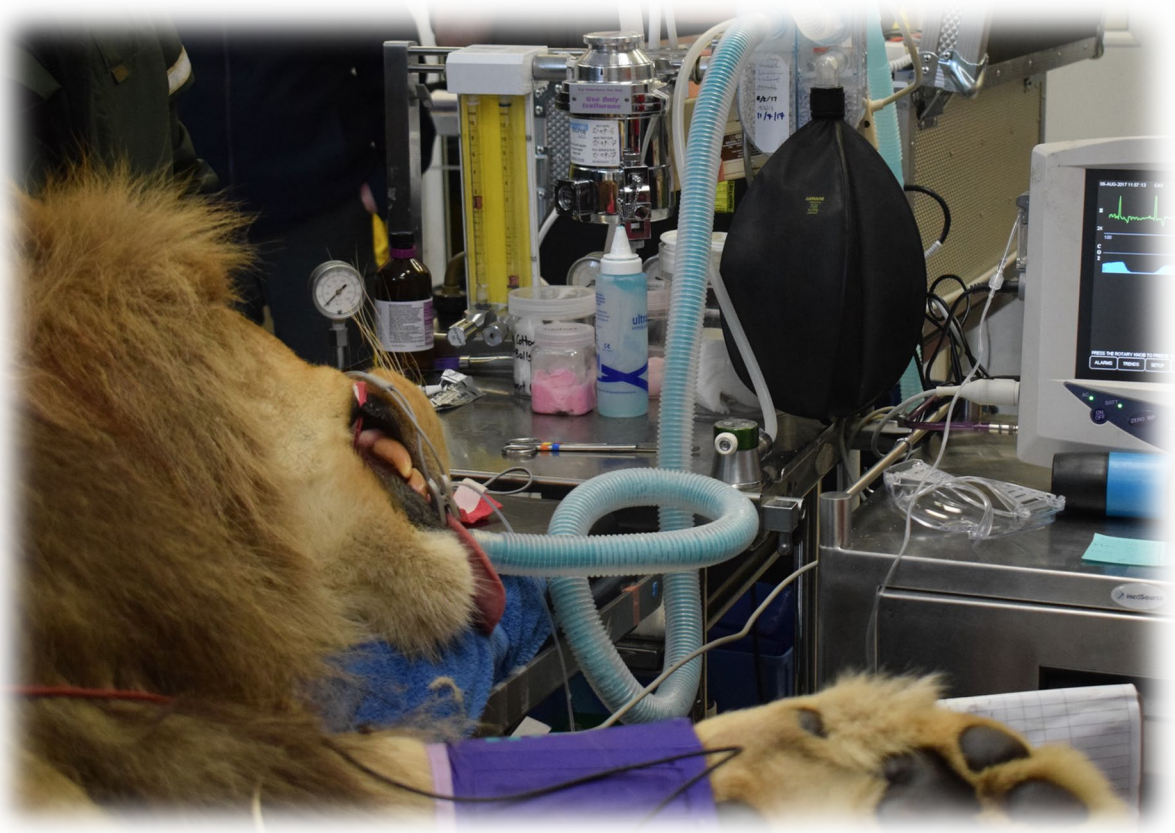
# Comparative assessment of physiological homeostasis in zoo mammals under general anaesthesia



**A thesis presented in partial fulfilment of the requirements for the degree of**

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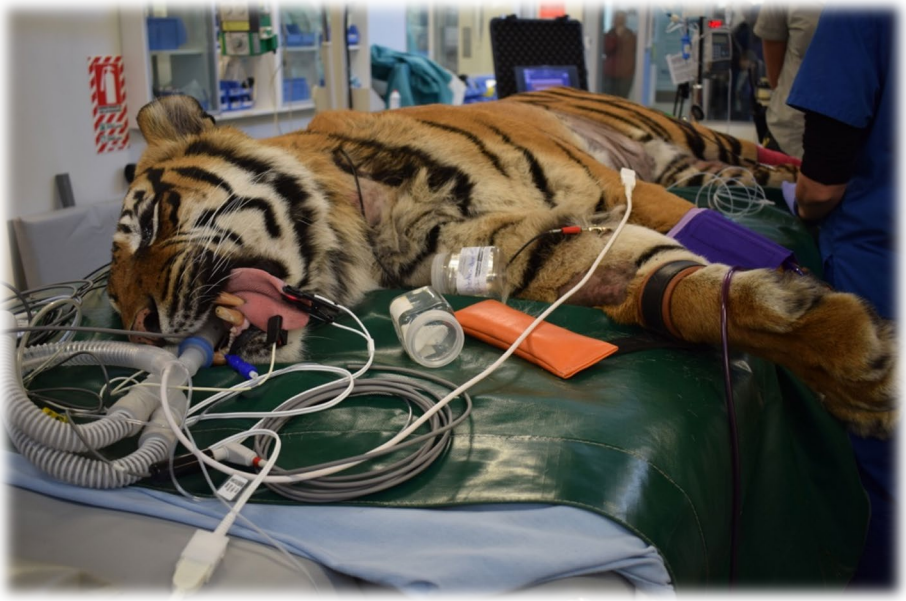
**Abstract:**



During the anaesthesia of dangerous zoo animals, additional anaesthetic risks to patients arise from precautions required to assure human safety. Evaluating the safety of anaesthetic approaches for these animals is not straightforward, with challenges associated with identifying disturbances to physiological homeostasis, attributing these disturbances to particular interventions and relating them to morbidity endpoints. In zoo animal anaesthesia, more reliance is necessarily placed on observational studies to quantify risks related to immobilisation regimens and to refine and improve the ways anaesthesia is subsequently delivered.

This study applied an opportunistic approach, using readily accessible monitoring methods to investigate general changes to physiological homeostasis occurring under zoo anaesthesia in twenty-six individuals. This included comparative analysis of nine large mammal species, of diverse phylogeny and dietary ecology. In eleven large felids representing three different *Felidae* species included in the study, assessment was then undertaken of changes in metabolic acid-base status, the contribution of anions that are not normally measured to these changes and their possible association with mean arterial blood pressure preceding blood sampling episodes.

Strong evidence was found for time effects during anaesthesia on a variety of the measures of homeostasis that were monitored. Within the broad species ranges that were apparent in the homeostatic parameters measured, many unexpected findings were manifest in the way that different species and/or different conspecific individuals responded to anaesthesia. Within a species, differences were sometimes apparent in spite of relatively minor variations in the immobilisation protocols used, as was evident within the *Panthera* genus. Clinically significant alterations noted included blood pressure changes, changes to ventilation, possible ventilation-perfusion mismatches, alterations in acid-base status and occasional but concerning instances of acidaemia, hypoglycaemia, hypoxaemia and hyperkalaemia. All animals survived anaesthesia and did not show any apparent morbidity, limiting our ability to determine the pathological effects, if any, of the changes seen. Based on extrapolations from other mammals, some disturbances may have caused pathology or mortality if they had become more sustained or progressive.

Extracellular (blood) base deficits exceeding -7mmol/L were common in the anaesthetised large felids, suggesting that these animals commonly displayed a state of metabolic acidosis under anaesthesia based on proposed definitions from domestic felids. However, there were marked species differences in acid-base status under anaesthesia, and further analysis showed that the drivers of the changes also varied between species. Although strong cautionary caveats are associated with the low power of the analysis, the findings suggest that inter-species extrapolations of acid-base physiology will be flawed, indicating a strong need for readily accessible species-specific reference ranges.

There was no evidence for an association between strong ion gap and mean arterial pressure, but blood pressure was actively managed in most of the anaesthetics which may have confounded these results. Further study of tissue perfusion states than those provided by mean arterial blood pressure will be required to evaluate if a relationship exists between strong ion gap levels and corresponding states of blood flow in zoo animal anaesthesia. Nevertheless, this study has demonstrated that strategic point of care clinical pathology tests and blood gas analysis provide practical opportunities to

minimise and even prevent many physiological changes, with the potential to diminish risk to patients without placing human safety at additional risk.

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**Permissions and Ethics Approval:**

Ethics approvals for this study have been granted from the respective Animal Ethics Committees of Auckland and Wellington Zoos and from Massey University Animal Ethics Committee under MUAEC permit 17/50. Special permission for the inclusion of non-human hominids in the study was granted by the Director General of the New Zealand Ministry for Primary Industries.

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To Africa's wonderful people and to the continent's awesome wildlife: in its own quiet way, the work behind this thesis is dedicated to you, for the enormous inspiration that you have given to me throughout my life. I look forward immensely to the prospect of returning home to Kenya sometime and to being reunited with my parents and my brothers, to whom I also owe so much.

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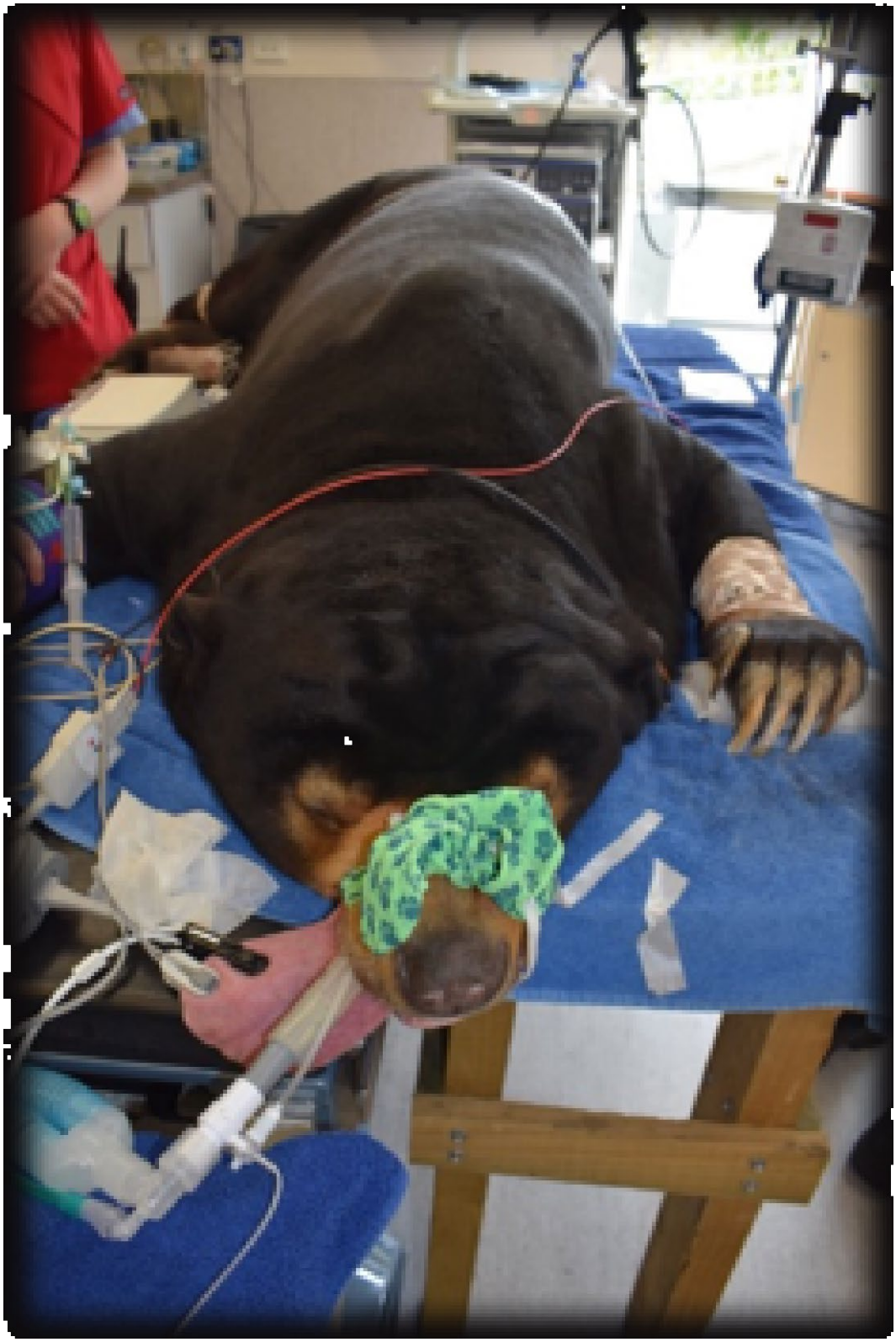
**Chapter One: Introduction and Literature Review:**



## 1.1 Introduction:

Zoo animal veterinary care involves particular challenges with the provision of anaesthesia to dangerous wildlife. Such challenges include inabilities to carry out full pre-anaesthetic health assessments, a dearth of scientific information supporting particular anaesthetic practices, precautionary use of high doses of potent immobilising drugs without ability to titrate them, unpredictable drug uptake associated with remote drug delivery, dispositions of wild animals placing them at greater risk of self-injury, inabilities to approach patients during high risk periods of anaesthesia and the physio-anatomical challenges associated with anaesthetic monitoring and support for animals of extremes of body size and aspect.

The goal of anaesthetic management in a zoological setting is to improve patient anaesthetic safety without compromising human safety and although discoveries of new neuro-modulatory agents have revolutionised the safety of veterinary anaesthesia (Taylor 2014), there are still significant risks to zoo patients associated with delivering and maintaining anaesthesia using existing therapies (Lamont and Grimm 2014, Ozeki and Caulkett 2014). A clear understanding of the determinants of anaesthetic safety permits anaesthetic risk to be better mitigated using technologies available, which is important as technological breakthroughs in the delivery of anaesthesia are not frequent (Pronovost et al 2004, Lamont and Grimm 2014). A framework for developing this understanding is to identify the changes to homeostasis that occur during zoo mammal anaesthesia and to examine the risk factors associated with them, as a basis for gaining further insight into how and why they develop.

## 1.2 Definition of Anaesthesia:

Anaesthesia is traditionally defined as ‘a drug-induced absence of sensation’ (Heavner 2001). Better mechanistic understanding has since broadened this definition (Eger and Sonner 2006) to encompass the various attributes of an anaesthetic state and the synergism of different drugs used to achieve this (Tonner 2005), as enshrined in the concept of ‘balanced anaesthesia’ (Lundy 1942). Key components of balanced

anaesthesia include loss of consciousness, muscle relaxation, analgesia and the suppression of stress responses (Hubbell and Muir 2009). In this thesis, the term 'anaesthesia' is used to embrace the combined neuro-modulatory effects of drugs used to provide balanced anaesthesia in zoo animals. Its use therefore applies to agents used for anxiolysis, chemical restraint, and analgesia as well as the general and dissociative anaesthetics. This inclusive definition is applied because the combined effects of these neuro-modulatory agents are integral to determining associated outcomes (Hubbell and Muir 2009, Hernandez 2014).

In zoo animal anaesthesia, these outcomes may include the loss and subsequent return to consciousness and mobility in a time frame that allows human safety, with minimal detrimental effects on the anaesthetised animal. These effects include short term but acutely hazardous changes in homeostasis, such as hypothermia, hypoxia or hypercapnia, and long term effects on health such as muscle damage, gastrointestinal ulceration or decreased renal function.

## 1.3 Overview of Homeostatic Disturbances and Risks Associated with Anaesthesia:

General anaesthesia involves controlled, reversible, drug induced intoxication of the central nervous system, hereinafter 'CNS' (Trim, Clarke and Hall 2014, Taylor 2014). Depending on pre-anaesthetic clinical states and on peri-anaesthetic management, induction and maintenance of anaesthesia may constitute a threat to the life of a patient (Hall and Clarke 1991, Trim, Clarke and Hall 2014, Taylor 2014). Most of the effects of neuro-modulatory agents on organ function are mediated through their depression or alteration of neurological and neuro-endocrine control of such organs, involving alteration of synaptic activity and membrane conduction in brain centres and associated neurological and neuro-endocrine pathways (Hall and Clarke 1991). Every organ system may be affected in this way (Hall and Clarke 1991) and lethal anaesthetic overdose results from cessation of such controls rather than direct induction of brain death (Musizza and Ribarac 2010).

The CNS control of functions altered by neuro-modulatory agents is complex (Trim, Clarke and Hall 2014) and mechanism of actions and processes by which the molecular and cellular actions of these drugs translate into neuro-modulation is still poorly understood (Urban 2008, Musizza and Ribarac 2010). Anaesthesia itself is a particularly complex state: anaesthetics produce separate sub-states, probably at different areas of the CNS (Musizza and Ribarac 2010), each in a manner specific to particular agent types (Perouansky et al 2012).

Potential impacts on homeostasis arise not just from general depression of neurological function; those occurring following the abolition of CNS inhibition or impaired coordination may be similarly profound. Examples from the veterinary zoological domain include excitatory responses to potent or high opioid dosage in felids and horses (KuKanich and Weise 2015) and excitatory and ataxic phases associated with lighter planes of anaesthesia (Meyer 2015), risking patient self-injury and hyperthermia. Complex interactions between the sympathetic nervous system, the endocrine system and muscular activity may also contribute towards the development of the different syndromes of capture myopathy (Hernandez 2014, Breed et al 2019).

Wide dose-dependence, varying routes of administration, individual idiosyncrasies (Trim, Clarke and Hall 2014), drug interactions (Hall and Clarke 1991) and pre-anaesthetic clinical and behavioural states (Lamont and Grimm 2014) all influence the pharmacokinetics and pharmacodynamics of each of the various neuro-modulatory agents. Furthermore, marked variations in the way that different species tend to respond to these agents and marked variations in the way that each species tend to process them (Forsyth 1992, Ozeki and Caulkett 2014) adds yet another layer of complexity to the range of expected responses arising from their use within the domain of veterinary anaesthesia.

The most common conditions affecting the course of veterinary anaesthesia involve the cardiovascular and respiratory systems (Trim, Clarke and Hall 2014). Almost all drugs that elicit anaesthesia, particularly inhalational anaesthetics (such as isoflurane, sevoflurane and halothane) and to a lesser extent injectable anaesthetics (such as

barbiturates, propofol and steroid anaesthetics) affect cardiovascular function by inducing dose-dependent myocardial depression, mediated through a range of mechanisms including negative chronotropy, inotropy and possible conduction disturbances (Lees 1991). They also alter vascular reactivity directly or via reduced autonomic activity (Hall and Clarke 1991), causing spasmolytic vasodilation and affecting compensatory auto-regulatory blood flow responses. These effects may lead to hypotension and redistribution of blood flow, with potential for hypoxic injury (Noel-Morgan and Muir 2018).

Some agents have stimulatory effects on certain cardiovascular functions, potentially inducing hypertension. Examples in a veterinary zoological context are potent narcotics (Swan 1993, Burroughs et al 2014), cyclohexamines (Cerveny and Sleeman 2014), high doses of alpha-2 agonists (Ozeki and Caulkett 2014) and different combinations thereof as, for example, documented in canids (Larsen and Kreeger 2014), ursids (Caulkett and Fahlman 2014) and ungulates (Ball and Hofmeyr 2014). When chronic or extreme or sustained, hypertension may predispose to cerebral and pulmonary oedema and haemorrhage, retinopathy, choroidopathy and renal injury (Brown et al 2007, Haskins 2015). Select agents, such as alpha-2 agonists, may have biphasic stimulatory and depressive cardiovascular effects (Scheinin et al 1989, Grimm et al 2001, Saleh et al 2005, Trim, Clarke and Hall 2014), with changing haemodynamic impacts.

Most anaesthetic agents (including cyclohexamines) and many sedative, opioid analgesic and hypnotic agents (including dexmedetomidine, midazolam, fentanyl and remifentanil) have varying dose-dependent depressive effects on ventilation and respiratory performance (Goudra and Farid 2013), often secondary to their influences on neurogenic and myogenic activity. Bradypnoea may occur with excessive anaesthetic depth, apnoea is common following induction of anaesthesia and some irregular breathing patterns observed during anaesthesia directly reflect medullary depression (Trim, Clarke and Hall 2014, Haskins 2015). All anaesthetics tend to depress chemoreceptor responses to carbon dioxide (Lamont and Grimm 2014) and loss of the intercostal component of breathing with deepening anaesthesia exacerbates poor chemoreceptor responses to carbon dioxide elevations (Hall and Clarke 1991), leading

to carbon dioxide accumulation without assistance to ventilation. The hypoxic drive to breathing is also abolished by certain anaesthetics such as halothane, in some species (Hall and Clarke 1991). Through various mechanisms, particularly following anaesthetic-induced reductions in functional residual lung capacity (Hall and Clarke 1991), ventilation-perfusion mismatches may develop and most inhalant agents may interfere with hypoxic pulmonary vasoconstriction, exacerbating such mismatching (Lamont and Grimm 2014). Furthermore, cardiovascular and respiratory depressive effects may be interconnected through arrays of mechanisms, for example through the effects of positive end pressure ventilation on reducing venous return and resultant cardiac output (Hall and Clarke 1991).

One of the effects which may contribute in particular to a multitude of other homeostatic derangements is the multifaceted impact of chemical restraint and anaesthesia on thermoregulation (Haskins 2015). The risk of such derangements depend on pre-existing morbidities and the extent of thermal dysregulation. Hypotension, myocardial ischemia and arrhythmias may be potentiated by hypothermia (Ko and Krimins 2014) and its occurrence is also associated with elevated risks of anaesthetic overdose, prolonged anaesthetic recovery and possibly impaired coagulation and predisposition to post-operative wound infection (Grimm 2015). Severe hyperthermia may cause multiple organ dysfunction and organ failure (Haskins 2015).

Homeostatic alterations induced by anaesthesia are almost invariably the aggregate result of multiple pathophysiological processes because of the dependence of higher metazoan cellular health on the integrated function of different organs. Since normally self-regulating physiological components are either suspended or radically altered by neuro-modulatory agents (Ludders and McMillan 2017), body systems under anaesthesia have been described as ‘tightly coupled’ (Gaba et al 1987) because changes in one component very rapidly affect others, with cascading domino-like effects. For their ability to induce profound cascading effects most immediately threatening to life, the neurological, cardiovascular, respiratory and thermoregulatory systems are prioritised in anaesthetic monitoring (Hall and Clarke 1991, Hubbell and Muir 2009). Metabolic disorders may well reflect the aggregate effects of multiple system

dysfunctions and acid-base disorders, electrolyte imbalances and alterations in glycaemic states provide salient examples of such potentially life-threatening homeostatic disturbances that also warrant close monitoring, particularly in at-risk patients (Trim, Clarke and Hall 2014).

Anaesthetic agents have multiple and varied effects on the gastrointestinal tract, some of which may be particularly significant to patient prognosis. Most reduce gastrointestinal motility and may be associated with emesis, regurgitation and gastroesophageal reflux (Adams et al 2015). Ruminants are particularly prone to anaesthesia induced regurgitation and ileus (McDonell and Kerr 2015) and the cardiopulmonary consequences of gastric tympany may rapidly become life threatening.

Regurgitation is also the most prevalent documented peri-anaesthetic gastrointestinal complication in small animal veterinary practice (Brodbelt et al 2015). Various significantly adverse morbidities may be associated with this passive process, including aspiration pneumonia and ulcerative oesophagitis and stricture (Wilson and Shih 2015). The risk of mucosal injury from gastroesophageal reflux may be greater than evident from times where reflux is observed (Brodbelt et al 2015, Wilson and Shih 2015). Contributing causes are many and given the lack of overt clinical signs at occurrence (Wilson and Shih 2015), these conditions are not well characterised in zoological medicine.

A rapid transition through the intermediate stages of anaesthesia is vital to reduce aspiration risk from reflex emesis. In humans (Dutton 2013), non-human hominids (Murphy 2015) and other monogastrics such as felids, canids and ursids (Caulkett and Fahlman 2014) as well as ruminants such as caprines (Tranquili and Grimm 2015), antelopines (Ball and Hofmeyr 2014) and giraffids (Citino and Bush 2014), emesis or regurgitation is associated with laryngeal or pharyngeal stimulation under light planes of anaesthesia during induction. Chemo-emesis is also a common side effect of opioids and alpha-2 agonists in certain taxa such as canids and felids (Swan 1993, Kreeger and Arnemo 2012, Lamont and Grimm 2014) and while usually occurring prior to the abolition of swallowing reflexes, residual ingesta that remains in proximity to the glottis

may still pose a risk for aspiration.

Often considered of less importance in the immediate term than the cardiovascular and respiratory systems, other organ systems are nevertheless critically important to general cellular health and function in the peri-anaesthetic period. Hepatic and renal function in particular may be variably impacted by anaesthesia, directly or via haemodynamic disturbances and alterations in their neuro-endocrine control (Papper 1953, Gelman 1987, Mercatello 1990, Motayagheni et al 2017). Their functional capabilities are more the focus of pre-anaesthetic consideration where possible (Hubbell and Muir 2009) and are usually inferred during anaesthesia from amalgamated measures used to monitor more immediately vital organ systems (Hall and Clarke 1991). Nevertheless, the pharmacokinetic effects of altered renal or hepatic function (together with other important biotransformation organs such as lungs) may profoundly alter the course of anaesthesia through affecting the actions and eliminations of neuro-modulatory drugs (Sear 1987, Hiraoka et al 2005).

Procedural errors and equipment malfunctions during provision of anaesthesia may also have far-reaching impacts on homeostasis. The most prevalent complications in a large Australian study of human anaesthetic morbidity involved airway injury and aspiration pneumonia, arising respectively from difficult intubations and inadequate airway securement, with occurrences and sequels most manifest during intubation and after removal of the endotracheal tube (McNicol and Mackay 2010). Another particularly important procedural source of risk is error in clinical reasoning and anaesthetic decision making (Ludders and McMillan 2017), whereby anaesthetists' errors in their responses to information from anaesthetic monitoring may further complicate the anaesthesia rather than resolve the disturbance to homeostasis. Often there may be more than one possible explanation for particular clinical abnormalities identified during anaesthesia, with the likelihood of each being influenced by complex interactions between the effects of the anaesthetic agent, the patient's pre-anaesthetic health status and conditions that may develop during anaesthesia. The clinical discipline of anaesthesia is therefore both a learned practical art and a science (Taylor 2014, Trim, Clarke and Hall 2014), drawing on both experience and on research to improve safety.

## 1.4 Particular Challenges Associated with Zoo Animal and Wildlife Anaesthesia:

The practicalities of anaesthetising captive wildlife often involve enhanced risks to the patient relative to human and domestic animal anaesthesia and also to the staff involved in the procedure. These risks may be further accentuated in free-ranging wildlife.

### 1.4.1 Disposition of Wildlife:

The often dangerous disposition of wildlife demands application of precautionary principles for human safety. Associated risks of anaesthesia to the animal patient may be enhanced by such emphasis on human safety, through accentuation of dose-dependent homeostatic impairments (Hatt and Jurado 2012) and associated prolonged recoveries (Hernandez 2014). Although there is no published data pertaining to the prevalence or incidence of human morbidity or mortality associated specifically with zoo animal and free-ranging wildlife restraint and anaesthesia, occupation-related fatalities have been reported to occur (Langley and Hunter 2001) and in one survey, albeit now somewhat dated, 61% of zoo veterinarians reported themselves to have been victims of major animal-induced trauma at some point during their career (Hill et al 1998). Furthermore, injuries to staff sustained by dangerous animals which have spontaneously awoken from anaesthesia have occurred (M Kock pers comm, D Cooper pers comm). Nevertheless, occupational safety has become a particularly important focus in the provision of zoo animal veterinary care, including for anaesthesia. This is evidenced by the development and implementation of comprehensive protocols and measures for dangerous animal crisis management (Murphy 2012), widespread legal emphases on responsibility towards safety and in developments in health and safety planning and program implementation (Hammond 2019).

To allow human operators safe interactions with captive and free-ranging wildlife, Forsyth et al (1999) noted at the time that relatively high dosages of immobilising agents may be administered to animals such as tigers (in comparison with sedation of domestic species of shared taxa, especially when doses are allometrically adjusted) and this

observation is still largely valid for dosages often applied in current zoo practice involving large felids and other dangerous animals. Besides the precautionary principle (Hatt and Jurado 2012), part of the reason for these high doses may relate to the need to overcome the patient's psychogenic response to restraint (Carregaro et al 2016). There is rarely proper opportunity to titrate such drugs to effect in zoo medicine (Ozeki and Caulkett 2014, Lamont and Grimm 2014), yet potent or concentrated formulations are often administered (Ozeki and Caulkett 2014, Caulkett and Arnemo 2015). Particular uncertainties may surround drug bioavailability, especially with remote drug application depending on full intramuscular delivery (Caulkett and Arnemo 2015, Hernandez 2014) and with the use of oral premedication; supplementation in the face of uncertainty may risk overdoses.

Sudden awakening from anaesthesia with few premonitory signs is a documented risk in various species, particularly involving the use of alpha-2 agonists (for examples, see Burroughs et al 2014, Lamberski 2015 and Murphy 2015) and actual occurrences have been reported in less recent literature, involving medetomidine-ketamine combinations commonly used at the time in large felids (Jalanka and Roeken 1990, Miller et al 2003). As a result, such concerns may incline zoo veterinarians to err towards caution in the anaesthetic 'plane' maintained in species considered as particularly dangerous by zoological institutions (Hatt and Jurado 2012). The risk incurred as a result is that CNS functions may be depressed beyond what is necessary for anaesthesia.

The nervous, flighty disposition of wild animals places them at significant risk of self-injury prior to full immobilisation, necessitating fast-acting (and therefore often potent) drugs be delivered at the higher end of the dose range in an endeavour to induce anaesthesia rapidly (Ozeki and Caulkett 2014). Overdosing is even considered by some authors to be relatively less unsafe to many patients than under-dosing (particularly in free-ranging wildlife), because extended induction times may significantly increase stress and associated complications (Haigh 1990, Arnemo et al 2014). Prey species housed in zoological collections may be more at risk of self-injury and capture myopathy, particularly with added confinement, human approach, physical restraint and associated unnatural fear and stresses (Chalmers and Barrett 1982, Paterson 2014).

Significant advancements have, however, been made in relation to the reduction of pre-anaesthetic stress responses (leading to pre-anaesthetic states more conducive to safer anaesthesia), particularly with the application of patient training techniques based on positive reinforcement (see, for example, Reichard 2008 and Whittaker and Laule 2012), the strategic use of neuroleptic or psychotropic drugs such as sedatives and tranquilisers (see, for example, Kaandorp 2005 and Tynes 2014) and the general application of holistic approaches towards animal care which better address the social and psychological aspects of patient wellbeing (Kagan et al 2015, Mellor 2016).

### 1.4.2 Challenges Associated with Anaesthesia of Megafauna:

In general, the larger the animal, the greater the anaesthetic risks likely to be involved (Trim, Clarke and Hall 2014). In equine practice, for example, a wider range and higher prevalence of perioperative and postoperative complications are evident compared with small animals (Brodbelt et al 2015), with many of these discrepancies being associated with anaesthesia. Equids are particularly susceptible to anaesthesia-related cardiovascular collapse (Muir and Hubbell 2009), myopathies, neuropathies (Duke et al 2006, Trim, Clarke and Hall 2014) and significant ventilation-perfusion mismatches (Hall and Clarke 1991). Gravitational influences, in which the weight of the animal bears on organs and results in restricted breathing or compression of vasculature, are important in the pathogenesis of many of these conditions (Lamont and Grimm 2014, Trim, Clarke and Hall 2014). Anaesthesia of the megafauna encountered in zoo animal practice may face even greater gravitational challenges than domestic equids and these are combined with challenges posed by the peculiar physiological and anatomical considerations associated with greater extremes of body size and body aspect.

The varied nature of these challenges are well documented for many large terrestrial, semi-aquatic and aquatic mammals. Texts advising on restraint and anaesthesia of *Giraffidae*, *Rhinoceridae*, *Elephantidae*, *Hippopotamidae*, *Tapiridae* and *Bovidae* highlight the importance of taking steps to mitigate risks of local weight-focused myopathies and neuropathies (Stegmann et al 2014, Miller and Buss 2014, Miller et al 2014, Wolfe 2015, Zimmerman and Hernandez 2015). These pathologies are

nevertheless avoidable even under prolonged anaesthesia in high risk animals such as black rhinoceros and elephant (Burroughs et al 2014, Horne and Loomis 2014, Radcliffe and Morkel 2014) and they occur as a result of general or local inadequacies in providing for tissue oxygenation requirements (Meltzer and Kock 2014). In terms of blood flow, giraffes are thought to show particular myocardial susceptibility to the effects of hypoxia due to significantly reduced cardiac output under anaesthesia (Citino and Bush 2014, Masterton 2020), especially because their inability to auto-regulate cranial blood pressure under anaesthesia may induce profound reflex bradycardia and resultant hypotension (Geiser et al 1992, Citino and Bush 2014, Masterton 2020). Furthermore, giraffe kidneys maintain extremely high renal interstitial hydrostatic pressures as a likely adaptation to protect against the high arterial driving pressures that are physiologically normal for this taxon, with physiologically low effective renal plasma flow as a result (Bie et al 2012). As such, it may be hypothesised that renal blood flow in giraffes may become particularly compromised by major reductions in arterial blood pressures.

In terms of the respiratory system, larger species may be at greater risk of developing both hypoxaemia and hypercapnia, due to difficulties associated with achieving adequate ventilation and due to greater tendencies towards ventilation-perfusion mismatching under anaesthesia (Muir and Hubbell 1991). Hypoxaemia and hypercapnia may become particularly manifest in species with poor lung compliance under anaesthesia due to thoracic muscle rigidity or due to the immense pressure of abdominal contents on the diaphragm and sternum (*Elephantidae*: Burroughs et al 2014, *Rhinoceridae*: Wenger et al 2007, Horne and Loomis 2014, Radcliffe and Morkel 2014). Particular challenges may be associated with the anaesthesia of species with a reduced ability to respond to hypercapnia because of their relatively limited residual lung volumes in relation to body size (*Elephantidae*: Burroughs et al 2014, *Giraffidae*: Mitchell and Skinner 2011, Delk et al 2019). In terms of amelioration of these conditions, there are challenges associated with minimising the negative cardiovascular effects arising from positive pressure ventilation in animals exceeding one hundred kilogrammes in bodyweight (Simpson 2007). Furthermore, given the propensity for high fractions of inspired oxygen to induce varying degrees of atelectasis under anaesthesia (Lumb 2007),

much work still needs to be performed to determine optimal inspired oxygen fractions in zoological species in different clinical situations.

In terms of the challenges associated with extremes of body aspect, giraffes provide the most salient example in this regard, with their long neck and legs making them extremely prone to head and limb injuries sustained during anaesthetic induction and recovery (Burroughs et al 2014, Citino and Bush 2014). At a different part of the aspect spectrum, there is marked potential for hyperthermia in species with lower surface area to volume ratios (*Elephantidae*, *Hippopotamidae*: Miller et al 2014; *Rhinoceridae*: Miller and Buss 2015; *Pinnipedia*: van Bonn 2015). In cetaceans, in which bodyweight is normally supported by buoyancy forces exerted by the water column, their removal from water immediately induces significant and potentially life-threatening compromises to cardiopulmonary and thermoregulatory function and these compromises are further exacerbated by chemical restraint and anaesthesia (Dold and Ridgeway 2014).

### 1.4.3 Constraints Facing Preparedness in Zoo Anaesthesia:

Safe and effective anaesthesia is particularly reliant on thorough pre-anaesthetic patient assessment and preparation (Bednarski et al 2011) in order to identify and provide optimal anaesthetic management of pre-existing conditions. Such requirements are addressed well in human clinical anaesthesia (see, for example, Pollard and Kitchen 2018) and reasonable provision for it is possible in domestic animal veterinary medicine.

For safety reasons, rarely is there the opportunity to provide the same depth of evaluation in zoo animal medicine (Caulkett and Arnemo 2015, Hernandez 2014, Divers 2014), although holistic approaches to veterinary care that draw on a multi-disciplinary approach to health (see, for example, EAZA 2014 and Meehan 2015), recognition of subtle clinical and behavioural changes by keepers (see, for example, AZA 2012 and AZA 2017), application of various technologies (see, for example, Al-Naji et al 2019) and patient training based on positive reinforcement (see, for example, McNally 2017 and Murphy and Danforth 2019) have improved capacities for pre-anaesthetic health

assessment. Nevertheless, Cerveny and Sleeman (2014) state that unknown diseases may easily go undetected and Ball and Hofmeyr (2014) note that wild animals may still mask significant disease, with both of these cautions likely having similar validity in current wildlife anaesthesia practice. Furthermore, there are often only a few pre-anaesthetic baselines against which the range and extent of anaesthetic impacts may be assessed.

Significant technological developments and knowledge advancements have been made in the art and science of exotic animal, zoo and wildlife anaesthesia over recent decades, particularly towards monitoring and understanding the pathophysiological effects of anaesthesia (see, for example, Fahlman 2008), the development of safer anaesthetic protocols even for particularly high risk species such as giraffe (see, for example, Bush et al 2002, Citino and Bush 2014, Burroughs et al 2014, Bertelsen 2015, Delk et al 2019 and Masterton 2020) and the provision of anaesthetic support (see, for example, Comolli et al 2019). These advances have drawn upon progress made in human and domestic animal anaesthesia and from research and sharing of knowledge and experiences in the zoological veterinary arena.

In many zoological species there is, however, often still a paucity of information to provide scientific support for anaesthetic techniques (Ozeki and Caulkett 2014, Caulkett and Arnemo 2015), with pharmacodynamics studies of different protocols tending to dominate the empirical evidence base. Of particular relevance in this regard, one of the core requirements for anticipating the effects of anaesthesia is knowledge of the pharmacology and side effects of drugs used for its induction and maintenance (Cracknell 2008, Hubbell and Muir 2009). Yet in zoo animal medicine, rarely have detailed pharmacokinetic studies been performed in the species involved (Hunter 2010, Hernandez 2014). In practical terms, the number of options available for achieving balanced anaesthesia may also be more limited in zoo animal medicine because particular drug combinations have to be given together to counter each drug's side effects, leaving less facility for more controlled, sequential premedication and induction. Before the completion of procedures and the conscious decision to awake a dangerous animal, anaesthetists may also be cautious to administer antagonists to the reversible

components of these combinations (such as alpha-2 agonists), as unexpected patient awakening or loss of balanced anaesthesia may be elicited. As a result, there is the potential for prolonged exposure to the unwanted side effects of such drugs. A personal observation is that for human safety reasons, a cautious approach may also be taken towards trialling alternative or complementary anaesthetic maintenance approaches such as total or supplementary use of intravenous anaesthesia.

## 1.5 Assessing Zoo Animal Anaesthesia:

### 1.5.1 Practical Constraints Facing Anaesthetic Monitoring and Response in Zoo Anaesthesia Practice:

Unlike in other domains of anaesthetic practice, particularly limited anaesthesia monitoring may be undertaken in zoo animal and free-ranging wildlife settings and there may also be limited scope for timely corrective interventions, especially during the high risks periods prior to induction and post endotracheal tube removal (Lamont and Grimm 2014, Hernandez 2014).

Ideally, anaesthetic monitoring should be undertaken continuously from the moment of safe approach (Ozeki and Caulkett 2014) until it is no longer safely feasible, ideally conforming in manner and extent to recommendations made by professional bodies (for examples, see ACVA 1995, ACVA 2009, Robertson et al 2018 and Grubb et al 2020). To limit the need to repeat anaesthesia, there may be a tendency in zoo veterinary practice to carry out several diagnostic activities or health assessments with each anaesthetic event, in order to make the most of opportunities provided by them. A personal observation is that besides the need for patient transport, the frequent patient re-positioning associated with the pursuit of these activities may potentially interfere with anaesthetic vigilance, especially if the practicalities of placing and maintaining monitoring equipment and undertaking monitoring are deemed to unjustifiably protract procedure time and thus incur potential additional risk to the patient.

### 1.5.2 Detecting Homeostatic Disturbances: Technical and Interpretative Challenges:

Clinical reasoning and decision-making are critical skills in anaesthesia, permitting rapid diagnosis and application of appropriate and timely therapy (Ludders and McMillan 2017). To respond to anaesthetic changes appropriately, the anaesthetist must have a good understanding of the physiological and pathophysiological processes involved for each species. In zoological medicine, such foundations may be lacking. For example, reference ranges for clinical pathology parameters are poorly established for many species (Stacy and Hollinger 2018). With the anatomical and physiological variation apparent in such a wide range of species (Hernandez 2014), clinical errors may arise from inter-species extrapolations (Caulkett and Arnemo 2015). Anaesthetic equipment and techniques designed for use in medical and domestic veterinary fields also require adjustment, with interpretation having to be extrapolated to the unique and diverse anatomy and physiology of different taxa (Divers 2014). Particular challenges arise, for example, when monitoring equipment is applied to very large or very small patients (Ozeki and Caulkett 2014).

Many of the significant interpretative challenges facing zoo animal anaesthetists are universal to anaesthetic monitoring, but they may be more complicated in a zoo setting. One of the key objectives of anaesthetic monitoring is to permit accurate assessment of the adequacy of tissue perfusion (Ozeki and Caulkett 2014) and various advanced modalities have been developed to complement the clinical parameters used to assess its adequacy in human medicine. Many of the associated haemodynamic monitoring methods are invasive and often not practical to use in routine clinical situations, particularly in zoological medicine where time of anaesthesia is often limited. Therefore, reliance is placed instead on indirect macro-circulatory measures as the basis for making approximations of cardiovascular function. These include basic measures of heart rate, blood pressure and pulse pressure, together with mucous membrane colour, capillary refill time and temperature gradients. By cross referencing between these measures, a judgement is made about the likelihood that vital organs are being adequately perfused and oxygenated (Ozeki and Caulkett 2014). The limitations that are associated with a reliance on these measures have important implications for the accuracy of clinical

judgements based upon them. Foremost of these is the reliance on blood pressure as a poor proxy both for cardiac output and tissue perfusion (Wagner 2005, Lawson and Hutton 2012). Furthermore, macro-circulatory measures may not reflect the adequacy of oxygen delivery to different organs (Lawson and Hutton 2012), particularly as blood flow afforded to them is likely to be differentially distributed to reflect bodily importance of function (Bonanno 2011).

Many commonly employed clinical indicators of the perfusion of particular organs that may be of use in conscious states, such as measures of cognitive function and urine output, often bear limited or no relation to organ function during anaesthesia (Noel-Morgan and Muir 2018). Other more general measures, such as the assessment of mucous membrane colour and capillary refill time and vigour, do retain utility as general measures of perfusion but they may not necessarily reflect the reality of critical organ blood flow (Haskins 2015). A discussion by Chawla and Wilson (2007) stated that a clinically robust intra-operative measure of renal function does not yet exist. As such, if this still holds true, less specific and more indirect measures will need to be applied to assess the likely health and functioning of organs and monitor the effectiveness of resuscitative efforts on organs such as the kidneys and gastrointestinal tract under anaesthesia.

The control of anaesthetic depth is equally fundamental to safe anaesthesia (Hubbell and Muir 2009), with the judiciousness of the anaesthetist being one of the most important influences in determining patient anaesthetic outcomes. The accurate assessment of patient anaesthetic depth is not straightforward (Trim, Clarke and Hall 2014) and particularly with the added complexities associated with multimodal methods used to achieve balanced anaesthesia, it is recognised as a current challenge in medicine (Musizzi and Ribarac 2010). Significant inter-individual and procedure related variations exist in responses to anaesthetic drugs (Musizzi and Ribarac 2010) and interspecies variations add further dimension to these challenges in the veterinary context (Hubbell and Muir 2009). Physical signs such as blood pressure, heart rate, heart rate variation and respiratory rates often provide inaccurate, non-specific or too variable measures of anaesthetic depth (Trim, Clarke and Hall 2014) and the classical signs of depth of

anaesthesia based on Geudel's observations may vary with species and reflect only momentary measures under situations where depth may be changing rapidly (Trim, Clarke and Hall 2014, Raue et al 2019). These challenges make it a refined art to gauge and pitch depth to safely accord with anticipated levels of patient stimulation, incurring risk that anaesthesia may be conducted at levels deeper than is often necessary (Trim, Clarke and Hall 2014).

Assessment of lung function is the third major foundation of anaesthetic monitoring. As lungs function as a cardio-respiratory unit, this demands the ability to assess how well lung perfusion and ventilation are matched. Monitoring based on respiratory rates and tidal minute volumes may be useful for detecting trends and can signal changes in the underlying status of the patient but their value alone is limited because they do not provide information about physiologic dead space and functional alveolar ventilation (Haskins 2015). These more detailed parameters require assessment of alveolar or blood gas measures (Bourgoin et al 2017). Capnography, which is the graphic display or recording of expired carbon dioxide concentration versus time or expired volume during a respiratory cycle (Pypendop 2015), is a valuable monitoring tool to identify whether ventilation is adequate and whether cardiac output may be changing (Williamson et al 1993), providing trending information to indicate the development of excessive alveolar dead space (Haskins 2015) and thus prompting the anaesthetist to search for underlying causes. However, as the clinical status of a patient changes, so too may dead space and the relationship between end tidal carbon dioxide and arterial partial pressure of carbon dioxide (PaCO2) may become less predictable. In this situation, periodic serial blood gas determination needs to be undertaken to calibrate the two, particularly when changes in pulmonary function are indicated by end-tidal carbon dioxide or capnography changes (McSwain et al 2010).

Current best practice in human anaesthesia is that monitoring is carried out by combining these techniques with more sophisticated methods where necessary. These include monitoring of systemic and peripheral haemodynamics, microcirculation and tissue oxidation (Kipnis and Vallet 2016), use of electro-encephalographic sensors and mathematical algorithms for assisting with anaesthetic depth estimation (Musizza and Ribarac 2010) and the application of various means to serially assess gas exchange,

respiratory system mechanics and patients' readiness for withdrawal from positive pressure ventilation (Kipnis et al 2012).

In veterinary anaesthesia and, in particular, zoo animal anaesthesia, anaesthetic monitoring is often much more basic, increasing the risk of adverse effects and introducing more uncertainly about whether procedures are being conducted within acceptable margins of safety for the patient. In many cases, this also means that anaesthesia safety and success is assessed simply and crudely by the nature of recovery from anaesthesia and survival of the animal beyond the immediate procedure. Lack of knowledge about the disturbances to homeostasis which are occurring and lack of understanding of the reasons for them make it more difficult to make safety-enhancing refinements to anaesthetic procedures.

## 1.6 Quantifying and Mitigating Risk in Zoo Anaesthesia:

### 1.6.1 Overview of Methods Currently Used to Quantify Anaesthetic Risk:

Being able to quantify anaesthetic safety and identify the risk factors most strongly associated with it are important steps in anaesthetic risk mitigation. These risk factors provide a basis for more informed anaesthetic decision making and for refining protocols or exploring novel approaches to enhancing anaesthetic safety. Their most effective contribution to risk mitigation arises from the better causal and mechanistic understanding they may provide about the homeostatic disturbances occurring under anaesthesia.

In human medicine, the tenets of evidence-based medicine have been advanced to promote more robust comparative evaluation of different treatments (Sackett et al 1996, Sackett et al 1997). Some critics consider these tenets to have limited application to the domain of anaesthesia (Horan 1997). Besides, there is neither the scope nor quantity of data, nor the rigorous and systematic approach demanded of its collection (as set out by Myles et al 1999) to permit such full critical appraisal of different approaches in zoo anaesthesia.

Nonetheless, it is still critical to scrutinise the strength of evidence to support the safe and effective application of different anaesthetic approaches (Masters et al 2007). This is particularly relevant to choices involving the various ways to achieve balanced anaesthesia, as published protocols are widely disseminated and may be widely applied without the full context of their evaluation. Any lack of safety substantiation may be particularly relevant to patients who are already physiologically compromised, for whom the homeostatic effects of pre-existing morbidities may be exacerbated by anaesthesia (Hall and Clarke 2014).

Critical determinants of the strength of evidence for studies evaluating the safety of treatments are the outcomes measured (Guyatt et al 1995). Mortality measures provide the most definitive representation of anaesthetic procedural safety and records of fatality provide one of three key inputs for describing and quantifying the safety of zoo anaesthesia in the Species360® Zoo Information Management System (ZIMS). A wide body of information pertaining to peri-anaesthetic mortality is now available in ZIMS and in the veterinary zoological literature and historically, the extensive use of certain protocols with minimal or no associated mortality has been stated as an argument to support and promote their safe use (for example, see Jalanka and Roeken 1990). Even in less dated, peer-scrutinised literature, there is the acknowledgement that safety of wildlife capture and immobilisation methods are sometimes still evaluated on the basis of mortality rather than on physiological responses (Fahlman 2008).

Two of the most important limitations of using mortality based outcomes to quantify and compare anaesthetic risk are the inherent difficulties of defining 'anaesthetic mortality' (Jones 2001, Brodbelt et al 2015, Haller et al 2009) and the variations in study designs applied to their derivation (Masters et al 2007), especially since associations between anaesthetic events and outcomes do not necessarily imply cause and effect (Dyson et al 1998). Nevertheless, information from published peri-anaesthetic studies do suggest a much greater risk of peri-anaesthetic mortality in many domestic animals relative to humans. In human anaesthesia, peri-anaesthetic mortality rate estimates range from 0.01% to 0.001% (Irwin and Kong 2014, cited by Dugdale and Taylor 2016). By comparison, estimates in dogs and cats vary between 0.2% and 0.6% (Brodbelt et al

2015) and those associated with equine anaesthesia range from 0.08% to 1.8% (Dugdale and Taylor 2016).

There have been few systematic attempts to evaluate the relative safety of zoo anaesthetic procedures in a manner comparable to the studies undertaken in human and domestic veterinary anaesthesia. The only comprehensive study of peri-anaesthetic mortality under zoo conditions published to date is a retrospective review involving great apes undertaken by Masters et al (2007), in which the derived peri-anaesthetic mortality was 1.35%. Given this apparently high risk of mortality relative to humans, dogs and cats, if these estimates do provide a reflection of the safety of zoo anaesthesia more widely, there is obviously some cause for concern. The authors concluded that only prospective studies may permit the systematic analysis of each death incident required to judge its likely relation to anaesthesia. Furthermore, there was uncertainty whether the high reported rates of peri-anaesthetic complications documented by this study were associated with increased risk of mortality and the same uncertainty could equally apply to whether potentially significant subclinical or undetected injuries were occurring as well. Such uncertainties emphasise the importance of seeking earlier, more sensitive and more specific outcomes than mortality for assessing anaesthetic safety.

Measures of morbidity provide other means for assessing anaesthetic safety and risk, although their application to evidence-based anaesthesia has been questioned by some (Horan 1997). Extensive morbidity information is generated from comprehensive requirements for monitoring anaesthetic performance in human anaesthesia (McNicol and Mackay 2010) and strong efforts to standardise their definition make comparison between studies more meaningful (Myles et al 2016). In the veterinary literature, peri-anaesthetic morbidities are far less comprehensively documented (Brodbelt et al 2015) and in a much less recent prospective study, monitoring limitations were cited as a major reason for difficulties of ensuring consistent detection of adverse events in the veterinary practice setting (Dyson et al 1998).

The monitoring and documentation of morbidity also appears to be limiting in zoo animal practice. ZIMS, for example, provides only limited scope for capturing the nature

and quantifying the significance of morbidities encountered during zoo anaesthetic procedures, although facility exists for ranking their perceived gravity into one of three semi-quantitative and subjectively delineated categories. Database users are thus able to draw on only three broad outcome-based measures (mortality, gravity of complication and abnormalities of recovery) to make judgements about the safety of particular drug regimens and there is no facility within the database to enable discrimination of patient and anaesthetic factors contributing to the data. These limitations associated with the characterisation of morbidity emphasise the importance of monitoring homeostatic changes in relation to risk factors as another basis for generating insight about safety and its determinants in the zoo anaesthesia setting.

### 1.6.2 Associating Homeostatic Disturbances with Anaesthetic Risk: Focus of the Zoological Anaesthesia Literature:

The evidence base for substantiating the safety of zoo animal anaesthesia protocols documented in the scientific literature is largely predicated on the ability to assess and evaluate how these protocols may affect homeostatic processes most closely linked to the patient's immediate survival. To this end, reproducible and reliable means of assessing anaesthetic depth are required for comparison of different anaesthetic regimens (Whelan and Flecknell 1992) and evaluations also depend on the ability to perform extensive assessments of cardiovascular and respiratory performance (Hubbell and Muir 2009) in order to provide some gauge of the adequacy of tissue perfusion and oxygenation. With advances in point of care blood testing (see, for example, Radcliffe et al 2015) and laboratory analytical techniques, opportunities have opened for more comprehensive assessments of a wider range of homeostatic alterations to complement the more routine monitoring using physiological variables in zoo anaesthesia.

Much of the literature pertaining to the anaesthesia of wild and captive mammals has been devoted towards evaluation of different immobilisation regimens and (much more infrequently) to extended anaesthesia protocols. Of the sixty-nine non-review articles directly related to mammalian anaesthesia published in the Journal of Zoo and Wildlife Medicine between 2010 and October 2020, forty-one involved such evaluations. Over

half of these studies did broaden characterisation of cardiovascular and respiratory performance beyond routine physiological measures by using serial blood gas assessments (involving two or more repeated measures) as part of the evaluation and many of these involved arterial measures to improve understanding of pulmonary performance. Of particular note, very few of these studies took full advantage of readily accessible testing technology to widen concurrent and serial assessment to include other parameters of homeostasis and provide further measures of the likely general states of the cellular environment. For instance, only five of the regimen studies (Boesch et al 2011 - white-tailed deer; Fahlman et al 2011 - brown bears; Black et al 2020 – nyala; Eggers et al 2020 – black-footed cats; Hewlett et al 2020 – warthogs;) detailed serial characterisation of acid-base status and only two studies (Boesch et al 2011 – white-tailed deer; Gerlach et al 2017 - impala) additionally measured serial creatinine or creatine kinase, respectively to track changes in azotaemia status and to quantify muscle cell injury.

Of the remaining studies, nineteen involved targeted assessments of very specific homeostatic disturbances occurring under anaesthesia, their focus being specifically restricted to investigations of hyperkalaemia in large felids (McEntire et al 2020) or to investigations of echocardiographic performance or other very specific cardiovascular and cardiopulmonary alterations in various taxa, such as investigations into the effects of anaesthesia on renal blood flow in cheetahs (Stagegaard et al 2017). A further nine studies have either specifically evaluated the safety and effectiveness of particular anaesthetic monitoring techniques or have evaluated a very specific means (such as specialised ventilation) of providing additional patient support.

### 1.6.3 The Evidence Base Used to Substantiate the Safety of Immobilisation-Anaesthetic Protocols in Zoo Practice: Large Felids as a Case Illustration:

A review of the anaesthetic management of select captive large felids provides a largely reflective illustration of the published approaches used and the strength of evidence they generate in the evaluation of safety of zoo anaesthetic regimens, with many of the findings relating similarly to other taxa. Of the published anaesthetic regimens involving

the management of large felids in captivity, most document procedures of short duration. They do, nevertheless, illustrate how integral the sedative protocol is in contribution to balanced anaesthesia, particularly as the immobilisation protocol may profoundly reduce general anaesthetic requirements for the first sixty to eighty minutes after being administered (Nam et al 2013, cited by Buck et al 2017).

Of the ten non-review publications of studies involving the anaesthesia of captive tigers published between 1997 and October 2020, only two of these studies (Curro et al 2004, Clarke-Price et al 2015) were analytical, being very low power cohort studies comparing two different drug regimens. The remainder (Forsyth et al 1999, Miller et al 2003, Steeil et al 2013, Lewis et al 2014, Reilly et al 2014, Laricchiuta et al 2015, Larsson et al 2017, McEntire et al 2020) involved even lower power observational (case series) or retrospective studies, of which only half provided reasonably comprehensive serial assessment of a range of homeostatic parameters; the focus of the remainder had other specific objectives. Only a single study (Lewis et al 2014) investigated association between drug pharmacokinetics and measured outcomes. Using immediate survival and quality of recovery from anaesthesia as measures of post-emergence outcome, all but one study specified such outcomes by either describing or scoring them. Of the 144 anaesthetic procedures involving tigers covered by these publications, five cases of anaesthetic mortality were documented and eight cases required cardiopulmonary resuscitative interventions to survive (Steeil et al 2013, Reilly et al 2014, McEntire et al 2020), although some were multi-species studies where the species effected were sometimes not specified and some of the same mortality or resuscitation cases may have been repeatedly included in different publications. Furthermore, four post-anaesthetic recoveries involved significant or protracted complications (Lewis et al 2014, McEntire et al 2020), three of which involved profound post-anaesthesia lethargy in individuals treated for hyperkalaemia with intravenous insulin (McEntire et al 2020).

Five of the publications provide important illustrations of how absence of mortality or demonstrated morbidity provide poor reflections of possibly significant risks facing patients or operators. Although somewhat dated, Forsyth et al (1999) documented the occurrence of profound cardiovascular and respiratory depression using medetomidine

dosages recommended at the time for tigers (0.03 mg/kg) and in one case, a tiger awoke from sedation and dissociative anaesthesia following patient stimulation when 'mid-range' medetomidine (0.025 mg/kg) was combined with a lower dose (1.66mg/kg) of ketamine (Miller et al 2003). More recently, three of the publications (with the acknowledgement, in some cases, of repeated inclusion of the same animals) described the occurrence of a number of electrocardiographic abnormalities during anaesthesia (although the timing of their development were not always specified), sometimes associated with but not necessarily resulting from hyperkalaemia (Steeil et al 2013, Reilly et al 2014, McEntire et al 2020).

Only two of the ten studies provided for serial integrated assessment of cardiovascular status based on measurement of mean arterial pressure, heart rate and continuous electrocardiographic monitoring. Only one study included enough data for serial assessments of cardiovascular status based on these parameters combined with serial acid base, lactate and azotaemia marker measurements. Just two permitted serial estimates of anaesthetic depth (either by invasive blood pressure measurement or from frequently recorded Guedel-type staging of anaesthetic depth). Only one provided data to evaluate (to some extent) the serial relationship between cardiovascular status and anaesthetic depth. Finally, only one enabled reasonable assessment of ventilation and cardiopulmonary parameters using serial measurement of arterial blood gases, end tidal carbon dioxide and pulse oximetry. The study by Curro et al (2004) is the only one providing enough scope for more integrated, serial evaluation of all these homeostatic parameters together. In common with half the studies, the investigation by Curro et al (2004) also involved only short procedures where anaesthetic maintenance agents were not used. As an example of the relative paucity of research on the use of intravenous anaesthetics as primary maintenance agents in the *Panthera* genus, no studies have yet evaluated the use of propofol in tigers.

On the basis of the quality of recovery, the regimens which were applied appear to perform safely but given the limited scope of the studies to identify homeostatic derangements and associate them with risk factors, the evidence base to substantiate the safety of important procedural considerations such as the choice of dosage of

anaesthetic protocol in captive tigers has been furthered only to a limited extent since the study by Forsyth et al (1999). There is clearly still much to learn about the effects of various drugs used in anaesthesia on biochemical, cardiovascular and other clinical parameters in captive tigers. Similar remarks apply to other large felids, for whom it is most evident that there is a particular paucity of research pertaining to detailed evaluation of approaches used to maintain anaesthesia (Buck et al 2017). Just as with tigers, almost all of the associated studies published between 1997 and October 2020 involving captive cheetahs and lions were undertaken with procedures of short duration. In the only study comparing the use of propofol in lions with other induction protocols (Epstein et al 2002), the physiological parameters measured were too limited and the procedures too short to properly evaluate its safety as a maintenance agent.

To date, the most robust study permitting safety comparisons of propofol and isoflurane maintenance protocols involved an investigation of their cardiopulmonary effects in cheetahs (Buck et al 2017). The authors of this study expressly recognise the difficulties associated with comparing these regimens (given possible lack of equi-potency between them). Furthermore, by undertaking serial arterial blood gas monitoring and relating the findings to macro-circulatory and acid-base measures, this study marks an important step forwards in providing better serial characterisation of the relative adequacy of ventilation and perfusion under the regimens which were compared, but there is still much scope for wider investigations into the homeostatic effects which may occur. There is also need for further research to better inform about the most judicious use of maintenance agents and their combination with other approaches to achieve and maintain balanced anaesthesia in large felids.

## 1.7 Comprehensive Serial Monitoring to Improve Safety Evaluation of Zoo Animal Anaesthesia:

The challenges associated with assessing risk and substantiating the safety of zoo anaesthesia practices do not detract from the importance of finding ways to minimise their impact on physiological homeostasis and to better match anaesthetic approaches to the particular demands of intended interventions. There is no facility to perform

controlled trials in a zoo context and knowledge will need to come from observational studies to develop safety enhancing procedural refinements pertaining to both the regimens used and the subsequent delivery of anaesthesia.

Because many safety-related evaluations have involved drug regimen evaluations based on a limited range of measured homeostatic parameters, there is often uncertainty about the wider range of homeostatic alterations which patients may be experiencing. The evidence from human anaesthesia suggests that the more information gathered on physiological functions during zoo animal anaesthesia, the better will be the ability to identify changes in homeostasis that affect morbidity, long term health or mortality. Most importantly, the broader the range of parameters which can be tracked together and cross referenced in accordance with physiological principles, the better the prospect of being able to explain the changes which occur. To date, the full range of analytes available with blood gas analysers have rarely been fully exploited to monitor changes with much serial intensity in zoo animal anaesthesia.

Consideration of the risk factors associated with such changes is an equally important part of this explanatory process and the more considered the risk factors, the more informed will be the risk mitigation process. While studies of different anaesthetic regimens provide critical contributions to the overall promotion and enhancement of anaesthetic safety, an equally important priority in risk mitigation is to better understand the factors most determinant of anaesthetic risk in practice. Few studies in the zoo medical literature have assessed how well or otherwise patients are supported in everyday zoo anaesthetic circumstances and investigation of the factors influencing such adequacy should extend beyond the regimens used to include other procedural aspects. The working hypothesis is that current protocols for zoo animal anaesthesia are causing physiologically important homeostatic disturbances that are not detected by routine anaesthetic monitoring used in zoo settings. Identifying and prioritising these changes in homeostasis under anaesthesia could be used to improve zoo animal anaesthesia.

To address these knowledge gaps, more systematic and comprehensive serial collection and monitoring of a wider range of readily measurable parameters reflecting homeostasis should be applied to evaluate the safety of zoo anaesthesia as it occurs in practice. By identifying which homeostatic parameters tend to change over the course of anaesthesia and assessing the factors that are reflected in the scope and extent of these changes, the process should lay the foundations for more targeted investigations aimed at better understanding the causes and pathogenesis of the homeostatic disturbances which are apparent. A better mechanistic understanding of these disturbances should then provide a stronger evidence base for recommending practical refinements to anaesthesia management which contribute to enhanced safety.

## 1.8 Research Aims, Objectives and Applications:

The aims of the research presented in this thesis were:

- to identify disturbances to physiological homeostasis occurring in larger species of mammals under anaesthesia in zoo practice, in order to advance understanding about the safety of anaesthetic practices for zoo patients, and
- to gain further insight into how and why such disturbances occur.

The practical application of this research is that a better understanding of the causes and mechanisms associated with the disturbances identified should permit identification of more effective measures to mitigate them, generating recommendations of both a monitoring and procedure-related nature.

These research aims were addressed in this thesis by pursuing the following objectives:

1. To apply readily accessible techniques to serially monitor and describe the prevalence and extent of physiological disturbances occurring in large mammals during zoo anaesthesia practice;
2. To initiate examination of risk factors associated with these disturbances;
3. To describe and characterise serial alterations in metabolic acid-base and unmeasured ion status occurring in large felids during anaesthesia;

4. To assess whether unmeasured anion values are associated with mean arterial blood pressures in the anaesthetic period preceding blood sampling events;

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**Chapter Two: Comparative effects of anaesthesia in large zoo mammals:**

## 2.1 Introduction:

The anaesthesia of many zoo mammals is frequently complicated by their dangerous nature, which often results in limited pre-anaesthetic evaluation, remote delivery of anaesthetic agents and limited opportunities for intensive monitoring during the induction and recovery phases of anaesthesia. There is also a wide variety of species involved, often for which veterinary knowledge is relatively rudimentary. The end result is that zoo mammal anaesthesia is suspected to have higher rates of complications and mortality than for human or domestic animal procedures. Hence, better mitigation of risks that can be managed should be a priority for enhancing the safety of zoo animal anaesthesia.

Evaluation of the safety of anaesthesia in the zoological literature is dominated by pharmacodynamics studies involving or comparing particular immobilisation regimens. Much less published attention has been given to critically evaluating the wider aspects of anaesthesia provision and to evaluating patient physiology under anaesthesia in everyday zoo veterinary practice. To identify ways to enhance safety using existing drugs and technologies, both the regimens used and the risk factors associated with their application must be considered together if a better mechanistic understanding is to be developed of how and why disturbances to physiological homeostasis occur under anaesthesia. This assessment process is particularly relevant to compromised patients, for whom the effects of pre-existing morbidities may be greatly exacerbated by anaesthesia (Clarke and Trim 2014).

Even with empirical contributions from extensive pharmacodynamics studies, major knowledge gaps still remain which limit informed choices in the immobilisation and anaesthesia practices used in zoological medicine (Ozeki and Caulkett 2014). For example, these studies are largely predicated on evaluation of the effect of different regimens on the cardiovascular and respiratory system but often a lack of more specific monitoring and a variety of interpretative issues influence how effective these measures are for evaluating fundamental processes such as tissue perfusion and lung function (Noori and Seri 2008, Hubbell and Muir 2009). It may also be particularly difficult to

directly compare regimens, given the challenges associated with making valid comparisons about anaesthetic depth (Whelan and Flecknell 1992, Musizza and Ribarac 2010, Clarke and Trim 2014). The restricted physiological focus of these studies also raises uncertainty about the range of other effects on the animals' physiological homeostasis these regimens may be inducing, including the potential for subclinical injury of long-term health significance. Rarely, too, have pharmacodynamics studies in zoological species been undertaken concomitantly with pharmacokinetic studies (Hunter 2010, Hernandez 2014), making it difficult to refine protocols on the basis of abilities to relate physiological effects to the bio-distribution of drugs used in different approaches.

With limited recourse to controlled clinical trials in a zoo context, more opportunistic means are required to assess safety pertaining both to the regimens used and the subsequent delivery of anaesthesia. Refinements of anaesthetic regimens may include fine dosage adjustments, trialling of alternate drug combinations for balanced anaesthesia and reduction of anaesthetic drug maintenance requirements (particularly the application of remifentanil constant rate infusions for their potential anaesthetic dose-sparing benefits), as well as exploration of total or supplementary intravenous anaesthesia (TIVA, SIVA) to reduce dependence on inhalational anaesthetics for maintenance. Refinements to the delivery of anaesthesia may include the critical evaluation of the anaesthetic depth at which patients are maintained, the optimal manner and timing of alpha-two adrenergic agonist reversal, the use of vasoactive drugs based on better understanding of their haemodynamic effects in different species (Mama 2019) and the optimisation of ventilation support and inspired oxygen fractions provided to different patients.

To address these knowledge gaps, much more systematic and comprehensive serial monitoring of a range of readily measurable parameters reflecting homeostatic processes needs to be applied to evaluate the safety of zoo anaesthesia as it occurs in practice. To date, few studies have taken full advantage of point of care blood gas analysers to serially monitor patients, although some authors have exploited them to incorporate advanced cardiopulmonary and acid-base assessments into their regimen

evaluations (Zeiler et al 2015, Gerlach et al 2017, Zeiler and Meyer 2017a, 2017b, Buck et al 2017b). Concurrent use of an increasingly wide range of laboratory-based measures of physiological homeostasis is also becoming more accessible.

As a preliminary basis for investigating how zoo animals fare under anaesthesia, the aim of this study was to combine these readily accessible techniques and apply them with routine monitoring to assess the range, frequency and extent of disturbances to physiological homeostasis that are occurring in zoo anaesthetic practice in a range of large mammal species. Measures which reflect cardiovascular status, ventilation, acid-base balance, electrolyte status and cellular injury were chosen in this initial focus. The working hypothesis is that current protocols used for zoo animal anaesthesia are causing physiologically important homeostatic disturbances that are not detected by routine anaesthetic monitoring used in zoo settings. Identifying, understanding the causes of, and prioritising these changes in homeostasis under anaesthesia could be used to improve zoo animal anaesthesia and patient safety.

## 2.2. Materials and Methods:

### 2.2.1 Study Design, Inclusion Criteria, Patient Recruitment:

Zoo anaesthetic procedures were monitored at two New Zealand zoos for the occurrence and course of significant alterations to physiological homeostasis identifiable using clinical techniques, accessible anaesthetic monitoring equipment and analysis of blood analytes using point of care and laboratory technologies. The study used a prospective cohort design per criteria of Vandenbroucke et al (2007) and Dekkers et al (2012). Inclusion was restricted to eutherians exceeding twenty kilogrammes in bodyweight and patient recruitment was opportunistic, governed by zoo veterinary staff decisions on which animals required anaesthetic interventions over a two-year timeframe. The ancillary monitoring described in this study was undertaken as soon as practical following intramuscular immobilising drug delivery (defined as time =0), continuing until endotracheal tube removal or final sedative/opioid reversal. The extent of monitoring and its timing varied between animals, depending on safety and accessibility to the animals at the time. The research was undertaken under zoo practice

conditions, although many findings from ancillary monitoring for the research were immediately available to the attending anaesthetists.

### 2.2.2 Physiological Variables: Equipment and Measurement:

Anaesthetic monitors (Surgivet Advisor® Vital Signs Monitor, Smiths Medical, Dublin, OH 43017, USA or Tafonius® Ventilator/Monitor, Vetronics, Abberkerswell, UK) were used in 35 procedures (n=27 and n=8 respectively) to measure rectal/oesophageal temperature (T°), non-invasive (NIBP) or invasive (IBP) mean arterial pressure (MAP), heart rate (HR), end-tidal carbon dioxide (ETCO2) and respiratory rate (RR). Blood-pressure cuff widths accorded as close as possible to 40 per cent of patient appendage circumferences (per recommendations of Clarke and Trim 2014) but location of cuff placement varied. The measurements of this physiological data, included in the results below, corresponds to nearest times to blood sampling.

### 2.2.3 Blood Sampling:

Venous (n=104) or arterial (n=23) blood samples were collected opportunistically from saphenous, jugular, cephalic, coccygeal, auricular or dorsal pedal vessels. Samples were drawn into a 3ml non-heparinised syringe using 18G needles or via catheterised vessels, latterly discarding the first 1-3mls of blood drawn up. Venous samples were then placed into lithium heparin anticoagulant tubes (BD microtainers, BD Diagnostics, Franklin Lakes, NJ, USA) and plain serum tubes (BD microtainers, BD Diagnostics, Franklin Lakes, NJ, USA). Venous heparinised samples were analysed at earliest opportunity using point of care analysis (mean 17, median 4, range 0-390 minutes), samples being chilled at 2 °C if holding time exceeded 10 minutes. The samples in serum tubes were chilled to 2 °C, left to clot for 30 minutes and centrifuged at approximately 1000g for 10 minutes. The serum was extracted and stored at -80°C until further analysis. Arterial samples were drawn from catheterised dorsal pedal or auricular arteries. Discarding the first 1-3mls of blood drawn, a further 1-3mls of blood was then collected using a 3ml non-heparinised syringe and analysed immediately from the same unpressurised and capped non-heparinised 3ml syringes, by point of care testing. Remaining arterial blood drawn up was managed as per venous blood.

### 2.2.4 Analytical Equipment Used and Analytes Measured:

A point of care blood gas analyser (EPOC®, Siemens Healthineers, Erlangen, Germany, EPOC® BGEM cartridges, Epocal Inc, Ottowa, ON, Canada) was used for all procedures, save for one procedure using a different blood gas analyser (i-STAT Portable Clinical Analyzer, Heska Corporation, Waukesha, WI, USA with CG4+ and CG8+ cartridges). Measured and machine calculated parameters included blood acidity (pH), partial pressure of carbon dioxide (pCO2), concentration of total carbon dioxide (cTCO2), bicarbonate ion concentration (cHCO3), standard base excess (cBEecf); sodium (Na), potassium (K), calcium (Ca), chloride (Cl), lactate (lact), anion gap including potassium (cAGK), partial pressure of oxygen (pO2), percent saturation oxygen (sO2%), haematocrit (Hct), haemoglobin (cHgb), glucose (Gluc) and creatinine (EPOC Crea).

Serum was analysed in two batches by wet biochemistry using at a commercial laboratory (IDEXX, School of Veterinary Science, Massey University, Palmerston North, New Zealand). Analytes included blood urea nitrogen (BUN), sulphyl-dimethyl-arginine (SDMA), creatinine (NZVP Crea), total protein (TP), albumin (Alb), globulin (Glob), albumin: globulin ratio (A:G), creatine kinase (CK) and phosphate (PO4-). These analytes were run on stored serum in batches to limit inter-assay variation and, hence, collection to analysis intervals varied (1-18 months).

### 2.2.5 Statistical Analysis:

All statistical analysis was carried out using SPSS v25 (IBM, Armonk, NY, USA). A general linear model (GLM) was carried out to assess for differences between species using time from immobilisation as a covariate and patient identity as a random factor. Where there was evidence of an effect (Pillai's trace $p<0.05$) of time from immobilisation or between species, *posthoc* analysis was performed using a K matrix. Given the restricted sample sizes, the analytes assessed were categorised as follows and analysed as separate GLMs:

1. Physiological variables (T°, MAP, HR, ETCO2 and RR)
2. Blood gas analysis (pH, pCO2, cTCO2, cHCO3, cBEecf, Na, K, Ca, Cl, Lact, cAGK, pO2, sO2%, Hct, cHgb, Gluc and EPOC Crea).
3. Serum biochemistry (BUN, SDMA, NZVP Crea, TP, Alb and Glob, Alb: Glob ratio,

CK, PO4-).

## 2.3 Results:

The results include the analysis from twenty-six different zoo mammals who underwent anaesthetic procedures of varying duration (27-451 minutes) involving different induction/maintenance regimens (*data sheet supplied in Appendix A1*). The procedures occurred over an eighteen-month period, with representation of nine different species from three broad dietary groups, involving animals ranging in age from 1-39 years (Table 2.1).

***Table 2.1:*** *Anaesthetic events included in the study: Species inclusion, ranges in duration of anaesthesia, and ages of patients.*

| Species | Scientific Name | Number of Anaesthetic Events | Mean Anaesthetic Duration (Minutes) | Anaesthetic Duration: Range (Minutes) | Mean Age (Years) | Age Range (Years) |
|---|---|---|---|---|---|---|
| African Lion | *Panthera leo* | 6 | 295 | 217 to 451 | 17 | 13 to 19 |
| Sumatran Tiger | *Panthera tigris sumatrae* | 2 | 221 | 221 to 222 | 9 | 8 to 10 |
| African Cheetah | *Acinonyx jubatus* | 3 | 159 | 140 to 189 | 4 | 2 to 8 |
| Malayan Sun Bear | *Helarctos malayanus* | 2 | 250 | 150 to 349 | 17 | 13 to 22 |
| Common Chimpanzee | *Pan troglodytes* | 5 | 102 | 58 to 212 | 27 | 7 to 39 |
| Hamadras Baboon | *Papio hamadryas* | 3 | 167 | 140 to 183 | 11 | 7 to 15 |
| Lowland Nyala | *Tragelaphus angasii* | 3 | 178 | 27 to 285 | 2 | 1 to 2 |
| Southern White Rhino | *Ceratotherium simum* | 1 | 117 | 117 | 17 | 17 |
| Plains Zebra | *Equus burchelli* | 1 | 117 | 117 | 26 | 26 |

Every patient survived the anaesthetic procedures without apparent post-anaesthetic complications. However, aspiration of ingesta was narrowly avoided in one particular

patient and particularly prolonged recoveries were reported following four procedures (notably including all geriatric felids maintained under propofol anaesthesia). Furthermore, much inter- and intra-individual variation was apparent in the homeostatic parameters measured. A sufficient number of procedures were monitored to evaluate the influence of the species involved and the time since administration of immobilising drugs as possible factors accounting for this overall variation. There were an insufficient variety and number of procedures to permit examination of the influences of drug and dosage regimens as covariates in the model and this was also the case for many other factors that were considered *a priori* as possible determinants of variation in the parameters measured (notably animal age, bodyweight, positioning of animal, anaesthetic maintenance protocol, ventilation method and extent of anaesthetist experience).

There was very strong evidence for species differences in virtually all the homeostatic parameters measured (Table 2.2). The *post hoc* analysis (Table 2.3) displays the variety of inter-species differences that were seen across the analyses of physiological parameters, blood gas analyses and serum biochemical analyses.

There are many interesting inter-species variations that warrant closer analysis. Regarding the physiological variables, relative hypotension and higher heart rates were recorded in baboons (Table 2.3, Figures 2.1a and 2.1b), while cheetahs were relatively markedly hypertensive, also recording relatively higher heart rates (Table 2.3, Figures 2.1a and 2.1b) concomitant with strong evidence for having relatively lower lactate levels (Table 2.3). Combining physiological and blood gas measures, both these species also demonstrated relatively elevated end-tidal carbon dioxide and pCO2 levels, despite recording relatively elevated respiratory rates (Table 2.3, Figures 2.1c, 2.2b and 2.1d). Levels of pCO2 were somewhat stratified by species, with rhino pCO2 levels often being exceeded by many smaller species (Figure 2.2b). Likewise, lions also displayed relatively lower end-tidal carbon dioxide and pCO2 levels (Table 2.3, Figures 2.1c and 2.2b).

Regarding electrolyte and metabolite measures, baboons and cheetahs displayed markedly lower and higher blood potassium levels respectively (Table 2.3, Figure 2.3b). In cheetahs, relative elevations in blood glucose levels were apparent (Table 2.3), while

lion glucose levels lay significantly below the group mean (Table 2.3). Species could largely be grouped according to azotaemia marker levels and while physiologically intuitive to an extent, felids nevertheless demonstrated profoundly elevated creatinine levels in relative terms (Table 2.3, see also *data supplied in Appendix A1*). In terms of markers of cellular injury, lion CK levels stood out as being relatively low, while cheetah, nyala and baboon levels were relatively high (Table 2.3, Figure 2.5). Surprisingly, no CK elevation was apparent over time during the rhino anaesthesia (Figure 2.5).

As an overall reflection of how all animals fared as anaesthesia progressed, very strong ($p<0.001$) to strong ($p<0.01$) evidence for time effects were also evident for many of the homeostatic parameters measured in this study (Table 2.2). Of the physiological parameters, there was very strong evidence ($p<0.001$) of an effect of time on body temperature (Table 2.2), with animals appearing to tend towards mild hypothermia as anaesthesia progressed (Figure 2.1e). Likewise, there was also strong evidence for changes over time involving physiological measures of respiratory performance, (including end tidal CO2 and RR, Figures 2.1c and 2.1d). In contrast, there was insufficient evidence ($p>0.1$) for time effects on those physiological parameters which broadly reflect cardiovascular performance (MAP, HR, Figures 2.1a and 2.1b).

Of the blood gas and acid-base analytes measured or calculated, there was extremely strong evidence ($p<0.0001$) for an effect of time from immobilisation on pC02 levels and thus for the respiratory component of acid base-status (Figure 2.2b). The same was the case for the metabolic component of acid base status (BE; Figure 2.2c). An overall trend of reducing base deficit (BD) or, alternatively, increasing BE over time was apparent in most species, including all felids (Figure 2.2c). Integrated measures combining the performance of metabolic and respiratory processes in determining acid-base status (Bicarbonate, pH; Table 2.2, Figures 2.2d and 2.2a) also showed strong evidence of an effect of time from immobilisation.

Of the electrolyte analytes measured that represent important ionic determinants of pH and BE (Stewart 1983), chloride levels showed strong evidence of time effects (Table 2.2 and Figure 2.3a), while sodium and anion gap did not. Nor did lactate, a metabolite with the potential to influence anion gap and therefore BE.

***Table 2.2:*** *The results of a GLM assessing changes in physiological parameters of zoo mammals under anaesthesia (a) Evidence for species effect (b) Evidence for effect of time since immobilisation. p <0.001 indicates very strong evidence for effect; p<0.01 strong evidence; p<0.05 moderate evidence, p<0.1 weak evidence; p>0.1 insufficient evidence.*

| | **a)** Species Effect | | | **b)** Time Effect | | |
|---|---|---|---|---|---|---|
| **Parameter** | **df** | **F** | **p-value** | **df** | **F** | **p-value** |
| **Physiological Parameters** | | | | | | |
| Body Temperature (Degrees Celsius) | 8 | 5.863 | <.001 | 1 | 28.058 | <.001 |
| Mean Arterial Pressure (mm Hg) | 8 | 6.426 | <.001 | 1 | .107 | .744 |
| Heart Rate (Beats/Min) | 8 | 12.823 | <.001 | 1 | .620 | .434 |
| End Tidal CO2 (mm Hg) | 8 | 26.993 | <.001 | 1 | 8.847 | .004 |
| Respiratory Rate (Breaths/Min) | 8 | 5.775 | <.001 | 1 | 6.334 | .014 |
| **Blood Gas (EPOC) Parameters** | | | | | | |
| pH | 4 | 68.213 | <.001 | 1 | 16.946 | <.001 |
| pCo2 (mmHg) | 4 | 66.204 | <.001 | 1 | 38.658 | <.001 |
| cTCO2 (mmol/L) | 4 | 79.179 | <.001 | 1 | 17.962 | <.001 |
| cHCo3- (mmol/L) | 4 | 87.340 | <.001 | 1 | 17.593 | <.001 |
| BE (ecf) (mmol/L) | 4 | 99.982 | <.001 | 1 | 7.820 | .008 |
| Na+ (mmol/L) | 4 | 23.843 | <.001 | 1 | .971 | .330 |
| K+ (mmol/L) | 4 | 7.272 | <.001 | 1 | 1.519 | .225 |
| Ca++ (mmol/L) | 4 | 8.596 | <.001 | 1 | 2.055 | .159 |
| Cl- (mmol/L) | 4 | 97.433 | <.001 | 1 | 9.063 | .005 |
| Lactate (mmol/L) | 4 | 6.799 | <.001 | 1 | .162 | .689 |
| AG K (mmol/L) | 4 | 7.040 | <.001 | 1 | .494 | .486 |
| pO2 (mm Hg) | 4 | 2.978 | .030 | 1 | .082 | .776 |
| cSO2 (%) | 4 | 2.545 | .054 | 1 | .100 | .754 |
| Hct (%) | 4 | 6.301 | <.001 | 1 | 5.231 | .028 |
| cHgb (g/dL) | 4 | 6.537 | <.001 | 1 | 6.189 | .017 |
| Glucose (mmol/L) | 4 | 3.624 | .013 | 1 | .146 | .705 |
| Creatinine EPOC (micromol/L) | 4 | 30.093 | <.001 | 1 | .234 | .631 |
| **Laboratory Clinical Pathology Parameters** | | | | | | |
| BUN (Urea) (mmol/L) | 6 | 36.950 | <.001 | 1 | 1.282 | .263 |
| SDMA (microgrammes/dL) | 6 | 18.050 | <.001 | 1 | .009 | .926 |
| Creatinine wet chemistry (micromol/L) | 6 | 54.106 | <.001 | 1 | .604 | .441 |
| Total Protein (g/L) | 6 | 8.731 | <.001 | 1 | 24.453 | <.001 |
| