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# Advances in Wildlife Immobilisation and Anaesthesia

Clinical and Physiological Evaluation  
in Selected Species

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## Advances in Wildlife Immobilisation and Anaesthesia Clinical and Physiological Evaluation in Selected Species

### Abstract

Improvement of chemical capture is an important part of wildlife conservation and animal welfare to minimise distress for the animals and the risk of morbidity and mortality. The aim of this thesis was to improve wildlife immobilisation and anaesthesia by studying physiological variables and clinically evaluate different drug combinations and methods of capture in selected species. Reversible anaesthetic protocols were developed for use in free-ranging lions and four species of South-East Asian primates. Capture and anaesthesia of free-ranging wolverines, brown bears, black and white rhinoceros were physiologically evaluated. The effect of intranasal oxygen supplementation on arterial oxygenation was assessed in brown bears and rhinoceros. Partial reversal of the opioid effect and different body positions were evaluated in rhinoceros.

Capture methods used included darting from a helicopter or the ground, and physical restraint followed by drug injection. Medetomidine-ketamine was used in wolverines and medetomidine-zolazepam-tiletamine in primates, lions and brown bears. Rhinoceros were immobilised with a combination of an opioid, azaperone and an  $\alpha_2$ -agonist. Body temperature and cardiorespiratory variables were monitored in all animals. Arterial blood samples were analysed to interpret pulmonary gas exchange and acid-base status in carnivores and rhinoceros.

Low doses of medetomidine-zolazepam-tiletamine rapidly anaesthetised primates and lions, and reversal with atipamezole resulted in a smooth and calm recovery. Physiological alterations varied with different protocols and species and included changes in body temperature, respiratory and heart rates, gas exchange and acid-base balance. Hypoxaemia was recorded in all rhinoceros and most carnivore species. The major contributor to hypoxaemia was likely ventilation-perfusion mismatch including shunt. In rhinoceros, hypoventilation contributed to an impaired gas exchange and the animals remained hypoxaemic despite partial reversal. However, in black rhinoceros arterial oxygenation was higher during sternal compared to lateral recumbency. Capture-induced lactic acidaemia was recorded in carnivores and rhinoceros. Intranasal oxygen supplementation improved arterial oxygenation.

In conclusion, this thesis increases the understanding of the effects of capture and anaesthesia in several wildlife species. Physiological derangements were identified, potential causative factors were investigated and methods for improvement were evaluated.

*Keywords:* acid-base status, anaesthesia, arterial blood gases, capture, hypoxaemia, immobilisation, oxygen supplementation, wildlife.

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*Quiet time spent out in nature, observing wildlife,  
has the power to uplift the human spirit.*

John Muir (1838-1914)

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Fahlman, Å., Bosi, E.J. and Nyman, G. (2006). Reversible anaesthesia of South-East Asian primates with medetomidine, zolazepam, and tiletamine. *Journal of Zoo and Wildlife Medicine* 37(4), 558-561.
- II Fahlman, Å., Loveridge, A., Wenham, C., Foggin, C., Arnemo, J.M. and Nyman, G. (2005). Reversible anaesthesia of free-ranging lions (*Panthera leo*) in Zimbabwe. *Journal of the South African Veterinary Association* 76(4), 187-192.
- III Fahlman, Å., Arnemo, J.M., Persson, J., Segerström, P. and Nyman, G. (2008). Capture and medetomidine-ketamine anaesthesia in free-ranging wolverines (*Gulo gulo*). *Journal of Wildlife Diseases* 44(1), 133-142.
- IV Fahlman, Å., Arnemo, J.M., Swenson, J.E., Pringle, J., Brunberg, S. and Nyman, G. Physiologic evaluation of capture and anesthesia with medetomidine-zolazepam-tiletamine in brown bears (*Ursus arctos*). (*Submitted*)
- V Fahlman, Å., Edner, A., Wenger, S., Foggin, C., Buss, P., Hofmeyr, M., and Nyman G. Pulmonary gas exchange and acid-base status in immobilised black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*). (*Manuscript*)

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## Abbreviations

BE	base excess (actual)
CNS	central nervous system
GABA	$\gamma$ -aminobutyric acid
$FiO_2$	fraction of inspired oxygen
$HCO_3$	bicarbonate (actual)
HR	heart rate
i.m.	intramuscular
IUCN	the International Union for the Conservation of Nature and Natural Resources
NMDA	<i>N</i> -methyl- <i>D</i> -aspartate
$P(A-a)O_2$	alveolar-arterial oxygen tension difference
$PAO_2$	partial pressure of alveolar oxygen
$PaO_2$	partial pressure of arterial oxygen
$PaCO_2$	partial pressure of arterial carbon dioxide
$P_B$	barometric pressure
$P_{H_2O}$	saturated vapour pressure for water at 37°C
$P_I O_2$	partial pressure of inspired oxygen
RQ	respiratory quotient
RR	respiratory rate
$SaO_2$	arterial haemoglobin oxygen saturation calculated by i-STAT®1
s.c.	subcutaneous
$SpO_2$	arterial haemoglobin oxygen saturation measured by pulse oximetry

## Introduction

Wild animals have fascinated people throughout history. Many wildlife species have spiritual and religious significance in different cultures around the world. Expressions like “as brave as a lion” and “as strong as a bear” are commonly used to describe human attributes. The diversity, the beauty, the power, and the mystery of wild animals leave no-one untouched, whether it is out of fascination or fear.

Wild animals were an important source of protein already for ancient human populations. Many people still rely upon wild game or bush meat as part of their diet. When capturing wild animals, early hunters used physical restraint, but for several thousand years chemical restraint was practiced for hunting by the use of blow pipe darts or arrows prepared with poison (Nielsen, 1999). Live capture of wild animals by chemical immobilisation was introduced in the 1950's. Since then numerous new capture methods, darting equipment and drug protocols have been developed and used for different wildlife species.

The word immobilise can be defined as “to make immovable, to render incapable of being moved”. Immobilisation of an animal is possible through physical or chemical restraint. Chemical immobilisation can vary from light sedation to unconsciousness depending on the drugs and doses used. General anaesthesia includes reversible loss of sensation and loss of consciousness. Hence, an anaesthetised animal is immobilised whereas an immobilised animal may or may not be anaesthetised.

Immobilisation of free-ranging wildlife is often carried out as part of management and conservation, including snare removal, translocation, disease surveillance and fitting of radio-collars for research studies. In

captivity, immobilisation of wild animals is often required for health examination and for diagnosis and treatment of disease. Immobilisation of free-ranging wild animals is often conducted under the most difficult circumstances in remote locations. Since health status and body weight is usually unknown when darting wild animals, the drugs should preferably have a wide safety margin. For remote darting, it is necessary to use potent concentrated drugs that can be delivered in small volumes. Free-ranging animals often require higher drug doses than captive wildlife due to a high level of excitement and stress associated with capture in the wild. A rapid induction is critical to reduce the risk of the animal getting injured in the terrain or losing sight of the animal in dense bush or in darkness. The use of drugs with effects that are reversible by an antagonist is an advantage in case of emergencies and facilitates a quick recovery at the end of the procedure. In the wild it is important for the animal to quickly regain mobility and the ability to defend itself.

### What is safe wildlife capture?

Wildlife species react in different ways to the many methods of capture and chemical restraint that are being used. Already 40 years ago, Arthur M. Pearson from the Canadian Wildlife Service in Yukon wrote:

”Because different species vary greatly in their response to particular drugs it is important that basic research be carried out for each species to determine the most suitable drug and the optimum dosages.”

This is still true. Pearson found that recommended doses of different drug combinations used in grizzly bears (*Ursus arctos*) and black bears (*U. americanus*) were higher than required, creating an unnecessary risk to the bears (Pearson *et al.*, 1968). However, this was based on mortality rate only and no physiological data was presented. Even in recent peer-reviewed literature, capture methods or drug combinations are reported as safe based mainly on mortality, with no or limited description of physiological responses (Arnemo *et al.*, 2006; Webb *et al.*, 2008).

There is more to Good Anaesthesia...  
than Life or Death

“No Death is no longer the criteria for good anaesthesia.  
There is a lot between living and dying.”

James S. Gaynor, DVM, MS

The goal of anaesthesia is to reduce the physiological stress that occurs during the procedure and to ensure optimal tissue oxygenation. Physiological stress is a common result of depression of the respiratory and cardiovascular systems, which will lead to less efficient tissue perfusion.

Safe handling of wildlife should not only warrant low mortality but also low morbidity and ensure stable physiology. The capture event and the immobilising drugs can influence the animal's welfare by altering physiological and biochemical variables. Patterns of physiological disturbance vary with method of capture, drugs and species (Cattet *et al.*, 2003a; Kock *et al.*, 1987; Marco & Lavin, 1999; Powell, 2005). Detailed knowledge on physiological effects is needed to be able to prevent or treat complications.

For many wildlife species there is limited or no information on their physiology and possible drug protocols that can be used for immobilisation. Since extrapolation between species can result in complications, it is imperative to assess the use of different drugs and doses in each species. Improvement of wildlife capture and immobilisation are important parts of wildlife conservation and animal welfare.

## Natural history of selected species

World wide wildlife species are threatened with extinction. There are over 5,400 described species of mammals in the world, ranging in size from the 2-g Etruscan shrew (*Suncus etruscus*) to the over 100-ton blue whale (*Balaenoptera musculus*). Over 1,000 mammalian species were listed as threatened in 2007 on the Red List of threatened species by the International Union for the Conservation of Nature and Natural Resources (IUCN, 2007). Globally, on-going conservation and research projects include capture and immobilisation of thousands of wild mammals every year.

### Primates

The order Primates is a large and diversified group consisting of 233 species, ranging from 120-g pygmy marmosets (*Cebuella pygmaea*) to 180-kg gorillas (*Gorilla gorilla*). Most primates are arboreal and live in tropical forests. The primary threat to free-ranging primates is widespread habitat destruction. Habitat fragmentation by logging and forest fires reduce primate populations and make them prone to genetic drift and inbreeding (Ancrenaz *et al.*,

2007). Primate populations are also declining due to the illegal animal trade, the poaching of animals for food and their persecution as agricultural pests (Davies, 1986).

Four species of South-East Asian primates are included in this thesis: Bornean orangutan (*Pongo pygmaeus pygmaeus*), Bornean gibbon (*Hylobates muelleri*), long-tailed macaque (*Macaca fascicularis*) and pig-tailed macaque (*Macaca nemestrina*) (Fig. 1). Orangutans are classified as endangered on the IUCN Red List and are facing a very high risk of extinction in the wild in the near future (Ancrenaz *et al.*, 2007). Pig-tailed macaques are listed as vulnerable, whereas long-tailed macaques and Bornean gibbons are at lower risk but are likely to qualify for a threatened category in the near future (Eudey & Members of the Primate Specialist Group, 2000).

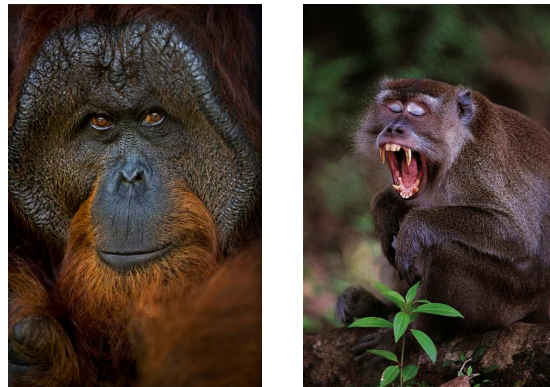


Photo: Mattias Klum

Figure 1. Free-ranging Bornean orangutan (left) and long-tailed macaque (right).

Orangutans are the largest of the arboreal mammals and their diet consist of fruits and more than 500 plant species. Free-ranging orangutans currently exist only in a few areas of tropical rain forest on the islands of Borneo and Sumatra in Malaysia and Indonesia. The majority of wild remnant populations are located outside protected areas. Within the state of Sabah in Malaysia, orangutans that are stranded in forest fragments are captured for translocation to protected habitat (Andau *et al.*, 1994; Kilbourn *et al.*, 1997). At Sepilok Orangutan Rehabilitation Centre in Sabah, orphaned and injured orangutans, as well as other primate species, are raised and treated before release into the wild. Anaesthesia is often required for health examination and treatment of captive-held primates and for translocation of free-ranging orangutans.

## Carnivores

The order Carnivora includes 233 species, ranging in size from the 30-g least weasel (*Mustela nivalis*) to the 600-kg polar bear (*Ursus maritimus*) (Macdonald, 2001). Wild carnivores are able to adapt to all major types of habitats and occur naturally on every continent except Antarctica and Australia. This thesis includes three species of carnivores: lions (*Panthera leo*), wolverines (*Gulo gulo*) and brown bears (*Ursus arctos*).

