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Evaluation of two different doses of butorphanol-medetomidine-midazolam for anaesthesia in free-ranging versus captive black-footed cats (*Felis nigripes*)

by

Birgit Eggers

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Supervisor: Dr. Leith Meyer

Co-Supervisor: Dr. Adrian Tordiffe

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DECLARATION

I, Birgit Eggers, do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisors, I have received no assistance.

This dissertation is presented in partial fulfillment of the requirements for the degree Master of Veterinary Medicine (Fer.) at the University of Pretoria.

I declare that neither the substance, nor any part of this dissertation has previously been submitted by me for a degree at this or any other tertiary institution.

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Date: 31st March 2016

Signed: 

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SUMMARY

The black-footed cat (*Felis nigripes*) is the rarest, smallest wild felid species of southern Africa and is categorized as vulnerable on the IUCN Red List 2012 (Sliwa 2008). These cats are solitary, nocturnally active predators with a high metabolic rate. In order to fuel this metabolism they hunt all night, catching and consuming approximately one fifth of their body weight. In order to facilitate research studies and sample collection a reliable, safe, fully reversible anaesthetic drug combination for use in the field is imperative to enable the cats to resume their hunting as quickly as possible post anaesthesia. Both the captive and the free-ranging populations face many survival challenges, including renal amyloidosis, and the use of an anaesthetic drug combination that does not compromise renal function would be of major benefit. To date, there is no published data on the physiological effects of any anaesthetic protocol in this species. The butorphanol-medetomidine-midazolam combination has been safely and successfully used in a variety of domestic and other wild animal species, and recently it has been used in black-footed cats in the field and zoo setting.

We used the butorphanol-medetomidine-midazolam drug combination to anaesthetise black-footed cats, both in captivity and in the wild. As the drug doses currently used in wild free-ranging animals are approximately twice as high as those required in captive animals, concerns have been raised regarding the physiological safety of using a higher dose of this anaesthetic combination in these animals. My aim was to document, evaluate and compare the anaesthetic and physiological effects induced by the use of two different doses of the butorphanol-medetomidine-midazolam combination to effectively and safely anaesthetise captive and free-ranging black-footed cats.

Due to the differing physiological stressors associated with the capture methods in the free-ranging black-footed cats these animals were divided into two groups, namely those that were dug out of burrows, and those that had been caught after a short intense chase. Once captured, all the cats were hand injected into a hind limb muscle group with the anaesthetic drug combination. Injections for different mass categories had been prepared prior to capture and the doses that were given were based on quick visual estimates of an animal's mass. In total, 23 black-footed cats, nine captive and 14 free-ranging, were anaesthetised. Times to induction, quality of anaesthesia and times to recovery after reversal of the effects of the anaesthetic drugs were recorded. Physiological variables recorded every 10 minutes during anaesthesia were heart rate, respiratory rate, SpO_2, $ETCO_2$, rectal temperature, systolic, mean and diastolic non-invasive blood pressures. Three central venous blood samples, taken at 20-minute intervals during anaesthesia, were analyzed for pH, lactate and glucose concentrations and $cTCO_2$ and base excess were determined.

Anaesthetic induction and recoveries were quick, smooth and uneventful with mean times of 5.9 minutes and 4.5 minutes respectively. We determined that even higher doses than our original target doses were required for induction and maintenance of a 45 minute anaesthesia. The quality and depth of anaesthesia were optimal for the minor sampling procedures performed, with the majority of cats maintaining a deep level of anaesthesia (Score 5) for 45 minutes before the antagonist combination was administered. Cardiac variables remained within normal physiological limits throughout the anaesthesia. Mild hyperventilation, moderate hypoxaemia and a mild to moderate lactic acidosis were initially recorded in the chased free-ranging animals. The respiratory and acid-base variables in the other two groups of cats were within clinically acceptable limits. A gradual decrease in body temperature, despite the animals being placed on hot water bottles, was recorded in all the cats and thus a better means to mitigate this heat loss is recommended. Hyperglycaemia was recorded in all black-footed cats, and the fear, anxiety and exertion of the chase would have contributed to the hyperglycaemia induced by the use of the α_2 adrenoreceptor-agonist medetomidine in the anaesthetic drug combination.

Intramuscular injection of butorphanol, medetomidine and midazolam in black-footed cats induces rapid and smooth induction and is a safe and effective anaesthesia in both captive and free-ranging animals, despite higher doses used in free-ranging animals. The method used to capture free-ranging animals had a greater influence on the physiological stability of the anaesthetised animals than did the dose of drugs used. If black-footed cats exert themselves during a chase, before they receive this anaesthetic drug combination, they develop mild to moderate hypoxaemia, tissue hypoxia and metabolic lactic acidosis during the anaesthesia. Therefore, oxygen supplementation during anaesthesia is recommended in black-footed cats if they are chased directly before induction.

1. INTRODUCTION

The black-footed cat (*Felis nigripes*) is the rarest, smallest wild felid species of the southern African sub-region. It inhabits the arid, central parts, which have an annual rainfall of 100-500mm. It is a strictly nocturnal, shy and solitary animal, becoming aggressive when cornered. Agricultural habitat encroachment with its' concomitant indiscriminate pesticide use, baiting and trapping of perceived "problem" predators, hunting dog packs and increased exposure to domestic and feral cats with potential disease transmission, are all risks threatening the survival of the free-ranging population of black-footed cats (Olbricht and Sliwa 1997).

A single long-term field study has shown the density of adult cats to be particularly low (Sliwa 2008). As of 2002, black-footed cats are categorized as vulnerable on the IUCN Red List (2012), and are included on CITES Appendix 1 (Sliwa 2008). There is a dearth of published information on black-footed cats, particularly from animals in the wild, and currently the Black-Footed Cat Working Group is conducting multidisciplinary, long-term research on the biology, distribution, ecology, health and reproduction of these animals (Sliwa *et al*. 2011).

In order to ensure the genetic diversity and survival of the species, black-footed cats are held *ex situ* in a number of zoos and breeding facilities in the United States of America, Europe and South Africa (Stadler 2012). However, these captive animals also have inherent problems with a high percentage of early mortality, low breeding success, skewed sex-ratios, and amyloidosis (Olbricht and Sliwa 1997; Stadler 2012; Terio *et al*. 2008; Zimmermann *et al*. 2011), which have all contributed to decreasing numbers of captive-held cats worldwide, with only 74 animals registered in the International Studbook for black-footed cats in 2011 (Stadler 2012).

Currently, the Black-Footed Cat Working Group are taking samples from both captive and wild populations to determine the presence of amyloidosis, and semen is collected for cryopreservation and future assisted reproductive techniques (Stadler 2012). Similar samples are taken from captive populations across the world. In order to get these and other samples animals need to be safely anaesthetised. No studies on anaesthesia in this species have been documented to date. Therefore evaluating and publishing the effects and safety of currently used anaesthetic protocols is important. Currently the reversible butorphanol-medetomidine-midazolam anaesthetic combination is used in the field in black-footed cats, but little is known about the use of this drug combination in this species.

The butorphanol-medetomidine-midazolam drug combination was used in a few of the free-ranging black-footed cats in the 2012 capture season (pers. comm. Dr. Nadine Lamberski), with no apparent side effects or deaths. However, as the drug doses in wild, free-ranging

animals is approximately 2x higher than in their captive counterparts (Gunkel and Lafortune 2007; Sladky *et al*. 2000; Williams *et al*. 2003), concerns were raised about the possible negative physiological effects of these high doses.

Therefore, the aims of this study were:

1. To evaluate and document the safety and effectiveness of the butorphanol-medetomidine-midazolam anaesthetic combination in black-footed cats.

2. To evaluate the physiological side effects of this anaesthetic combination in captive and free-ranging black-footed cats and determine whether these effects are exacerbated when higher doses of the drugs are used in free-ranging animals.

2. LITERATURE REVIEW

The aim of any anaesthetic protocol used in free-ranging wild animals in which short-term chemical capture followed by release are the objective, is to find a synergistic, miscible drug combination which induces a rapid, safe and smooth anaesthesia of good quality, adequate depth and length, with minimal side effects, smooth recoveries, and which is fully reversible (Burroughs *et al*. 2012; Gunkel and Lafortune 2007; Wenger *et al*. 2010).

An anaesthetic combination of butorphanol (a partial mixed opioid agonist/antagonist), medetomidine (an α2 adrenoreceptor-agonist) and midazolam (a benzodiazepine) has been studied in a variety of domestic (Bierman *et al*. 2012; Verstegen and Petcho 1993) and wild (red fox - *Vulpes vulpes*; Bertelsen and Villadsen 2009, wild dogs – *Lycaon pictus*; Fleming *et al.* 2006, patas monkeys - *Erythrocebus patas*; Kalema-Zikusoka *et al*. 2003, cheetahs - *Acinonyx jubatus*; Lafortune *et al*. 2005, lions - *Panthera leo*; Wenger *et al*. 2010, and ring-tailed lemurs - *Lemur catta*; Williams *et al*. 2003) species, with good results in all the desired criteria. As these drugs appear to work synergistically, individual drug doses can be reduced, and the side effects that are normally associated with each drug on its' own are ameliorated (Bush *et al*. 2012; Gunkel and Lafortune 2007). Also of great importance is that this combination is fully reversible.

Traditionally, most anaesthetic protocols in wild felids have included a cyclohexylamine. In larger felids Zoletil®, a combination of the cyclohexylamine tiletamine, and the benzodiazepine zolazepam (Posner and Burns 2009a), has been used extensively, either alone or in combination with an α2 adrenoreceptor-agonist, like medetomidine. In the smaller felid species, ketamine, which is three times less potent than tiletamine, is combined with either medetomidine or xylazine, both α2 adrenoreceptor-agonist drugs. Cyclohexylamines produce a dissociative, cataleptoid anaesthesia, characterized by muscle hypertonicity and rigidity and spontaneous involuntary muscle movements (Posner and Burns 2009a). They provide good analgesia and have minimal respiratory depressant effects. They do however increase noradrenaline release, both by central adrenergic stimulation and by decreasing its' uptake (Posner and Burns 2009a), resulting in hypertension, an increased heart rate and increased myocardial oxygen demand (Bierman *et al*. 2012; Dobromylskyj 1996; Posner and Burns 2009a). In combination with α2 adrenoreceptor-agonists a smooth induction with good analgesia, muscle relaxation and anaesthetic depth is achieved, however vomition, respiratory depression with hypoxia and transient apnoea, hypertension, bradycardia and cardiac arrhythmias are frequent side effects seen when this combination is used (Bierman *et al*. 2012; Dobromylskyj 1996; Posner and Burns 2009a; Zeiler *et al*. 2014). Sudden arousals and post-anaesthesia ataxia (Bierman *et al*. 2012; Kalema-Zikusoka *et al*. 2003; Larsen *et al*. 2002; Verstegen and Petcho 1993; Wenger *et al*. 2010) are a major disadvantage with

ketamine, whereas recoveries with Zoletil are prolonged and potentially stormy (Gunkel and Lafortune 2007).

Cyclohexylamine-induced sympathetic stimulation can add to the expected increase in circulating catecholamines induced by the stress of capture and can exacerbate hypertension even further. Amyloidosis with renal failure has retrospectively been proven as a major cause of death in captive black-footed cats (Terio *et al*. 2008), and has recently been recorded by Zimmermann et al. (2011) in a free-ranging animal. As cyclohexylamines induce hypertension and are excreted mostly unchanged by the kidneys in cats (Posner and Burns 2009a), their use as part of an anaesthetic protocol in black-footed cats could be undesirable especially if an animal is affected by amyloidosis. Furthermore, there is no antidote to cyclohexylamines, therefore the use of these drugs in the field is not optimal.

Although a combination of ketamine, midazolam and butorphanol has been studied in domestic cats and is advocated by Gunkel and Lafortune (2007) for use in small, healthy wild felids, it is only useful for short, non-invasive procedures. Determining the health of a free-ranging animal by means of a clinical examination before any drugs are administered is not possible, and any underlying disease can therefore not be ascertained.

Research is ongoing to find safer and fully reversible anaesthetic combinations, especially for free-ranging wildlife situations, where adverse drug reactions are not that easily dealt with and vulnerability to predation on anaesthetic recovery and release are a real threat. The butorphanol-medetomidine-midazolam combination is proving to be a safe reversible combination in the different species in which it has been studied so far. Anaesthetic agents are normally studied and tested in domestic animals and results are extrapolated to wildlife for practical, and where endangered wildlife is concerned, ethical reasons. However, although extrapolation is useful it is important to determine the specific physiological responses of a species to these agents, as there appear to be species-specific responses, particularly in felids, to all anaesthetic drug combinations. As the circumstances under which these drugs are administered differ between domestic and wild species, the efficacy and pharmacodynamics may also differ.

Butorphanol, as a partial mixed opioid agonist/antagonist, provides dose-dependent analgesia and sedation through agonism at the spinal and supraspinal kappa-receptors, and partial agonism at the mu-receptors (Kukanich and Papich 2009). Although its affinity for mu-receptors is high, it has a low efficacy at these receptors (Trescot *et al.* 2008). Used alone butorphanol, like all opioids, causes respiratory depression, however its ability to improve or reverse the respiratory depression induced by other opioid drugs, while still maintaining analgesia is believed to be caused by a partial mu-receptor antagonism (Bush *et al*. 2012; Kukanich and Papich 2009). Cardiovascular effects of opioids differ in different species, and

differ in the severity of their effects according to their different potencies, but are generally minimal in domestic animals (Kukanich and Papich 2009), especially in appropriate clinical doses. Opioids are predominantly used for analgesia in domestic animals, whereas in wildlife they are important immobilizing drugs used for capture. They are commonly used as adjuncts with sedatives and anaesthetics due to their additive and synergistic sedative and hypnotic effects (Bush *et al*. 2012; Kukanich and Papich 2009). Butorphanol is a weak immobilizing opioid which when used on its own, sometimes only causes a standing sedation from which an animal can quickly be aroused. The effects of butorphanol are enhanced by the adjunctive use of tranquilizers or sedatives, which improves the quality of the immobilization and allows for individual doses of the combined drugs to be reduced with the concomitant reduction of their potential side effects (Bush *et al.* 2012). Thermoregulation is affected by opioids, but is species dependent. In cats, hyperthermia is a common side effect of opioid administration, as are hyper-excitement, dysphoria, mydriasis and constipation (Kukanich and Papich 2009).

Naltrexone is a pure opioid antagonist, acting at all the opioid receptors (Kukanich and Papich 2009). Reversal of the effects of opioid agonists is rapid and long lasting and naltrexone is the antagonist of choice for complete opioid reversal, especially in a free-ranging environment, where a rapid return to full mental alertness and the physical flight-or-flight response is essential for survival of wild animals from predation.

Medetomidine is a selective and potent α2 adrenoreceptor-agonist, providing profound analgesia and sedation, good muscle relaxation, anxiolysis and immobilization, without the loss of consciousness (anaesthesia) (Bierman *et al*. 2012; Posner and Burns 2009b). These effects are dose dependent (Bierman *et al*. 2012; Posner and Burns 2009b). The activation of α2 adrenergic receptors reduces the release of noradrenaline at the presynaptic nerve endings resulting in sedation and the inhibition of afferent pain pathways (Posner and Burns 2009b). As an α2 adrenergic agonist, medetomidine potentiates the effect of other sedatives and significantly reduces their dose (Posner and Burns 2009b). Side effects of concern are the biphasic cardiovascular response, with the initial phase caused by the profound peripheral vasoconstriction with potentially severe hypertension and reflex bradycardia, and the second phase caused by decreasing systemic vascular resistance and possible inhibition of the baroreceptor reflex, with resultant hypotension (Posner and Burns 2009b), as well as atrioventricular blockade. Depression of respiratory rate and thermoregulation, vomition and hyperglycemia are also side effects of concern (Posner and Burns 2009b). Different species appear to have varying sensitivity to α2 adrenergic agonists, resulting in differing effects and side effects (Posner and Burns 2009b). A major advantage of using α2 adrenoreceptor-agonists like medetomidine, is that their effects can be completely reversed by using atipamezole, a potent, specific and highly selective α2 adrenoreceptor-antagonist. Atipamezole is rapidly absorbed, and results in smooth and relatively fast recoveries (Posner and Burns 2009b).

