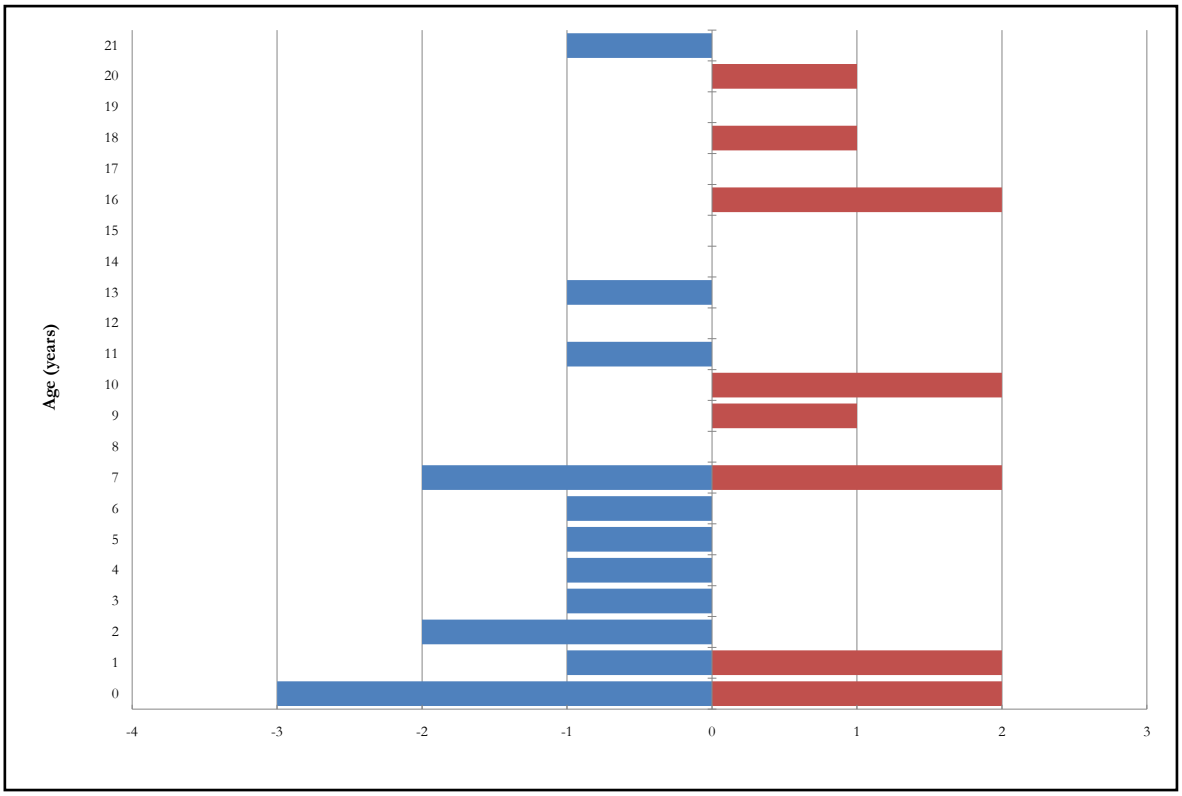


Figure 51. Age Pyramid for Arabian leopard held at BCEAW.

In August 2009 there were 740 leopards listed in ISIS (Table 7), of which 270 (36% of the population) have no subspecies listed or were recorded as hybrids. The remaining purebred leopards (470) represent 12 subspecies. It should be noted that these figures are based on the number of zoological institutions listed in ISIS. Not all *Panthera pardus* spp. holders are listed in ISIS so this data is not representative of the entire population of the leopards but does gives a worldwide overview of the captive population.

Table 7: Number of leopards registered with ISIS (recorded on 27 August 2009).

Leopards listed in ISIS	Males	Females	Unknown	No. of individuals
Panthera pardus	119	141	6	266
Hybrids	2	2	0	4
P. p. delacouri	9	14	0	23
P. p. fusca	1	1	0	2
P. p. japonensis	22	29	0	51
P. p. kotiya	40	32	0	72
P. p. leopardus	2	4	0	6
P. p. ciscaucasicus	2	0	0	2
P. p. melas	2	1	0	3
P. p. nimr	22	13	0	35
P. p. orientalis	83	78	2	163
P. p. pardus	1	2	0	3
P. p. saxicolor	51	51	0	102
P.p. shortridgei	4	2	0	6
TOTAL	**360**	**372**	**8**	**740**

It is immediately obvious that the data in ISIS conflicts with current taxonomic classifications as there are more subspecies listed in ISIS (12) than the accepted nine (Uphyrina *et. al.* 2001). This is due to user interpretation as ISIS does not edit or restrict taxonomic classification when entering data.

In 2008 a survey conducted by Marc Damen found that there are 423 leopards, from eight subspecies and 77 hybrid animals, held in 115 EAZA member institutions. Four subspecies represent 73% of the total captive population in EAZA, namely *P. p. kotiya* (12%) with 51 individuals, *P. p. japonensis* (9%) with 39 leopards, *P. p. saxicolor* (25%) with 104 individuals and *P. p. orientalis* (27%) with 115 leopards. All four of the subspecies are recommended and are currently managed in an European Endangered Species Program (EEP).

A fifth subspecies, the Arabian leopard (*P. p. nimr*), with 28 leopards (9%) is not currently part of an official EEP program but is managed as a monitored population and is recognised as an important subspecies within EAZA.

The remaining 86 leopards (20%) are either not from one of the four recommended subspecies (9) or are hybrids (77). It is recommended the hybrid or leopards of doubtful origin undergo DNA profiling and proved to be hybrids and/or unwanted subspecies they should be phased out, to allow institutions to focus on recommended subspecies.

Species Management Programmes

Leopard
Panthera pardus spp.

International Studbook Keeper: Ms. Olivia Walter, Federation of Zoological Gardens of Great Britain & Ireland.
Register for North Chinese leopard, Sri Lankan leopard, Amur leopard, Persian leopard and Arabian leopard.
conservation.fedzoo@zsl.org

North Chinese leopard
Panthera pardus japonensis

Coordinator & EAZA Studbook Keeper: Dr Michael Flügger, Hamburg
fluegger@hagengeck.de

Sri Lankan leopard
Panthera pardus kotiya

Coordinator & EAZA Studbook Keeper: Mr Thierry Jardin, Lisieux, CERZA Zoo
lisieux@cerza.com

Amur leopard
Panthera pardus orientalis

Coordinator & EAZA Studbook Keeper: Ms. Sarah Christie, London
sarah.christie@zsl.org

AZA Studbook Keeper: Mr. Chris Pfefferkorn, Portland
pfefferkornc@metro.dst.or.us

JAZA Studbook Keeper: Dr. Akira Narita, Sapporo
akira.narita@city.sapporo.jp

Persian leopard
Panthera pardus saxicolor

Coordinator & EAZA Studbook Keeper: Dr Martina Raffel, Munster
raffel@allwetterzoo.de

JAZA Studbook Keeper: Dr Akira Narita, Sapporo
akira.narita@city.sapporo.jp

ARAZPA Studbook Keeper: Mr David Pepper-Edward,
Sydney.dpepper@zoo.nsw.gov.au

Arabian leopard
Panthera pardus nimr

Monitored subspecies

Studbook Keeper: Dr Jane Budd, Breeding Centre for Endangered Arabian Wildlife, Sharjah.
breeding@epaa-shj.gov.ae

Individual identification and marking

Individuals of many species, including leopards, can be identified by their natural marks alone (Rice and Kalk, 1996). Spots, blotches, patterns and colour are genetically controlled, while scars, notches etc. are acquired through the course of life. Using natural markings as an

identification system can however be very subjective and therefore requires careful description and recording (written, photographic or diagrammatic). It is a very useful system to use in small collections with easily distinguished individuals.

An ideal identification system should provide lifelong identification and should be 1) inexpensive, 2) humane, 3) unobtrusive and 4) easy to use. The use of transponders/microchips as a permanent marking system fulfils most of these requirements and is widely used by zoological institutes worldwide. The transponder system consists of 3 components, an implantable identification microchip, an applicator to inject the microchip and a scanner/reader.

Each transponder code provides the animal with unique identification for life. A disadvantage of this identification system is that it has a short reading distance and animals generally require restraint to read the number. Transponder systems manufactured by different companies are not compatible. Standardisation of transponder equipment is an issue that has been addressed within the zoo community (Rice and Kalk, 1996) but has not yet been resolved. Standard implantation sites at the base of the left ear or over the left scapula are advised. Reproductive implants should always be implanted on the animal's right side (Rice and Kalk, 1996).

Newborn animals should be marked/identified as soon as possible but there are some constraints as to when this can be safely done to avoid maternal neglect. To reduce the number of manipulations of suckling cubs, insertion of the transponder can be carried out at the same time as vaccination and deworming.

References

Rice C.G. and Kalk P. 1996. *Identification and Marking Techniques* in: Wild Mammals in Captivity. Chicago University Press, Chicago, USA.

International Species Informations System. August 2009. Retrieved 12 September 2009, from http://app.isis.org/abstracts/abs.asp

Appendix 1

European Felid Tag Necropsy Protocol (July 1998)

Section 1: Animal details

Collection/owner: _____

_____

Address: _____

_____

Subspecies: _____

_____

Studbook N°: _____ ISIS N°: _____

Transponder/Microchip N°: _____

In-house identity: _____ Sex: _____

Date of birth: _____ Age: _____

Origin: _____

Weight: _____

Date of death: _____ Date of necropsy: _____

Necropsy number: _____

Reproductive history: Proven breeder: _____

Contraception: _____

Necropsy performed by: _____

History (include clinical signs and circumstances of death): _____

Clinical history attached? Yes / No

ARKS record attached? Yes / No

Section 2 Gross findings

Fill in details of gross necropsy findings in section below, or circle one of NAD (No Abnormality Detected) or NE (Not Examined).

General condition:
(Nutritional condition, physical condition, condition of carcass – fresh, decomposed etc) NAD / NE

Skin:
(NB: In the case of a neonate, examine the umbilical stump and surrounding tissues) NAD / NE

Musculoskeletal system:
(Bones, joints, muscles) NAD / NE

Body cavity:
(Fat stores, abnormal fluids) NAD / NE

Lymphoreticular system:
(Spleen, lymph nodes, lymphatic, thymus) NAD / NE

Respiratory system:
(Nasal cavity, larynx, trachea, lungs, regional LN's. In neonates note whether lungs float or sink in formal saline) NAD / NE

Cardiovascular system:
(Heart, pericardium, blood vessels) NAD / NE

Digestive system:
(Mouth, teeth, oesophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes. In the case of a neonate, note whether milk is present in the stomach). NAD / NE

Urinary system:
(Kidneys, ureters, urinary bladders, urethra) NAD / NE

Reproductive system:
(Testes/ovaries, uterus, vagina, penis, prepuce, accessory glands, mammary glands, placenta) NAD / NE

Endocrine system:
(Adrenals, thyroid, parathyroid, pituitary) NAD / NE

Nervous system:
(Brain, spinal cord, peripheral nerves) NAD / NE

Sensory organs:
(Ears, eyes) NAD / NE

Section 3: Laboratory Tests and Diagnoses

Laboratory Tests:
Give details of all specimens submitted for bacteriology, virology, parasitology and histopathology. Please attach reports of these tests to the completed form.

Bacteriology: Report attached Yes / No

Virology: Report attached Yes / No

Parasitology: Report attached Yes / No

Histopathology: Report attached Yes / No

Other: (Specify) Report attached Yes / No

Preliminary diagnosis:

FINAL DIAGNOSIS:

Section 4: Tissues for storage.

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

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

Tissues	Area	Taken (Yes / No)
Adrenal:	Entire gland with transverse cut.	
Brain:	Sliced longitudinally along midline.	
Heart:	Longitudinal section of atrium, ventricle and valves from each side.	
Intestines:	Duodenum, jejunum, ileum, caecum, colon. Open along long axis.	
Kidney:	Section of cortex, medulla and pelvis from each kidney.	
Liver:	2 sections from 2 lobes with capsule and gall bladder.	
Lung:	Sections from several lobes including a bronchus	
Lymph nodes:	Cervical, anterior mediastinal, bronchial, mesenteric and lumbar with a transverse cut.	
Pancreas:	Samples from 2 areas.	
Peripheral nerve:	3cm section of sciatic nerve.	
Skeletal muscle:	Cross section of thigh muscle.	
Skin:	3cm length of full thickness abdominal skin.	
Spleen:	Cross section including capsule.	
Spinal cord:	Section from cervical, thoracic and lumbar cord.	
Stomach:	Cardia, antrum and pylorus.	
Testis/Ovaries:	Entire with transverse cut	
Thyroid:	Intact including parathyroid	
Urinary bladder:	Cross section.	
Uterus:	Entire with longitudinal cut into lumen.	

Details of other tissues stored:

Appendix 2

TES "Easy Blood" BUFFER FOR DNA

TES "EASY BLOOD" buffer for DNA:
100mM Tris, 100mM EDTA, 2% SDS.

This buffer, when mixed in equal parts with fresh blood (collected into an anticoagulant, preferably EDTA), will lyse the red and white blood cells but protect the DNA and inhibit the nucleases and micro organisms. This solution is used in field situations where no centrifugation or refrigeration is available. Once the samples are back in the laboratory, refrigeration or freezing is recommended for long term storage.

(1) From stock solutions:	for 100ml	for 500ml
Water	50ml	250ml
0,5M Tris HCl pH 7,5	20ml	100ml
0,5M Na2 EDTA	20ml	100ml
20% SDS	10ml	50ml

Appropriate quantities can be dispensed into vials for transport to the study site; Use a large enough vial to allow room to add an equal volume of blood.

(2) From dry chemicals:

Tris base	(MW=121,2)	1,2gm/100cc
EDTA Na2	(MW=372,2)	3,7gm/100cc
2% SDS		2,0gm/100cc

Add water to final volume of 100cc
(The resulting pH will be around 8,0)

Alternatively, the components can be weighed into plastic vials for transport and latex mixed with the appropriate amount of water at the study site. Be careful with this because it requires weighing out microgram amounts of each chemical (enough for 2 - 5ml/vial).

Appendix 3

Weights to two hand-reared female Sri Lankan leopards at the CERZA Zoo.

Day	Shaven Weight (g)	Unshaven Weight (g)			
1	400	425	29	2110	2120
2	585	610	30	2150	2160
3	680	710	31	2190	2200
4	850	880	32	2200	2230
5	775	785	33	2250	2230
6	950	1010	34	2300	2390
7	930	1025	37	2520	2430
8	895	1005	40	2740	2705
9	980	1130	43	3140	3010
10	1050	1160	46	3130	3330
11	1110	1200	49	3750	3510
12	1165	1230	52	4000	3790
13	1170	1230	55	4450	3890
14	1230	1300	58	4750	4390
15	1370	1420	61	5100	4700
16	1500	1560	64	5500	5170
17	1510	1570	67	5900	5700
18	1550	1620	70	6120	6200
19	1580	1645	77	6400	6350
20	1600	1640	80	6600	6720
21	1650	1700	83	6690	6990
22	1700	1800	86	6750	7050
23	1810	1825	89	6950	7150
24	1850	1850	92	7250	7300
25	2000	1900	95	7460	7450
26	2000	1950	98	7780	7730
27	1970	1990	101	8200	8100
28	2000	2050			

Appendix 4

Normal Blood Values of a leopard (*Panthera pardus* spp.)

	He	Ca	P	Na	K	Cl	Fe	Mg	Cr	UA	GL	CH	TR
Panthera pardus spp.	124	2.5	1.7	152	4.0	119	22	0.7	212	0.01	7.2	4.5	0.3

He: Haemoglobin
Ca: Calcium
P: Phosphorus
Na: Sodium
K: Potassium
Cl: Chloride
Fe: Iron
Mg: Magnesium
Cr: Creatinine
UA: Uric Acid
GL: Glucose
CH: Cholesterol
TR: Triglyceride

Appendix 5

Composition of different milk used in zoological institutions.

Appendix 5.1: Maternal milk composition.
Appendix 5.2: Esbilac composition.
Appendix 5.3: KMR feline milk composition.
Appendix 5.4: Pet-Ag's zoological milk matrix composition.
Appendix 5.5: Milkodog composition.

Appendix 5.1:

Maternal milk composition

Here are two composition of the leopards' milk. One is calculated in the fed basis and the other in the dry matter basis.

Fed basis:	
Dry matter	19,4%
Crude protein	11,1%
Crude fat	6,5%
Carbohydrates	4,2%

Dry matter basis:	
Solids	19,6%
Crude protein	57,2%
Crude fat	33,5%
Carbohydrates	21,6%

Appendix 5.2:

Esbilac composition (www.petag.com)

H210
Esbilac® (Powder and Liquid)

Product Description and Indications for Use: This is a milk replacement for puppies or a nutritional supplement for older dogs and nursing mothers.

Dosage and Administration: Mix one measure of powdered Esbilac with two measures of warm water. Warm reconstituted Esbilac to room or body temperature and feed 2 tablespoons (30ml) per 4 oz.(115g) of body weight daily. Small or weak puppies should be fed every 3 - 4 hours, and larger or older puppies should be fed every 8 hours. The daily feeding amount should be divided into equal portions for each feeding. Increase or decrease the amount fed to meet the individual requirements for each puppy. Weigh each puppy daily to be sure it is gaining weight and being fed enough. When feeding liquid Esbilac, the volume to feed puppies is the same as the reconstituted powder.

Nutritional information:

Crude protein	33%
Crude fat	40%
Crude fiber	0%
Ash	7.75%
Moisture	5%

Pregnant and lactating mothers should be fed 2 teaspoons of Esbilac powder or 2 tablespoons of Esbilac liquid per 5lbs. of body weight every day until 2 weeks after whelping. Growing puppies, show animals, and older dogs should receive 1 teaspoon Esbilac powder or 1 tablespoon of Esbilac liquid per 5lbs. body weight daily.

Packaging: Powdered Esbilac comes in 12 and 28oz. cans, a ¾oz. pouch, and in 5lb. bags. Liquid Esbilac comes in 8 and 12.5oz. cans.

Precautions and Side Effects: If possible, all puppies should first receive the mother's milk for at least two days. The mother's natural colostrum provides extra nutrition and temporary immunity from certain diseases that are not provided in supplements.

Storage and Shelf Life: Shake liquid Esbilac before use, store it in the refrigerator after opening, discard it after 72 hours, and do not freeze it. Powdered Esbilac may be refrigerated for 3 months. If the powder has been reconstituted, it can be refrigerated for up to 24 hours.

Drug Type: O-T-C

Manufacturer: Pet-Ag

Appendix 5.3:

Composition of KMR milk replacer.

KMR® Powder for Kittens

Ingredients: Whey Protein Concentrate, Casein, Dried Skimmed Milk, Vegetable Oil, Butter Fat, Corn Syrup Solids, Egg Yolk, Monocalcium Phosphate Lactose, L-arginine, Lechithin, Calcium Carbonate, Potassium Chloride, Choline Chloride, Potassium Phosphate Monobasic, Dicalcium Phosphate, Magnesium Carbonate, Taurine, Potassium Phosphate Dibasic, Magnesium Sulfate, Ferrous Sulfate, Vitamin E Supplement, Zinc Sulfate, Dipotassium Phosphate, Silico Aluminate, Niacin Supplement, Ascorbic Acid, Copper Sulfate, Vitamin A Supplement, Vitamin B12 Supplement, Calcium Pantothnate Manganese Sulfate Vitamin D3 Supplement, Ethylenediamine Dihydroiodide, Folic Acid, Riboflavin, Thiamine Hydrochloride, Pyroxine Hydrochloride, Biotin, and Mono and Diglycerides.

Nutritional Information:

Guaranteed Analysis

Crude Protein	40.0% Min
Crude Fiber	0.0% Max
Crude Fat	27.0% Min
Ash	7.0% Max
Moisture	5.0% Max

KMR® Liquid for Kittens

Ingredients: Water, Skimmed Milk, Soy Oil, Sodium Caseinate, Calcium Caseinate, Butter, Egg Yolk, Lecithin, Calcium Carbonate Precipitated, L-arginine, Potassium Chloride, Potassium Phosphate Monobasic, Choline Chloride, Magnesium Sulfate, Carrageenan, Potassium Phosphate Dibasic, Ascorbic Acid, Taurine, Ferrous Sulfate, Zinc Sulfate, Vitamin E Supplement, Vitamin A Supplement, Copper Sulfate, Niacin Supplement, Calcium Pantothenate, Vitamin B12 Supplement, Manganese Sulfate, Thiamine Hydrochloride, Riboflavin, Vitamin D3 Supplement, Folic Acid, Potassium Iodide, Pyridoxine Hydrochloride, Potassium Citrate.

Appendix 5.4:

Pet-Ag's zoological milk matrix composition

MILK MATRIX 42/25 Typical Nutritional Analysis			
Composition per 100 grams powder			
Energy kcal	461.0	Fiber, g	0.0
Protein g	45.2	Moisture g	2.7
Fat g	28.6	Ash g	6.3
Carbohydrate g	17.0		
Minerals per 100 grams powder			
Calcium mg	1320.0	Iron mg	4.4
Phosphorus mg	1000.0	Copper mg	1.4
Potassium mg	710.0	Zinc mg	8.0
Sodium mg	450.0	Manganese mg	1.2
Magnesium mg	70.0	Total Chloride mg	808.0
Vitamins per 100 grams powder			
Vitamin A I.U.	3238.0	Pantothenic Acid mg	3.8
Vitamin D3 I.U.	782.0	Vitamin B6 mg	0.4
Vitamin E I.U.	11.0	Choline mg	210.0
Thiamin mg	0.6	Folic Acid mg	0.8
Riboflavin mg	1.4	Vitamin B12 mcg	3.6
Niacin mg	4.9	Biotin mcg	14.0
Amino Acids per 100 grams powder			
Lysine g	3.11	Valine g	2.64
Arginine g	2.00	Histidine g	1.19
Methionine g	1.26	Alanine g	1.36
Cystine g	0.39	Aspartic Acid g	3.65
Tryptophane g	0.55	Glutamic Acid g	9.84
Threonine g	1.93	Glycine g	0.82
Isoleucine g	2.08	Proline g	4.89
Leucine g	3.80	Serine g	2.52
Phenylalanine g	2.03	Tryosine g	2.13
		Taurine g	0.04
Fatty Acids per 100 grams powder			
8:0 Caprylic Acid g	<1.0	16:1 Palmitoleic Acid g	0.00
10:0 Capric Acid g	<1.0	18:0 Stearic Acid g	1.22
12:0 Lauric Acid g	2.81	18:1 Oleic Acid g	6.84
14:0 Myristic Acid g	0.84	18:2 Linoleic Acid g	10.79
16:0 Palmitic Acid g	4.09	18:3 Linolenic Acid g	1.33
Guaranteed Analysis			
Crude Protein %, min.	42.0	Moisture %, max.	5.0
Crude Fat %, min	25.0	Ash %, max	7.0
Crude Fiber %, max.	0.0		

INGREDIENTS

Vegetable oil (preserved with BHA. BHT, propyl gallate and citric acid), casein, dried skimmed milk, egg yolk, calcium carbonate precipitated, potassium phosphate monobasic, l-arginine, dried corn syrup, calcium hydroxide, salt, lecithin, monocalcium phosphate, sodium hydroxide, choline chloride, potassium chloride, magnesium carbonate, taurine, magnesium sulfate, vitamin A supplement, zinc sulfate, vitamin E supplement, iron sulfate, niacin supplement, copper sulfate, calcium pantothenate, vitamin B12 supplement, manganese sulfate, vitamin D3 supplement, folic acid, riboflavin, thiamine hydrochloride, calcium iodate, pyridoxine hydrochloride.

Zoologic A product line of PetAg, Inc. 1-800-323-0877

MILK MATRIX 30/55 Typical Nutritional Analysis

Composition per 100 grams powder

Energy kcal	595.0	Fiber, g	0.0
Protein g	31.3	Moisture g	3.6
Fat g	55.8	Ash g	7.0
Carbohydrate g	3.1		

Minerals per 100 grams powder

Calcium mg	1090.0	Iron mg	11.0
Phosphorus mg	753.0	Copper mg	1.0
Potassium mg	805.0	Zinc mg	6.0
Sodium mg	575.0	Manganese mg	1.4
Magnesium mg	88.0	Total Chloride mg	741.0

Vitamins per 100 grams powder

Vitamin A I.U.	3656.0	Pantothenic Acid mg	7.0
Vitamin D3 I.U.	1023.0	Vitamin B6 mg	0.4
Vitamin E I.U.	34.4	Choline mg	498.0
Thiamin mg	0.4	Folic Acid mg	0.3
Riboflavin mg	0.9	Vitamin B12 mcg	6.0
Niacin mg	7.6	Biotin mcg	31.1

Amino Acids per 100 grams powder

Lysine g	2.15	Valine g	1.85
Arginine g	0.99	Histidine g	0.86
Methionine g	1.08	Alanine g	0.60
Cystine g	0.11	Aspartic Acid g	2.39
Tryptophane g	0.38	Glutamic Acid g	6.01
Threonine g	1.32	Glycine g	0.58
Isoleucine g	1.47	Proline g	3.07
Leucine g	2.41	Serine g	1.65
Phenylalanine g	1.55	Tryosine g	1.39

Fatty Acids per 100 grams powder

8:0 Caprylic Acid g	0.02	16:1 Palmitoleic Acid g	1.57
10:0 Capric Acid g	0.06	18:0 Stearic Acid g	6.94
12:0 Lauric Acid g	0.10	18:1 Oleic Acid g	23.31
14:0 Myristic Acid g	0.78	18:2 Linoleic Acid g	4.91
16:0 Palmitic Acid g	12.71	18:3 Linolenic Acid g	0.20

Guaranteed Analysis

Crude Protein %, min.	30.0	Moisture %, max.	5.0
Crude Fat %, min	55.0	Ash %, max	8.0
Crude Fiber %, max.	0.25		

INGREDIENTS

Animal fat (preserved with BHA and citric acid), casein, dicalcium phosphate, condensed whey, vegetable oil, calcium carbonate, lecithin, potassium chloride, choline chloride, magnesium sulfate, vitamin E supplement, vitamin A supplement, zinc methionine, ferrous sulfate, calcium pantothenate, vitamin B12 supplement, niacin supplement, manganese sulfate, copper sulfate, vitamin D3 supplement, riboflavin, thiamine mononitrate, pyridoxine hydrochloride, menadione sodium bisulfite complex, folic acid, calcium iodate, biotin, sodium selenite, mono and diglycerides.

Zoologic A product line of PetAg, Inc. 1-800-323-0877

Appendix 5.5:

Composition of Milkodog

Milk composition: per 100 g (Milkodog)

Crude protein	28%
Crude fat	26%
Crude ash	5.8%
Crude cellulose	2%
Humidity	3.5%
Iron	1.5mg
Copper	0.03mg
Zinc	3.5mg
Vitamin A	1000UI
Vitamin D_3	800UI
Vitamin E	40UI
Vitamin B_1	0.25mg

(Source: Plan d'élevage artificiel pou des panthères du Sri Lanka. CERZA.)

Appendix 6

Composition of different vitamin and mineral supplements used in zoological institutions.

Appendix 6.1:

Composition of the SA-37 vitamins and minerals supplement

Product Class: Vitamins, minerals, trace elements
Target Species: Cats, dogs and cage birds.
Active ingredients: Vitamins
Description: Feed supplement containing vitamins, minerals, essential fatty acids, proteins, oil and cellulose to keep dogs and cats in good condition. Available in a formulation with or without probiotics.
Formulation: Tablet or powder.
Indications: To be given during the periods of stress or convalescence and in growing puppies, kittens and in pregnant or lactating bitches and cats.
Method of administration: By oral route mixed through the food.
Presentation: Jars containing 100 or 250 tablets, or 250g or 1kg. Powder each.
Storage: At room temperature.

Composition:	**Per tablet**	**Per gram powder**
Vitamins:		
Vitamin A	1550IU	800IU
Vitamin B complex		
Vitamin B1 (Thiamine)	180µg	100µg
Vitamin B2 (Riboflavine)	450µg	250µg
D-calcium pantothenate	375µg	200µg
Nicotinic acid (Niacin)	1.9mg	1mg
Folic acid	30µg	20µg
Biotin	3.6µg	2µg
Vitamin B6 (pyridoxyne hydrochloride)	195µg	100µg
Vitamin B12	6.5µg	3.6µg
Vitamin C (Ascorbic acid)	11.0mg	6.0mg
Vitamin D3	155IU	80IU
Vitamin E	803µg	550µg
Vitamin K3	5.3mg	2.8mg
Minerals:		
Calcium (Calcium propionate)	10.4mg	5.8mg
Phosphorus (Calcium hydrogen phosphate)	18mg	10mg
Potassium (Potassium chloride)	3.4mg	1.9mg
Iron (Ferrous carbonate)	18.9mg	10.5mg
Iodine (Potassium iodide)	225µg	120µg
Copper (Copper sulphate)	2.25mg	1.2mg
Cobalt (Cobalt sulphate)	330µg	180µg
Manganese (Manganese sulphate)	1.26mg	700µg
Zinc (Zinc oxide)	540µg	300µg
Micro-nutrients:		
Choline chloride	22mg	12mg
Lecithin	84mg	41mg
Micro-organisms:	30 million cfu	20 million cfu
Anti-oxydants:		
Ethoxyquine	2.8µg	1.4µg
BHT	180µg	100µg

Appendix 6.2:

Composition of CARMIX supplemen

Carmix: Vitamin and Mineral concentrate for meat eating mammals, birds and reptiles

Ground products used to produce Carmix include opened grains, vegetable oils, dairy products, inactivated yeast, vitamins, minerals and trace elements.

Average Analysis:

Raw Protein	18.5%
Raw Fat	10.5%
Ash	30%
Moisture	5.5%
Carbohydrates	35%

Vitamins per kg:

Vitamin A	275.00IU
Vitamin D3	45.00IU
Vitamin E	500mg
Vitamin C	1.000mg
Vitamin B1	200mg
Vitamin B2	200mg
Vitamin B12	0.4mg
d-Pantothenic acid	650mg
Nicotinic acid	725mg
Biotin	5mg
Folic acid	22mg
Vitamin B6	200mg
Methionine	100mg
Choline	5.550mg

Minerals and trace elements per kg :

Calcium	115g
Phosphor	3.5g
Magnesium	5.5g
Sodium	1.6g
Potassium	8.0g
Iron	375mg
Copper	150mg
Zinc	780mg
Mangenese	980mg
Cobalt	8.5mg
Iodine	45mg

Carmix is fed at 2% equivalent of the total ration fed, a 500g ration would therefore be fed with 10g of Carmix sprinkled on to the meat.

Appendix 6.3:

Composition of Osteo-Form supplement

Osteo-Form

Advantages

- Calcium-phosphorus ratio of 1.8 to 1 to aid in correcting deficiencies without excess phosphorus
- Vitamins A, D and C, all involved in bone and cartilage metabolism
- Aids in healing fractures
- For young puppies of large breeds during rapid growth periods
- Ideal for pregnant and lactating bitches

VET-A-MIX offers a complete line of quality, research-proven chewable nutritional supplements and drug dosage forms. These tablets are formulated in a palatable flavored base and are readily, even eagerly, accepted by dogs and cats.

Vet-A-Mix products are available only through licensed veterinarians.

Calcium and phosphorous supplement for dogs and cats.

VET-A-MIX®

LLOYD, Inc.
800-831-0004
www.lloydinc.com

Osteo-Form

Indications:
For use as an aid in the prevention of dietary deficiencies of calcium, phosphorus and vitamins A and D in dogs and cats.

Administration:
Feed free choice, from the hand, or crumble and mix into the food.

Dosage: As an aid in the prevention of dietary deficiencies, give 1 to 2 tablets per 5 kilograms (11 pounds) of body weight per day.

Note: Two Osteo-Form chewable tablets are equivalent to one slightly rounded teaspoonful of Osteo-Form SA Powder

Caution:
When using Osteo-Form chewable tablets as a maintenance dietary supplement, calcium and phosphorus from other food sources should be considered.

1103

Guaranteed Analysis per Tablet:
(All values are minimum quantities unless otherwise stated.)
Minerals:
Calcium (min 18.5% - max 22.2%) . . . . . 600 mg
Phosphorus (min 10.0%) . . . . . 335 mg
Vitamins:
Vitamin A . . . . . 750 IU
Vitamin D_3 . . . . . 75 IU

Ingredients:
Dicalcium phosphate, calcium carbonate, torula dried yeast, extracted glandular meal, bone ash, lactose, starch propylene glycol, animal liver meal, bone meal steamed, magnesium stearate, ascorbic acid, vitamin A palmitate, ethoxyquin, cholecalciferol, BHT.

KEEP OUT OF REACH OF CHILDREN

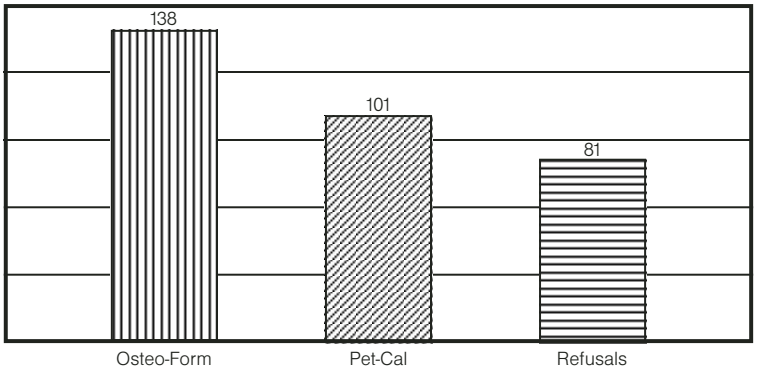
VET A MIX®

P. O. Box 130 • Shenandoah, Iowa 51601 U.S.A.
800-831-0004 • www.lloydinc.com

Compare Osteo-Form to the Pet-Cal Formulation

Ingredient	Vet-A-Mix Osteo-Form	SK Beecham Pet-Cal®
Calcium	600	600
Phosphorus	335	464
Vitamin A	750	—
Vitamin D_3	75	400
Vitamin C	10	—

Canine Palatibility Comparison



Number of tablets accepted free choice during a twenty-day palatability trial

Appendix 6.4:

Composition of Spectrall Plus supplement

Manufactured for:

PRN PHARMACAL

SPECTRALL PLUS™

Directions:
Dogs and Cats: 1 teaspoon per 20 lbs. of body weight daily. For dogs and cats 10 lbs. and under: ½ teaspoon daily.

Ingredients:		
Each ounce contains		
Unsaturated Fatty Acids	**25%**	**Riboflavin** **2 mg**
Dried Food Yeast	**25%**	**Thiamine** **45 mcg**
Dried Milk Solids	**25%**	**Vitamin B12** **8 mcg**
Biotin	**0.6 mg**	**Ascorbic Acid** **60 mg**
Vitamin A	**3,500 I.U.**	**Folic Acid** **0.4 mg**
Vitamin D	**1,750 I.U.**	**Iodine** **150 mcg**
Vitamin E	**10 I.U.**	**Iron** **18 mg**
Choline	**60 mg**	**Copper Gluconate** **2 mg**
Niacin	**12 mg**	**Zinc Gluconate** **210 mg**
Inositol	**6 mg**	**Chlorpheniramine Maleate** **2 mg**
Pantothenic Acid	**3 mg**	

Net weight 12 oz. per can, 12 cans per case.

Keep out of reach of children.
For veterinary use only.

Appendix 6.5:

Composition of Raubtierzusatzmehl supplement

Raubtierzusatzmehl

„Raubtierzusatzmehl" Ergänzungsfuttermittel, Wirkstoffkonzentrat – Raubtiere / Fleischfresser

Gehalt an Inhaltsstoffen:
24,0 % Rohprotein
20, 0 % Rohasche
6,0 % Rohfaser
5,0 % Rohfett

Gehalt an Zusatzstoffen je kg:

100.000 IE Vitamin A	1.500 mg Cholinchloid
8.000 IE Vitamin D3	277 mg Pantothensäure
2.800 mg Vitamin E	476 mg Nikotinsäure
35 mg Vitamin B1	29 mg Folsäure
100 mg Vitamin B1	9.000 mcg Biotin
1.730 mcg Vitamin B12	110 mg Kupfer

Zusamensetzung:
Weizenmehl; Biertreber, Bierhefe, BHT, pflanzl. Raff. Fette; Monodicalciumphosphat, ELWANA-Vormischung: Spurenelemente, Vitamine
Fütterungsempfehlung: Etwa 7% der Gesamtration beimischen und über die Fleischstücke streuen:
Fütterungshinweis: Dieses Ergänzungsfuttermittel darf wegen der gegenüber Alleinfuttermittel höheren Gehalte an Vit. D3 nur an Flesichfresser bis zu 20% Tagesration verfüttert werden.

Hersteller: ELWANA Biochemie GmbH
Schweriner Str. 35, 19306 Neustadt – Glewe
Tel/Fax: 038757-22313

Appendix 6.6:

Composition of the Fel-Titan Vitamins and Minerals supplements (per tablespoon).

Major Minerals :	
Calcium	58mg
Phosphorus	450mg
Sodium	125mg
Chlorine	
Magnesium	
Potassium	
Trace minerals :	
Iron	20mg
Copper	4mg
Manganese	15mg
Zinc	20mg
Cobalt	0.125mg
Iodine	0.2mg
Selenium	0.045mg
Taurin	40mg
Karnitin	17.5 mg
Fluorine	
Vitamins :	
Retinol	
Vit A	2 500 000 IU
Cholecalciferol	
D3	250 000 IU
D1 Alphatocopherol	
E	52.5mg
B1 (Thiamin)	2.5mg
B2 (Riboflavin)	2.25mg
B3 (Niacin)	
B6 (Pyridoxine)	1.5mg
B12	0.025mg
C (Ascorbic Acid)	13.75mg
K3	0.4mg
Folic Acid	1.25mg
Nicotinic Acid	15.0mg
Pantothenic Acid	7.5mg
Choline	500mg
Inositol	
Biotin	0.55mg
p-Aminobenzoic Acid	
Beta-Carotene	

Appendix 6.7:

Composition of the Diafarm Maintenance Cat supplement (per kg).

Major Minerals:	
Calcium	0.0135mg
Phosphorus	0.01mg
Sodium	0.0044mg
Chlorine	
Magnesium	0.0008mg
Potassium	0.0036mg
Trace minerals:	
Iron	120mg
Copper	6mg
Manganese	50mg
Zinc	104mg
Cobalt	4mg
Iodine	2mg
Selenium	0.30 mg
Taurin	
Karnitin	
Fluorine	
Vitamins :	
Retinol	
Vit A	17 000 IU
Cholecalciferol	
D3	2 000 IU
D1 Alphatocopherol	
E	110mg
B1 (Thiamin)	8mg
B2 (Riboflavin)	9mg
B3 (Niacin)	
B6 (Pyridoxine)	7.2mg
B12	0.055mg
C (Ascorbic Acid)	850mg
K3	2mg
Folic Acid	1.50 mg
Nicotinic Acid	80mg
Pantothenic Acid	32mg
Choline	479mg
Inositol	
Biotin	0.70 mg
p-Aminobenzoic Acid	
Beta-Carotene	

Appendix 6.8:

Composition of the Kolmarden vitamineral blandning

Kolmårdens Djurpark, *Vet. B.O.Röken, 95-06-14*

MINERAL-VITAMIN-TILLSKOTT ÅT KÖTTÄTANDE DJUR

Pulverform		Rovdjursbl.	Rovdjursbl.	Kafomavit
Analysgaranti	*per ml*	*Kvarnby*	*Kvarnby*	*Dogman*
		per ml	*ml*	*per 1,3g*
Mineraler		*Gammal*	**Ny!!**	
		t.o.m. 1994	fr.o.m. 95-06	
Kalcium	*mg*	250	250	260
Fosfor	*mg*	100	100	130
Ca/P-kvot		2,5	2,5	2
Magnesium	*mg*	2	2	0
Vitaminer				
Vit A	*IE*	400	400	250
Vit D_3	*IE*	20	20	27
Vit E	*mg*	2	2	1,5
Vit B_1	*mg*	0,2	0,2	0,1
Vit B_{12}	*mikrog*	1	1	0
Vit C			10	0
Div B-vit	*mikrog*	0	0	1315
Spårelement				
Kobolt	*mikrog*	10	10	9
Koppar	*mikrog*	500	500	29
Mangan	*mikrog*	500	500	230
Zink	*mikrog*	1000	1000	570
Jod	*mikrog*	200	200	0
Se	*mikrog*	20	20	15
Fe	*mikrog*	0	0	15
Aminosyror				
Taurin	*mg*	0	20	0
Densitet	*g/l*	1,1	1,1	?
Pris	*kl moms*		30:-, 600:-	471:-/5 kg
Förpackning		30 kg	1 kg, 30 kg	5 kg

Dos: *Köttätande djur o fåglar: 1 ml per 100 g kött, 10 ml per kg kött!* **Obs!** *Rovdjursblandning saknar Fe och koksalt!*

This supplement contains no salt or iron.

Appendix 6.9:

Composition of the Mazuri Carnivore Supplements

Mazuri® Carnivore Supplement 57UX

With vitamin B_{12} & Taurine
(Available at our TestDiet Unit (765)966-1885/info@testdiet.com)

Description
For all carnivorous species in need of added taurine. To supplement raw meat diets.

Product Form
Meal

Catalog #0008081

Approximate Nutrient Composition

Taurine, %	9.9
Calcium, %	35.8
Iodine, ppm	4.6
Thiamin, ppm	13
Riboflavin, ppm	10
Niacin, ppm	40
Pantothenic Acid, ppm	60
Folic Acid, ppm	3.0
Pyridoxine, ppm	9.8
Vitamin B_{12}, mcg/kg	100
Vitamin A, IU/kg	449,000
Vitamin D_3 (added), IU/kg	3,000
Vitamin E, IU/kg	300
Vitamin K (as menadione), ppm	1.5

Ingredients
Calcium carbonate, taurine, monosodium glutamate, vitamin A acetate, dl-alpha tocopheryl acetate (source of vitamin E), menadione dimethylpyrimidinol bisulfite (vitamin K), vitamin B_{12} supplement, calcium pantothenate, nicotinic acid, thiamin mononitrate, pyridoxine hydrochloride, riboflavin, calcium iodate, cholecalciferol (vitamin D_3), folic acid.

Directions for use
Supplement at a rate of 3 to 5 grams Mazuri Carnivore Supplement, 57UX, per 100 pounds body weight per day (1 to 1 2/3 teaspoons per 100 pounds of body weight per day or 1/4 to 1/2 teaspoon per 25 pounds of body weight per day). Supplementation level is somewhat dependent on the raw meat diet. Diets which include whole animals and/or organ meat may be supplemented with 3 grams per 100 pounds body weight per day. Diets which are primarily composed of skeletal muscle should be supplemented with 5 grams per 100 pounds body weight per day.

Average Feed Weights

Measurement	Kg of Diet
1 tsp	2.93
1 tbsp	8.91
¼ cup	32.4
½ cup	64.2
1 cup	131.9

Quality Controlled by PMI Nutrition International, a subsidiary of America's oldest and largest animal nutrition company.

Nutrient composition is based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analyses will vary accordingly.

Mazuri® Carnivore Supplement 58QC

For Slab Meat
(Available at our TestDiet Unit (765)966-1885/info@testdiet.com)

Description
For all carnivorous species. To supplement raw meat diets without bones.

Product Form
Meal **Catalog #0053173**

Approximate Nutrient Composition

Taurine, %	5.0
Calcium, %	19.2
Zinc, ppm	1,200
Manganese, ppm	150
Copper, ppm	160
Iodine, ppm	20
Iron, ppm	500
Thiamin, ppm	200
Riboflavin, ppm	200
Niacin, ppm	500
Pantothenic Acid, ppm	125
Folic Acid, ppm	16
Pyridoxine, ppm	200
Biotin, ppm	5.0
Vitamin A, IU/kg	198,000
Vitamin D3 (added), IU/kg	40,000
Vitamin E, IU/kg	8,000
Vitamin C, ppm	5,000
Vitamin K (as menadione), ppm	50

Ingredients
Calcium carbonate, cooked chicken, taurine, menadione dimethylpyrimidinol bisulfite (vitamin K), dl-alpha tocopheryl acetate (vitamin E), l-ascorbyl-2-polyphosphate (vitamin C), zinc sulfate, copper sulfate, nicotinic acid, vitamin A acetate, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, calcium pantothenate, cholecalciferol (vitamin D_3), calcium iodate, folic acid, biotin.

Directions for use
Mazuri Carnivore Supplement for Slab Meat is designed to be added at 2.0% of wet weight of slab meat (without bone).

Average Feed Weights

Measurement	Kg of Diet
1 tsp	3.44
1 tbsp	9.11
¼ cup	35.5
½ cup	75.7
1 cup	135.2

Quality Controlled by PMI Nutrition International, a subsidiary of America's oldest and largest animal nutrition company.

Nutrient composition is based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analyses will vary accordingly.

Mazuri® Carnivore Supplement 58QB

For Whole Prey
(Available at our TestDiet Unit (765)966-1885/info@testdiet.com)

Description
For all carnivorous species. To supplement raw meat diets that contain bones.

Product Form
Meal **Catalog #0053172**

Approximate Nutrient Composition

Taurine, %	20
Zinc, ppm	4,800
Manganese, ppm	600
Copper, ppm	640
Iodine, ppm	80
Thiamin, ppm	800
Riboflavin, ppm	800
Niacin, ppm	2,000
Pantothenic Acid, ppm	500
Folic Acid, ppm	64
Pyridoxine, ppm	800
Biotin, ppm	20
Vitamin C, ppm	20,000
Vitamin A, IU/kg	800,000
Vitamin D_3 (added), IU/kg	300,000
Vitamin E, IU/kg	31,500
Vitamin K (as menadione), ppm	200

Ingredients
Cooked chicken, taurine, menadione dimethylpyrimidinol bisulfite (vitamin K), dl-alpha tocopheryl acetate (vitamin E), l-ascorbyl-2-polyphosphate (vitamin C), zinc sulfate, copper sulfate, nicotinic acid, manganese sulfate, vitamin A acetate, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, calcium pantothenate, cholecalciferol (vitamin D_3), calcium iodate, folic acid, biotin.

Directions for use
Mazuri Carnivore Supplement for Whole Prey is designed to be added at 0.5% of wet weight of whole prey.

Average Feed Weights

Measurement	Kg of Diet
1 tsp	2.57
1 tbsp	7.42
¼ cup	26.8
½ cup	56.1
1 cup	103.8

Quality Controlled by PMI Nutrition International, a subsidiary of America's oldest and largest animal nutrition company.

Nutrient composition is based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analyses will vary accordingly.

Appendix 6.10:

Composition of Sofcanis supplement:

Sofcanis composition:

Minerals:	
Phosphorus	5.5%
Calcium	11%
Magnesium	0.12%
Amino Acid: per kg	
Choline HCl	5g
Methionine	5g
Vitamins: (per kg)	
A	160000 UI
D3	10000 UI
E	160mg
C	800mg
B1	40mg
K3	32mg
B2	80mg
B6	20mg
B12	0.4mg
H	0.8mg
PP	800mg
Calcium pantothenic acid	400 mg
Trace elements: (per kg)	
Iron	180mg
Cobalt	18mg
Manganese	900mg
Zinc	70mg
Iodine	60mg
Copper	25mg
Medium content:	
Crude protein	3.5%
Crude fat	0.15%
Crude cellulose	0.1%
Crude ash	35%
Humidity	9%

Dosage following the body weight (BW)	Needs
5 g per 10 kg BW	Care, fur and vitality,
5 g per 5 kg BW	Growth,
5 g per 5 kg BW	Pregnancy and lactation.

Appendix 7

Composition of different feline diets used in zoological institutions.

Appendix 7.1: Mazuri Exotic Feline.
Appendix 7.2: Toronto Zoo Feline Diet.
Appendix 7.3: Composition of the Zupreem exotic feline diet.
Appendix 7.4: Composition of the Dallas Crown carnivore prepared diet.
Appendix 7.5: Composition of the special beef feline diet of Nebraska brand.

Appendix 7.1:

Composition of the Mazuri Exotic Feline

Mazuri® Exotic Feline - Large 5M53

Description
Mazuri® Exotic Feline - Large Diet is a constant formula diet supplying complete life-cycle nutrition for reproduction, lactation, growth and maintenance.

Features and Benefits
- **Complete nutrition** - No supplementation required.
- **High protein and high energy** - Promotes good body condition.
- **Contains fish oil** - A rich, natural source of omega-3 fatty acids.
- **Palatable** - Even adult exotic felines accept this dry diet.
- **Dry diet** - Shelf stable; no need to store frozen.

Product Form **Catalog #0001484**
- Extruded pellet: 1/2" length x 1 1/8" x 1/2" diameter

Guaranteed Analysis

Crude protein not less than	35.0%
Crude fat not less than	14.0%
Crude fiber not more than	4.0%
Moisture not more than	12.0%

Ingredients
Poultry by-product meal, corn gluten meal, ground brown rice, dried beet pulp, porcine meat meal, dehulled soybean meal, ground corn, poultry fat preserved with ethoxyquin, porcine animal fat preserved with BHA, poultry digest, wheat germ, phosphoric acid, soybean oil, fish oil, brewers dried yeast, dried whey, salt, calcium carbonate, dried egg product, lecithin, taurine, choline chloride, potassium chloride, pyridoxine hydrochloride, DL-methionine, menadione dimethylpyrimidinol bisulfite (vitamin K), thiamin mononitrate, folic acid, d-alpha tocopheryl acetate (natural source vitamin E), cholecalciferol (vitamin D_3), ferrous sulfate, inositol, biotin, vitamin A acetate, vitamin B_{12} supplement, ethoxyquin (a preservative), riboflavin, calcium pantothenate, nicotinic acid, L-lysine, copper sulfate, manganous oxide, zinc oxide, calcium iodate, ferrous carbonate, zinc sulfate, cobalt carbonate, sodium selenite.

Feeding Directions
Use following equation for feeding large felines:

Feed intake (kcal/day) = 140 x body weight $^{0.75}$.
Feed intake of 5M53 (kg/day) = Feed intake (kcal/d) / 352

In general, using these equations, recommended feed intake will be 1-3% of body weight per day.

Animals that are growing, reproducing (particularly during late stages of pregnancy), or lactating lactation will have higher needs.

While these equations are a useful starting point, it is always best to feed to body condition and keep animals at optimal body condition even if that means feeding more, or often less, than the equation would predict. Keep in mind that if animals are supplemented with other foods, the amount of dry diet will be reduced.

Average Feed Weights (note that average feed weights may vary due to method of measuring)

Measurement	g of Diet
Each	2.32
½ cup	44.0
1 cup	87.9

5M53 Mazuri® Exotic Feline - Large

Approximate Nutrient Composition

NUTRIENTS

Protein, %	**36.6**
Arginine, %	1.98
Cystine, %	0.53
Glycine, %	2.07
Histidine, %	0.60
Isoleucine, %	1.38
Leucine, %	3.09
Lysine, %	1.80
Methionine, %	0.80
Phenylalanine, %	1.46
Tyrosine, %	0.77
Threonine, %	1.24
Tryptophan, %	0.28
Valine, %	1.67
Taurine, %	0.24
Fat, %	**14.0**
Omega-3 Fatty Acids, %	0.39
Omega-6 Fatty Acids, %	1.94
Fiber (Crude), %	**4.0**
Neutral Detergent Fiber, %	9.0
Acid Detergent Fiber, %	5.7
Starch%	16.0
Metabolizable Energy*, kcal/kg	3,470

MINERALS

Ash, %	**8.1**
Calcium, %	1.80
Phosphorus, %	1.25
Phosphorus (non-phytate), %	1.0
Potassium, %	0.65
Magnesium, %	0.12
Sodium, %	0.35
Chlorine, %	0.54
Iron, ppm	466
Zinc, ppm	112
Manganese, ppm	77
Copper, ppm	24
Cobalt, ppm	0.82
Iodine, ppm	1.9
Chromium, ppm	0.08
Selenium, ppm	0.56

VITAMINS

Thiamin, ppm	81
Riboflavin, ppm	22
Niacin, ppm	124
Pantothenic Acid, ppm	26
Choline, ppm	1,845
Folic Acid, ppm	12
Pyridoxine, ppm	14.8
Biotin, ppm	0.46
Vitamin B_{12}, mcg/kg	220
Vitamin A, IU/kg	15,400
Vitamin D_3 (added), IU/kg	3,800
Vitamin E, IU/kg	300
Vitamin K (as menadione), ppm	3.2

* Metabolizable energy determined using modified Atwater factors (3.5 kcal/g protein, 8.5 kcal/g fat, 3.5 kcal/g carbohydrate), based on NRC of Dogs and Cats, 2007.

Quality Controlled by PMI Nutrition International, a subsidiary of America's oldest and largest animal nutrition company.

Nutrient composition is based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analyses will vary accordingly.

Appendix 7.2:

Composition of the Toronto Zoo Feline Diet

MILLIKEN MEAT PRODUCTS LTD.

SPECIALIZING IN CAPTIVE FELINE DIET

TORONTO ZOO FELINE DIET
TORONTO ZOO CANINE DIET
TORONTO ZOO SMALL CARNIVORE DIET

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PRODUCTS

Toronto Zoo Feline Diet

The Toronto Zoo Feline Diet is the result of more than 25 years of research and development. This diet is formulated to be fed to captive felines. At the Toronto Zoo, the felines are also fed bones and/or whole animals twice weekly to maintain general health status.

Ingredients

Horse meat, Cellulose, Tricalcium Phosphate, Toronto Zoo Carnivore Supplement, Vitamin E, Fatty Acid Supplement, Taurine. No bone or meat meal is included in this diet.

Product Standards

Horse meat originates from animals slaughtered in plants according to the Canadian Food Inspection Agency (CFIA) standards. All meat is 100% muscle; no by-products are included, such as organs, bones, cartillage and connective tissue.

Typical Analysis for Feline Diet

CALCULATED ANALYSIS (Dry Matter Basis)		
Moisture	% (maximum)	70
Protein	% (minimum)	65
Fat	% (minimum)	15
Crude Fibre	% (maximum)	
Calcium	% (minimum)	1.7
Phosphorus	% (minimum)	1.6
Magnesium	%	0.08
Iron	ppm	130
Zinc	ppm	100
Vitamin A	IU/g	23
Vitamin D	IU/kg	
Vitamin E	IU/kg	344
Taurine	%	0.2

The Toronto Zoo Diets are prepared exclusively by:

Milliken Meat Products Ltd.
3447 Kennedy Road, Unit 1
Scarborough, Ontario
Canada
M1V 3P1

Orders
Phone: (416) 299-9600
Fax: (416) 299-5305
Contact: Mr. Orlando De Rosa

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Packaging
Frozen 2 kg. (4.5 lbs.) bags, 10 bags per box

Storage
Keep frozen until needed. DO NOT re-freeze thawed meat.

Appendix 7.3:

Composition of the Zupreem Exotic Feline Diet

Exotic Feline Diet
Canned

ZuPreem®

Product Description

ZuPreem® Exotic Feline Diet Canned is formulated to be a nutritionally complete diet designed as the sole diet for carnivores such as non-domestic canines, hyenas, and exotic felines, such as servals, bobcats, lynx, cougars and caracals.

- **100% complete nutrition for growth, maintenance and reproduction life stages**
  No supplements required or recommended
- **Fed to exotic felines for more than 30 years**
  Time tested and proven efficacy
- **High-energy density with mineral restriction**
  Meets higher energy demands of felines while supplying a lower level of ash to discourage urolith formation
- **Fortified with taurine**
  Meets requirement of felines for this essential amino acid that promotes a healthy heart and eyes
- **Stringent quality control standards**
  Fixed formulation provides consistent dependable nutrition
- **High-quality ingredients**
  Improves digestibility and delivers important nutrients
- **Canned product**
  Longer shelf-life and easier to store than dry or frozen diets
- **Formulated by veterinarians and nutritionists**
  Provides precise and balanced nutrition for optimum energy and health

Product Characteristics

Meat-based loaf canned product.
Shelf-life: Three years from date of manufacture.

Size	Item #
14 oz. can (24)	6910

Exotic Feline Diet
Canned

Feeding Instructions

ZuPreem® Exotic Feline Diet Canned is energy dense. Most adult carnivores should eat approximately one can of ZuPreem Exotic Feline Diet Canned per 30 lbs. of body weight. Feeding the proper quantity helps the individual animal achieve optimum body mass without thinness or obesity. Growing or lactating animals should be fed free choice.

Replace with fresh canned diet if food becomes dried out. Cover and refrigerate unused canned food for up to five days. If refrigerated, return the diet to room temperature prior to feeding. If the food has become dry during refrigeration, moisten the food with warm water and mix thoroughly to return the food to its original consistency before feeding.

Freshly opened product is always more palatable than older refrigerated product, especially to exotic felines that are highly dependent on their acute sense of smell.

Animals can be converted to ZuPreem Exotic Feline Diet Canned by replacing 10% of the current diet with the ZuPreem diet. Over a period of 10 days, increase the percent of ZuPreem Exotic Feline Diet Canned by 10% each day until 100% of the diet is ZuPreem Exotic Feline Diet Canned. Care should be taken to ensure the animal is consuming food during this conversion process. If the animal refuses to eat for two consecutive meals during this process, return to the original diet for a period of three days and begin the process again. **An exotic carnivore should not be starved to force it to convert to a new diet.** Success with reluctant feeders has been experienced by slightly heating the diet. Mix the heated diet thoroughly to make sure there are no hot spots before feeding. The temperature of the diet should be raised only slightly to avoid oral trauma and harming the animal.

Do not feed to cattle or other ruminant animals.

Note – As an indicator of health, always monitor for changes in weight and body condition. Provide adequate amounts of food to maintain body condition, preventing excessive thinness or obesity.

Always provide clean, fresh water.

Ingredients

Chicken, Liver, Water, Meat by-products, Poultry by-product meal, Animal fat, Ground corn, Powdered cellulose, Iodized salt, Potassium chloride, Taurine, Choline chloride, Iron oxide, Zinc oxide, Manganous oxide, Cobalt carbonate, Calcium iodate, Sodium selenite, Vitamin D_3 supplement, Vitamin E supplement, Thiamine, Niacin, Calcium pantothenate, Pyridoxine hydrochloride, Riboflavin, Folic acid, Biotin, Vitamin B_{12} supplement.

Guaranteed Analysis– As fed

Crude Protein	Min.	16.0%
Crude Fat	Min.	14.0%
Crude Fiber	Max.	1.0%
Moisture	Max.	63.0%
Ash	Max.	3.0%
Calcium	Min.	0.4%
Phosphorus	Min.	0.3%
Taurine	Min.	0.05%

ZuPreem®

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Appendix 7.4:

Composition of the Dallas Crown carnivore prepared diet

Nutrient Specifications for Meat-based Frozen Carnivore Diet

Application: This product is a frozen, fresh meat diet for use in the feeding of captive carnivores. It may be used as the sole diet for felids. In addition, provision of knucklebones to chew on, two or more times per week, may aid in promoting oral health.

Ingredients: The following ingredients, used as indicated, constitute a satisfactory diet.

INGREDIENT	% BY WEIGHT
Horse meat or horse meat trimmings (15% fat)	94.10
Solka Floc (wood cellulose)	3.00
Calcium phosphate, tribasic (31.6% Ca, 17.3% P)	1.25
Sodium chloride	0.30
carnivore trace element premix	0.50
Carnivore vitamin premix	0.50
Choline chloride (60% choline)	0.15
Taurine	0.10
Stablized L-ascorbyl-2-polyphosphate (Roche Rovimix® Dry - 15% vitamin C activity)	0.10
As is	100.00
Dry matter	40.00

When added to the diet at the percentages indicated above, the trace element and vitamin premixes should have the following composition:

CARNIVORE TRACE ELEMENT PREMIX		
Element	**Amount in Premix**	**Acceptable Form**
Iodine	80 ppm	Ethylenediamine dihydriodide Calcium periodate Calcium periodate Calcium idodate Potassium iodate
Copper	640 ppm	Copper sulfate
Manganese	400 ppm	Maganese sulfate
Selenium	4 ppm	Sodium selenite

CARNIVORE VITAMIN PREMIX		
Vitamin	**Amount in Premix**	**Acceptable Form**
Vitamin A	800,000 IU/kg	Stabilized retinyl acetate Stabilized retinyl palmitate
Vitamin D3	160,000 IU/kg	D-activated animal sterol

Vitamin E	32,000 IU/kg	D,L-alpha tocopheryl acetate D-alpha tocopheryl acetate
Vitamin K	200 ppm	Menadione sodium bisulfite complex Menadione dimethylpyrimidinol bisulfate
Thiamin	800 ppm	Thiamin mononitrate Thiamin hydrochloride
Riboflavin	800 ppm	Riboflavin
Pantothenic acid	500 ppm	D-calcium pantothenate
Niacin	4,800 ppm	Nicotinic acid Nicotinamide
Vitamin B6	800 ppm	Pyridoxine hydrochloride
Biotin	20 ppm	D-biotin
Folacin	64 ppm	Folic acid

Nutrient	**NRC**	**AAFCO**	**Min**	**Max**	**Expected**
Moisture, %				70	66
Crude protein, %	24	30	30		56
Arginine, %	1	1.25			4.8
Histidine, %	0.3	0.31			2.3
Isoleucine, %	0.5	0.52			2.8
Leucine, %	1.2	1.25			4.3
Lysine, %	0.8	1.2			4.3
Meth + Cyst, %	0.75	1.1			uknown
Methionine, %	0.4	0.62			3.4
Phe + Tyr, %	0.85	0.88			unknown
Phenylalanine, %	0.4	0.42			1.9
Taurine, %	0.04	0.1-0.2			0.3
Threonine, %	0.7	0.73			2.5
Tryptophan, %	0.15	0.25			0.3
Valine, %	0.6	0.62			2.9
Crude fat, %		9	10	40	20
Linoleic acit, %	0.5	0.5	0.5		unknown
Arachidonic acid, %	0.02	0.02			unknown
Crude fiber, %				3	3.0
Acid detergent fiber, %				5	5.0
Ash, %				8	7.8
Calcium, %	0.8	1	0.8	1.6	1.3
Phosphorus, %	0.6	0.8	0.6	1.2	1.2
Magnesium, %	0.04	0.08	0.05	0.09	0.09
Potassium, %	0.4	0.6	0.5		0.5
Sodium, %	0.05	0.2	0.2		0.5
Chloride, %	0.19	0.3			0.3

Iron, ppm	80	80	80		183
Copper, ppm	5	5-15	5		17
Iodine, ppm	0.35	0.35	1		1
Zinc, ppm	50	75	75		110
Manganese, ppm	5	7.5	7.5		20
Selenium, ppm	0.1	0.1	0.1	2	0.5
Vitamin A, IU/kg	3333	9000	10000		14000
Vitamin D3, IU/kg	500	750	1000		2400
Vitamin E, IU/kg	30	30	200		470
Vitamin K, ppm	0.1	0.1	1		2.5
Thiamin, ppm	5	5	7		15
Riboflavin, ppm	4	4	6		17
Vitamin B6, ppm	4	4	6		28
Niacin, ppm	40	60	60		226
Pantothenic acid, ppm	5	5	10		15
Folacin, ppm	0.8	0.8	0.8		1
Biotin, ppm	0.07	0.07	0.1		0.29
Vitamin B12, ppm	0.02	0.02	0.03		0.1
Vitamin C, ppm					470
Choline, ppm	2400	2400	2000		2700

a National Research Council 1986, Nutrient Requirements of Cats. National Academy of Sciences, Washington, DC.

b AAFCO, 1991, Official Publication, Association of American Feed Control Officials, Inc., GA Dept. of Agr., Plant Food, Feed and Grain Div., Capitol Square, Atlanta, GA 30334

Appendix 7.5:

Composition of the special beef feline diet of Nebraska brand

NEBRASKA BRAND

"PREMIUM" BEEF FELINE DIET (frozen)

INGREDIENTS

Beef , Solka Floc, dicalcium phosphate, calcium carbonate, **Nebraska Brand Vitamin Premix (**vitamin E supplement, niacin, d-biotin, vitamin A acetate, riboflavin, pyridoxine, folic acid, d-calcium pantothenate, thiamine mononitrate, vitamin D-3 supplement, menadione sodium bisulfate complex - source of vitamin K activity**)**, **Nebraska Brand Trace Mineral Premix (**zinc oxide, manganese oxide, copper oxide, mineral oil, sodium selenite, calcium iodate**)**, choline chloride, taurine, salt

GUARANTEED ANALYSIS		As Fed	DM
Moisture……	(max)	65.00%	
Crude Protein……	(min)	18.00%	51.45%
Crude Fat……..	(min)	12.00%	34.2%
Crude Fiber……..	(max)	1.50%	4.20%
Ash……...	(max)	4.50%	12.80%
Calcium……	(min)	0.60%	1.70%
Phosphorus……..	(min)	0.50%	1.40%
Vitamin A……IU/kg	(min)	3500	10000
Vitamin D……IU/kg	(min)	550	1571

PACKAGING

8 frozen 5 pound casings per box

CENTRAL NEBRASKA PACKING, INC.

PO BOX 550 ~ NORTH PLATTE, NEBRASKA 69103-0550

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EMAIL: info@nebraskabrand.com WEB PAGE: www.nebraskabrand.com

NEBRASKA BRAND

PREMIUM BEEF FELINE DIET

	Premium Beef Feline Diet	
	As Is Basis	Dry Matter Basis
Nutrient		
Moisture, %	64.00	
Protein, %	18.10	50.20
Ash, %	3.5	9.70
Crude Fat, (maximum)%	18.00	50.00
Crude Fat, (minimum) %	12.00	33.30
Crude Fiber, %	1.0	2.70
Metabolizeable Energy Carnivore, Kcal/kg	1280	3555.5
Lysine, %	1.5	4.10
Methoinine, %	0.50	1.30
Metoinine + Cystine, %	0.75	2.00
Threonine, %	0.85	2.30
Arginine, %	1.42	3.90
Histidine, %	0.70	1.90
Isoleucine, %	0.80	2.20
Leucine, %	1.40	3.80
Phenylalanine, %	0.60	1.60
Tryptophan, %	0.15	0.40
Valine, %	0.95	2.60
Taurine, %	0.20	0.50
Vitamin A, IU/kg	4100	11388
Vitamin E, IU/kg	165	458
Vitamin D, IU/kg	400	1111
Vitamin K, ppm	1.0	2.70
Thiamin, ppm	4.7	13.00
Vitamin B12, ppm	0.04	0.10
Choline Chloride, ppm	825	2291
Calcium, %	0.70	1.90
Total Phosphorus, %	0.55	1.50
Magnesium, %	0.04	0.10
Sodium, %	0.20	0.50
Chloride, %	0.19	0.50
Potassium, %	0.38	1.00
Iron, ppm	68.0	188.80
Zinc, ppm	33.0	91.60
Copper, ppm	5.3	14.70
Manganese, ppm	9.6	26.60
Selenium, ppm	0.23	0.60

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CENTRAL NEBRASKA PACKING, INC.

P.O. BOX 550 / NORTH PLATTE, NE 69103-0550

Web Page: www.nebraskabrand.com Email: info@nebraskabrand.com Fax: 308-532-2744

Appendix 8

Different diets used at EAZA zoological institutes

This list is not intended to suggest that the diets used in zoological institutions are ideal, it is simply intended as an overview of the various diets and supplements that are being used in zoological institutes throughout Europe.

- 1kg of beef meat (two days a week), ½ rabbit (one day a week), 1 kg of chicken (two days a week), 4 rats. All these are supplemented with a teaspoon per feeding of SA-37 vitamins and minerals supplement (See Appendix 6.1).
- 2.5kg of beef sometimes with roe deer or rabbit (six days a week), supplemented with 2% of meat weight of Carmix (See Appendix 6.2).
- 0.60kg of lean meat with bone (four days a week), tripe (1 day a week), 0.20kg lean meat for enrichment (7 days a week). All these are supplemented with 2% of meat weight of Carmix (3/4) and CaCo3 (1/4) (See Appendix 6.2).
- 2kg of beef meat for males and 1.5kg for females (five days a week) supplemented with a tablespoon per 100 pounds of a mixture of Osteo-Form and Spectrall Plus (Appendices 6.3 and 6.4).
- 1 - 2kg of lean beef meat, or 1 large rabbit (without organs), or a piece of goat, or large rats or large hamsters (six days a week). All these are supplemented with 20g per animal per feeding of "Raubtierzusatzmehl" (See Appendix 6.5).
- 3kg of beef with bones (six days a week), supplemented with 10 tablespoons per feeding per animal of Fel-Titan (See Appendix 6.6).
- 2.5kg of horse beef and rabbits for the male and 6.5kg for the female and 3 cubs (four days a week). Supplementation is Diafarm Maintenance for cats as 3g/cat/feed. (See Appendix 6.7).
- 1 - 2.5 kg of beef meat with bone (four days a week), supplemented with 1ml per 100g of meat of Kolmarden vitamineral blandning. (See Appendix 6.8).
- 1.80kg of horse, beef or rabbit for males and 0.80kg for females (six days a week). All these are supplemented with 16g/feed of SDS Carnivore Supplement (See Appendix 6.10).
- 3kg of beef or horse meat for male and 2.5 - 3kg for female, supplemented with rabbits, chicken, rats and quail as available (five days a week). These meals are supplemented with approximately 8g of Mazuri Carnivore Supplement per feed. (Appendix 6.10).
- 3.25kg of Commercial Toronto Zoo Feline Diet (six days a week) and two ox tails on the seventh day (See Appendix 7.2).

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