The African lion is classified as vulnerable on the IUCN Red List and its future is uncertain (Bauer & Nowell, 2004). Major threats to the African lion are loss of habitat and prey base and persecution (Cardillo *et al.*, 2004). Today, most free-ranging lions live in isolated protected populations with increased risk of inbreeding (Bauer & Van Der Merwe, 2004). Field anaesthesia of lions is necessary for various reasons, e.g. fitting of radio-collars for research studies, disease surveillance and control of problem animals.

The wolverine is a member of the family Mustelidae which also includes badgers, otters and weasels (Fig. 2). Wolverines have a circumpolar distribution and inhabit forest and arctic tundra of the northern hemisphere (Pasitschniak-Arts & Larivière, 1995). The wolverine is classified as vulnerable by IUCN (Mustelid Specialist Group, 1996) and endangered on the national red list in Sweden where it has been a protected species since 1969 (Gärdenfors, 2005). In 2004 the Swedish wolverine population was estimated to consist of 450 animals (Gärdenfors, 2005). The main prey for wolverines in Sweden is the semi-domesticated reindeer (*Rangifer tarandus*), which puts the wolverine in conflict with the interests of the reindeer herding Sámi people (Persson, 2003). This conflict continually leads to illegal harvest of wolverines and demands for increased hunting quotas. Ongoing research projects studying the ecology of wolverines in Scandinavia involve anaesthesia for radio-marking of about 30 animals per year.

The brown bear is one of the world's most extensively distributed terrestrial mammal and the most widely distributed ursid (Fig. 2). Although not a threatened species, many populations of brown bears are small and isolated and in several countries where the species used to exist it is now extinct (Bear Specialist Group, 1996). The brown bear population in Scandinavia was almost exterminated at the end of the 19<sup>th</sup> century and the low point was around 130 bears. With successful conservation efforts the

numbers have increased and the latest population estimate was 2,900 brown bears in 2007 (J. Kindberg & J.E. Swenson, personal communication). At present hunting of brown bears is allowed throughout most of their range in Sweden. Every year around 100 brown bears are anaesthetised for ecological studies in the Scandinavian Brown Bear Research Project.



Photo: Staffan Tamm

Figure 2. In Scandinavia, free-ranging adult wolverines weigh 8–15 kg (left) and adult brown bears weigh 50–340 kg (right), depending on sex and season.

### Rhinoceros

Rhinoceros belong to the order Perissodactyla (odd-toed ungulates), which includes three families: Equidae, Tapiridae, and Rhinocerotidae. Perissodactyls are hind-gut fermenters and range in size from the 150-kg mountain tapir (*Tapirus pinchaque*) to the over 2-ton white rhinoceros (*Ceratotherium simum*). There are five species of rhinoceros, which are further divided into 11 subspecies. One of the main threats to free-ranging rhinoceros is poaching for the international rhino horn trade for traditional use in Chinese medicine and ornamental use in the Middle East. The black rhinoceros (*Diceros bicornis*) was the most numerous of the world's rhinoceros species throughout most of the 20<sup>th</sup> century. Hunting and habitat loss have reduced the population from a probable several hundred thousand to approximately 3,700 by 2005 (Emslie, 2006). Even though intensive anti-poaching efforts have had encouraging results and the numbers of black rhinoceros are slowly increasing, the species is critically endangered (African Rhino Specialist Group, 2003). The black rhinoceros (hook-lipped) is smaller than the white rhinoceros, with an adult body weight of around 1 ton. Black rhinoceros are browsers and occur in a variety of habitats, whereas white rhinoceros (square-lipped) are grazers that live in grassland and savannah woodlands. Conservation efforts for the southern white rhinoceros (*Ceratotherium simum* spp *simum*) have been very successful. This subspecies was on the brink of extinction by the end of the 19<sup>th</sup> century

when only around 20 animals remained in South Africa. After years of protection and many translocations, the subspecies had grown to near 14,500 animals by 2005 (Emslie, 2006). Today the southern white rhinoceros is listed as near threatened by the IUCN and there is limited sport hunting of surplus males (African Rhino Specialist Group, 2003).

### Physiological effects of capture, immobilisation and anaesthesia

Physical exertion after helicopter pursuit and resistance to handling during manual restraint result in increased stress and can lead to elevated body temperature, oxygen depletion and lactic acid production. Under other circumstances hypothermia may develop.

#### *Thermoregulatory changes*

**Hyperthermia** commonly develops when capturing wild animals in high ambient temperatures, although not unusual also in animals captured in cold climates due to muscular activity during the induction period. Stress alone can induce hyperthermia because increased adrenaline levels cause redistribution of blood flow by vasoconstriction, and a decreased blood flow to the skin impairs heat loss. Thick fur may further increase heat stress. Even mild hyperthermia greatly increases metabolic rate and circulatory demands. At body temperatures above 42°C cellular hypoxia result in multi organ failure that can be life-threatening (Tranquilli *et al.*, 2007). Acidosis, disseminated intravascular coagulation, non-cardiogenic pulmonary oedema, convulsions and cardiac arrhythmia may also occur. Since oxygen consumption exceeds oxygen supply in hyperthermic animals, oxygen supplementation to meet the increased demand is advantageous.

**Hypothermia** can develop due to drug-induced alterations on thermoregulation or result from immobilisation in a cold environment (Broadstone, 1999). However, also in warmer climates there is a risk of hypothermia, especially in small animals due to a greater body surface area in relation to size. The stress response elicited by hypothermia result in redistribution of blood flow by peripheral vasoconstriction and in tachycardia and hypertension. Hypothermia can cause an increased risk of wound infection, impaired wound healing and deficiencies in coagulation. If severe, cardiac arrhythmia and death may occur. During recovery from hypothermia, shivering can increase oxygen consumption by as much as 400 to 500% and anaesthetic recovery can be prolonged (Broadstone, 1999).



### *Impairment of pulmonary gas exchange and acid-base balance*

Immobilising drugs used in wildlife commonly interfere with the normal respiratory function, which can lead to respiratory depression (hypoventilation), hypoxaemia (inadequate amount of oxygen in the blood) and respiratory acidosis. The amount of carbon dioxide in the blood increases (**hypercapnia**) if the alveolar ventilation is inadequate, and if the metabolic rate is increased such as during hyperthermia (DiBartola, 2006). Mild to moderate hypercapnia may be beneficial because it stimulates the sympathetic nervous system and supports cardiovascular function (positive inotropic effects and vasoconstriction) and enhances the release of oxygen from haemoglobin into the tissues. On the other hand, severe hypercapnia may lead to haemodynamic instability, tachyarrhythmia, impaired diaphragmatic contractility and coma (DiBartola, 2006).

Hypercapnia and lactic acid accumulation lead to a decrease in pH and during **acidosis** the strength of the heart contractility is reduced. The capture myopathy syndrome can develop in an animal that becomes oxygen-depleted because of severe physical exertion (Spraker, 1993). Tissue hypoxia and oxygen-debt may result in lactic acidosis, which predispose the animal to muscle fatigue, cellular death and organ failure (Spraker, 1993). This can lead to significant morbidity or mortality, with death occurring within minutes or hours of capture, or even up to weeks later.

**Hypoxaemia** may develop due to a low inspired oxygen concentration (high altitude, low barometric pressure) or hypoventilation, or due to intrapulmonary causes, i.e. ventilation-perfusion mismatch, shunt, or diffusion impairment (Tranquilli *et al.*, 2007). Hypoxaemia can lead to insufficient oxygen delivery and inadequate oxygen levels in the body (**hypoxia**). Tissue hypoxia rapidly leads to cell damage in the most sensitive organs; the brain, heart, kidney and liver. Brain cell death can occur within minutes and tissue necrosis can result in multi organ damage.

Oxygen supplementation during immobilisation has been recommended for several large wildlife species (Caulkett *et al.*, 2000a; Caulkett *et al.*, 2000b; Honeyman *et al.*, 1992; Osofsky, 1997; Portas, 2004; Raath, 1999; Schumacher *et al.*, 1995). However, methods of oxygen delivery and flow rates have been evaluated in only a few wildlife species (Bush *et al.*, 2004; Dunlop *et al.*, 1994; Heard *et al.*, 1986; Mich *et al.*, 2008; Read *et al.*, 2001; Schumacher *et al.*, 1997).

Body positioning during anaesthesia can also impair the pulmonary function, with a higher risk of hypoxaemia in heavy animals, as shown in horses (Gleed & Dobson, 1988). Recommendations for positioning of immobilised rhinoceros in sternal or lateral recumbency vary between authors (Kock *et al.*, 1995; Radcliffe & Morkel, 2007). Anaesthetised bears have been recommended to be positioned in sternal recumbency (Caulkett, 2007), but there is a lack of data to confirm the optimal position in many wildlife species.

### **Evaluation of clinical and physiological effects of chemical capture**

Irrespective of the environmental situation, monitoring stress response and level of unconsciousness is essential when immobilising or anaesthetising animals, to avoid excessively light or deep levels of anaesthesia. Anaesthetic depth and analgesia must be provided to each animal in relation to the procedure performed and the extent of handling. The choice of anaesthetic protocol and monitoring devices should be based on professional experience. Close observation of physiological variables during anaesthesia is essential to be able to recognise potentially harmful cardio-respiratory changes. Continuous monitoring enables proactive rather than reactive manipulations. In the event of anaesthetic complications, vigilance and knowledge on how to institute corrective measures is imperative to reduce the risk of morbidity or mortality. In the many different wildlife species, the collection of base-line data of physiological variables is necessary to establish species-specific reference values, which is the essential basis to then recognise clinically important alterations.

Basic monitoring carried out during anaesthesia commonly includes monitoring of body temperature, heart rate and respiratory rate. These vital signs can easily be monitored without expensive equipment. However, respiratory rate can vary widely and a normal rate does not equal adequate ventilation. Moreover, even an increase in respiratory rate might not compensate for a decrease in tidal volume, resulting in hypercapnia.

Many hypoxic patients go undetected and untreated. Cyanosis is an important sign of hypoxaemia, but if the animal is anaemic severe hypoxaemia may be present without any easily visible change in the colour of the mucous membranes. In contrast, mucous membranes may appear cyanotic due to peripheral vasoconstriction even when arterial oxygen

tension is adequate, for example when  $\alpha_2$ -agonists are used. The fact that hypoxaemia, like hypercapnia and acidosis, is often clinically silent, necessitates the use of monitoring equipment to measure the effectiveness of pulmonary gas exchange and to evaluate acid-base balance.

#### **Pulmonary gas exchange and acid-base status**

Arterial oxygenation can be measured by pulse oximetry or arterial blood gas analysis. Pulse oximetry is an inexpensive, non-invasive method for continuous measurement of arterial haemoglobin oxygen saturation and it is part of minimum standard of care during anaesthesia of humans. However, poor performance of pulse oximeters may occur due to movement or bright ambient lighting. In addition, a reduced peripheral blood flow because of vasoconstriction, hypotension, hypovolemia and hypothermia can also affect the pulse oximetry function. Pulse oximeters are calibrated based on data from human volunteers and the accuracy can vary between different models, probe sites and species (Matthews *et al.*, 2003). Pulse oximetry tends to underestimate oxygen saturation at high ranges and overestimate oxygen saturation at lower values. Therefore, readings must be interpreted critically, especially at low values – when monitoring is clinically important (Hanson & Nijhuis, 1997).

Arterial blood gas analysis is considered the gold standard for assessment of ventilation and enables calculation of the alveolar-arterial oxygen tension difference. The partial pressure of oxygen and carbon dioxide can be used as key factors for oxygen uptake in the lungs and alveolar ventilation, respectively. The acid-base status can be assessed by measuring pH and lactate and by calculated values of base excess and bicarbonate. Lactate usually serves as a marker for the adequacy of tissue oxygenation but can also reflect stress induced glycolysis (Wolfe & Martini, 2000). Blood gases and acid-base status are valuable means for evaluation of the physiological effects that different capture methods and drugs have on wild animals (Suzuki *et al.*, 2001).

#### **Clinical pharmacology of wildlife capture drugs**

A wide variety of drugs are used for immobilisation and anaesthesia of wildlife species. The individual animal response as well as the species-specific reaction to a given drug can vary greatly. For example, the potent opioids commonly used for immobilisation of ungulates, elephants and rhinoceros can produce severe respiratory depression in primates and

excitation in feline species. Today wild animals are usually immobilised with a combination of drugs to enable lower drug dosing due to synergistic effects, to counteract side effects and to permit reversal of the immobilisation.

#### *Primates and carnivores*

The most widely used anaesthetic drugs in wild carnivores and primates are the dissociative anaesthetics ketamine and tiletamine. Although the exact mechanism of dissociative anaesthetics is unknown, antagonism of the *N*-methyl-*D*-aspartate (NMDA) receptor is believed to account for most of the analgesic, amnesic, psychomimetic and neuroprotective effects of ketamine (Kohrs & Durieux, 1998). Ketamine is less potent and has a shorter duration of action than tiletamine. Disadvantages of anaesthesia with ketamine or tiletamine alone include poor muscle relaxation, convulsions, excessive salivation and the lack of a specific antagonist (Kreeger & Arnemo, 2007). To reduce side effects, tiletamine is available only in combination with the benzodiazepine tranquiliser zolazepam. Benzodiazepines produce anticonvulsant and hypnotic effects through their interaction with  $\gamma$ -aminobutyric acid (GABA), the most common inhibitory neurotransmitter in the central nervous system (CNS) (Klein & Klide, 1989). Anxiolytic and muscle relaxant effects are produced through interaction in glycine-mediated inhibitory pathways in the brain and spinal cord (Klein & Klide, 1989). Zolazepam has a longer duration of action than tiletamine in domestic cats, which can lead to prolonged recovery periods. On the contrary, dogs metabolise zolazepam more rapidly than tiletamine, which may lead to rough recoveries due to residual tiletamine effects (Lin *et al.*, 1993). The combination of zolazepam-tiletamine has been used for immobilisation of many species of wild carnivores and primates (Fowler & Miller, 2003; Kreeger & Arnemo, 2007). Induction is rapid but recovery is usually prolonged. The recovery period can be shortened by reversing the effects of zolazepam with a benzodiazepine receptor antagonist, such as flumazenil or sarmazenil, as described in lions and cheetahs (Stander & Morkel, 1991; Walzer & Huber, 2002).

When combining a dissociative anaesthetic with a sedative, a tranquilliser, or both, muscle relaxation is improved and the dose of the dissociative anaesthetic can be reduced (Hayama *et al.*, 1989; Jalanka & Roeken, 1990; Lewis, 1993; Röken, 1997). Frequently used sedatives are the  $\alpha_2$ -adrenoceptor agonists ( $\alpha_2$ -agonists) xylazine, detomidine and medetomidine, of which medetomidine has the greatest potency and is the

most selective for the  $\alpha_2$ -receptors (Klein & Klide, 1989).  $\alpha_2$ -agonists produce sedative, muscle relaxant and analgesic effects by stimulating  $\alpha_2$ -receptors that exist pre- and postsynaptically in tissues throughout the body. For reversal of the effects of  $\alpha_2$ -agonists, atipamezole, yohimbine and tolazoline can be used. Tolazoline is a relatively non-selective  $\alpha$ -receptor antagonist, whereas atipamezole has the highest selectivity for  $\alpha_2$ -receptors (Klein & Klide, 1989).

Ketamine in combination with xylazine or medetomidine is commonly used for many wildlife species (Kreeger & Arnemo, 2007). However, in lions the large volume of ketamine that is required for an effective dose when combined with xylazine can necessitate repeated drug administration (Herbst *et al.*, 1985; Van Wyk & Berry, 1986). Another important disadvantage is sudden recoveries, as reported in bears and lions anaesthetised with ketamine in combination with medetomidine or xylazine (Arnemo *et al.*, 2006; Cattet *et al.*, 1999a; Jalanka & Roeken, 1990; Quandt, 1992; White *et al.*, 1996). In potentially dangerous species, the slower recovery from zolazepam-tiletamine is safer from a personnel perspective, but the lack of a reversal agent for tiletamine is a disadvantage during free-ranging conditions. When immobilising free-ranging orangutans with zolazepam-tiletamine, the induction time is shorter and the dart volume smaller than when xylazine and ketamine are used, but recovery is longer (Andau *et al.*, 1994; Kilbourn *et al.*, 1997).

When zolazepam-tiletamine is combined with medetomidine, the dose of zolazepam-tiletamine can be reduced by at least 50% in many species (Cattet *et al.*, 1999c; Kreeger & Arnemo, 2007; Röken, 1997). Recovery from anaesthesia can be shortened by reversing the effects of medetomidine with atipamezole. The combination of medetomidine and zolazepam-tiletamine has not been reported in South-East Asian primates and has been only briefly described in free-ranging lions (Bengis & Keet, 2000; Grobler, 1997). In Scandinavia, the drug combination has been used for over 1,250 capture events when helicopter darting free-ranging brown bears between 1992 and 2008, but physiological effects have not yet been reported. Similarly, the combination of medetomidine and ketamine has been used in Scandinavia since 1998 for anaesthesia in free-ranging wolverines, but there is a lack of knowledge of the physiological effects of capture and anaesthesia in the species. The few studies published on wolverine immobilisation report only body temperature or no physiological

variables at all (Ballard *et al.*, 1982; Golden *et al.*, 2002; Hash & Hornocker, 1980).

### *Rhinoceros*

For immobilisation of free-ranging rhinoceros, highly potent opioids are required to enable drug dosing suitable for remote darting and to ensure quick induction times. Etorphine is more commonly used than carfentanil, as the latter has a longer duration of action. The newer fentanyl derivative thiafentanil is rapid-acting and has a short duration of action in different wildlife species (Janssen *et al.*, 1993; Stanley *et al.*, 1988). Etorphine, thiafentanil and carfentanil are pure opioid agonists which mediate their effects through opioid receptors ( $\mu$ ,  $\delta$ , and  $\kappa$ ) distributed in the CNS and peripherally (Grimm & Lamont, 2007). The  $\mu$ -receptors mediate most of the analgesic and sedative effects as well as several side-effects, including respiratory depression. Opioids are regularly used in combination with a sedative, a tranquilliser, or both, to enhance the quality of immobilisation and to decrease opioid requirements. For rhinoceros, the  $\alpha_2$ -agonists xylazine and detomidine and the butyrophenone tranquilliser azaperone are frequently used. The effect of azaperone is primarily through blockade of dopamine receptors, but it has also some antagonistic effects on  $\alpha_1$ -receptors (Tranquilli *et al.*, 2007).

Adverse side effects during immobilisation of rhinoceros include hypoxaemia, acidosis, and hypertension (Bush *et al.*, 2004; Hattingh *et al.*, 1994; Heard *et al.*, 1992). To improve respiration in immobilised rhinoceros, several drugs have been used, including nalorphine and nalbuphine and the respiratory stimulant doxapram (Hattingh *et al.*, 1994; Kock, 1992; Kock & Morkel, 1993; Kock *et al.*, 1995; Raath, 1999; Reuter & Winterbach, 1998). Partial opioid agonists and mixed agonist-antagonists have varying effects at different opioid receptors and the effect can be dose dependent (Grimm & Lamont, 2007). Nalorphine in low doses has been described to act as a competitive antagonist that blocks most actions of morphine, whereas higher doses are analgesic and mimics the effects of morphine (Rang *et al.*, 2007). These effects probably reflect an antagonistic action of nalorphine on  $\mu$ -receptors, coupled with a partial agonist action on  $\kappa$ - and  $\delta$ -receptors. In white rhinoceros, the use of nalorphine for partial reversal of the effects of etorphine is reported to clinically improve respiratory rate and depth, and haemoglobin oxygen saturation measured by pulse oximetry (Kock *et al.*, 1995). However, oxygen saturation determined by arterial blood gas analysis improved only marginally in white rhinoceros

after nalorphine administration, and severe hypoxaemia persisted during immobilisation (Bush *et al.*, 2004).

## Aims

The general aim of this thesis was to improve wildlife immobilisation and anaesthesia by studying physiological variables and clinically evaluate different drug combinations and methods of capture in selected species of wildlife.

The specific aims were to

- develop reversible anaesthetic protocols including medetomidine-zolazepam-tiletamine in four species of South-East Asian primates (Study I) and in free-ranging African lions (Study II), and in each species evaluate
  - the cardiorespiratory effects of the drug combination
  - anaesthetic reversal with atipamezole
- evaluate physiological variables, including pulmonary gas exchange and acid-base status, in
  - free-ranging wolverines anaesthetised with medetomidine and ketamine (Study III)
  - free-ranging and captive brown bears anaesthetised with medetomidine and zolazepam-tiletamine (Study IV)
  - black and white rhinoceros immobilised with a drug combination of an opioid, an  $\alpha_2$ -agonist and azaperone (Study V)
- assess the effect of the following on arterial oxygenation
  - partial reversal of the opioid effects (Study V)
  - intranasal oxygen supplementation (Study IV and V)
  - different body positions (Study V)





# Materials and Methods

## Study areas and animals

The fieldwork for this thesis was carried out in four countries on three different continents: Malaysia in South-East Asia, Zimbabwe and South Africa in Southern Africa and Sweden in Northern Europe (Fig. 3). Four species of primates, three species of carnivores and two species of rhinoceros were included in the studies (Table 1). The animals were anaesthetised as part of routine work (Study I), on-going research projects (Study II, III and IV) or management and health purposes (Study V). A variety of procedures were performed such as physical examination, intradermal tuberculin testing, snare removal, translocation, fitting of radio-collars, and surgery for intraperitoneal radiotransmitters (Study III and IV).

### *I. Primates*

The primate study was performed within the state of Sabah in Malaysia, on the island of Borneo. A total of 23 anaesthetic procedures involving 18 individual primates were evaluated. Twenty procedures involved temporarily captive-held primates at Sepilok Orangutan Rehabilitation Centre and three procedures were translocation of free-ranging orangutans within the state of Sabah. The age of the animals varied from infants to adults. Ambient temperature ranged from 30–33°C during the procedures.

### *II. Lions*

Twenty-one anaesthetic events of 17 free-ranging adult and subadult lions were evaluated in Hwange National Park and Malilangwe Wildlife Reserve in Zimbabwe. Ambient temperature ranged from 15–34°C.

### III. Wolverines

Twenty-five anaesthetic events of 24 free-ranging wolverines (12 adults, 12 juveniles) were studied in and around Sarek National Park, north of the Arctic Circle in Sweden. Anaesthesia was carried out at altitudes of 500–1,300 m above sea level with an ambient temperature of  $-5$  to  $+25^{\circ}\text{C}$ . The wolverines were anaesthetised as part of the Swedish Wolverine project with approval from the Ethical Committee on Animal Experiments in Umeå, Sweden.



Figure 3. Study areas in Northern Europe, Southern Africa and South-East Asia.

### IV. Brown bears

This study included the anaesthesia of 52 free-ranging and six captive brown bears. Of the free-ranging bears, 49 were anaesthetised in the county of Dalarna and three in Sarek National Park, Sweden. Free-ranging brown bears (27 adults, 6 subadults, 19 yearlings) were anaesthetised for ecological studies in the Scandinavian Brown Bear Research Project, with approval given by the Ethical Committee on Animal Experiments in Uppsala, Sweden. The six captive bears (3 subadults, 3 yearlings) were anaesthetised at Orsa Bear Park, a zoo in Dalarna. Ambient temperature ranged from  $2$ – $23^{\circ}\text{C}$ .

## V. Rhinoceros

The study included 26 black and three white rhinoceros immobilised in various Wildlife Reserves and Conservancies in Zimbabwe, and 14 white rhinoceros immobilised in Kruger National Park in South Africa. Of these, one black and two white rhinoceros were boma-held (i.e. in enclosures), while the rest were free-ranging. Ambient temperature ranged from 20–36°C in Zimbabwe and from 11–28°C in South Africa.

Table 1. Species, number of individuals (*n*) and procedures, sex and body mass of the animals included in the different studies in this thesis.

Study and species	<i>n</i>	♂ / ♀	Number of procedures	Body mass (kg)
I. Bornean orangutan ( <i>Pongo pygmaeus p.</i> )	12	4 / 8	Captivity 13 Free-ranging 3	3.9 – 45.5 20.0 – 26.0
I. Bornean gibbon ( <i>Hylobates muelleri</i> )	2	- / 2	Captivity 2	2.9 – 3.0
I. Long-tailed macaque ( <i>Macaca fascicularis</i> )	2	2 / -	Captivity 3	2.6 – 3.4
I. Pig-tailed macaque ( <i>Macaca nemestrina</i> )	2	2 / -	Captivity 2	2.2 – 3.7
II. African lion ( <i>Panthera leo</i> )	17	5 / 12	Free-ranging 21	105.0 – 211.0
III. Wolverine ( <i>Gulo gulo</i> )	24	8 / 16	Free-ranging 25	3.4 – 15.2
IV. Brown bear ( <i>Ursus arctos</i> )	58	17 / 41	Free-ranging 52 Captivity 6	12.0 – 241.0 37.0 – 87.0
V. Black rhinoceros ( <i>Diceros bicornis</i> )	26	18 / 8	Free-ranging 25 Captivity 1	not recorded not recorded
V. White rhinoceros ( <i>Ceratotherium simum</i> )	17	10 / 7	Free-ranging 15 Captivity 2	1,238 – 2,290 <sup>a</sup> not recorded

<sup>a</sup> Body mass of adult white rhinoceros immobilised in South Africa.

## Drugs, drug delivery and capture methods

For anaesthesia, medetomidine was used in combination with zolazepam-tiletamine in primates, lions and brown bears, and in combination with ketamine in wolverines. All primates and carnivores were weighed during anaesthesia and their actual drug doses were calculated in mg/kg (Table 2).

Table 2. *Drugs and actual mean doses (range)<sup>a</sup> in mg/kg used in primates and carnivores.*

Study and species, number of procedures	Medetomidine <sup>b</sup>	Zolazepam- tiletamine <sup>c</sup>	Ketamine <sup>d</sup>
I. Bornean orangutan			
• Captivity 13	0.02 <sup>a</sup>	1.1 (0.9-1.3)	-
• Free-ranging 3	0.02 <sup>a</sup>	1.3 (1.2-1.3)	-
I. Bornean gibbon			
• Captivity 2	0.02 <sup>a</sup>	0.9 (0.8-0.9)	-
I. Long-tailed macaque			
• Captivity 3	0.04 (0.02-0.05)	1.5 (0.9-1.9)	-
I. Pig-tailed macaque			
• Captivity 2	0.06 (0.05-0.06)	2.0 (1.7-2.3)	-
II. African lion			
• Free-ranging 21	0.04 (0.03-0.06)	0.7 (0.4-1.3)	-
III. Wolverine			
• Free-ranging 25			
- adults	0.37 (0.26-0.44)	-	9.4 (6.6-11.0)
- juveniles	0.14 (0.10-0.21)	-	7.5 (5.2-11.0)
IV. Brown bear			
• Free-ranging 52			
<i>Single dart (n=36):</i>			
- adults & subadults	0.08 (0.05-0.13)	4.1 (2.3-6.6)	-
- yearlings	0.07 (0.04-0.10)	3.2 (1.5-5.2)	-
<i>2 or 3 darts (n=16):</i>			
- adults & subadults	0.12 (0.06-0.20)	6.0 (3.1-10.0)	-
- yearlings	0.12 (0.10-0.14)	6.1 (5.0-7.2)	-
• Captivity 6			
- subadults	0.02 (0.02-0.03)	2.2 (1.9-2.7)	-
- yearlings	0.03 <sup>a</sup>	1.5 (1.3-1.7)	-

<sup>a</sup> If no range is presented, all animals received the same dose.

<sup>b</sup> Domitor<sup>®</sup>, 1 mg medetomidine HCl/ml, or Zalopine<sup>®</sup>, 10 mg medetomidine HCl/ml, Orion Pharma Animal Health, Turku, Finland. Concentration used depending on dose, species and drug delivery method.

<sup>c</sup> Zoletil<sup>®</sup>100, or Zoletil forte vet, Virbac RSA (Pty) Ltd, Halfway House, South Africa, and Virbac S.A., Carros, France. Prepared by dissolving the powder in sterile water to a total drug concentration of 100 or 200 mg/ml.

<sup>d</sup> Narketan<sup>®</sup>10, 100 mg ketamine HCl/ml Chassot, Dublin, Ireland.

For immobilisation of rhinoceros, a drug combination of an opioid (etorphine or thiafentanil) and azaperone was used for all animals. In Zimbabwe, an  $\alpha_2$ -agonist (detomidine or xylazine) was added to the combination. Drug doses, age and sex of free-ranging black and all white rhinoceros are presented in Table 3 and 4. The boma-held black rhinoceros (subadult male) was immobilised three times with 1.7 mg etorphine and 30-40 mg azaperone. When darting free-ranging rhinoceros, hyaluronidase was added at 1,250-8,000 IU per dart (lyophilised powder, 5,000 IU/vial, Kyron Laboratories (Pty) Ltd., Benrose, South Africa).

Table 3. Age, sex, and drug doses used in 25 free-ranging black rhinoceros in Zimbabwe. A combination of an opioid, azaperone, and an  $\alpha_2$ -agonist was used for immobilisation. Nalorphine was used for partial reversal.

Species			Black rhinoceros		
Age			adult	subadult	calf
Number of animals and sex			12♂, 5♀	2♂, 1♀	3♂, 2♀
<u>Immobilising drugs</u>					
Etorphine <sup>b</sup>	(mg)	N <sup>a</sup> =23	3.2-4.3	2.7-2.8	1.0-2.2
Thiafentanil <sup>c</sup>	(mg)	N= 2	3.0	2.0	-
Azaperone <sup>d</sup>	(mg)	N=25	70-90	35-50	15-30
Detomidine <sup>e</sup>	(mg)	N= 5	3.5-4.5	2.5	1.0
Xylazine <sup>f</sup>	(mg)	N=19	20-45	20	10-13
<u>Partial reversal drug</u>					
Nalorphine <sup>g</sup>	(mg)	N=23	1.0-8.0	2.0-5.0	4.0-6.0

<sup>a</sup>N = number of immobilisation procedures the drug was used in.

<sup>b</sup>M99<sup>®</sup>, 9.8 mg etorphine HCl/ml, Novartis South Africa (Pty) Ltd., Kempton Park, South Africa.

<sup>c</sup>A3080, 10 mg thiafentanil oxalate/ml, Wildlife Pharmaceuticals Inc., Fort Collins, Colorado, USA.

<sup>d</sup>Stresnil<sup>®</sup>, 40 mg azaperone/ml, Janssen Animal Health, Halfway House, South Africa.

<sup>e</sup>Domosedan<sup>®</sup>, 10 mg detomidine HCl/ml, Novartis South Africa (Pty) Ltd.

<sup>f</sup>Rompun<sup>®</sup>, 500 mg xylazine powder/vial, Bayer, Leverkusen, Germany.

<sup>g</sup>Nalorphine<sup>®</sup>, 20 mg nalorphine hydrobromide/ml, Kyron Laboratories (Pty) Ltd, Benrose, South Africa.

### I. Primates

For captive primates at Sepilok Orangutan Rehabilitation Centre, the drugs were administered i.m. by hand syringe while physically restraining the animals. Free-ranging orangutans in trees were darted from the ground at a

distance of 8-15 m by using a dart rifle powered by air pressure from a foot pump (Telinject Vario 4V Rifle, Telinject USA Inc., Saugus, California, USA).

### *II. Lions*

All lions were darted from a vehicle at distances of 10-33 m by using a CO<sub>2</sub> powered rifle (Dan-Inject CO<sub>2</sub> Injection Rifle Model JM Special, Dan-Inject SA, Skukuza, South Africa). Up to three lions were darted and kept under anaesthesia on the same occasion. The initial aim was to administer 0.06 mg/kg medetomidine and 1.45 mg/kg zolazepam-tiletamine, followed by a gradual decrease of the doses based on observed reactions. For the first six lions, drug doses were prepared after estimation of the body weight once the lions were sighted. Thereafter, lions were given drug doses according to sex: males received 6-8 mg medetomidine and 80-100 mg zolazepam-tiletamine whereas females received 4-6 mg medetomidine and 50-80 mg zolazepam-tiletamine. Actual doses in mg/kg are presented in Table 2.

### *III. Wolverines*

On eight occasions adult wolverines were darted from a helicopter and on three occasions from the ground. The darting distance was 1-6 m and a CO<sub>2</sub> powered rifle (Dan-Inject, Børkop, Denmark) was used. Two adult wolverines were dug out of rendezvous sites (a site where juveniles are left while the female forage), captured by hand with a snare pole and hand-injected i.m. with the drugs. A standard dose of 4 mg medetomidine and 100 mg ketamine was used in adult wolverines (total dose per animal). Juvenile wolverines were dug out of rendezvous sites and captured with a snare pole. Up to four wolverines were captured on the same occasion, and juveniles were kept separately in canvas bags until the drugs were administered and the animals were anaesthetised. After estimation of body weight or being weighed in a canvas bag, juveniles were hand-injected i.m. with 0.1 mg/kg medetomidine and 5-10 mg/kg ketamine. Drug doses in mg/kg according to actual body mass are shown in Table 2. Surgery for implantation or replacement of intraperitoneal radiotransmitters was performed in eight adult and 12 juvenile wolverines.

For analgesia in wolverines and brown bears undergoing surgery for intraperitoneal radiotransmitters, 4 mg/kg carprofen (Rimadyl<sup>®</sup>, Orion Pharma Animal Health, Turku, Finland) was administered s.c. pre-operatively. To minimise the risk of wound infection, procaine

benzylpenicillin and benzathine benzylpenicillin were injected intramuscularly (i.m.) at 100,000 IU/kg (PENI-kél L.A. 15+15, Kela Laboratoria NV, Hoogstraten, Belgium).

#### IV. Brown bears

Doses of medetomidine and zolazepam-tiletamine used in brown bears are presented in Table 2. Free-ranging bears were darted from a helicopter at a distance of 4-6 m by using a CO<sub>2</sub> powered rifle (Dan-Inject, Børkop, Denmark). When capturing family groups, the yearlings were darted before the mother. After successful darting of the first yearling, it was observed from the helicopter at a high altitude throughout the induction period, prior to darting of the next family member. Surgery for implantation of intraperitoneal radiotransmitters was performed in 12 adult brown bears and 14 yearlings. Captive bears were darted in their indoor quarters at the zoo.

Table 4. Age, sex, and initial drug doses used in 15 free-ranging and two boma-held white rhinoceros in South Africa and Zimbabwe. Etorphine and azaperone was used for immobilisation of all animals, and in Zimbabwe an alpha<sub>2</sub>-agonist was added to the combination to two calves. For partial reversal, nalorphine in combination with diprenorphine was used.

Species	White rhinoceros			
Age	adult		calf	
Number of animals and sex	8♂, 6♀		2♂, 1♀	
Country	South Africa		Zimbabwe	
<u>Immobilising drugs</u>				
Etorphine <sup>b</sup>	(mg)	N <sup>a</sup> =17	4.0-5.0 (8.0) <sup>i</sup>	1.5-2.0 (3.6) <sup>i</sup>
Azaperone <sup>d</sup>	(mg)	N=17	40 (80)	20-35 (40)
Detomidine <sup>e</sup>	(mg)	N= 1	-	1.5
Xylazine <sup>f</sup>	(mg)	N= 1	-	22
<u>Partial reversal drugs</u>				
Nalorphine <sup>g</sup>	(mg)	N=17	10	6-10
Diprenorphine <sup>h</sup>	(mg)	N=17	1.2	0.5-0.6

<sup>a-g</sup> See Table 3.

<sup>b</sup> M5050<sup>®</sup>, 12 mg diprenorphine hydrochloride/ml, Novartis South Africa (Pty) Ltd.

<sup>i</sup> Four free-ranging adults and one calf required darting with a second drug dose due to initial subcutaneous injections or a malfunctioning dart; total dose is presented in italics in brackets. Free-ranging and boma-held adults received the same initial doses of etorphine and azaperone.



## *V. Rhinoceros*

Free-ranging rhinoceros were darted from a helicopter whereas captive-held rhinoceros were darted from the ground in bomas. For rhinoceros in Zimbabwe, Cap-Chur<sup>®</sup> syringes (Palmer Cap-Chur Inc., Powder Springs, Georgia, USA) were fired from a powder charge rifle (Pneu-Dart Inc., Williamsport, Pennsylvania, USA). For rhinoceros in South Africa, “Kruger darts” were fired from a modified shotgun. Drug doses are presented in Table 3 and 4.

### **Partial reversal of the opioid effect**

In rhinoceros, mixed opioid agonist-antagonists were injected in an ear vein in attempt to improve respiration by partial reversal of the effect of etorphine or thiafentanil (Study V). Black rhinoceros were given small incremental doses of nalorphine (Table 3) at the discretion of the veterinarian in charge; the drug was usually given if the pulse oximetry readings were below 85%. To white rhinoceros, nalorphine in combination with diprenorphine was given as soon as possible after recumbency (Table 4).

### **Oxygen supplementation**

Intranasal oxygen was administered at a flow rate of 2-5 L/min to two captive and five free-ranging brown bears in response to marked hypoxemia, hyperthermia, or both (Study III). Oxygen was provided from a portable cylinder via a tube inserted approximately 2-5 cm into the nasal cavity of the bears (Fig. 4). Two black and five white rhinoceros received intranasal oxygen at a flow rate of 5-15 L/min, depending on body size (Study V). The tube was inserted into the nasal cavity to the level of the medial canthus of the rhinoceros' eye (Fig. 4).

### **Reversal drugs**

For reversal of the effects of medetomidine, atipamezole (Antisedan<sup>®</sup>, 5 mg atipamezole HCl/ml, Orion Pharma Animal Health) was administered i.m. at 5 times the dose of medetomidine in primates, wolverines and brown bears. For lions, atipamezole was administered i.m. at 5 times the dose of medetomidine during eight anaesthetic events, and thereafter at 2.5 times the medetomidine dose.

During translocation of black rhinoceros, 30-50 mg nalorphine i.v. was administered for loading into transport containers. For reversal of immobilisation in the field or at the boma, black rhinoceros were given

diprenorphine i.v. at 2.2–3.5 times the etorphine dose, or naltrexone (Trexonil<sup>®</sup>, 50 mg naltrexone HCl/ml, Wildlife Pharmaceuticals Inc., Fort Collins, Colorado, USA) i.m. at 20 times the thiafentanil dose. In white rhinoceros, 12–24 mg diprenorphine i.v. was administered for loading into transport containers, and naltrexone i.v. at approximately 20 times the etorphine dose was used for reversal in the boma or in the field.



*Figure 4.* Intranasal oxygen supplementation to an adult female brown bear anaesthetised for radio-collaring in Sweden (left). Blood sampling from an auricular artery of an adult white rhinoceros receiving intranasal oxygen during immobilisation for translocation in South Africa (right).

## Anaesthesia

### Induction

Times from drug injection or darting to first sign of sedation and to recumbency (induction time) were recorded.

### Physiological variables

In all species, body temperature, respiratory rate, heart rate and haemoglobin oxygen saturation were monitored throughout anaesthesia. Blood pressure was measured in adult orangutans (Study I). Arterial blood gas analysis was performed in carnivores and rhinoceros (Study II–V).

### *Body temperature*

Rectal temperature was monitored with a digital thermometer with continuous reading and a measurement range of 28.9–42.2°C (Welch Allyn Diatec 600, Welch Allyn, Inc., Skaneateles Falls, New York, USA). In primates, tympanic temperature was measured using a tympanic thermometer for the ear (Braun ThermoScan Instant Thermometer IRT

1020, Thermoscan Inc., San Diego, California, USA). To prevent hypothermia (rectal temperature  $<37.0^{\circ}\text{C}$ ), wolverines and yearling brown bears were placed on an insulated blanket (Fjellduken<sup>®</sup>, Jerven AS, Odda, Norway), unless their rectal temperature was elevated. To prevent or treat hyperthermia ( $>40.0^{\circ}\text{C}$ ), one or several of the following cooling measures were taken: providing shade, fanning, cooling with water or snow, intravenous fluid, and intranasal oxygen supplementation.

#### *Respiratory rate and heart rate*

Respiratory rate was monitored by observation of chest movements, or by sensing the periodic exhalation of air from the nostrils. Heart rate was monitored by pulse oximetry, by palpation of peripheral pulse or by auscultation of the heart.

#### *Haemoglobin oxygen saturation*

Haemoglobin oxygen saturation ( $\text{SpO}_2$ ) was monitored continuously by pulse oximetry with the pulse oximeter probe attached to the tongue on carnivores (Nellcor NPB-40 Handheld Pulse Oximeter, Nellcor Inc., Pleasanton, California, USA and Tuffsat<sup>®</sup> Pulse Oximeter, Datex-Ohmeda Inc., Madison, Wisconsin, USA). In rhinoceros, the pulse oximeter probe was placed on the ear after scraping the skin on both sides of the ear with a scalpel blade until cartilage was seen. In primates, the pulse oximeter probe was attached to the tongue, a finger or a toe, and a pulse oximeter that required electricity was used (Engström Eos Pulse Oximeter, Gambro Engström AB, Bromma, Sweden).

#### *Blood pressure*

Systolic arterial blood pressure was measured oscillometrically in only adult orangutans (Study I). A non-invasive device with a cuff width of 140 mm was used (Omron Digital Blood Pressure Monitor Model HEM-400 C, Omron Corporation, Tokyo, Japan). The cuff was placed over the left brachial artery; the cuff width was approximately half the circumference of the limb.

#### *Blood sampling and analysis*

Blood samples were collected for analysis of arterial blood gases, acid-base status, and selected haematological and plasma variables (Study II-V). One to five samples were collected per animal at different intervals after drug administration depending on the ongoing procedure.

Arterial samples were collected anaerobically by using self-filling arterial syringes with heparin (PICO™70, Radiometer Copenhagen, Brønshøj, Denmark) (Fig. 5). In carnivores, the femoral pulse was palpated in the groin and the needle was introduced percutaneously into the femoral artery, confirmed by pulsating blood. Firm pressure was applied to the sample site for 2 min post-sampling to avoid bleeding. In rhinoceros, samples were collected from the artery on the medial aspect of the ear (Fig. 4).



*Figure 5.* The i-STAT®1 analyser used for field analysis of arterial blood samples (left). Storage of the i-STAT®1 during fieldwork to ensure optimal temperature conditions (right).

All samples (whole blood) were processed immediately in the field using a portable analyser and cartridges (i-STAT®1 Portable Clinical Analyser and i-STAT® cartridges CG4+, 6+, CG8+, Abbott Laboratories, Abbott Park, Illinois, USA) (Fig. 5). Because the i-STAT®1 analyser only operates in +16 to +30°C, during hot days it was kept with freeze blocks in a polystyrene foam box in an insulated cooler bag. In cold climate, a warm water bottle in the polystyrene foam box prevented the analyser becoming too cold (Fig. 5).

The analysis included measured values for pH, partial pressure of arterial carbon dioxide (PaCO<sub>2</sub>), partial pressure of arterial oxygen (PaO<sub>2</sub>), lactate, haematocrit, sodium (Na), potassium (K), chloride (Cl), urea, ionised calcium and glucose. Blood gas values and pH were corrected to the rectal temperature. Calculated values were provided for actual base excess (BE), actual bicarbonate (HCO<sub>3</sub>), arterial haemoglobin oxygen saturation (SaO<sub>2</sub>) and haemoglobin.

The alveolar-arterial oxygen tension difference, at standard temperature (37°C), was estimated according to the equation (Study III-V):

$$P(A-a)O_2 = PAO_2 - PaO_2$$

$$PAO_2 = P_I O_2 - (PaCO_2/RQ)$$

$$P_I O_2 = F_I O_2 \times (P_B - P_{H_2O})$$

$P(A-a)O_2$  = alveolar-arterial oxygen tension difference

$PAO_2$  = partial pressure of alveolar oxygen

$P_I O_2$  = partial pressure of inspired oxygen

RQ = respiratory quotient

$F_I O_2$  = fraction of inspired oxygen (0.21)

$P_B$  = barometric pressure

$P_{H_2O}$  = saturated water vapour pressure at 37°C (47 mmHg)

For carnivores, the respiratory quotient was assumed to be 0.8 (primarily protein metabolism), whereas for rhinoceros it was assumed to be 1.0 (primarily carbohydrate metabolism). The strong ion difference (SID) was calculated as  $(Na+K)-(Cl+lactate)$  (Study III-V)(DiBartola, 2006).

Hypoxaemia was defined as mild ( $PaO_2$  60-80 mmHg), marked ( $PaO_2$  40-60 mmHg) or severe ( $PaO_2$  <40 mmHg). Acidaemia was defined as a pH <7.35, and marked acidaemia if pH <7.20. Hypocapnia was defined as a  $PaCO_2$  <35 mmHg and hypercapnia was defined as mild ( $PaCO_2$  45-60 mmHg) or marked ( $PaCO_2$  >60 mmHg).

### Recovery

Time from drug injection or darting until administration of the reversal drug was recorded in all animals. The time for reversal was determined during each anaesthetic event, depending on the procedure being performed. In most animals, times from reversal until first signs of recovery and, if obvious, to full recovery were recorded. In lions, time from reversal until head up, sternal, standing and walking were also recorded. After reversal, wolverines and brown bears were left undisturbed to recover in lateral recumbency. Post-anaesthetic survival was followed up in free-ranging radio-marked animals by radio-tracking, whereas animals held in captivity were inspected daily.

## Statistical analysis

The Procedure Mixed was used for statistical analyses in all studies (SAS<sup>®</sup> System 9.1, SAS Institute Inc., Cary, North Carolina, USA) (Kirk, 1995; Littell *et al.*, 2006). Physiological data from captive-held orangutans and free-ranging lions were analysed statistically with analysis of variance (ANOVA) with repeated measures (Study I and II). For animals anaesthetised twice, only the first anaesthetic event was included in the analyses. Paired measurements of rectal and tympanic temperature in orangutans were compared using the intra-class correlation coefficient model 2 (ICC 2). For wolverine data, a two-way ANOVA with repeated measures on one factor was used (Study III). Data from free-ranging brown bears were analysed using a three-way mixed model with the fixed factors Sex, Age group, Time after darting, and the interaction Age group\*Time after darting (Study IV). For data from free-ranging white rhinoceros in lateral recumbency, a one-way ANOVA with repeated measures was used (Study V) to evaluate the effect of partial reversal. For free-ranging black rhinoceros, each physiological variable was studied as change per minute by using a one-sample t-test (Study V). Differences in arterial blood gases and pH between body positions (sternal versus lateral recumbency) and age groups (adults, subadults and calves) were analysed in black rhinoceros with a two-way main effects ANOVA. Spearman Rank Order Correlation ( $r_s$ ) was used to evaluate the correlation between different variables. A  $p$ -value  $<0.05$  was considered significant in all analyses.



## Results and Discussion

The first part of this thesis (Study I-II) included development and assessment of new reversible anaesthetic protocols for use in four species of South-East Asian primates and in free-ranging African lions. The following studies (III-V) included detailed physiological evaluation of different capture methods and drug combinations used by ongoing research projects and management programs for free-ranging wolverines and brown bears in Scandinavia and black and white rhinoceros in Southern Africa. In addition, methods for improvement of arterial oxygenation were evaluated.

### Development of reversible anaesthetic protocols in South-East Asian primates and African lions

The intent in using medetomidine in combination with zolazepam-tiletamine was to allow use of a low dose zolazepam-tiletamine and thus avoid prolonged recovery induced by zolazepam-tiletamine, when reversing the effects of medetomidine with atipamezole. The drug protocol should also be suitable for remote darting and provide adequate duration of anaesthesia, preferably without risk of spontaneous recoveries and the need for repeated dosing. When developing a protocol with these drugs for use in new species or capture situations, such as darting of free-ranging animals, it is valuable to review the literature regarding doses used in related species or captive conspecifics. Initial drug doses were determined by extrapolating published doses of medetomidine-zolazepam-tiletamine in captive animals (Röken, 1997) and doses of zolazepam-tiletamine used in free-ranging orangutans and lions (Kilbourn *et al.*, 1997; Stander & Morkel, 1991). Compared to when using zolazepam-tiletamine alone in these species, initially approximately half that dose was used in combination with medetomidine. In the lion study, the drug doses were gradually decreased



according to observed reactions during recovery and the lowest doses used were 0.027 mg/kg medetomidine and 0.38 mg/kg zolazepam-tiletamine. Interestingly, the medetomidine dose is similar and the zolazepam-tiletamine dose is even lower than doses recommended to captive lions (Röken, 1997), although free-ranging animals commonly need higher doses than captive animals (Kreeger & Arnemo, 2007). In contrast, medetomidine doses over 0.160 mg/kg have been used in combination with ketamine in free-ranging lions (Quandt, 1992).

The low doses of medetomidine-zolazepam-tiletamine reliably induced anaesthesia with a rapid and smooth induction in free-ranging lions and in all four species of primates (Table 5). In free-ranging orangutans, the induction was favourable compared to when using zolazepam-tiletamine alone because the animals were able to grip branches as they descended from the tree, instead of falling unconsciously. One free-ranging orangutan, which required additional drug dosing to induce anaesthesia, developed hyperthermia and apnoea but was successfully resuscitated.

Table 5. *Range of induction times and physiological variables in four species of South-East Asian primates<sup>a</sup> and in African lions anaesthetised with medetomidine-zolazepam-tiletamine.*

	South-East Asian primates		African lions
1 <sup>st</sup> sign of sedation	1-3 min		2-8 min
Recumbency	1-7 min		3-10 min
	Orangutans	Gibbons & macaques	
RR (breaths/min)	22-120	20-56	14-34
HR (beats/min)	64-114	68-160	39-84
Rectal temp. (°C)	34.7-40.0	37.0-40.1	37.6-40.9
SpO <sub>2</sub> (%)	82-94	88-99	85-96
SAP (mmHg)	71-148	NR	NR

RR = respiratory rate; HR = heart rate; SpO<sub>2</sub> = haemoglobin oxygen saturation measured by pulse oximetry; SAP = systolic arterial blood pressure measured non-invasively; NR = not recorded.

<sup>a</sup> Bornean orangutan, Bornean gibbon, long-tailed macaque and pig-tailed macaque.

The duration of anaesthesia was a minimum of 1 hr in all lions and this predictable working time is an advantage for personnel safety when working with dangerous animals. Only one lion showed spontaneous movements before reversal, but the lion was left undisturbed for 10 min, after that it was possible to resume the procedure without additional drug dosing.

Handling that includes changing position of the lion should preferably take place early during anaesthesia, since moving the animal during the later part of anaesthesia might stimulate recovery. In captive-held and free-ranging orangutans similar drug doses were used, and in two of three free-ranging orangutans these doses were sufficient to induce recumbency and anaesthesia. However, the duration of anaesthesia was short and supplemental drug doses were required 9-29 min after darting. Of the captive-held primates, voluntary movements were recorded in four orangutans and one gibbon 19-40 min after initial drug injection. Three of these orangutans required supplemental drug doses in order to complete the ongoing procedures.

#### Field guidelines

4-6 mg medetomidine (0.030 mg/kg) and  
50-80 mg zolazepam-tiletamine (0.40 mg/kg)  
is recommended for 1-2 hr anaesthesia  
of an adult or subadult male or female lion.

Free-ranging lions in Southern Africa have commonly been anaesthetised with a total dose of 500 mg zolazepam-tiletamine alone, but a tenth of that dose is sufficient when used in combination with medetomidine.

After completion of the lion study in Zimbabwe, the developed drug protocol has been used for anaesthesia of free-ranging lions also in Botswana, Mozambique and South Africa. The drug combination has been used for successful mass capture and translocation of a pride of 10 lions (C. Wenham, personal communication). Four adult females were darted with a total dose of 6 mg medetomidine and 100 mg zolazepam-tiletamine, whereas six cubs received 1 or 2 mg medetomidine and 30 or 50 mg zolazepam-tiletamine, depending on size. During the transport, only one adult female required an additional drug dose due to signs of spontaneous recovery. At the final destination, 6 hr after initial darting, atipamezole was administered at 2.5 times the medetomidine dose and the lions were walking within 15 min.

Atipamezole was administered to primates 23-54 min after initial drug injection, or the last supplemental drug dose, and to lions 46-165 min after darting. Recovery was smooth and calm in orangutans, gibbons, long-tailed macaques and lions, whereas excitement and muscle tremors were observed in the two pig-tailed macaques. The first signs of recovery were recorded

within 3–30 min after reversal in primates as well as lions. The two gibbons were fully recovered 11 and 20 min after reversal, respectively. Most orangutans were fully recovered or only lightly sedated within 8–42 min after reversal. Lions stayed sternal until they were able to get up at the first attempt and thereafter walk in a coordinated manner. The three lions that received zolazepam-tiletamine doses  $>1$  mg/kg had smooth but prolonged recoveries and did not walk until 50–166 min after reversal. In contrast, most lions that received zolazepam-tiletamine doses  $<1$  mg/kg were walking within 30 min. In lions, the atipamezole dose was changed from 5 times the medetomidine dose to 2.5 times, which was adequate for reversal. The lower dose reduces costs and the risk of tachycardia, which has been documented in domestic cats and captive lions given higher doses of atipamezole (Tomizawa *et al.*, 1997; Verstegen *et al.*, 1991).

Advantages of the medetomidine-zolazepam-tiletamine protocol in primates and lions include a small drug volume for darting, a reliable induction of anaesthesia, and reversibility with atipamezole. Physiological variables are presented in Table 5 (page 40) and discussed in the next section and in each study (I–II).

## Physiological evaluation of different capture methods and drug combinations

### *Body temperature*

Hyperthermia (rectal temperature  $>40.0^{\circ}\text{C}$ ) was recorded in captive-held long-tailed macaques (2 of 2) and in free-ranging orangutans (1 of 3), adult wolverines (11 of 12), juvenile wolverines (1 of 12) and brown bears (24 of 52). No captive bears, gibbons or pig-tailed macaques developed hyperthermia. Rectal temperatures  $>39.0^{\circ}\text{C}$  were recorded during immobilisation in black rhinoceros (11 of 26) and white rhinoceros (4 of 17). During 11 of 21 lion captures the rectal temperature increased  $>39.0^{\circ}\text{C}$  and despite cooling the temperature reached  $>40.0^{\circ}\text{C}$  in four lions.

Hyperthermia occurred in individuals captured using all methods described in this thesis: helicopter darting, ground darting and physical restraint followed by hand-injection. Thus, in addition to physical exertion or resistance to handling, effects of the immobilising drugs, stress and ambient temperature probably all contributed to the development of

hyperthermia. Aggressive treatment of hyperthermic animals was necessary to prevent further temperature increase during anaesthesia, which emphasizes the importance of physiological monitoring throughout the procedure. Notably, hyperthermia was recorded in animals during anaesthesia in ambient temperatures as low as +3°C, whereas hypothermia developed in animals during ambient temperatures of +33°C.

Body temperature decreased over time in orangutans, wolverines and brown bears whereas it increased in lions. Juvenile wolverines and yearling brown bears had lower rectal temperatures than adults and subadults. Hypothermia (rectal temperature <37.0°C) was recorded in three captive-held orangutans, in one adult and two juvenile wolverines, in two free-ranging yearling brown bears and two captive brown bears. Rectal temperatures <37.0°C were recorded during immobilisation of black (2 of 26) and white rhinoceros (6 of 17), however, the temperature was within the normal range reported in rhinoceros (Citino & Bush, 2007; Fowler & Miller, 2003).

#### *Respiratory rate and heart rate*

Respiratory rate decreased over time in brown bears and adult white rhinoceros, whereas it was stable throughout anaesthesia in orangutans, lions and wolverines, although individual variations occurred. Lions immobilised with medetomidine-zolazepam-tiletamine (Study II) had respiratory rates similar to lions immobilised with zolazepam-tiletamine alone (Stander & Morkel, 1991). Throughout immobilisation of white rhinoceros (Study V), the respiratory rate was decreased compared to reference values in standing, unrestrained conspecifics (8-16 versus 16-23 breaths per minute, respectively) (Citino & Bush, 2007).

Heart rates were stable throughout anaesthesia of orangutans and lions, although individual variations occurred (Table 5, page 40). In wolverines as well as brown bears and white rhinoceros, immobilised with three different protocols, there was a decrease in heart rate over time. Juvenile wolverines and yearling brown bears had higher heart rates than adults and subadults, which may partly be explained by a higher metabolic rate in smaller animals. Heart rates in lions (Study II) were similar to those reported during immobilisation with higher doses of medetomidine (0.100 mg/kg) in combination with ketamine (3-4 mg/kg), and lower than during immobilisation with zolazepam-tiletamine alone (Quandt, 1992; Stander & Morkel, 1991). Bradycardia, defined as a 20% reduction below resting heart

rate (Sedgwick & Martin, 1994), was recorded in seven lions and in one juvenile and six adult wolverines. Medetomidine commonly induces bradycardia, which however can be offset by the sympathomimetic properties of tiletamine and ketamine (Jalanka & Roeken, 1990; Sinclair, 2003).

In immobilised white rhinoceros, the heart rate remained more than twice as high compared to values reported in unrestrained white rhinoceros (80–156 versus 32–42 beats per minute (bpm), respectively) (Citino & Bush, 2007). Tachycardia likely developed as a result of physical activity before recumbency in combination with sympathomimetic effects of etorphine, hypercapnia and hypoxaemia during immobilisation. The higher tachycardia rates recorded initially in free-ranging white rhinoceros (130–156 bpm) compared to in the two boma-held white rhinoceros (110 bpm) probably reflects the contribution of physical exertion before recumbency. Bush *et al* (2004) reported that in white rhinoceros immobilised with lower doses of etorphine, heart rate was elevated despite alleviation of hypoxaemia through oxygen supplementation. Since these animals were hypercapnic, this may indicate that hypercapnia contributed more than hypoxaemia to the development of tachycardia.

#### *Blood pressure*

Systolic arterial blood pressure decreased in orangutans during anaesthesia and values below 80 mmHg were recorded in four individuals. A decrease in blood pressure associated with medetomidine use has been reported in other primate species and can be controlled by intravenous fluid administration (Capuano III *et al.*, 1999; Horne, 2001).

#### **Influence on pulmonary gas exchange**

Low SpO<sub>2</sub> values in some orangutans and lions anaesthetised with medetomidine-zolazepam-tiletamine indicate hypoxaemia and motivate further in-depth study on pulmonary gas exchange in these species. The mean values for PaO<sub>2</sub> in wolverines and brown bears (Table 6) were within the range that is normal for most species (80–100 mmHg) or the range for mild hypoxaemia (PaO<sub>2</sub> 60–80 mmHg) (Tranquilli *et al.*, 2007). Nevertheless, a marked hypoxaemia was recorded during anaesthesia of individual wolverines (PaO<sub>2</sub> 51–57 mmHg) and free-ranging and captive brown bears (PaO<sub>2</sub> 47–59 mmHg). When evaluating results and publishing studies, the focus should not only be on mean values since for the individual animal it may be critical to detect and correct hypoxaemia.

Table 6. Pulmonary gas exchange<sup>d</sup> in brown bears anaesthetised with medetomidine-zolazepam-tiletamine and in wolverines anaesthetised with medetomidine-ketamine, all free-ranging. Mean  $\pm$ SD (range) is presented.

Physiological variable	<u>Brown bears</u>			<u>Wolverines</u>		
	adults & subadults	yearlings	juveniles	adults	juveniles	juveniles
<b>SpO<sub>2</sub></b> <sup>b</sup> (%)	Early	91 $\pm$ 5 (83-97)	91 $\pm$ 5 (85-96)			
	30 min	88 $\pm$ 5 (80-98)	89 $\pm$ 4 (81-96)	15-30 min	85 $\pm$ 3 (80-89)	82 $\pm$ 5 (74-89)
	60 min	90 $\pm$ 4 (81-98)	90 $\pm$ 5 (75-97)	45-60 min	90 $\pm$ 3 (86-93)	90 $\pm$ 3 (86-95)
<b>SaO<sub>2</sub></b> <sup>c</sup> (%)	Early	90 $\pm$ 3 (83-95)	90 $\pm$ 6 (78-95)			
	30 min	88 $\pm$ 5 (73-94)	88 $\pm$ 6 (71-94)	15-30 min	89 $\pm$ 1 (88-91)	87 $\pm$ 5 (76-92)
	60 min	89 $\pm$ 5 (69-94)	92 $\pm$ 3 (83-96)	45-60 min	92 $\pm$ 2 (89-96)	93 $\pm$ 2 (90-95)
<b>PaO<sub>2</sub></b> <sup>d</sup> (mmHg)	Early	91 $\pm$ 13 (63-110)	76 $\pm$ 11 (56-89)			
	30 min	75 $\pm$ 12 (50-96)	71 $\pm$ 11 (47-82)	15-30 min	81 $\pm$ 6 (69-88)	66 $\pm$ 9 (51-79)
	60 min	76 $\pm$ 10 (49-89)	78 $\pm$ 8 (59-87)	45-60 min	75 $\pm$ 13 (62-100)	69 $\pm$ 4 (63-73)
<b>PaCO<sub>2</sub></b> <sup>e</sup> (mmHg)	Early	39 $\pm$ 6 (28-47)	39 $\pm$ 3 (35-44)			
	30 min	42 $\pm$ 6 (33-54)	40 $\pm$ 4 (34-48)	15-30 min	38 $\pm$ 4 (32-47)	41 $\pm$ 2 (38-45)
	60 min	43 $\pm$ 5 (32-52)	42 $\pm$ 3 (37-47)	45-60 min	41 $\pm$ 4 (34-47)	43 $\pm$ 3 (39-48)

<sup>a</sup> SpO<sub>2</sub> = haemoglobin oxygen saturation measured by pulse oximetry; SaO<sub>2</sub> = arterial haemoglobin oxygen saturation (calculated value);

PaO<sub>2</sub> and PaCO<sub>2</sub> = partial pressures of arterial oxygen and carbon dioxide (measured values, temperature corrected).

<sup>b</sup> Significant difference over time in wolverines. <sup>c</sup> Significant difference over time in both species.

<sup>d</sup> Significant difference over time in brown bears and between age groups in wolverines.

A mild to severe hypoxaemia was recorded in white rhinoceros ( $\text{PaO}_2$  31-73 mmHg) but also in black rhinoceros ( $\text{PaO}_2$  39-77 mmHg). Severe hypoxaemia has previously been reported in white rhinoceros immobilised with different opioid-based protocols (Bush *et al.*, 2004; Hattingh *et al.*, 1994), but this is the first study presenting arterial blood gases in free-ranging black rhinoceros, showing that severe hypoxaemia can occur in both species.

Table 7. Alveolar-arterial oxygen tension difference, altitude and barometric pressure during immobilisation of selected wildlife species.

Species	Altitude (m)	$P_b$ (mmHg)	Time after drug injection	$P(A-a)O_2$ (mmHg)
<b>Wolverines</b>				
- adults & yearlings	500-1,300	638-716	15-30 min	27±8
			45-60 min	11±15
<b>Brown bears</b>				
• captivity	500	705-710	30 min	20±10
			60 min	13±9
• free-ranging	300-725	690-746		
- adults & subadults			30 min	36±9
			60 min	32±7
- yearlings			30 min	35±9
		60 min	23±10	
<b>Black rhinoceros</b>				
- sternal adults	~350	~730	15 min	29±9
- lateral adults	~350	~730	15 min	44±7
<b>White rhinoceros</b>				
- lateral adults	~350	724-746	11 min	40±7
			16 min	38±7
			24 min	45±6

$P_b$  = barometric pressure;  $P(A-a)O_2$  = alveolar-arterial oxygen tension difference (at 37°C).

The alveolar-arterial oxygen tension difference indicates an impaired oxygen exchange [ $P(A-a)O_2 > 10$  mmHg] in wolverines, brown bears and both rhinoceros species (Table 7). Impairment of the pulmonary gas exchange may be of both extrapulmonary origin, such as reduced  $P_1O_2$  (high altitude, low barometric pressure) or hypoventilation, and intrapulmonary origin, i.e. ventilation-perfusion mismatch, shunt, or

diffusion impairment (Tranquilli *et al.*, 2007). Arterial oxygenation varies with altitude according to the alveolar gas equation. Altitude (low  $P_{iO_2}$ ) was responsible for ~30% of the reduction in  $PaO_2$  in wolverines, ~20% in free-ranging brown bears and even less in the rhinoceros (Fig. 6). In all species, the major contributor to hypoxaemia was of intrapulmonary origin (Fig. 6), most probably ventilation-perfusion mismatch, shunt, or both.

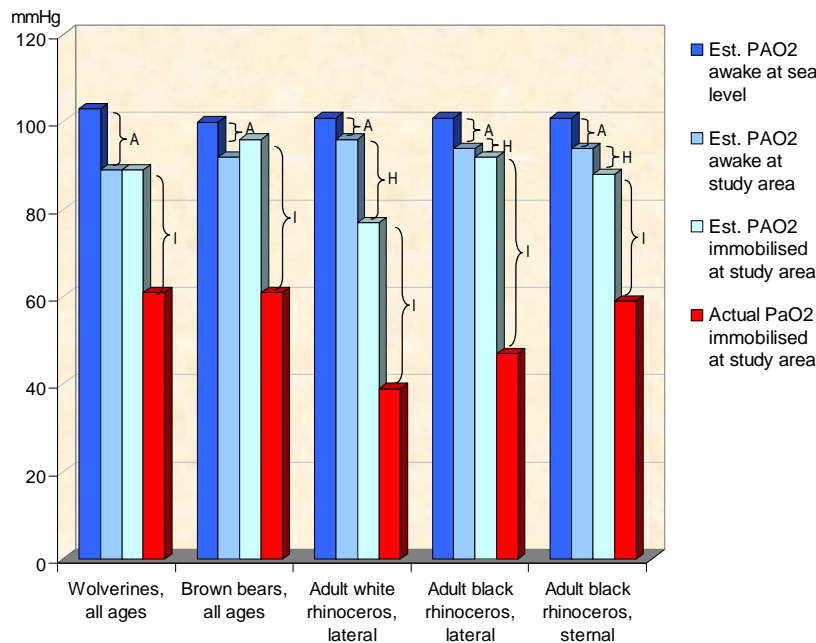


Figure 6. Mean alveolar-arterial oxygen tension difference estimated at standard temperature (37°C) 15-30 min after induction of immobilisation in free-ranging wild animals. Influence on arterial oxygenation ( $PaO_2$ ) by altitude (A), intrapulmonary factors (I) and hypoventilation (H).  $PAO_2$  = partial pressure of alveolar oxygen;  $PaO_2$  = partial pressure of arterial oxygen.

The impairment of gas exchange was more severe in free-ranging than in captive brown bears (Table 7). Interestingly, ventilation-perfusion mismatch and / or shunt contributed to the increase in the  $P(A-a)O_2$  to a higher degree in free-ranging bears (~80%) than in captive bears (~60%). This suggests that the degree of impairment of pulmonary gas exchange in bears could be dose dependent, because lower drug doses were used for bears in captivity (Table 2, page 28). Specifically, the impairment in bears was likely related to the medetomidine, as  $\alpha_2$ -agonists can increase the pulmonary vascular pressure and disturb the matching of pulmonary perfusion in



relation to ventilation, resulting in a reduction in PaO<sub>2</sub> (Marntell *et al.*, 2005). Since the medetomidine doses used in wolverines were very high (up to 0.44 mg/kg), it would be interesting to evaluate whether a lower dose would improve arterial oxygenation in this species.

The PaCO<sub>2</sub> increased over time in wolverines and brown bears (Table 6, page 45), although in wolverines it remained within the range that is normal for most species (35–45 mmHg), indicating adequate ventilation (Tranquilli *et al.*, 2007). The mild hypercapnia recorded in some free-ranging brown bears may have developed as a result of hypoventilation, an increased metabolism due to hyperthermia, or both. In contrast, a marked hypercapnia was recorded in more than 50% of the black rhinoceros and in all white rhinoceros (Study V), which were the only two species immobilised with opioid-based protocols. Hypoventilation contributed to a greater degree to the increase in P(A-a)O<sub>2</sub> in white than in black rhinoceros (Fig. 6). These results indicate that white rhinoceros are more sensitive than black rhinoceros to the respiratory depressant effects of opioids, as previously suggested (Bush *et al.*, 2004). However, other causes such as intercostal muscle rigidity and upper airway obstruction can also contribute to hypoventilation.

Amongst black rhinoceros with the lowest oxygen tensions and with a marked hypercapnia were the two rhinoceros immobilised with thiafentanil. Thiafentanil was used in two free-ranging black rhinoceros to evaluate the induction time, as a rapid induction is important to reduce the risk of injury, exertion and hyperthermia when capturing rhinoceros in rough terrain or during hot conditions. Recumbency occurred in both rhinoceros within 4–5 min, which is similar to induction times reported when using thiafentanil alone or in combination in other wildlife species (Citino *et al.*, 2001; Grobler *et al.*, 2001). Further studies on thiafentanil doses and physiological effects in black rhinoceros are needed.

#### Partial reversal

All but two black rhinoceros experienced hypoxaemia during immobilisation despite nalorphine administration (Study V). Adult white rhinoceros remained markedly hypoxaemic after partial reversal with both nalorphine and diprenorphine. In laterally recumbent white rhinoceros, there was no significant improvement in PaO<sub>2</sub> after partial reversal whereas PaCO<sub>2</sub> decreased significantly; however, most rhinoceros remained markedly hypercapnic (Table 8). The lack of improvement in arterial

oxygenation might be due to suboptimal doses of the partial reversal drugs or due to severe ventilation-perfusion mismatch or shunt during lateral recumbency. The slight but significant decrease in PaCO<sub>2</sub> indicates improved ventilation, which may be due to administration of the partial reversal drugs or a result of time, as the effect of etorphine wears off. A control group could have told the difference.

The interactions between opioids with differing receptor binding profiles are complex with the potential to produce additive, synergistic, or antagonistic effects (Grimm & Lamont, 2007). The clinical effects produced by co-administration of opioid agents with differing receptor binding profiles likely depend on many factors, including the specific drugs, doses and species involved. This is an area within wildlife immobilisation that deserves further attention.

Table 8. *Effect of partial reversal with 10 mg nalorphine and 1.2 mg diprenorphine on pulmonary gas exchange during immobilisation of adult white rhinoceros in lateral recumbency.*

Variable	Before partial reversal	Time after partial reversal	
		5 min (3-7 min)	12 min (9-15 min)
SaO <sub>2</sub> (%)	54±8 (39-64)	62±13 (41-77)	68±13 (58-87)
PaO <sub>2</sub> (mmHg)	35±3 (32-40)	40±8 (29-52)	43±12 (34-62)
PaCO <sub>2</sub> (mmHg)	69±5 (59-75)	68±8 (59-78)	60±9 <sup>a</sup> (49-72)
<i>n</i>	10	7	5

<sup>a</sup>Significant difference from the first and second sample.

### Oxygen supplementation

Intranasal oxygen supplementation proved to be a simple and efficient method to improve arterial oxygenation during immobilisation of brown bears and rhinoceros. During field work in remote areas, delivery of the minimum required flow rate is an advantage so the oxygen cylinder will last as long as possible. For brown bears, oxygen was initially administered at a flow rate of 5 L/min, but because their PaO<sub>2</sub> increased well above the expected normal range of 80-100 mmHg, the flow rate was subsequently decreased to the following bears (Table 9). A flow rate of 2 L/min, given to brown bears weighing 12-120 kg, was sufficient to improve the PaO<sub>2</sub> to an adequate level.

Table 9. *Effects of intranasal oxygen supplementation on pulmonary gas exchange<sup>a</sup> during medetomidine-zolazepam-tiletamine anesthesia of brown bears. Oxygen was administered to these bears due to a marked hypoxemia (PaO<sub>2</sub> <60 mmHg) and / or hyperthermia.<sup>b</sup>*

Age, sex, body mass <sup>c</sup>	Flow rate (L/min)		SpO <sub>2</sub> (%)	SaO <sub>2</sub> (%)	PaO <sub>2</sub> (mmHg)	PaCO <sub>2</sub> (mmHg)
Subadult ♂ 87 kg	5	Pre-O <sub>2</sub> :	NR <sup>c</sup>	84	55	43
		<b>18 min of O<sub>2</sub>:</b>	<b>NR</b>	<b>100</b>	<b>192</b>	<b>45</b>
Subadult ♀ 62 kg	5	Pre-O <sub>2</sub> :	NR	86	58	46
		<b>2 min of O<sub>2</sub>:</b>	<b>87</b>	<b>98</b>	<b>113</b>	<b>47</b>
		<b>32 min of O<sub>2</sub>:</b>	<b>89</b>	<b>100</b>	<b>306</b>	<b>50</b>
Yearling ♀ 19 kg	2	Pre-O <sub>2</sub> :	NR	77	55	45
		<b>8 min of O<sub>2</sub>:</b>	<b>93</b>	<b>100</b>	<b>216</b>	<b>49</b>
Yearling ♀ 15 kg	2	Pre-O <sub>2</sub> :	91	83	59	48
		<b>25 min of O<sub>2</sub>:</b>	<b>94</b>	<b>100</b>	<b>290</b>	<b>51</b>
Yearling ♀ 12 kg	2	Pre-O <sub>2</sub> :	82	71	47	43
		<b>14 min of O<sub>2</sub>:</b>	<b>86</b>	<b>99</b>	<b>165</b>	<b>46</b>
		11 min post-O <sub>2</sub> :	88	81	55	49
Adult ♀ <sup>b</sup> 72 kg	2	Pre-O <sub>2</sub> :	90	78	58	48
		<b>16 min of O<sub>2</sub>:</b>	<b>83</b>	<b>98</b>	<b>133</b>	<b>47</b>
Adult ♀ 120 kg	2	Pre-O <sub>2</sub> :	NR	85	59	39
		<b>16 min of O<sub>2</sub>:</b>	<b>NR</b>	<b>97</b>	<b>109</b>	<b>46</b>
		10 min post-O <sub>2</sub> :	84	91	70	41
Adult ♀ <sup>b</sup> 93 kg	2	Pre-O <sub>2</sub> :	84	82	63	40
		<b>12 min of O<sub>2</sub>:</b>	<b>86</b>	<b>97</b>	<b>124</b>	<b>49</b>
		25 min post-O <sub>2</sub> :	91	90	77	43
Subadult ♀ <sup>b</sup> 65 kg	2	Pre-O <sub>2</sub> :	NR	91	88	38
		<b>40 min of O<sub>2</sub>:</b>	<b>95</b>	<b>96</b>	<b>110</b>	<b>44</b>

<sup>a</sup>SpO<sub>2</sub> = haemoglobin oxygen saturation measured by pulse oximetry; SaO<sub>2</sub> = arterial haemoglobin oxygen saturation (calculated value); PaO<sub>2</sub> and PaCO<sub>2</sub> = partial pressures of arterial oxygen and carbon dioxide (measured values, temperature corrected).

<sup>b</sup>Hyperthermia = rectal temperature >40.0°C.

<sup>c</sup>NR = not recorded because the pulse oximeter failed to produce a reading.

Intranasal oxygen administered at 5-10 L/min to black and white rhinoceros subadults and calves markedly improved the PaO<sub>2</sub> to over 90 mmHg, irrespective of their body position (Study V). In contrast, although a higher flow rate (15 L/min) was administered to two adult white rhinoceros in lateral recumbency, their PaO<sub>2</sub> increased but remained within the range for mild to marked hypoxaemia. Bush *et al* (2004) reported a mean PaO<sub>2</sub> of 104 mmHg in adult and subadult white rhinoceros that received oxygen via a nasotracheal tube at a flow rate of 15-30 L/min during sternal recumbency. Their higher PaO<sub>2</sub> may be due to tracheal insufflation, a higher flow rate, different drug doses and / or sternal recumbency. Compared to nasotracheal intubation, intranasal oxygen supplementation is an easier method of oxygen administration which is practical for field conditions.

After publication of the wolverine study, the medetomidine-ketamine protocol, in combination with intranasal oxygen supplementation at a flow rate of 0.5-2 L/min, has been used for anaesthesia of wolverines at 2,500 m elevation in the state of Montana, USA (R. Inman, personal communication). Haemoglobin oxygen saturation measured by pulse oximetry increased from 76% before oxygen supplementation to 90-100% when oxygen was provided, indicating that intranasal oxygen supplementation can improve arterial oxygenation in wolverines anaesthetised with medetomidine-ketamine.

Due to risks of developing hypoxaemia, as shown with arterial blood analysis, with the drugs and doses presented here (Study III-V), oxygen supplementation is strongly recommended to improve safety for immobilised rhinoceros, brown bears and wolverines. Occasionally low pulse oximetry readings indicate hypoxaemia also in some orangutans and lions, which probably will benefit from oxygen supplementation as well. Since the consequences of hypoxaemia may be difficult to measure, a negative impact on an organ system does not need to be proven before therapy can be initiated. If the potential for improving overall organ function outweighs the risks and disadvantages of the therapy, it should be given strong consideration (Tranquilli *et al.*, 2007). Since muscle tremors and hyperthermia increase the oxygen consumption, these are additional reasons to provide oxygen supplementation. In addition, flushing of the nasal cavities with oxygen is a safe and simple method to protect the brain against hyperthermal damage (Einer-Jensen *et al.*, 2002). Because hypoxemia

recurred when oxygen supplementation was discontinued in rhinoceros as well as brown bears, it is necessary to provide oxygen continuously throughout the immobilisation period. Intranasal oxygen supplementation probably requires different flow rates for different sized rhinoceros and for different body positions. However, supplemental oxygen becomes less effective in improving the PaO<sub>2</sub> if shunt flows are over 30% (Tranquilli *et al.*, 2007), which may develop in immobilised laterally recumbent adult rhinoceros. At shunt flows over 50%, oxygen supplementation will not be effective at all.

For grizzly bears anaesthetised with xylazine-zolazepam-tiletamine, an oxygen flow rate of 6-10 L/min improved hypoxaemia, based on clinical signs and pulse oximetry, however, arterial blood gases were not reported (Cattet *et al.*, 2003b). Hypoxaemia can be missed if arterial oxygenation is evaluated based on pulse oximetry and not arterial blood gases, as shown in brown bears (Study IV) and in polar bears (*Ursus maritimus*) anaesthetised with zolazepam-tiletamine (Cattet *et al.*, 1999b). Further blood gas studies are needed to determine optimal oxygen flow rates in the different wildlife species.

#### Body position

In black rhinoceros, arterial oxygenation was significantly higher during sternal than lateral recumbency, which is in line with previous reports in large and heavy animals (Gleed & Dobson, 1988). The limited number of white rhinoceros in sternal recumbency did not allow statistical analysis for comparison of different body positions in the species. However, all white rhinoceroses in lateral recumbency developed a severe hypoxaemia (Table 8, page 49). Interestingly, the highest P<sub>a</sub>O<sub>2</sub> values in adult white rhinoceros (73 mmHg) were measured during sternal recumbency, whereas individuals during standing immobilisation had lower P<sub>a</sub>O<sub>2</sub> values (43 and 51 mmHg, respectively) (Study V).

In conclusion, alterations in pulmonary gas exchange can vary with species, animal size, positioning during immobilisation and different drugs and doses, showing the importance of evaluating different anaesthetic protocols in the various wildlife species.

#### Acid-base alterations

Acidaemia was recorded during anaesthesia of all carnivore and rhinoceros species (Study II-V). A decrease in pH shifts the oxygen dissociation curve to the right and thus facilitates the release of oxygen from haemoglobin in

the tissues (Bohr effect). On the other hand, myocardial contractility decreases with acidosis and at a pH <7.2 there is a higher risk of arrhythmias (DiBartola, 2006). A pH <7.2 was recorded in free-ranging individual brown bears, wolverines and black and white rhinoceros. Since most free-ranging rhinoceros (Study V) developed not only hypercapnia but also elevated lactate concentrations, their low pH reflects a mixed respiratory and metabolic acidaemia. The imposition of respiratory acidosis on metabolic acidosis can lead to severe acidaemia and a poor outcome. Death due to cardiac arrest during immobilisation has been reported in a pregnant black rhinoceros with severe acidosis (Stegmann *et al.*, 2001).

Lactic acidosis can be defined as a pH <7.35 and a plasma lactate concentration greater than 5 mmol/L (DiBartola, 2006). Although capture myopathy has been described in several of the species included in this thesis (lion, black rhinoceros, different primates and bear species) (Basson & Hofmeyr, 1975; Cattet *et al.*, 2005; Joubert & Stander, 1990; Kaczensky *et al.*, 2003), there are no reports on the presumed underlying lactate elevations in these species. Rhinoceros darted in bomas (Study V), brown bears darted at the zoo (Study IV) and free-ranging lions darted from a vehicle (Study II) all had lactate concentrations below 4 mmol/L. None of these animals went through the intense physical exertion demonstrated during the other capture methods (helicopter darting, capture with snare pole). Elevated lactate concentrations were recorded in free-ranging brown bears, wolverines, and black and white rhinoceros, indicating capture-induced lactic acidaemia. In brown bears and white rhinoceros, lactate concentrations as high as 15–20 mmol/L were recorded after helicopter darting. In comparison, lactate concentrations in grey hounds and race horses can reach 30 mmol/L after a race (Ilkiw *et al.*, 1989; Rose *et al.*, 1988). In wolverines and brown bears, lactate concentration decreased over time whereas pH, base excess, bicarbonate and SID increased, indicating that anaesthesia did not cause any further imbalance of the acid-base status in these animals.

There was no correlation between lactate concentrations and the distances the animals moved before and after darting, as tested for in wolverines and brown bears darted from a helicopter. The time of intensive helicopter pursuit was seldom longer than 30 seconds for the actual darting. On the other hand, a radio-marked animal could be tracked and driven, sometimes without being observed from the air due to dense vegetation, for longer periods before there was an opportunity to dart the animal. The

magnitude of post-exercise changes in lactate and pH probably depends on a number of factors, of which exercise intensity seems more important than the duration and distance of the helicopter pursuit. As reported in zebras (*Equus burchelli*) and blesbok (*Damaliscus dorcas phillipsi*) which were chased rapidly over short distances, intense forced exercise resulted in a more severe acidaemia compared to in animals that were run longer distances at slower speed (Harthoorn & van der Walt, 1974; Harthoorn & Young, 1974). In addition, the extent of physical exertion is probably also influenced by the type of terrain, such as steepness and snow depth, and the ambient temperature. Lactate production may be species-specific depending on post-darting behaviour and muscle fibre type composition.

Capture-induced lactic acidaemia developed not only in animals after helicopter pursuit, but also in manually restrained wolverines, which were vigorously fighting the snare pole before they were anaesthetised. The highest lactate concentrations recorded in anaesthetised wolverines were 6.8 mmol/L after helicopter darting and 5.2 mmol/L after manual restraint. Statistically, lactate concentration did not differ significantly between adult wolverines darted from a helicopter and juveniles captured with a snare pole. Thus, the muscular activity related to resisting manual restraint seemed to result in a similar anaerobic response to the physical exertion related to helicopter darting of wolverines. Bush *et al* (1977) reported that physical restraint in primates, in the absence of immobilising drugs, led to hyperthermia and severe metabolic acidosis within 2–6 min, with pH values as low as 6.8 in highly excited animals. In contrast, captive primates immobilised with ketamine or zolazepam-tiletamine had a near normal acid-base balance and were handled more easily than the physically restrained animals (Bush *et al.*, 1977). Unless an animal is relatively tame and therefore calm during handling, chemical immobilisation is probably less stressful for a wild animal than handling during physical restraint alone. In Scandinavia, most free-ranging wild mammals that are handled for research purposes are chemically immobilised, but some species are handled during physical restraint only. The effects of physical restraint alone of wildlife is an area that deserves further attention from an animal welfare perspective, since handling of wild animals without chemical immobilisation can result in severe stress and physiological alterations.

## Animal welfare considerations

In Scandinavia, most people seem to understand the need to carry out research on free-ranging large carnivores, such as brown bears and wolves. However, capture of wild animals has raised concerns about animal welfare issues from animal ethics committees, animal rights groups, media, researchers, politicians, wildlife managers and the general public. Although mortality rates have been low, mortality is not a suitable indicator for animal welfare. This thesis has identified a number of physiological alterations during wildlife immobilisation and anaesthesia which may have effects on the individual animal following recovery. Long-term effects documented in different wildlife species include negatively altered movement rates and reproduction (Cattet *et al.*, 2008; Côté *et al.*, 1998). To determine the full impact of capture and handling, behavioural and physiological effects should be evaluated not only during handling but also post-capture. Ultimately, the welfare of the animal must always be the first priority and the value of the research must be worth the potential detrimental effect on the animals involved.

## Future research

Further study is needed to:

- determine optimal doses of medetomidine-zolazepam-tiletamine for anaesthesia in free-ranging orangutans
- evaluate pulmonary gas exchange and acid-base status in South-East Asian primates and African lions anaesthetised with medetomidine-zolazepam-tiletamine
- compare physiological variables from wolverines captured with different methods and improve arterial oxygenation during anaesthesia with medetomidine-ketamine
- improve pulmonary gas exchange during immobilisation of black and white rhinoceros, which can include determination of optimal body positioning of immobilised white rhinoceros and further evaluation of mixed agonists-antagonists for partial reversal of the opioid effects
- evaluate the use of thiafentanil for immobilisation of rhinoceros
- determine optimal flow rates of intranasal oxygen during immobilisation of different wildlife species





## Conclusions

Through in-depth monitoring, including analysis of arterial blood samples, various degrees of physiological alterations associated with capture, immobilisation and anaesthesia of wildlife were identified.

Study I and II:

- Effective reversible anaesthetic protocols were developed for free-ranging lions and four species of South-East Asian primates by the use of medetomidine-zolazepam-tiletamine. Low doses reliably induced anaesthesia with a rapid and smooth induction and good muscle relaxation. Respiratory and heart rates were stable throughout anaesthesia. Prolonged recoveries were avoided by the administration of atipamezole for anaesthetic reversal.

Study I-V:

- Both hypo- and hyperthermia, unrelated to ambient temperature, were recorded in most species. The portable blood gas analyser enabled detailed physiological evaluation of capture and anaesthesia in tough conditions during remote field projects. Impaired arterial oxygenation and capture-induced lactic acidaemia were detected in wolverines, brown bears and both rhinoceros species. The major contributor to the impaired pulmonary gas exchange was of intrapulmonary origin, most likely ventilation-perfusion mismatch including shunt. In rhinoceros, hypoventilation also contributed to hypoxaemia. The severity of lactic acidaemia varied between species and capture methods.

Study IV-V:

- Despite partial reversal of the opioid effects, rhinoceros remained hypoxaemic. Intranasal oxygen supplementation proved to be a simple and effective technique for treatment of hypoxaemia in brown bears and rhinoceros. Arterial oxygenation was higher during sternal compared to lateral recumbency in black rhinoceros.

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