Midazolam is a water soluble benzodiazepine derivative, with a sedating and anxiolytic effect in animals (Klein and Klide 1989; Posner and Burns 2009b). Benzodiazepines enhance the effect of gamma aminobutyric acid (GABA), a neurotransmitter and the main inhibitory mediator in the brain (Klein and Klide 1989; Posner and Burns 2009b). $GABA_A$ receptor inhibition specifically is responsible for the anticonvulsant, anxiolytic and sedative/hypnotic effects of benzodiazepines (Klein and Klide 1989; Posner and Burns 2009b). Benzodiazepines also interact with glycine, another inhibitory neurotransmitter found in the central nervous system, and this glycine-mediated inhibition of the central neurons of the brain and spinal cord is thought to result in anxiolysis and muscle relaxation (Klein and Klide 1989). Benzodiazepines are often combined with sedative agents like opioids or with anaesthetic agents such as cyclohexylamines, to increase their depressant effects on the central nervous system. In combination they provide muscle relaxation and enhance sedation. Benzodiazepines are short-acting and cause minimal cardiovascular or respiratory depression at normal doses (Posner and Burns 2009b). Although side effects are few, with respiratory depression or hypotension only occurring at high doses, benzodiazepines do enhance the cardiorespiratory depression caused by other sedative and anaesthetic agents. In cats, paradoxical effects of dysphoria, agitation and aggression are possible, but these are generally associated with benzodiazepines used as sole agents, or at high doses (Klein and Klide 1989; Posner and Burns 2009b) or after anaesthesia when the effects of other agents are reversed.

Flumazenil is a specific competitive benzodiazepine receptor antagonist (Klein and Klide 1989; Posner and Burns 2009b, Walzer and Huber 2002). It has a high affinity for the receptor and antagonism of benzodiazepine agonists is rapid. It reverses the sedation, muscle relaxation and any dose-dependent respiratory depression and hypotension caused by agonists (Klein and Klide 1989; Posner and Burns 2009b). It has a high safety margin, but the duration of action is short and resedation is a possibility (Klein and Klide 1989; Plumb DC 2011b). Flumazenil has almost no intrinsic activity (Posner and Burns 2009b).

The importance in determining species-specific responses to novel anaesthetic protocols is illustrated in the comparative findings using the combination of the anaesthetic drugs listed above in domestic cats (Bierman *et al*. 2012) with those found in cheetah (Lafortune *et al*. 2005) and in lions (Wenger *et al*. 2010). In domestic cats, using dexmedetomidine the active enantiomer of medetomidine, as the α2 adrenergic agonist, the authors found good sedation, decreased heart rates and blood pressure. Hypothermia was significant and recoveries were prolonged (87 ± 36 minutes to standing). By comparison, in the two wild felid studies this combination produced rapid, smooth inductions in both cheetahs (Lafortune *et al*. 2005) and lions (Wenger *et al*. 2010), but Lafortune et al. (2005) recommended that this combination only be used for shorter procedures in cheetahs, as sudden arousals were possible after 40

minutes, whereas in lion no spontaneous arousals were recorded with some animals remaining immobilized for almost 5 hours (Wenger *et al*. 2010). Lafortune et al. (2005), found that although it also caused bradycardia in cheetah, this species became hypertensive compared to domestic cats. Hyperthermia developed in lions as opposed to hypothermia in domestic cats. Unfortunately, blood pressure was not measured in the lion study, and temperature was not recorded in the cheetah study for comparisons of these findings between all three of the species.

A further consideration involving anaesthetic protocols in free-ranging wild animals is the need for higher doses of the anaesthetic drugs compared to those required in domestic or captive animals (Gunkel and Lafortune 2007). The metabolic rate of an animal is inversely related to its' size and weight, and as metabolic rate affects the pharmacokinetics of a drug, the small body size of black-footed cats compared to other domestic and wild felids is also equated with the need for higher doses (Gunkel and Lafortune 2007). Should the physiological effects of the higher butorphanol-medetomidine-midazolam doses in free-ranging black-footed cats prove to be within clinically acceptable physiological limits, this fully reversible combination will have major advantages over other anaesthetic protocols.

3. MATERIALS AND METHODS

3.1. Field sites and animals

3.1.1. Field sites

Two facilities where animals were captive, one at Loskop Dam Nature Reserve, Mpumalanga, and the second at the privately owned "uBhetyan-o-Africa Game Lodge", Mpumalanga were used for this study. Three sites where animals were free-ranging were also used. These sites included Benfontein Nature Reserve situated 10km SE of Kimberley in the central part of South Africa and owned by De Beers Consolidated Mines, and "Nuwejaarsfontein Farm" and "Taaiboschpoort Farm" privately owned and located 24km south of De Aar in the Northern Cape Province (Fig.1).

Figure 1: Map of South Africa showing the locations of the Captive (♦) and Free-ranging Black-footed (■) Cats

3.1.2. Study animals

Nine captive (Captive Group, cBFC1 - cBFC9) and fourteen free-ranging black-footed cats were included in the study (frBFC1 - frBFC14). The free-ranging animals were divided into those individuals that were dug out of burrows before anaesthesia (Dug-out Group, n= 6; frBFCs 1, 3, 5, 6, 9 and 10), and those that were chased and immediately caught before anaesthesia (Chased Group n = 8; frBFCs 2, 4, 7, 8, 11, 12, 13 and 14). From the captive group six animals were males and three were females. The free-ranging animals comprised of four males and two females in the Dug Out Group, and four males and four females in the

Chased Group. Both previously radio-collared individuals found using telemetry, and un-collared individuals, randomly caught at the field study sites were used. Although most of the black-footed cats studied were adults, two sub-adults were included in the Captive Group and the free-ranging Chased Group.

3.2. Experimental Design

Both the captive and the free-ranging black-footed cats were anaesthetised with a novel, fully reversible anaesthetic drug combination containing butorphanol-medetomidine-midazolam in a prospective unpaired comparative study. The dose of the drug combination was set to be twice as high in the free-ranging animals compared to the captive animals (Table 1).

Table 1: Captive vs. Free-ranging anaesthetic doses

	BUTORPHANOL	MEDETOMIDINE	MIDAZOLAM
CAPTIVE	0.2mg/kg	0.05mg/kg	0.1mg/kg
FREE-RANGING	0.4mg/kg	0.1mg/kg	0.2mg/kg

The time from injection to immobilisation and anaesthesia were recorded on individual data sheets, and once anaesthetised, predetermined physiological variables between the “lower dose captive” and the “higher dose free-ranging” individuals were recorded, assessed and compared.

In the free-ranging black-footed cats if an animal entered a burrow after being chased a cut-off time of <15 minutes for digging out a chased cat was used as the criteria to determine which of the two groups these cats would be allocated to i.e. cats chased into a burrow and dug out within 15 minutes of a chase were included in the Chased Group, others were included in the Dug Out Group.

The free-ranging black-footed cats were studied over two capture seasons in 2013 and 2014. Two of the animals were recaptured in subsequent capture seasons. In the first season (2013) these animals were caught and injected and were included in the Chased Group. In the second season (2014) they were tracked using telemetry and dug out of their burrows and were included in the Dug Out Group.

3.3. Experimental Procedures

3.3.1. Capture of the captive black-footed cats

Captive black-footed cats were captured and restrained in their enclosures using a fish landing net and immediately covered with a blanket. Cats caught this way generally ran for less than 60 seconds before being captured. They were then hand injected intramuscularly into an accessible hind limb muscle group, using a 1ml tuberculin syringe with 25G needle (Terumo Corp. Japan). The dose (lower dose) of the drug combination had already been drawn up prior to the capture of the individuals and was based on the known or estimated masses (see section on drug doses below). As soon as the cats were anaesthetised they were carried from their enclosure to a portable examination table for immediate weighing, sampling and data collection (Fig. 2).

Figure 2: A captive black-footed cat anaesthetised, blindfolded and ready for sampling and data collection on the portable examination table.

3.3.2. Capture of previously collared free-ranging black-footed cats

Black-footed cats previously collared were tracked and found using telemetry during daylight hours. Their burrows were identified and the cats were then dug out using shovels. Cats caught in this way were included in the Dug Out Group (n = 6). As soon as the burrow was opened enough they were caught and restrained using fish landing nets, covered with a blanket and hand-injected (higher dose) in the same manner as described for the captive cats. In the field, the tailgate of the capture vehicle was used as an examination table, and as soon as the cats were anaesthetised, they were carried to the vehicle for weighing, sampling and data collection.

3.3.3. Capture of un-collared free-ranging black-footed cats

Capture of un-collared free-ranging cats occurred at night, using a vehicle and manually operated spotlights. Once found, cats were briefly chased for a maximum of 500-800m until they stopped, squatted down and froze. Fish landing nets were used to confine the cats, which were then covered with a blanket and hand injected (higher dose) in the same manner as described for the captive black-footed cats. Cats caught in this way were included in the

Chased Group (n = 8). In the event of a cat escaping down a burrow during the chase, the burrow was identified using a semi-rigid drainpipe endoscope equipped with a light and camera with which the eyes of the cat could be seen and the position of an animal in the borrow could be determined (Fig. 3 and 4). All the other burrow openings in the immediate vicinity of the identified burrow were blocked using blankets to prevent the escape of the cat via another interconnecting burrow. The cat was then dug out using shovels. Two of the cats caught in this way were included in the Dug Out Group, while one cat was included in the Chased Group using the criteria described in the Experimental Design.

Figure 3: The semi-rigid endoscope being used to identify the position of a chased cat in the burrow into which it had escaped.

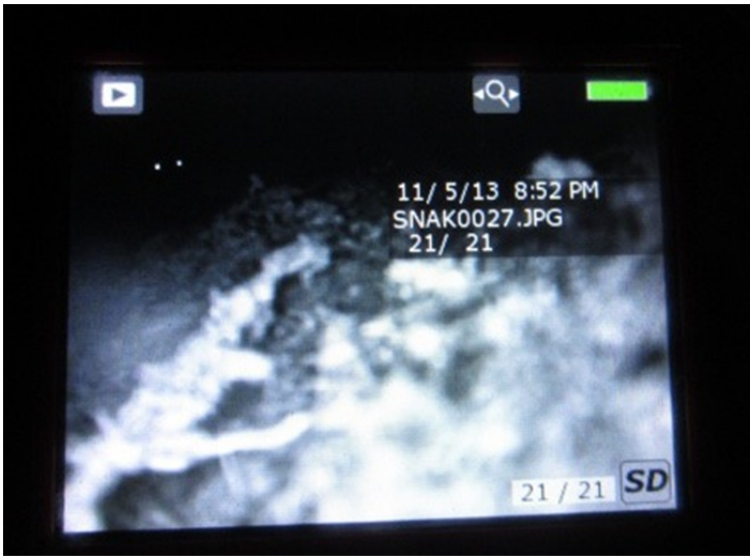

Figure 4: The illuminated eyes of the black-footed cat visible in the top left-hand corner on the screen of the semi-rigid endoscope.

3.3.4 Doses administered

The average mass of an adult free-ranging male black-footed cat ranges between 1.5-2kg, and that of an adult female between 1.2-1.5kg. We had prepared four standard doses of the drug combination for a 1,2kg, 1.5kg, 1.8kg and 2kg animal. We selected which of these initial doses to use based on the closest mass estimated at the time of capture. For the captive cats we used three initial standard doses of 2kg, 2.5kg and 3kg based on the closest mass previously measured or estimated.

We had prepared top-up doses of 0.5kg and 1kg, which were administered if required, once the cats had been weighed, and the depth of anaesthesia scored. Some animals in each of the groups were initially not adequately anaesthetised for sample and data collection (an anaesthetic score of 4 or 5, see Table 3 below) and therefore required a top-up dose of the anaesthetic drug combination. The time from the first drug injection and the dose of the top-up combination were recorded. A further top-up dose to achieve the desired anaesthetic score was required in one captive (cBFC2) and one chased free-ranging (frBFC8) cat, and these times were also recorded (Table 2).

Table 2: The mass of the black-footed cats and the dose of the drug combinations administered based on estimated mass before weighing.

Groups	Sex	Weight (kg)	Initial dose	*Top-up $Dose_1$ (kg)	**Time (secs)	*Top-up $Dose_2$ (kg)	**Time (secs)	#Total Dose Injected (kg)	‡Actual dose received (mg/kg)
Captive									
cBFC1	M	2.84	2.5	0.5	561			3.0	0,369
cBFC2	M	2.51	2.5	0.5	540	0.5	870	3.5	0,488
cBFC3	F	1.50	2.0					2.0	0,467
cBFC4	M	3.02	3.0					3.0	0,348
cBFC5	M	2.08	2.5					2.5	0,427
cBFC6	M	1.76	2.5					2.5	0,497
cBFC7	F	1.68	2.0					2.0	0,417
cBFC8	M	1.44	2.0					2.0	0,486
cBFC9	F	1.40	2.0					2.0	0,5
Dug Out									

frBFC1	M	1.67	2.0	1.0	1020			3.0	1,257
frBFC3	F	1.07	1.5					1.5	0,981
frBFC5* **	M	1.43	2.0	0.5	600			2.5	1,224
frBFC6	F	1.2	1.5					1.5	0,875
frBFC9	M	1.25	1.5					1.5	0,84
frBFC10	M	1.99	2.0	0.5	720			2.5	0,88
Chased									
frBFC2	M	1.9	2.0					2.0	0,737
frBFC4	F	1.15	1.2	0.5	420			1.7	1,034
frBFC7	M	1.35	1.5					1.5	0,778
frBFC8[##]	F	1.24	1.2	0.5	420	0.5	780	2.2	1,242
frBFC11	F	1.3	1.5					1.5	0,808
frBFC12	F	1.28	1.5					1.5	0,82
frBFC13	M	1.43	1.5					1.5	0,734
frBFC14[###]	M	2.05	1.8	0.5	660			2.3	0,786

***The top up doses of the combined anaesthetic drug combination required.**
****Times at which the top-ups were given after the initial drug combination in seconds. $Time_1$ is the time of the first additional dose and $Time_2$ is the time a second additional dose was administered to achieve adequate anaesthesia and sample collection.**
cBFC indicates the captive black-footed cats
frBFC indicates the free-ranging black-footed cats
***** indicates the animal that was injected with a top up dose after the first samples had been taken.**
[##] Indicates the animal that was injected after having been chased twice with a 20minute interval between the two chases.
[###]Indicates the animal that was chased the furthest at 800m before capture
[#]The Total dose injected is the dose of the drug combinations given in kg
[‡]The actual dose received is the total dose injected normalized with the actual body mass in mg/kg.

3.3.5. Blood collection

As soon as each individual was adequately anaesthetised to allow handling they were weighed and their eyes were flushed out using a sterile isotonic buffered solution (Eye Wash, Sterioptics, Hi-Tech Pharmacal Co., Inc, USA) to remove any dirt, especially in those individuals that had been dug out of burrows. Tear gel (Novartis SA Pty) was then inserted into the eyes to protect them from mechanical injury or drying out, and a blindfold was placed over the eyes to minimize any visual stimulation. Due to the small size of the animals and the large surface area to body mass ratio, the cats were placed on a jacketed hot water bottle, which was covered with a towel. An EPOC BGEM test card (Epocal Inc. Ottawa ON Canada) was inserted into the EPOC blood gas analyser (Epocal Inc. Ottawa ON Canada) and allowed

to calibrate for 3 minutes, so that the first blood sample could be analysed as soon as it had been taken. The neck over the jugular area of the cat was shaved using battery-operated clippers (Wahl®, USA) and the shaved area was disinfected using a sterile alcohol cleansing pad (Dis-Chem, RSA). A 1ml pre-heparinised syringe with 25G needle (Terumo Corp. Japan) was used to collect a venous blood sample from the jugular vein. Once taken, the blood sample was immediately injected into the BGEM card inlet port of the EPOC analyser and the results printed out using a bluetooth thermal printer (Woodley Diagnostics Ltd. UK). Once the first venous sample had been obtained, a full physical examination was undertaken and cardiorespiratory variables collected.

Three venous blood samples were obtained from each black-footed cat. The first sample (at time 0 minutes) was taken as soon as the individual was anaesthetised and had been weighed. The two subsequent samples were taken at 20 minutes and 40 minutes post induction. The EPOC BGEM test cards measured the venous pH, glucose and lactate. At the same time the $cTCO_2$ and Base excess were calculated by the EPOC analyser.

3.3.6. Monitoring of Animals

Each animal was anaesthetised for a minimum of 45 minutes prior to the administration of the antagonistic drugs. A total of five readings at 10 minute intervals during the anaesthetic period were recorded. The following parameters were recorded on the individual data sheets:

- Non-invasive Systolic, Diastolic and Mean Blood Pressures (mmHg) were determined using a SurgiVet 3 Parameter Advisor® Vital Signs Monitor (Smiths Medical, Smiths Group, UK) with a pressure cuff (3-6cm Neonatal Blood Pressure cuff with bayonette fitting, Critikon Classic-Cuf®, Sharn Veterinary., USA) which was placed between the elbow and the carpus to detect radial arterial blood pressure, with the limb positioned in such a way as to place the cuff as close to the level of the heart as possible. Prior to placement of the cuff, the leg was shaved to improve contact and the detection of the radial arterial blood pressure (Fig. 5).

- Peripheral arterial blood oxygen saturation (SpO_2 %) and heart rate (beats per minute) were determined using a pulse oximeter (Nonin 9847V, Nonin Inc. USA) with a lingual clip sensor (Nonin 9847V, Nonin Inc. USA, Fig.5) placed on the tip of the tongue of the animal. The heart rate determined from the pulse oximeter was confirmed by manual auscultation using a stethoscope (3M™ Littman® Stethoscopes).

- $ETCO_2$ (mmHg) was determined using an intranasal t-tube (nasal sample line, V1186, Smiths Medical, Smiths Group, UK) attached to the capnograph unit of the SurgiVet Monitor (Fig. 5).

 Respiratory rate (breaths per minute) was determined by manually counting the chest movements over a 60 second period and this rate was confirmed by the capnograph trace of the SurgiVet Monitor.

 Body Temperature (^{o}C) was measured using a digital thermometer (Model DT-K 1118, Clicks, RSA) placed inside the rectum.

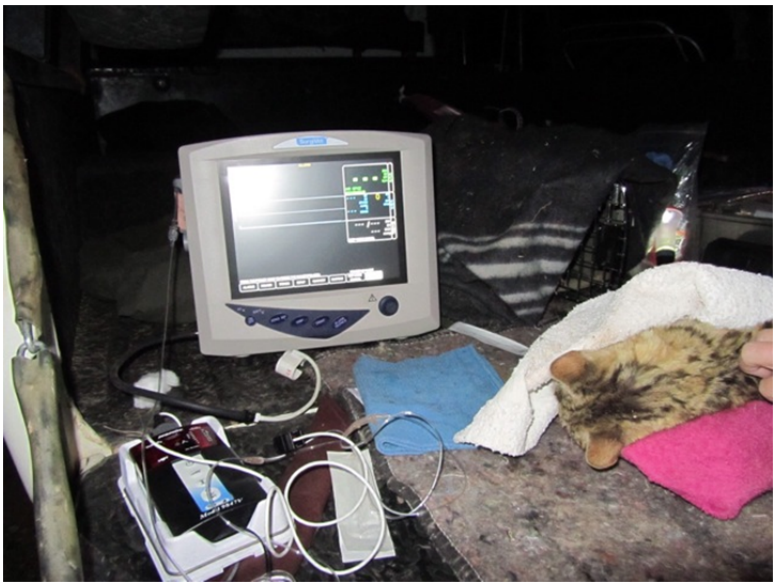

Figure 5: The SurgiVet Monitor with blood pressure cuff and intra-nasal t-tube attachment (not yet placed on the animal), Nonin Pulse oximeter and hot water bottle.

Due to various technical issues it was not always possible to obtain all the physiological variables for each black-footed cat at each time interval. Problems encountered included blockages of the intra-nasal t-tube and the occasional disconnection of the t-tube from the capnograph unit of the SurgiVet Monitor, poor contact between the blood pressure cuff and the skin of the animal and poor contact of the lingual clip sensor of the pulse oximeter.

3.3.7. Monitoring the effects of the anaesthetic drug combination

 Time to anaesthesia

The time to anaesthesia was the time taken from the hand injection of the animal to the animal becoming recumbent and non-responsive to handling and weighing procedures.

 Anaesthetic score

The depth of anaesthesia for each of the cats was categorized and recorded at 10 minute intervals at the times other physiological measurements were recorded. A descriptive scoring system, ranging from 1 (minimal effect) through to 6 (dangerously deep) was used to categorize anaesthetic depth. The degree of awareness, muscle movements, involuntary tail

movements, palpebral reflexes, jaw tone (assessed by checking muscle tone and resistance to opening the jaw wide) and response to painful stimuli (assessed by checking the deep pain limb withdrawal reflex by pinching a digit) were used to determine the anaesthetic score (Table 3).

Table 3: Description of the scoring system used to categorize the depth of anaesthesia in black-footed cats using the medetomidine-midazolam-butorphanol combination.

Anaesthetic score	Description
1. Minimal effect	Mild signs of sedation but the cat is awake and shows aggression when handled
2. Deep sedation	The cat has involuntary muscle movements, is responsive to painful stimuli and palpebral reflexes are present
3. Light anaesthetic plane	Muscle rigidity and involuntary tail movements are present, there is a slight response to painful stimuli and a delayed palpebral reflex
4. Medium anaesthetic plane	The cat is completely relaxed with no involuntary tail movements, jaw tone exists, no response to painful stimuli and no palpebral reflex can be elicited
5. Deep surgical anaesthetic plane	The cat is completely relaxed with no involuntary tail movements, the jaw is relaxed, there is no response to painful stimuli and no palpebral reflex can be elicited
6. Dangerously deep anaesthetic plane	There is no reflex activity at all and there is cardiorespiratory depression

 Time to recovery

After 45 minutes, the anaesthetic effects were reversed using a combination of the specific antagonistic drugs for the drugs used in the anaesthetic combination, namely naltrexone at 2x the butorphanol dose, atipamezole at 3x the medetomidine dose and flumazenil at 0.01-0.02x the midazolam dose (Table 4). The antagonists were combined into one syringe and injected intramuscularly into a hind limb muscle group.

The cats were then placed in a cat carrier with the hot water bottle and a towel, and the carrier was covered with a blanket. The cats were observed for signs of recovery, and the time from injection of the antagonists to complete recovery was recorded. Once completely awake, the captive cats were then released back into their enclosures. The free-ranging cats that had been dug out of burrows were either released back into the same burrow they had been dug out of, or a burrow as close to the original burrow as possible. The chased free-ranging cats were released into a burrow close to where they had been located or caught.

Camera traps were positioned outside the release burrows to monitor the behaviour of the animals on emergence from these burrows.

To provide consistency one person was responsible for evaluating the time from injection to anaesthesia, the depth of anaesthesia and the time to complete recovery.

Table 4: Captive vs. Free-ranging antidote doses.

	NALTREXONE	ATIPAMEZOLE	FLUMAZENIL
CAPTIVE	0.4mg/kg	0.15mg/kg	0.01mg/kg
FREE-RANGING	0.8mg/kg	0.3mg/kg	0.02mg/kg

3.4. Additional data collection

On capture, the sex of each black-footed cat was determined, the age was estimated and this information was recorded on the individual data sheets. In the free-ranging cats, a complete set of body measurements was taken and recorded on a separate data sheet for use in a separate study related to the biology of the species. The old collars of those cats that had been caught in the previous season were replaced with new collars. Vhf Collars were placed on all the newly caught black-footed cats. These cats were also microchipped.

3.5. Statistical Analysis

Data was analysed and compared using GraphPad Prism version 6.01 software. A two-way univariate analysis of variance (ANOVA) was used to compare the parametric physiological variables over time and between the Captive, Dug Out and Chased black-footed cat Groups. A post-hoc Tukey's multiple comparison test identified where the differences were between the three Groups, and a post-hoc Dunnet's multiple comparison test for comparison within each Group was used to identify where the differences occurred compared to the first sampling time point. Due to the small sample size it was not possible to test the data for normality so the data was treated as parametric or non-parametric based on an estimated data distribution of a variable in a normal cat population. Parametric variables analysed were heart rate, respiratory rate, $ETCO_2$, SpO_2, rectal temperature, systolic blood pressure, mean blood pressure, diastolic blood pressure, pH, base excess, glucose, $cTCO_2$. Lactate was treated as non-parametric and was log transformed (log10) before performing parametric analysis. Transformed data was used to calculate mean and standard deviations and this data was transformed back so that measured values could be depicted in the figures.

For non-parametric data (time to anaesthesia, general anaesthetic score and recovery times) a one-way ANOVA using the Kruskal-Wallis test was used to compare data between the

groups and post-hoc Dunn's multiple comparison test to compare data over time from the initial data.

In all tests a *p*-value below 0.05 was considered significant.

4. RESULTS

4.1 Environmental conditions during capture

Data from the cats in the Captive Group was collected on two days, the 27th & 28th of November 2013. On both days there was no cloud cover with ambient temperatures ranging between 21°C and 24°C.

The data from the free-ranging animals was collected between the 30th October and the 13th November in 2013, and during a second period between the 11th and the 24th November in 2014. In both capture periods the animals that had been caught and collared with vhf collars in the previous season were recaptured by tracking them to their burrows using telemetry and digging them out during daylight hours. The ambient temperatures during these days ranged between 16.5°C and 24°C, and there was no cloud cover. Four cats were recaptured in this manner in 2013 and two cats in 2014.

The remaining free-ranging cats were all captured at night. The ambient temperatures at night ranged between 10.4°C and 23°C. Apart from one rainy night, there was no cloud cover on any of the other nights.

4.2 Study animals

The masses of the males in the Captive Group were 2.28 ± 0.62 (range 1.44kg sub-adult to 3.02kg adult). The female masses were 1.53 ± 0.14 (range 1.4kg sub-adult to 1.68kg adult). In the free-ranging animals the male cats weighed 1.63 ± 0.3 (range 1.25kg to 2.05kg), and the females 1.21 ± 0.1 (range 1.07kg sub-adult to 1.3kg adult) (Table 2).

4.3 Induction and recovery times

The median and interquartile ranges of the time taken from injection until the animals were anaesthetised (induction time) for the Captive, Dug Out and Chased Groups were 364 s (IQR = 261-465), 312 s (IQR = 239-462) and 383 s (IQR = 213-565) respectively (Fig. 6). There was no difference in the induction times between the Groups (p= 0.33).

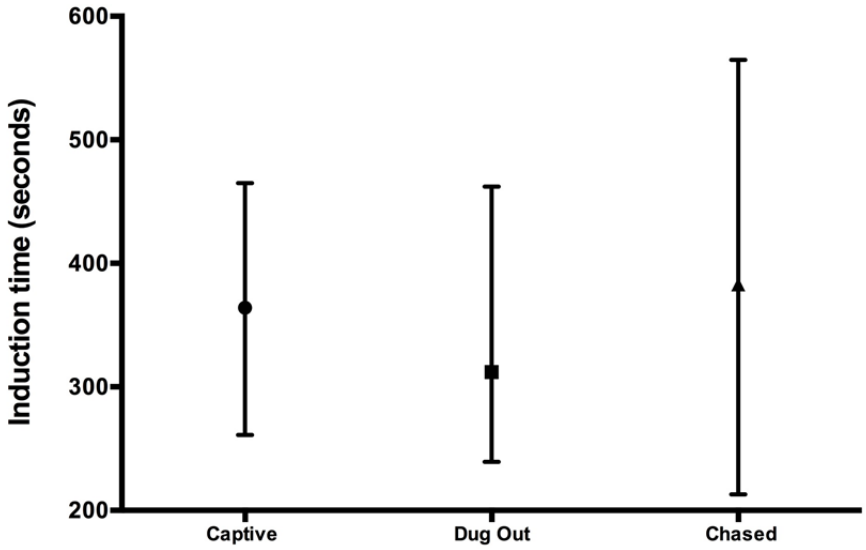

Figure 6: The medians with interquartile ranges of the anaesthetic induction times for black-footed cats in the Captive, Dug Out and Chased Groups. There were no differences in the induction times between these Groups (Kruskal-Wallis test).

The median and interquartile ranges of the time from administration of the antagonistic drug combination to the animals becoming fully conscious (recovery time) were 240 s (IQR = 240-270) in the Captive, 245 s (IQR = 104-375) in the Dug Out and 300 s (IQR = 300-345) in the Chased Groups (Fig. 7). There was no difference in recovery times between the three Groups (p= 0.43).

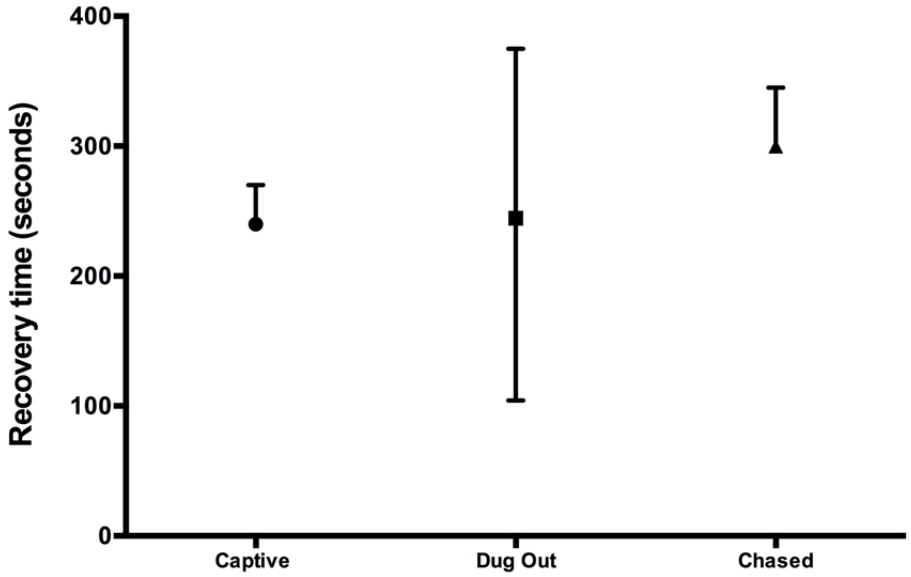

Figure 7: The medians with interquartile ranges of the recovery times for black-footed cats in the Captive, Dug Out and Chased Groups. There were no differences in the recovery times between these Groups (Kruskal-Wallis test).

4.4 Quality of anaesthesia

No animals in any of the three Groups reached a “dangerously deep” plane of anaesthesia (GA Score 6, Table 3) at any stage during the anaesthetic period. In total, eight black-footed cats required top ups. Only one animal required a top up dose (of 0.5 kg body mass) after the sampling and data collection had commenced (frBFC5 in the Dug Out Group). This dose was given 240s after the first blood sample had been taken (see Table 2), and was based on the cat having an anaesthetic score of 3 at this time (Table 3). Apart from this cat, which was injected after the initial sampling, the other seven cats (Captive Group cBFCs 1 and 2, Dug Out Group frBFCs 3 and 10 and frBFCs 4, 8 and 14 in the Chased Group) were given top-up doses before sampling and data collection commenced in order to reach a general anaesthetic score of 4 (Table 3). In the Captive Group the median anaesthetic score during anaesthesia was 5 (IQR 4.9-5). In the Dug Out Group, before frBFC5 received the top-up dose, it was 4.5 (IQR 3.75-5) and the median over the entire anaesthetic period was 4.9 (IQR 3.95-5). The Chased Group had a median score of 4.9 (IQR 4.3-5) over the anaesthetic period. There were no significant differences in the anaesthetic depth scores between the Groups at any time interval (0 minutes $p = 0.05$, 10 minutes $p = 0.31$, 20 minutes $p = 0.50$, 30 minutes $p = 0.22$ and 40 minutes $p = 0.93$).

4.5 Anaesthetic effects on the cardiovascular system

4.5.1 Heart rate

The average heart rate (beats.min^{-1}) for the Captive Group over the anaesthetic period was 84 ± 14 (range 56 -110), for the Dug Out Group it was 83 ± 17 (range 56 -122), and in the Chased Group the average heart rate was 104 ± 16 (range 68 -129). The heart rate in the Chased Group was higher at 0, 10, 20, 30 and 40 minutes compared to the Captive Group ($p < 0.05$). When compared to the Dug Out Group it was higher at 20, 30 and 40 minutes ($p < 0.05$). There was no difference in heart rate between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.97$). The heart rate in all the Groups did not change over time (Captive Group $p = 0.26$, Dug Out Group $p = 0.22$ and Chased Group $p = 0.28$, Fig. 8).

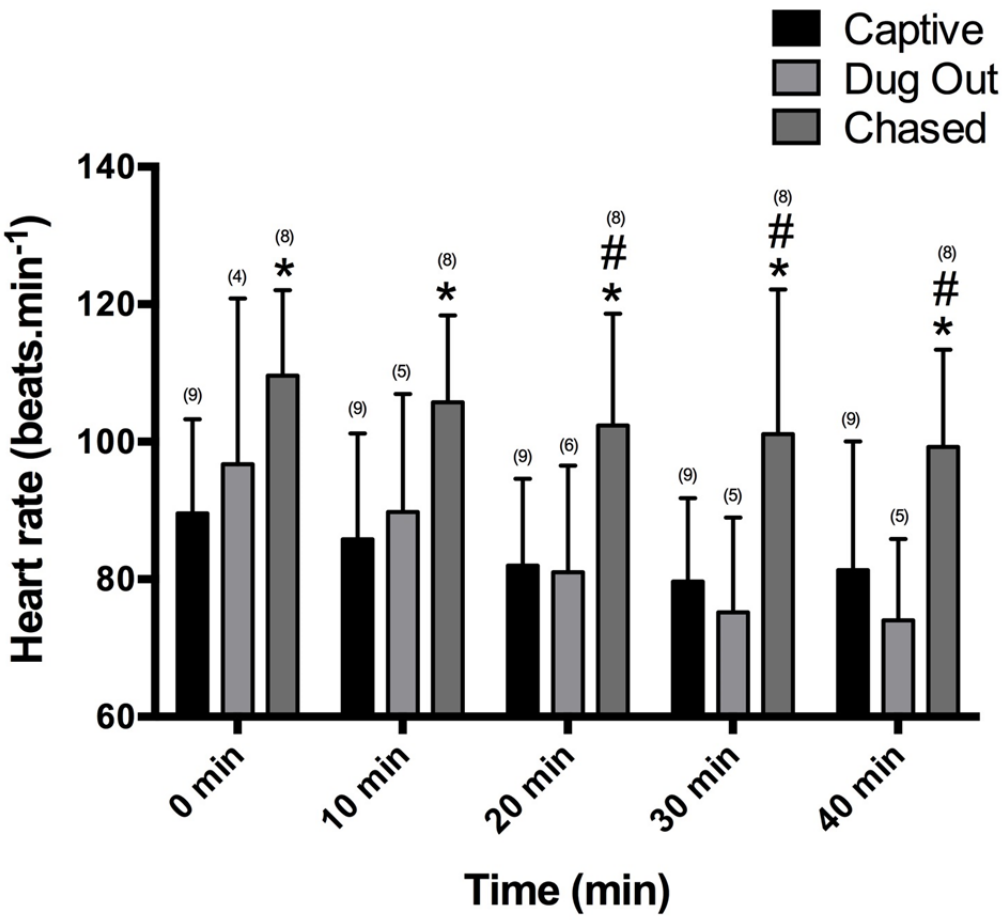

Figure 8: Mean and standard deviations of heart rate (beats.min^{-1}) from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group (p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.5.2 Systolic, mean and diastolic blood pressures

The average (± SD) systolic, mean and diastolic blood pressures in the Captive Group over the entire anaesthetic period was 129 ± 21 (range 94-169mmHg), 111 ± 20 (range 70-149mmHg) and 99 ± 19 (range 57-132mmHg) respectively. In the Dug Out Group they were 142 ± 11 (range 125-156mmHg), 120 ± 11 (range 103-142mmHg), and 111 ± 12 (range 92-135mmHg), and in the Chased Group the blood pressures were 132 ± 37 (range 75-243 mmHg), 109 ± 33 (range 54-214mmHg) and 98 ± 31 (range 44-200mmHg) respectively (Fig. 9). The blood pressures did not differ between the Groups throughout the anaesthesia (Systolic p = 0.44, Mean p = 0.37 and Diastolic p = 0.32). In the Captive Group the systolic (Fig. 9A) and mean blood pressure (Fig. 9B) were lower at 40 minutes compared to the initial

blood pressure at 0 minutes ($p < 0.05$). In the Chased Group the systolic, mean and diastolic pressures did not change over time ($p = 0.93$, 0.88 and 0.85 respectively). In the Dug Out Group there was insufficient data for comparison within the group over time.

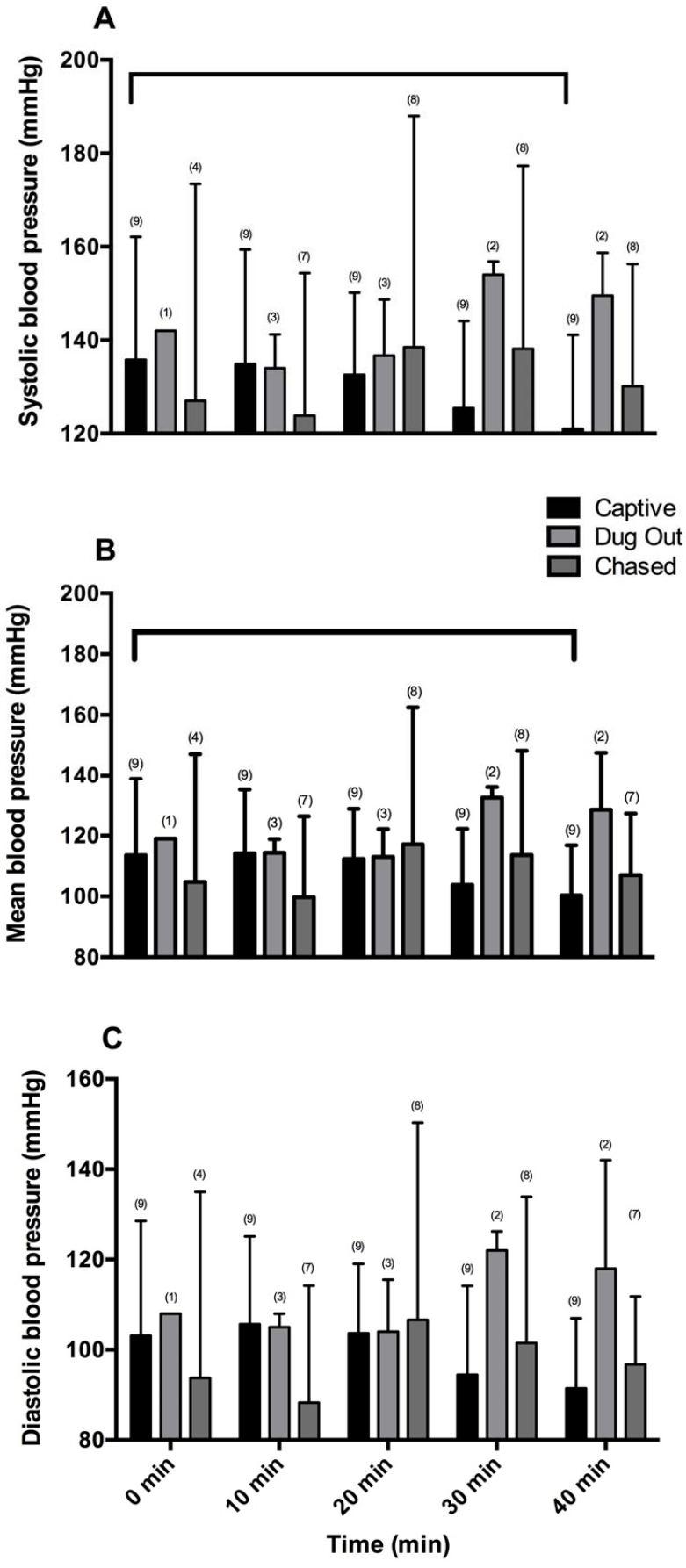

Figure 9: Mean and standard deviations of the systolic (A), mean (B) and diastolic (C) blood pressures from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The square bracket indicates significant differences in pressures at specific time points in a Group ($p < 0.05$, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.6 Anaesthetic effects on the respiratory system

4.6.1 Respiratory rate

In the Captive Group the average respiratory rate (breaths.min^{-1}) over the entire anaesthesia was 40 ± 10 (range 21- 70). The average in the Dug Out Group was 44 ± 16 (range 26 – 78) and in the Chased Group it was 47 ± 14 (range 21 – 74). The respiratory rate did not differ between the Groups throughout the anaesthetic period ($p = 0.05$), or within each Group over time (Captive Group $p = 0.07$, Dug Out Group $p = 0.9$ and Chased Group $p = 0.33$, Fig 10).

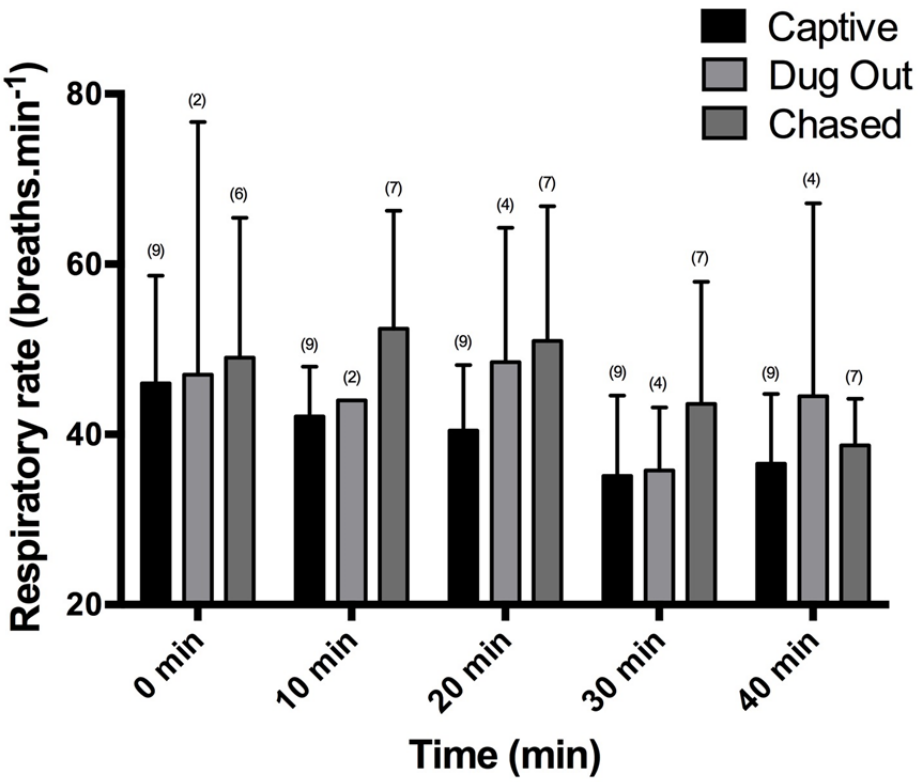

Figure 10: Mean and standard deviations of respiratory rate (breaths.min^{-1}) from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars.

(p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.6.2 Peripheral haemaglobin oxygen saturation (SpO_2)

The peripheral haemaglobin oxygen saturation (SpO_2 percentage) in the Captive Group over the entire anaesthetic period was 93 ± 4 (range 85 – 100%), in the Dug Out Group it was 95 ± 3 (range 84 – 100%) and in the Chased Group 89 ± 4 (range 80 – 96%). The SpO_2 was lower at 0 minutes in the Chased Group compared to the Captive Group (p = 0.02) and the Dug Out Group (p <0.01), and it was also lower at 20 minutes compared to the Dug Out Group (p <0.01). There was no difference in SpO_2 between the Captive and the Dug Out Groups over the anaesthetic period (p = 0.91). The SpO_2 did not differ within the groups over time (Captive Group p = 0.19, Dug Out Group p = 0.65 and Chased Group p = 0.19, Fig. 11).

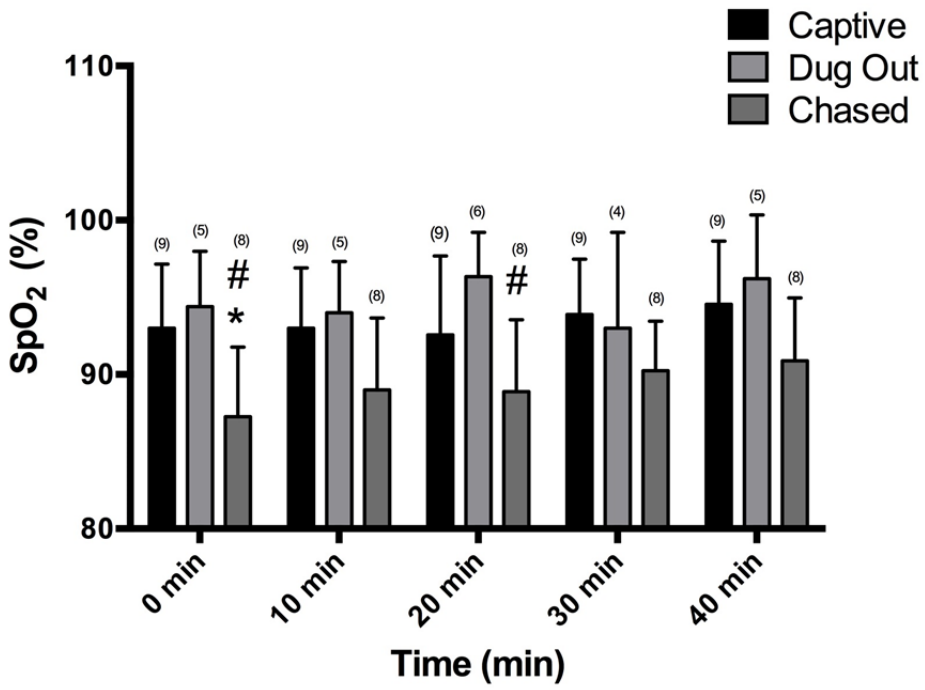

Figure 11: Mean and standard deviations of the S_pO_2 (%) from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group (p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.6.3 End Tidal Carbon Dioxide ($ETCO_2$)

The average $ETCO_2$ (mmHg) in the Captive Group over the anaesthetic period was 28 ± 6 (range 13 – 41mmHg). In the Dug Out Group it was 27 ± 5 (range 21 – 34mmHg) and in the Chased Group the average was 20 ± 7 (range 7 - 31mmHg). The $ETCO_2$ in the Chased Group was lower when compared to the Captive Group at 0 minutes ($p < 0.01$), 10 minutes ($p < 0.001$) and at 20 minutes ($p < 0.001$). It was also lower compared to that in the Dug Out Group at 20 minutes ($p < 0.05$). There was no difference between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.71$). The levels increased over time in the Chased Group and were significantly higher at 30 minutes than at 0 minutes ($p < 0.05$), and at 40 minutes than at 0 minutes ($p < 0.05$). The $ETCO_2$ levels also increased in the Captive Group and were significantly higher at 30 minutes than at 0 minutes ($p < 0.05$) and at 40 minutes than at 0 minutes ($p < 0.01$) (Fig.12).

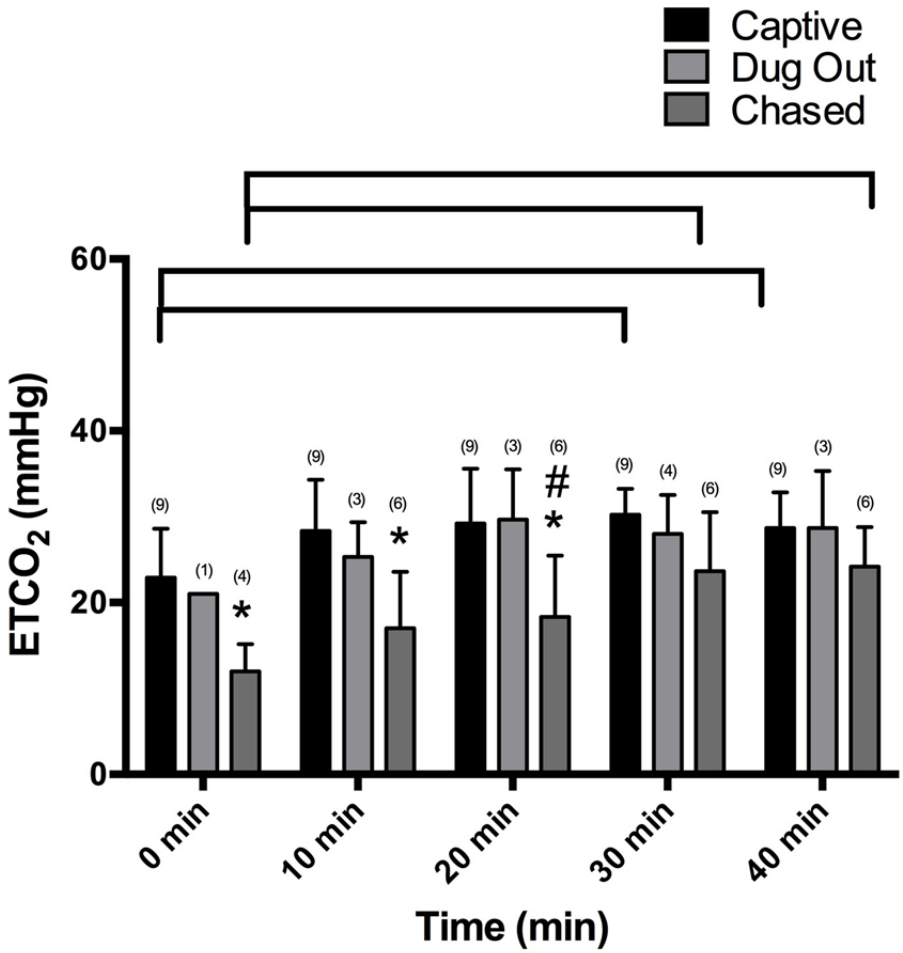

Figure 12: Mean and standard deviations of $ETCO_2$ (mmHg) from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the #

indicates a significant difference between the Chased Group and the Dug Out Group. The square bracket indicates a significant increase over time in $ETCO_2$ within the Captive Group and within the Chased Group compared to the initial $ETCO_2$ at 0 minutes ($p < 0.05$, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.7 Rectal Temperature

The average rectal temperature (^{o}C) in the Captive Group was 38.4 ± 0.9 (range 36.8 – 39.6 ^{o}C) over the anaesthetic period. In the Dug Out Group the average was 37.1 ± 0.7 (range 35.7 – 37.9 ^{o}C) and in the Chased Group it was 38.3 ± 1.2 (range 36.3 – 40.9 ^{o}C). The temperature in the Dug Out Group was lower than that in the Captive Group at each time point over the entire anaesthetic period (0 minutes $p = 0.03$, 10 minutes $p = 0.05$, 20 minutes $p = 0.02$, 30 minutes $p < 0.01$ and 40 minutes $p < 0.01$). Similarly, the Dug Out Group had lower temperatures than that of the Chased Group at 0 minutes ($p = 0.0006$), 10 minutes ($p = 0.03$) and at 20 minutes ($p = 0.03$). Within the Captive Group the temperature was lower at 10 minutes ($p = 0.02$), 20 minutes ($p = 0.0029$), 30 minutes ($p = 0.02$) and at 40 minutes ($p = 0.04$) compared to the initial temperature at 0 minutes. The Chased Group had lower temperatures at 30 minutes ($p < 0.01$) and at 40 minutes ($p < 0.01$) compared to the initial temperature at 0 minutes. There was no change in the Dug Out Group over the anaesthetic period ($p = 0.12$) (Fig. 13).

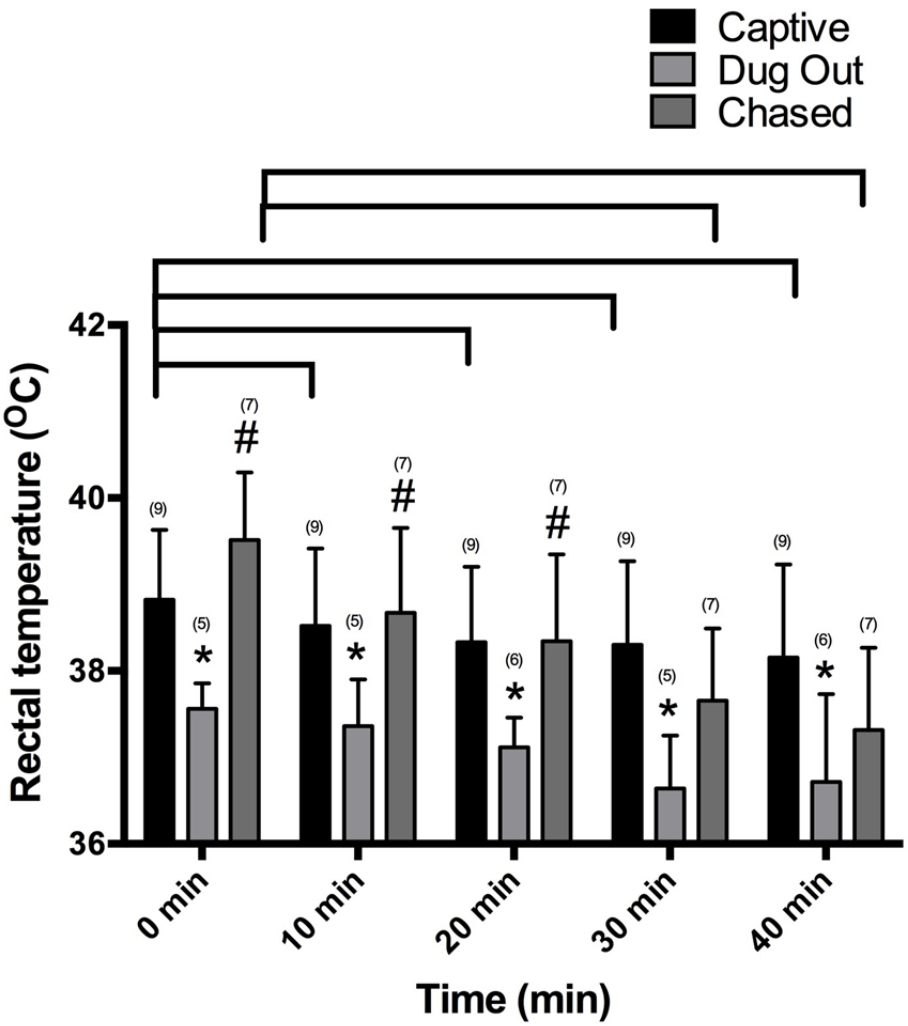

Figure 13: Mean and standard deviations of temperature (°C) from black-footed cats at 0, 10, 20, 30 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Captive Group and the Dug Out Group, and the # indicates a significant difference between the Dug Out Group and the Chased Group. The square brackets indicate significant decreases over time within the Captive Group and within the Chased Group compared to the initial temperature at 0 minutes (p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.8 Venous acid-base variables

4.8.1 pH

In the Captive Group the average blood pH was 7.3 ± 0.07 (range 7.10 to 7.42) over the anaesthetic period, and in the Dug Out Group it was 7.3 ± 0.06 (range 7.22 to 7.46), while in

the Chased Group the average pH was the lowest of the three Groups at 7.2 ± 0.16 (range 6.97 to 7.47). At 0 minutes the pH in the Chased Group was lower compared to that in the Captive Group ($p = 0.001$) and compared to that of the Dug Out Group ($p < 0.01$). There was no difference in pH between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.25$). Although the pH in the Chased Group tended to increase over time, this change was however not statistically significant ($p = 0.07$). The pH did not change within the Captive or the Dug Out Groups over time (Captive $p = 0.17$, Dug Out $p = 0.4$, Fig. 14).

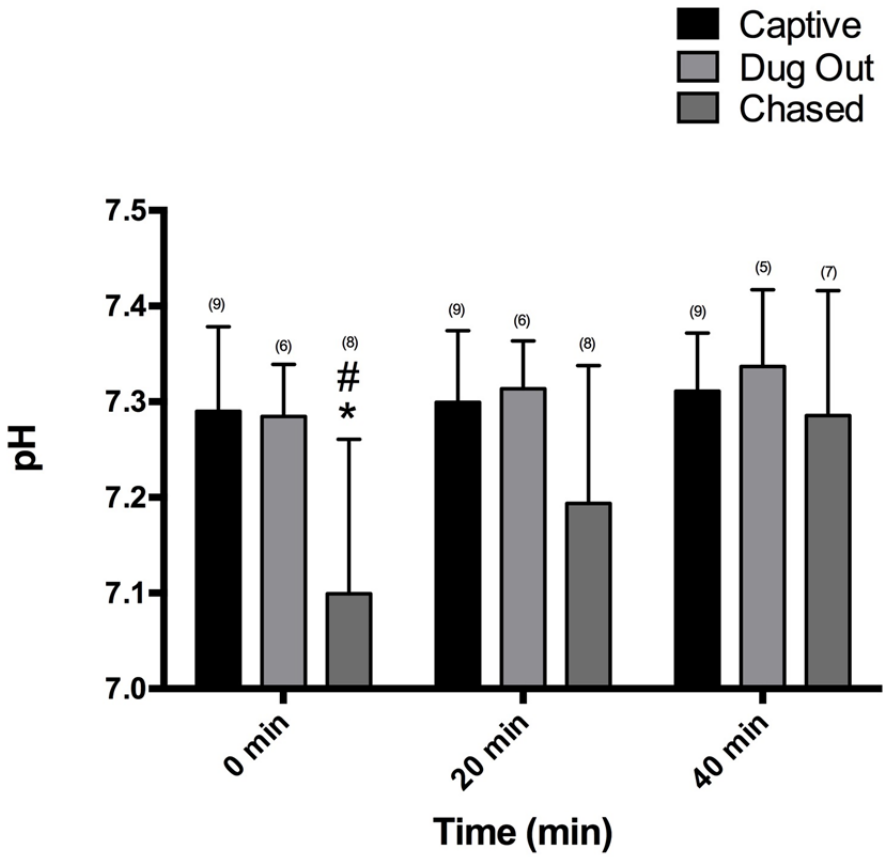

Figure 14: Mean and standard deviations of the blood pH from black-footed cats at 0, 20 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group ($p < 0.05$, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.8.2 Calculated Total Carbon Dioxide ($cTCO_2$)

The $cTCO_2$ (mmol/L) average for the Captive Group over the anaesthetic period was 20.1 ± 3.8 (range 10.6 – 24.6). The Dug Out Group had an average of 21.4 ± 3.8 (range 16.9 – 28.9) and in the Chased Group it was 15.6 ± 7.5 (range 7.0 – 32.9). At 0 minutes the $cTCO_2$ in the Chased Group was much lower compared to both the Captive Group ($p = 0.006$) and the Dug Out Group ($p = 0.001$). There was no difference between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.23$). At 40 minutes in the Captive Group and in the Chased Group, the $cTCO_2$ was higher than the initial level at 0 minutes ($p < 0.05$ in both Groups). There was no change in the Dug Out Group over the anaesthetic period ($p = 0.52$)(Fig. 15).

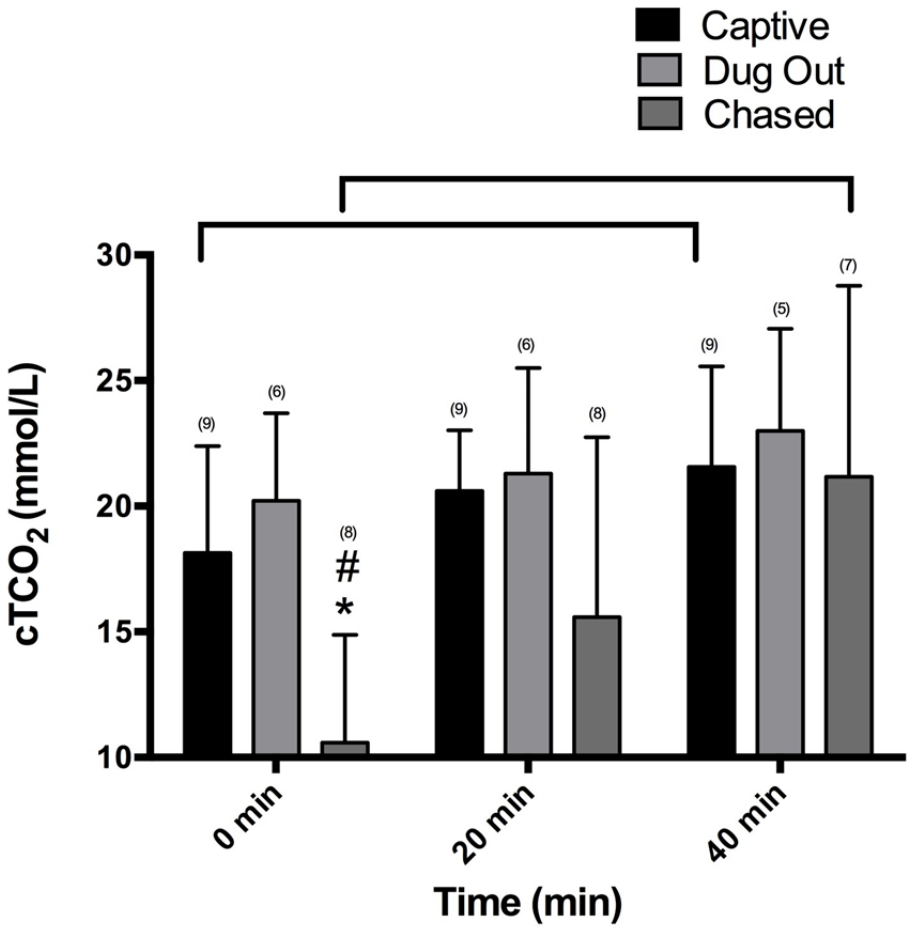

Figure 15: Mean and standard deviations of the $cTCO_2$ (mmol/L) from black-footed cats at 0, 20 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group. The square brackets indicate a significant increase within the Captive Group and within the Chased Group compared to the $cTCO_2$ at 0 minutes ($p< 0.05$, unpaired Two-

way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.8.3 Base Excess (BE)

Base Excess (mmol/L) in the Captive Group over the entire anaesthetic period was -7.5 ± 4.5 (range -20.1 to -1.0). The Dug Out Group average was -6.1 ± 4.6 (range -11.4 to 4.0) and that of the Chased Group was -13.6 ± 9.7 (range -25.0 to -7.8). The Chased Group had a lower mean than both the Captive Group ($p < 0.01$) and the Dug Out Group ($p < 0.01$) at 0 minutes. There was no difference between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.24$). At 40 minutes in both the Captive Group and in the Chased Group, the base excess was higher than the initial level at 0 minutes ($p < 0.01$) and ($p < 0.05$) respectively. There was no change in the Dug Out Group over the anaesthetic period ($p = 0.47$)(Fig. 16).

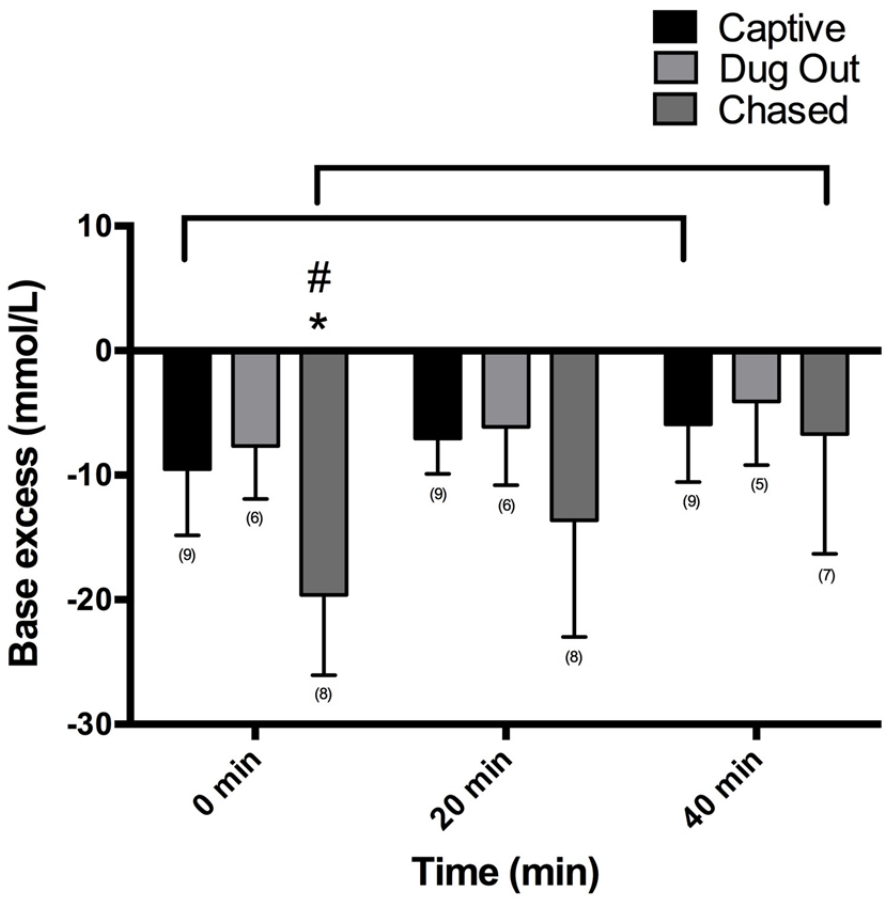

Figure 16: Mean and standard deviations of the base excess (mmol/L) from black-footed cats at 0, 20 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets below the bars. The * indicates a significant difference between the Chased Group and the Captive

Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group. The square bracket indicates a significant increase within the Captive Group between 0 and 40 minutes, and within the Chased Group between 0 and 40 minutes (p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

4.9 Biochemistry variables from blood samples

4.9.1 Glucose

The glucose (mmol/L) over the anaesthetic period in the Captive Group was 8.1 ± 3.3 (range 4.4 – 18.2), in the Dug Out Group 9.8 ± 3.4 (range 5.6 – 16.3) and in the Chased Group it was 8.1 ± 1.9 (range 4.9 – 11.4). The groups did not differ over the anaesthetic period (p = 0.13) and there was no difference within any of the groups over time (Captive Group p = 0.11, Dug Out Group p = 0.99 and Chased Group p = 0.46) (Fig. 17).

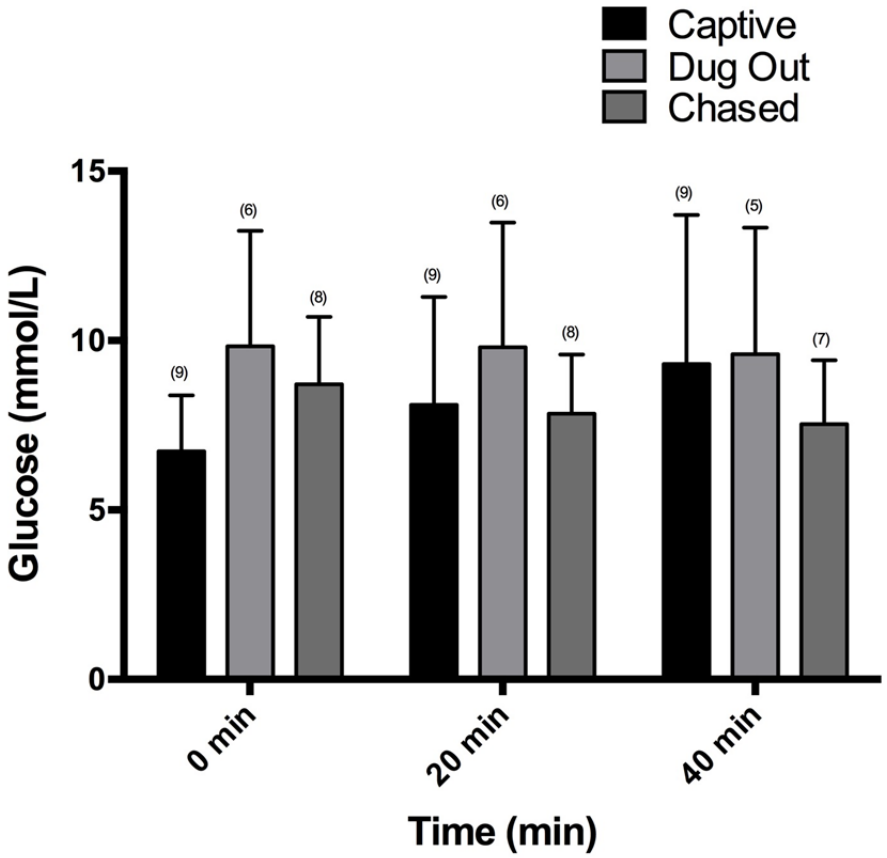

Figure 17: Mean and standard deviations of glucose (mmol/L) from black-footed cats at 0, 20 and 40 minutes during the anaesthesia period indicating no difference between the three Groups or within the Groups during the entire anaesthetic period (p< 0.05, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group

differences and Dunnett's multiple comparison test for time differences in each Group). The number of observations from the animals for each Group are given in brackets above the bars.

4.9.2 Lactate

The lactate concentration (mmol/L) in the Captive Group over the entire anaesthetic period was 1.96 ± 1.83 (range 0.58 – 7.44), in the Dug Out Group lactate was 2.89 ± 2.0 (range 0.58 – 7.24) and in the Chased Group it was 10.93 ± 4.28 (range 2.51 – 17.35). The lactate was higher at the measured time points in the Chased Group throughout the anaesthetic period when compared to the Captive Group - at 0 minutes ($p < 0.0001$) at 20 minutes ($p < 0.0001$) and at 40 minutes ($p < 0.0001$). It was also higher when compared to that in the Dug Out Group at 0 minutes ($p < 0.001$), at 20 minutes ($p < 0.0001$) and at 40 minutes ($p = 0.0017$). There was no difference between the Captive and the Dug Out Groups over the anaesthetic period ($p = 0.14$). In the Captive Group at 20 minutes and at 40 minutes the lactate was lower when compared to the initial lactate at 0 minutes ($p < 0.01$ and $p < 0.05$ respectively). In the Chased Group it was lower at 40 minutes compared to the initial level at 0 minutes ($p < 0.05$). There was no difference in the Dug Out Group over the anaesthetic period ($p = 0.16$) (Fig. 18).

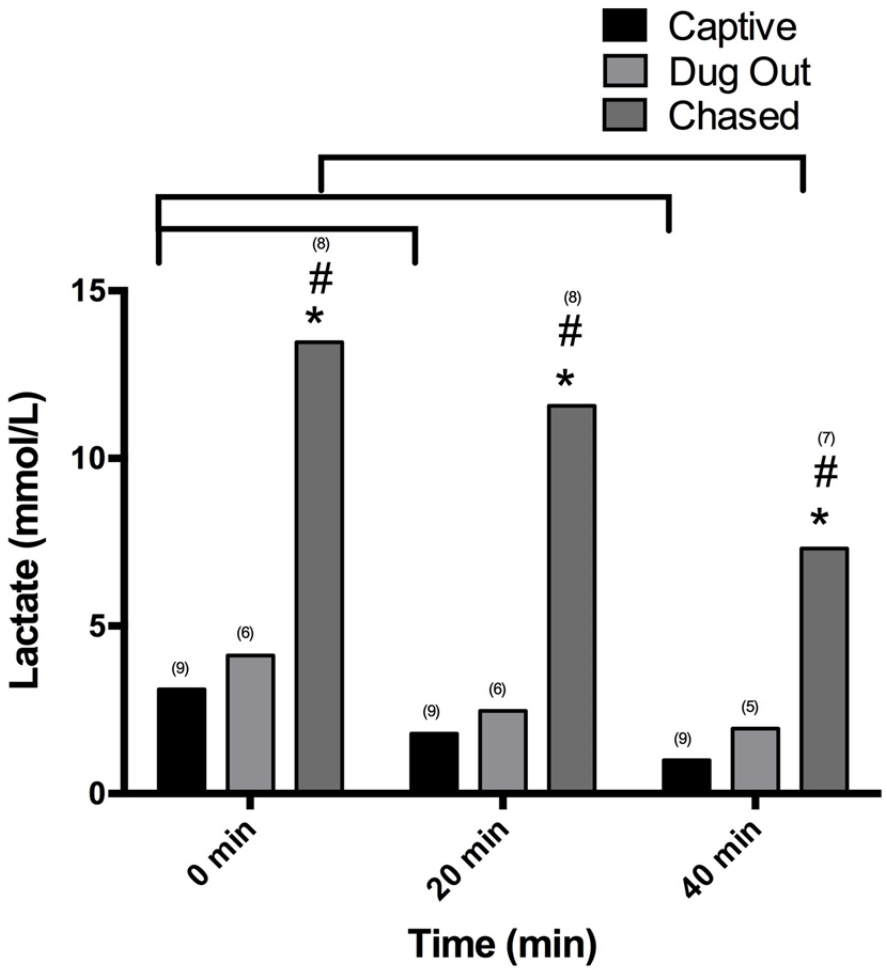

Figure 18: Mean and standard deviations of lactate (mmol/L) from black-footed cats at 0, 20 and 40 minutes during the anaesthesia period. The number of observations from the animals for each Group are given in brackets above the bars. The * indicates a significant difference between the Chased Group and the Captive Group, and the # indicates a significant difference between the Chased Group and the Dug Out Group. The square brackets indicate a significant decrease within the Captive Group between 0 and 20 minutes and between 0 and 40 minutes, and within the Chased Group between 0 and 40 minutes ($p < 0.05$, unpaired Two-way ANOVA with post-hoc Tukey's multiple comparison test for Group differences and Dunnett's multiple comparison test for time differences in each Group).

5. DISCUSSION

The butorphanol-medetomidine-midazolam combination resulted in rapid, smooth and uneventful induction into anaesthesia in the black-footed cats, with a mean time of 5.9 minutes across all three Groups. Of the 23 animals included in this study, five were given the correct target dose of the anaesthetic drug combination based on their subjectively estimated body mass, three were initially under-dosed and in 15 of the animals we had overestimated their mass and therefore they were relatively over-dosed. Eight of the animals required top-up doses in order to deepen the anaesthetic plane to facilitate sample collection (Table 2). Only one of these eight animals (frBFC 5 of the Dug Out Group) received its supplementary dose after the first blood sample had been collected (Table 2). These additional doses, and the high starting dose in many of the animals, indicate that doses even higher than our original target doses are required to maintain adequate anaesthesia for a period of 45 minutes. The animals that required the additional doses were either at a stage of deep sedation (GA Score 2, Table 3) or in a light anaesthetic plane (GA Score 3, Table 3) prior to top-up injection. None of the black-footed cats vomited on induction or experienced induction apnoea.

With the additional top-up doses the depth, quality and duration of the anaesthesia in all three Groups was good (GA score of 5, Table 3), with no sudden arousals and no animals reaching a dangerously deep level of anaesthesia (GA score of 6, Table 3) at any stage. Six of the black-footed cats were at a lighter plane of anaesthesia towards the end of the 45minute anaesthetic period, with three of these having received the correct initial target dose, further supporting our recommendations that higher initial doses are indicated.

Although our first two study animals were completely awake and fully mobile after receiving the antagonistic drug combination before their release into burrows, when camera traps left outside the burrows were later checked, they both appeared slightly ataxic on emergence. One animal was particularly unconcerned with its' surroundings and therefore a potential target for predation. As ataxia and disorientation are potential side effects of midazolam in domestic cats (Ilkiw *et al.* 1996; Kanda and Hikasa 2008b), the decision was taken to increase the flumazenil antagonist dose from 0.05x to 0.1x the midazolam dose. Thereafter there were no further concerns regarding post-emergence ataxia and sedation in the field and the recoveries in the rest of the cats were rapid, smooth and uneventful.

As far as we are aware normal reference ranges for the physiological variables in black-footed cats have not been determined or documented yet, and we based our evaluation of the cardiorespiratory effects and safety of this combination on those documented for small wild felids under anaesthesia (Gunkel and Lafortune 2007). The average heart rates in all the

Groups were well above those regarded as bradycardic (25-60 beats.min^{-1}) when an α_2 adrenoreceptor-agonist was used in other small felids (Gunkel and Lafortune 2007). Heart rates in the Chased Group were significantly faster than in the other two Groups over most of the anaesthetic period, and as there were no significant differences between the heart rates of the Captive and Dug Out Groups, the faster heart rates in the Chased Group were therefore most likely due to the method of capture rather than the dose of drugs used. The mean heart rates (83, 84 and 104 beats.min^{-1} – captive, dug out and chased animals respectively) were close to or within clinically acceptable limits (60-100beats.min^{-1}) for small felids under anaesthesia and above those of 25-60 beats. min^{-1} regarded as bradycardia in cats under the influence of an α_2 adrenoreceptor-agonist (Gunkel and Lafortune 2007). No rhythm abnormalities were detected on auscultation. The mean blood pressure values, measured non-invasively, were all within limits described by Gunkel and Lafortune (2007) of 60-150mmHg under anaesthesia. The free-ranging animals that received the higher dose of the anaesthetic drug combination had similar blood pressures compared to the captive cats, and therefore it did not appear that the higher doses influenced blood pressure. There was also no evidence of an α_2 adrenoreceptor-agonist induced biphasic blood pressure response in our study animals.

The respiratory variables of the animals in the Chased Group differed from those of the other two Groups over the initial half hour of the anaesthetic period. Although their respiratory rates appeared to be initially higher and to decrease as the anaesthesia progressed, these differences were not statistically different from the animals in the other two Groups (Fig. 10). The SpO_2 and $ETCO_2$ were significantly lower over this period in the chased animals when compared to those in the Captive and the Dug Out Groups (Fig. 11 and 12). The mean SpO_2 percentages in the animals in Chased Group were 89 ± 4% over the anaesthesia compared to 93 ± 4, and 95 ± 3 for the Captive and Dug Out Groups respectively. As the anaesthesia progressed the SpO_2 percentage increased, while the $ETCO_2$ tension increased significantly. The $ETCO_2$ tension in the Captive Group also significantly increased towards the end of the anaesthetic period (Fig. 12). The increased $ETCO_2$ tensions in both these Groups were close to being within normal limits at 40 minutes. The method of capture and the impact of the chase appeared to influence the respiratory variables in the different Groups rather than the difference in doses used between the captive and free-ranging animals.

Normal rectal temperatures in healthy domestic cats range between 38.1 and 39.2°C and should provide a valid reference range for wild felids (Gunkel and Lafortune 2007). Immediately post induction, the animals in the Captive Group had high normal body temperatures (38.8 ± 0.8), those in the Dug Out Group were hypothermic (37.6 ± 0.3) and in the Chased Group they were hyperthermic (39.5 ± 0.8). Animals in both the Captive and the Chased Groups became significantly hypothermic as the anaesthesia progressed, and the hypothermia worsened in the Dug Out animals. Although the anaesthetic drug combination

clearly affected the animals' ability to maintain normal body temperature, factors other than the dose of the drugs used appeared to influence the initial temperatures in the three Groups.

The animals from both the Captive and the Dug Out Groups had a mild degree of metabolic acidosis. The animals in the Chased Group had a moderate metabolic acidosis with pH, $cTCO_2$ and the base excess significantly lower and lactate significantly higher throughout most of the anaesthetic period compared to the animals in the other two Groups (Fig. 14, 15, 16 and 18). In the Chased Group the $cTCO_2$ and base excess increased and lactate decreased over time. The pH also increased to within normal limits. However, although the lactate concentrations had decreased in the chased animals, they were still elevated at 40 minutes (7.3 ± 3.3mmol/L) indicating that the tissues were possibly still in oxygen debt and metabolising anaerobically or that the initial lactate was being metabolised slowly. The mild acidaemia in the animals in the Captive Group also improved over time. The animals in the Dug Out Group had acid-base values that were constant throughout the anaesthesia and the closest to normal compared to the other Groups (Fig. 14, 15, 16 and 18).

All the black-footed cats in the study were mildly hyperglycaemic (means 8.1, 9.8 and 8.1 mmol/L – captive, dug out and chased animals respectively) compared to domestic cats (normal reference ranges EPOC analyser: 3.3 - 7.2mmol/L). Despite the higher doses of the anaesthetic drug combination used in the free-ranging cats there were no differences in these animals compared to the captive animals, which indicates that the higher doses used in the free-ranging animals did not exacerbate the hyperglycaemia.

The small size of the black-footed cats presented us with some challenges in our data collection. Firstly, palpation, visualisation and catheterisation of an artery proved to be extremely difficult in the field and therefore we were unable to collect arterial samples for blood gas and acid-base determination and hence we relied on the centrally obtained venous blood which could only give us measures of acid-base and other metabolic variables. As a surrogate for blood gas values we used peripheral oxygen haemaglobin percentages (SpO_2) as an indirect measure of arterial blood oxygenation and end tidal carbon dioxide partial pressures ($ETCO_2$) as an indirect measure of arterial blood carbon dioxide partial pressures (Sladky *et al.* 2000; Wenger *et al.* 2010; Zeiler *et al.* 2014). Despite using the smallest neonatal blood pressure cuff available (3-6cm Neonatal Blood Pressure cuff, Sharn Veterinary, USA) it was still slightly too large, and until we improved the contact of the cuff with the skin by shaving the leg of the animals we failed to obtain the first blood pressure readings for a number of the free-ranging cats, especially those of the Dug Out Group. Although this may have had an impact on the blood pressure results immediately post induction in these animals, we obtained sufficient readings from the other free-ranging cats for comparison with the captive animals over most of the anaesthesia.

Another limitation in our study was that the intra-nasal t-tube, which was connected to the capnography unit of the SurgiVet monitor, had a narrow bore and often became blocked with mucous. These blockages were not easy to remedy in the field, and therefore there were also missing $ETCO_2$ data points from some animals. In the Dug Out Group only one observation was made at 0 minutes, but we were able to record enough data from the other free-ranging animals for comparison with the captive animals over the remainder of the anaesthesia. Furthermore, the multidisciplinary research of the Black-Footed Cat Working Group required the collection of a number of other samples in between our blood sampling and data recordings, and at times we were not able to collect all our data which resulted in occasional gaps in the temperature, respiratory and heart rate data. However, we believe that these occasional lapses in data did not affect the scientific validity of the overall results we obtained.

Selecting safe and effective anaesthetic drug combinations in wild animals is challenging. Although extrapolation from domestic animals to wildlife is useful, species-specific responses to the drugs and their combinations, and the doses required vary greatly. Factors affecting specific anaesthetic drug responses include the health status of the animal, free-ranging as opposed to domestic or captive animals, size and metabolic rates, the manner in which an animal is caught and restrained and animal and species-specific pharmacodynamic and pharmacokinetic responses. The properties of an ideal anaesthetic drug combination in wild animals have previously been described (Burroughs *et al.* 2012; Swan 1993), and research into novel combinations is ongoing in the quest to fulfill as many of these properties as possible. Although the butorphanol-medetomidine-midazolam combination has been studied in both domestic dogs and cats (Bierman *et al.* 2012; Pypendorp *et al*. 1996; Pypendorp and Verstegen 1999; Verstegen and Petcho 1993) as well as in a variety of wild animal species including patas monkeys (Kalema-Zikusoka *et al.* 2003), ring-tailed lemurs (Williams *et al.* 2003), California sea lions (Spelman 2004), cheetah (Lafortune *et al.* 2005), red fox (Bertelsen and Villadsen 2009), wild dogs (Fleming *et al.* 2006), lion (Wenger *et al.* 2010) and Asian small-clawed otters (Fiorello *et al.* 2011), as far as we are aware its efficacy has not been studied in either captive or free-ranging black-footed cat populations.

We found that the butorphanol-medetomidine-midazolam combination in black-footed cats, like in many other species, was a safe and effective anaesthetic combination. However, a number of animals, both captive and free-ranging, required top-up doses in order to attain a level of anaesthesia deep enough to facilitate sample and data collection over 45 min (Table 2). Apart from frBFC5 of the Dug Out Group, which received its top up dose post induction, after the administration of any additional doses in the other animals in which they were required, the inductions in the black-footed cats were rapid, smooth and uneventful, and the quality and depth of the anaesthesia was good. The median induction times for all the study animals in the three Groups (Fig. 6) were all within the preferred 8 minutes desirable in free-ranging wild animals (Burroughs *et al.* 2012), and were comparable to other species

anaesthetised with this drug combination (Fleming *et al*. 2006; Lafortune *et al.* 2005; Wenger *et al*. 2010; Williams *et al.* 2003). Although there were no significant differences in the induction times between the animals in the three Groups (p = 0.33), the chased animals had the most variable induction times, from the quickest to the longest (6.4 minutes, IQR 3.6-9.4 minutes). The specific animal (frBFC8) in this Group, that had the longest induction time (12.5 minutes), had been chased twice within a short period of time (20 minutes), and although it had received the correct initial target dose for its mass once caught and restrained after the second chase, it required two further top up injections in order to reach a medium level of anaesthesia (GA Score 4, Table 3). Prolonged excitation, psychological stress and physical exertion potentially delay induction (Stegman and Jago 2006), and this is the likely explanation for the longer time to induction in this animal.

A rapid knockdown using higher doses is preferable, especially in the field, and is regarded as safer for both the animal and the capture team (Burroughs *et al.* 2012). As there is no literature on the use of this drug combination in black-footed cats we had to base our initial target doses on those used in domestic cats (Bierman *et al.* 2012; Plumb 2011a) and serval (*Leptailurus serval*) (Langan *et al.* study in 2000). The mean total doses (including the top-up doses) required for induction and maintenance of anaesthesia in the captive and free-ranging animals were 1.27x and 1.33x higher respectively compared to the original target doses we had set (Table 2). These higher doses did not appear to compromise the animals compared to those animals that had lower doses, therefore we recommend that the initial target doses in black-footed cats could be higher than the target doses we set and we believe that they could be as high as 1.5x our original target doses. This would mean an initial dose of 0.3mg/kg, 0.08mg/kg and 0.15mg/kg (combined total dose of 0.53mg/kg) for butorphanol, medetomidine and midazolam respectively in captive animals and in free-ranging animals, a dose of butorphanol 0.6mg/kg, medetomidine 0.15mg/kg and midazolam 0.3mg/kg (combined total dose of 1.05mg/kg). Vomition on induction with an α_2 adrenoreceptor-agonist is a common side effect in domestic small animals, especially in cats (Granholm *et al.* 2006; Posner *et al.* 2009b). We saw no sign of excessive salivation (a possible sign of nausea) or retching in any of our study animals and no emesis was recorded. Wenger *et al.* (2010) reported no vomition on induction in their lion study, and no vomition was recorded in cheetah (Lafortune *et al*. 2005), patas monkeys (Kalema-Zikusoka *et al*. 2003) and ring-tailed lemurs (Williams *et* al. 2003) using the same anaesthetic drug combination. However, in domestic cats (Zeiler *et al*. 2014) and in ring-tailed lemurs (Williams *et al*. 2003) in which ketamine was used with medetomidine or in combination with butorphanol and medetomidine respectively, nausea and vomition were recorded in these animals. Although opioids are also known to cause emesis, it is more likely to occur with the hydrophilic opioid drugs such as morphine and hydromorphine, and not with the lipophilic opioids such as butorphanol, fentanyl and etorphine. (Kukanich and Papich 2009; Pascoe and Steffey 2009). Midazolam is commonly used for its antiemetic effects, either alone or in conjunction with other drugs in human

medicine for pre- and post-operative nausea and vomition (Rodolà 2006; Kim *et al*. 2012). Although the exact mechanism of action, and whether this effect also occurs in cats are not known, it is probable that the synergistic effect of midazolam in this drug combination is the reason for the absence of the vomition normally induced by medetomidine. To reduce the likelihood of vomition, owners of domestic animals are requested to starve their animals for 24 hours prior to the use of an α_2 adrenoreceptor-agonist. Temporary starvation is clearly not possible in free-ranging cats and an anaesthetic protocol that does not result in emesis is a major advantage, especially in black-footed cats that hunt all night in order to maintain their high energy requirements.

Following the inductions, the quality and depth of the anaesthesia was well maintained in most of the study animals over the 45-minute period, with no sudden arousals. Wenger *et* al. (2010) reported similar findings in lions, although it appeared as though even longer anaesthetic durations were possible. In patas monkeys (Kalema-Zikusoka *et al*. 2003), the butorphanol-medetomidine-midazolam combination provided effective anaesthesia for 50-60 minutes. By comparison the authors of the cheetah study (Lafortune *et al.* 2005) proposed this anaesthetic drug combination for short procedures only in this species, as sudden arousals were likely to occur after 40 minutes. These species differences emphasize the need to evaluate an anaesthetic drug combination on a species-specific basis. Although the quality and length of the anaesthesia in our cats was ideal for our purposes, should a longer anaesthesia, or a more invasive and painful procedure be necessary, the addition of other drugs or a higher dose of the anaesthetic combination might be required.

As well as establishing the efficacy, our aim in this study was to evaluate the safety of the butorphanol-medetomidine-midazolam combination in black-footed cats, and to determine whether the higher doses required in the wild free-ranging animals exacerbated the physiological side effects of this anaesthetic protocol. We found minimal cardiovascular side effects in the black-footed cats, particularly those from the Captive and Dug-Out Groups. No normal reference values are available in small wild felids at rest, therefore, we compared the heart rates and blood pressures in our cats to those recorded by Gunkel and Lafortune (2007) for other small felids under anaesthesia, including those specifically under the influence of an α_2 adrenoreceptor-agonist. Based on this comparison we found no evidence of bradycardia, nor did we find this species to be either hyper- or hypotensive under the influence of the drug combination. Alpha$_2$ adrenoreceptor-agonist drugs, in particular, have profound effects on the cardiovascular and respiratory systems in domestic animals (Posner and Burns 2009b). Medetomidine in combination with ketamine has been the combination of choice for short procedures conducted under anaesthesia in domestic cats (Zeiler *et al*. 2014) and in small wild felids (Gunkel and Lafortune 2007). However, this combination can lead to severe hypertension, bradycardia, bradyarrhythmias and moderate to severe respiratory depression (Dobromylskyi 1996, Granholm *et al*. 2006). Despite not having a cyclohexylamine in the

butorphanol-medetomidine-midazolam combination, hypertension was found to develop in domestic dogs anaesthetized with this combination (Pypendorp *et al*. 1996), and the hypertension was mainly attributed to the effects of medetomidine. Domestic cats however were reported to develop hypotension when anaesthetized with the same combination (Bierman *et al*. 2012). During anaesthesia with the butorphanol-medetomidine-midazolam combination in wild animal species, Asian small-clawed otters (Fiorello *et al*. 2011), red foxes (Bertelsen and Villadsen 2009) and cheetah (Lafortune *et al*. 2005) all developed hypertension, whereas normotension was recorded in ring-tailed lemurs (Williams *et al*. 2003) and patas monkeys (Kalema-Zikusoka *et al*. 2003). The importance of determining species-specific responses to anaesthetic protocols is further illustrated in the comparative findings between these studies. Also, in contrast to lions (Wenger *et al.* 2010) and cheetahs (Gunkel and Lafortune 2007) no bradyarrhythmias were observed in our black-footed cats using the butorphanol-medetomidine-midazolam combination. As there is a potential predisposition for amyloidosis with resultant renal dysfunction in black-footed cats, the maintenance of normotension to maintain renal blood flow is a finding that further supports the use of this anaesthetic combination in this species.

We also evaluated the respiratory variables of the butorphanol-medetomidine-midazolam anaesthetic combination in this species. As there is no literature on the normal ranges of respiratory rates in anaesthetised small wild felids, we compared our observations with those of domestic cats (10-20 breaths.min^{-1} at rest) and servals under anaesthesia (range 17-37 breaths.min^{-1}, Langan *et al.* 2000). The mean respiratory rates in the black-footed cats in all the Groups were 40 - 47 beats min^{-1} (Fig. 10). We did expect the respiratory rates in the black-footed cats to be higher than other cat species due to their higher metabolism, as well as the consequence of the psychological and physiological stress induced by the capture and the effects of the anesthetic drugs. Although there were no significant differences in the respiratory rates between the three Groups over the anaesthetic period, the respiratory rates in the free-ranging chased cats were the highest over the first 30 minutes of the anaesthesia, with a mean (±SD) of 43 ± 14 breaths.min^{-1} at 30 minutes compared to 35 ± 9 and 36 ± 7 breaths.min^{-1} for the Captive and Dug Out Groups respectively.

At various time points over the same 30-minute period, the SpO_2 and the $ETCO_2$ concentrations were significantly lower in the Chased Group than those of either the Captive Group or Dug Out Group (Fig. 11 and 12). It was evident that the free-ranging cats that had been chased prior to capture were initially hyperventilating, indicated by hypocapnia (12 ± 3.2mmHg), but were moderately hypoxaemic, indicated by lower SpO_2 percentages (87.3 ± 4.5%) at the same time. These effects were most evident in the cat that had been chased the furthest at 800m (frBFC14 in the Chased Group, Table 2). This animal also had the fastest respiratory rate of the chased animals (range 48-74 breathes.min^{-}1) and the lowest SpO_2 percentages of all the animals throughout the anaesthesia (range 80-87%). Unfortunately, we

had no $ETCO_2$ data for this specific cat. The hyperventilation, hypocapnia and hypoxaemia in the cats in the Chased Group improved over the course of the anaesthesia. At 40 minutes, these cats were still hypocapnic ($ETCO_2$ of 24.2 ± 4 mmHg) but the hypoxaemia had resolved (SpO_2 of 91 ± 4 %). Normal and optimal SpO_2 in cats is greater than 90%, and below this threshold hypoxaemia occurs (Mosley and Gunkel 2007). The profound peripheral vasoconstriction associated with the use of α_2 adrenoreceptor-agonists has been deemed a potential cause of falsely low pulse oximeter SpO_2 readings by a number of authors (Fahlman *et al*. 2005; Gunkel and Lafortune 2007; Kalema-Zikusoka *et al*. 2003; Lafortune *et al*. 2005; Langan *et al*. 2000). However, in domestic cats (Zeiler *et al.* 2014), lions (Wenger *et al.* 2010) and red wolves (Sladky *et al.* 2000) SpO_2 readings obtained from pulse oximetry correlated well with PaO_2 readings obtained from arterial blood gases. The respiratory variables were the most stable and normal in the animals of the free-ranging Dug Out Group throughout the anaesthetic period, while in the Captive Group, the $ETCO_2$ concentrations increased towards the end of the anaesthetic period (Fig. 10,11 and 12).

We have no definitive data to conclusively explain the respiratory findings from the chased animals. It is conceivable that the initial paradoxical hypoxaemia with hypocapnia and hyperventilation in the chased cats could be explained by the initial increased oxygen utilisation in the muscles from the chase. If oxygen utilisation is greater than the lungs ability to oxygenate arterial blood, hypoxia can occur even if ventilation is normal or elevated, as it was in the chased cats. Opioids are known to cause respiratory depression (Swan 1993), resulting in hypoventilation, hypercapnia and hypoxaemia. Butorphanol is normally used to reverse the respiratory depression when potent opioids are used to immobilize herbivores (Bush *et al.* 2012, Haw *et al.* 2015), however when used on its own in other species such as primates it can cause respiratory depression (Bush *et al.* 2012). The effects of opioids on respiration are not just related to depression of ventilation, but also result from their effects on gas exchange (Meyer *et al.* 2015). The authors of a study using the opioid etorphine in goats determined that due to increased pulmonary vasoconstriction, pulmonary vascular resistance increased resulting in pulmonary hypertension. They speculated that this hypertension could result in pulmonary congestion and possibly oedema, which would reduce the movement of oxygen across the alveolar membrane. Furthermore, hypertension could also increase the speed of blood flow through the lungs. With increased speed there is less time for gaseous exchange and for oxygen to diffuse across the alveolar membrane. They concluded that pulmonary hypertension was the main cause of the hypoxia when opioids are used (Meyer *et al.* 2015). The mechanism of etorphine-induced pulmonary hypertension has not yet been elucidated. It is possible that the hypoxia we encountered in our chased cats could have been caused by these cardiopulmonary effects, however this is unlikely, as the same effects were not found to occur in the animals in the other two Groups. As butorphanol is not nearly as potent an opioid as etorphine, and as it is a partial agonist/antagonist with high affinity but low efficacy at the μ-opioid receptors (Trescot *et al.* 2008) it is also not likely to have contributed

to the respiratory picture we encountered in our cats to the same degree as etorphine was found to do in the goat study.

Although mild to moderate hypoxia was also reported in patas monkeys (Kalema-Zikusoka *et al.* 2003), ring-tailed lemurs (Williams *et al.* 2003) and in 14% of the lions in the Wenger *et al.* (2010) study, the paradoxical hypoxaemia with hypocapnia that we found in the chased black-footed cats, has not been reported in any other species anaesthetised with this butorphanol-medetomidine-midazolam combination. In lions anaesthetized with this combination there was evidence of ventilation perfusion mismatch (Wenger *et al.*2010). In patas monkeys, the authors were unsure whether the severe hypoxaemia they detected on pulse oximetry was due to the alpha$_2$ agonist-induced peripheral vasoconstriction, or whether the readings reflected hypoxaemia (Kalema-Zikusoka *et al.* 2003). However, as soon as oxygen supplementation was instituted in these animals, the SpO_2 concentrations returned to normal. As the moderate hypoxaemia seen in the animals of the Chased Group was not found in the cats of the Captive and the free-ranging Dug Out Groups, it is unlikely that the higher doses used were the cause of the hypoxaemia in the Chased Group. We found that the impact of the chase influenced the SpO_2 percentages in our study animals, rather than the higher doses required in the free-ranging animals. We found the butorphanol-medetomidine-midazolam anaesthetic combination to be safe in this species, however we recommend administering oxygen supplementation to ameliorate hypoxaemia in chased animals and any other animals where the SpO_2 percentages drop below the 90% SpO_2 threshold.

Initial rectal temperatures of the black-footed cats in the Dug Out Group were the lowest of all our study animals, followed by those in the Captive Group, with the temperatures of the cats in the Chased Group initially the highest (Fig. 13). The causes of an initial hyperthermia in wild animal species during capture are multifactorial. Although the long held belief that high environmental temperatures in conjunction with muscle heat production from exertion are the major causes of the initial hyperthermia (Meltzer and Kock 2012), Meyer *et al.* (2008) showed that psychological stress (fear-induced stress) had a greater role to play in the initial development of hyperthermia during capture. In our black-footed cats, the animals that were dug out of burrows during daylight hours were unable to escape and were mostly sedentary until they could be reached and injected and their metabolic rate would have been lower than those of both the captive and the free-ranging chased animals. Whereas the rapid increase in metabolism and the increased muscle contractions resulting from the intense stressful chase would most likely have initially generated a substantial amount of heat in the animals of the Chased Group; the animals in the Captive Group had also been chased, although not as intensely and for a much shorter distance. The initial body temperatures in our three Groups appeared to reflect the degree of exertion involved in the various methods of capture.

Alpha$_2$ adrenoreceptor-agonists depress thermoregulation (Posner and Burns 2009b) and opioids commonly induce hyperthermia in cats (Kukanich and Papich 2009). Once an animal has been captured, restrained and injected with anaesthetic or immobilizing drugs, the effects of these agents then start impairing the thermoregulatory abilities of the animals. At this point environmental temperatures, in conjunction with the length of anaesthesia and the surface area to body mass of the animal become major factors influencing body temperature (Meyer *et al.* 2008). Due to the large surface area to body mass ratio of black-footed cats we were more concerned about the potential for a rapid loss of body temperature under anaesthesia, especially under the cold ambient conditions of the nighttime captures. A hot water bottle was used in anticipation of this hypothermia in all of the Groups to try and mitigate any excessive heat loss. The temperatures in all the Groups decreased over the course of the anaesthesia, despite the use of hot water bottles. As all our cats became progressively more hypothermic, we believe that the poikilothermic effects of the drugs in the anaesthetic combination, as well as the large surface area to body mass ratio of the cats played a major role in their development of hypothermia post induction, despite our attempts to mitigate any heat loss. The risk for severe hypothermia, especially during cold nights in the field, is great in this species and we therefore recommend the use of equipment, such as electric blankets that can provide a constant source of heat to maintain normothermia.

The different circumstances at the time of capture followed by the poikilothermia induced by this anaesthetic drug combination, is illustrated in the varied body temperature effects reported in different species. Wenger et al. (2010) found hyperthermia in their lions, irrespective of whether the lions were mobile or sedentary after darting. Although Larsen et al. (2002) had incorporated diazepam as the benzodiazapine in their anaesthetic drug protocol, they also reported that the red wolves in their study became hyperthermic. In contrast, hypothermia developed in patas monkeys (Kalema-Zikusoka *et al.* 2003), ring-tailed lemurs (Williams *et al.* 2003) and small-clawed otters (Fiorello *et al.* 2011), while significant decreases in body temperature were reported in domestic cats (Bierman *et al.* 2012).

For analysis of acid-base balance and the metabolic status of our cats central venous blood from the jugular vein was collected. Venous blood is a more accurate reflection of the metabolic status of an animal (McGrotty and Brown 2013; Pypendorp and Verstegen 1999) and the values for lactate, pH, bicarbonate and base excess show a high level of agreement between those collected arterially and those collected centrally from the jugular vein (Middelton *et al.* 2006; Yildizdas *et al*. 2012). When using venous blood the $cTCO_2$, a calculated value, is used as a surrogate for bicarbonate (Centor RM 1990).

The peripheral vasoconstriction induced by the α_2 adrenoreceptor-agonist, medetomidine, would have resulted in a degree of hypoperfusion, tissue hypoxia, anaerobic metabolism and the production of lactate (McGrotty and Bilbrough 2013). Once tissue perfusion and

oxygenation is corrected, lactate concentrations rapidly return to normal as lactate is normally metabolized quickly (McGrotty and Bilborough 2013). During intense exercise stored glycogen in the skeletal muscles and in the myocardium is converted to glucose for the production of energy by way of various physiological pathways, the final steps being the conversion of pyruvate to hydrogen ions which are oxidized to produce ATP (energy). In hypoxaemic conditions, anaerobic metabolism and tissue hypoxia result in the production of lactic acid from pyruvate (Meltzer and Kock 2012). The accumulation of lactate concentrations in cells potentially leads to muscle damage with leakage of lactic acid, enzymes, electrolytes and myoglobin into the blood (Meltzer and Kock 2012). Hyperlactaemia results in a metabolic lactic acidosis (McGrotty and Bilbrough 2013). An initial degree of metabolic acidosis is common in domestic and wild species due catecholamine release resulting from the psychological and physiological stress of capture and increased lactate production from anaerobic metabolism in skeletal and heart muscles (Bierman *et al*. 2012; Larsen *et al*. 2002). Due to the reasons given above mild hyperlactaemia resulted in the initial mild acidosis seen in the captive animals which exercised briefly before capture, and in the free-ranging dug out cats, which, although sedentary prior to their capture, were exposed to the stress of being caught. A mild to moderate metabolic acidosis has also been reported in lions (Wenger *et al*. 2010) using capture procedures that did not involve a chase, and in frequently immobilized captive red wolves (Larsen *et al*. 2002) which were either confined or hand-netted prior to anaesthesia. In both these species the same, or a similar anaesthetic drug combination was used. We found that the initial severe hyperlactaemia and moderate metabolic acidosis in our free-ranging chased black-footed cats improved over the course of the anaesthesia, with decreased lactate and increased pH, $cTCO_2$ and base excess (Fig. 18, 14, 15 and 16). An increase in SpO_2 resulting in increased oxygen supply to the tissues, muscle relaxation and reduced oxygen demand induced by midazolam and medetomidine, the absence of conscious stress during the anaesthesia and the metabolism of plasma lactate would all have contributed to the overall improvement of the acidosis towards a normal pH in all of our study animals. Due to this improvement over the anaesthesia we do not believe that medical intervention is indicated to correct the initial acidosis, but the changes in the physiology of the chased animals highlights the potential for damage to occur in their tissues from hypoxia and acidosis. We therefore recommend close observation of the acid-base status of these animals, particularly if they have been chased. The higher drug doses required in the free-ranging wild animals did not appear to exacerbate the metabolic acidosis, and we found that the exertion of the chase was the major contributing cause of the lactic acidosis in the free-ranging animals of the Chased Group and not the higher doses required.

The captive and free-ranging animals were all mildly hyperglycaemic (Captive 8.1 ± 3.3, Dug Out 9.8 ± 3.4 and Chased 8.1 ± 1.9mmol/L; EPOC analyser – 3.3-7.2mmol/L, Fig. 17). Fear and anxiety are acute stressors that interrupt normal homeostasis and elicit the fight-or-flight response, stimulating the sympathetic nervous system with the release of catecholamines.

This response is almost immediate with the catecholamines stimulating glycogenolysis thus providing energy in the form of glucose (Kanda and Hikasa 2008a; Reeder and Kramer 2005). Alpha$_2$ adrenoreceptor-agonists also induce hyperglycaemia by inhibiting insulin release (Ambrisko *et al.* 2005). Although α_2 adrenoreceptor-agonists also suppress the release of catecholamines (Posner and Burns 2009b; Ambrisko *et al.* 2005) their overall metabolic effect is one of hyperglycaemia (Ambrisko *et al.* 2005; Kanda and Hikasa 2008a). There were no significant differences in glucose concentrations within or between the animals of the three Groups over the anaesthetic period, thus the higher combined dose of the drugs in the free-ranging animals did not influence the magnitude of the hyperglycaemia that developed. Although higher than the upper limit for domestic cats (EPOC analyser reference 3.3 – 7.2mmol/L), the glucose concentrations were not at a level considered to be pathological (>9.5 mmol/L – Rios and Ward 2008).

Once we had collected our data, the anaesthesia in the cats was reversed at 45 minutes. Following our decision to increase the flumazenil dose after the first two animals, all further recoveries in our study animals were rapid, smooth and uneventful without any signs of residual ataxia or disorientation. Flumazenil has a shorter half-life than midazolam, with the possibility of resedation when used at lower doses to reverse the effects of midazolam (Gunkel and Lafortune 2007; Plumb 2011b). At the flumazenil dose (0.01mg/kg for the captive animals and 0.02mg/kg for the free-ranging animals) we used in the majority of the cats, we did not notice any signs of resedation when we viewed their movements via the camera traps post recovery. The median time to recovery for all the black-footed cats was 4.4 minutes (IQR 3.59-5.5 minutes), with no differences between the three Groups (Fig. 7). There is no established dose of flumazenil per mg of midazolam for animals and the doses used in domestic and wild animal species vary greatly. In the domestic cat a dose of 0.01mg/kg, with the same dose repeated hourly should the short half-life of flumazenil result in resedation is suggested (Plumb 2011b). Repetitive dosing would clearly be impossible in free-ranging animals and would create an added stress in captive animals. In wild animals doses used have ranged between 0.0002-0.002 mg/kg in California sea lions (Spelman 2004), 0.003mg/kg in lions (Wenger *et al.* 2010), 0.006mg/kg in cheetah (Lafortune *et al.* 2005), 0.04mg/kg in wolves (Larsen *et al.* 2002), 0.2mg total dose in wild dogs (Fleming *et al.* 2006) and 0.06mg total dose in Asian small-clawed otters (Fiorello *et al.* 2011). In all of the above studies the authors reported rapid, complete recoveries without resedation or ataxia when flumazenil was included in the antagonistic drug combination. In lions Wenger *et al*. (2010) queried whether the low dose they had used was actually beneficial, but they did not compare the recoveries when it was not used. Spelman (2004) reported that animals in which they did not include flumazenil for midazolam anatgonism, remained mildly sedated for the rest of the day. The use of flumazenil is often deemed to be cost prohibitive and unnecessary. As our aim was to promote complete anaesthetic reversals in order to enable the black-footed cats to resume their hunting as soon as possible and to ensure their complete awareness of any

predatory threats, we are of the opinion that the benefits of including flumazenil, at a high enough dose to prevent resedation, outweighs the costs in these animals. Due to the small size of black-footed cats, we believe the cost of flumazenil was not that high in this study.

The initial capture of uncollared free-ranging black-footed cats is challenging. Techniques such as trapping have previously been tried with limited success. Identifying the optimal placements for the cage-traps is made difficult due to the cats' very low densities, their nocturnal activity and their use of various burrows. Furthermore, the prolonged stress induced by harsh environmental conditions and confinement, should an animal remain in a cage for a prolonged period of time, is concerning for black-footed cats or any other species that may be inadvertently caught. Unless future technology allows for the detection of the free-ranging cats during their daytime rest in burrows or dens, short intense chases will be required before the capture of these animals. Unfortunately, as we have shown, these chases predispose animals to developing hypoxaemia, tissue hypoxia and lactic acidosis resulting in the possible development of myocardial and skeletal myopathy and potentially other pathologies. The consequences of this capture method should be looked at more closely to ascertain the degree of pathophysiology that may develop, particularly in cardiac and skeletal muscle, and potential treatments should then be instituted and efficacy established. When black-footed cats are chased before capture, special attention needs to be given to the oxygenation, metabolic status and body temperatures of these animals post capture and corrective measures should be implemented as soon as possible. In addition to continuous supplementary oxygen we recommend that intravenous fluids should be given to improve tissue perfusion and oxygen delivery to the tissues. Continuous monitoring of body temperature should be a priority and electric blankets, as a constant source of heat, plus blankets to cover the animals should be used from early on in the anaesthesia especially for those cats caught and anaesthetised at night or on cold days.

We have shown that the butorphanol-medetomidine-midazolam combination is an effective anaesthetic, which causes rapid smooth inductions and maintains a good quality, depth and length of anaesthesia to allow for the collection of semen, blood and tissue samples. Collection of subcutaneous fat samples via biopsy incisions in the free-ranging population is ongoing in order to investigate the prevalence of amyloidosis in wild cats, and the possible genetic or familial predisposition of this species to this disease. Semen is collected for cryopreservation for assisted reproductive techniques in captive zoo populations and biobanking to preserve genetic diversity. This anaesthetic drug combination also allows for adequate time to place and test tracking telemetry collars in order to continue research into the biology, ecology, distribution and health of black-footed cats.

6. CONCLUSION

We have shown that the butorphanol-medetomidine-midazolam anaesthetic drug combination is safe, effective and reliable in black-footed cats in both captive and free-ranging animals. We have also shown that the higher doses required in the free-ranging animals do not exacerbate the physiological side effects of the drug combination. The method of capture, involving a short but intense chase in uncollared free-ranging animals, had a greater effect on their physiology than the higher doses of the drugs necessary for their anaesthesia. Side effects of concern include the poikilothermia induced by the anaesthetic drug combination, which caused rapid loss of body temperature and hypothermia, especially during the cold conditions of the nighttime captures. Whenever black-footed cats are anaesthetised with this combination in cold environments we recommend that animals be placed on an electric heating blanket and are covered with insulating blankets. We also recommend that body temperature is monitored continuously to ensure efficacy of heating and to identify and treat hyperthermia, if needed, especially directly after capture. In the chased animals the moderate hypoxaemia, tissue hypoxia and lactic acidosis with the possibility of cardiac and skeletal muscle myopathy were also of concern. We therefore recommend the use of oxygen supplementation, and if necessary, intravenous fluids in order to improve tissue perfusion and oxygen delivery to tissues.

As black-footed cats are predisposed to developing amyloidosis, it is important that any anaesthetic drug combination does not cause severe hypo- or hypertension. We have shown that the butorphanol-medetomidine-midazolam combination maintained blood pressure within a normal range for cats of a similar size. Cyclohexylamines often cause severe hypertension especially in cats (Posner and Burns 2009a), and therefore the exclusion of a cyclohexylamine from anaesthetic drug combinations used in black-footed cats has a distinct advantage. Of major benefit also is the ability to completely reverse the anaesthesia effects of this drug combination using the antagonists at our suggested doses.

We have shown that concerns regarding the exacerbation of any negative physiological side effects by using higher doses in free-ranging black-footed cats were unfounded. In fact, we have shown that even higher doses than those originally planned can safely and effectively be used in black-footed cats. Although the doses we originally planned to use caused adequate induction, in some animals top-up doses were required to maintain an adequate depth of anaesthesia. We therefore recommend that higher doses, at least 35% higher than what we planned, can be used to safely and effectively anaesthetise both captive and free-ranging black-footed cats for 45 minutes.

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ADDENDUM A

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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Animal Ethics Committee

PROJECT TITLE	**Evaluation of the physiological effects of a medetomidine / midazolam / butorphanol-induced anaesthesia in free-ranging black-footed cats *(Feline nigripes)* compared to those in captivity**
PROJECT NUMBER	**V084-13**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. B Eggers**

STUDENT NUMBER (where applicable)	**82224014**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet**

ANIMAL SPECIES	**Black-footed cats *(Feline nigripes)***	
NUMBER OF ANIMALS	**20**	
Approval period to use animals for research/testing purposes		**December 2013 –March 2014**
SUPERVISOR	**Dr. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	25 November 2013
CHAIRMAN: UP Animal Ethics Committee	Signature	

ADDENDUM B

UPSPACE SUBMISSION FORM FOR ELECTRONIC THESIS/DISSERTATION

TO BE COMPLETED BY STUDENT:

Surname Eggers	**Initials** B	**Title** Dr	
Student number 82224014	**Study completed in Month & Year**	April	2015
E-mail address blackegg@mweb.co.za	Should your e-mail address be made available on the UPSpace website? YES | NO		
Telephone number (031) 5628443	**Cell number** (074) 1973405		
Department Paraclinical Sciences, Pharmacology			
Faculty Veterinary Science, Onderstepoort	**Degree** MMedVet (Fer)		
Supervisor Dr. Leith Meyer	**Co-supervisor** Dr. Adrian Tordiffe		
Title of the study: Evaluation of two different doses of butorphanol-medetomidine-midazolam for anaesthesia in free-ranging versus captive black-footed cats (Felis nigripes)			
Please supply 5 keywords for the study:	1. Black-footed cats		
2. Butorphanol	3. Medetomidine		
4. Midazolam	5. Anaesthesia		

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SIGNATURE OF STUDENT : _____ **DATE:** 8th June 2015

TO BE COMPLETED BY SUPERVISOR:

Surname Meyer	**Initials** LCR	**Title** Prof
E-mail address for enquiries leith.meyer@up.ac.za		

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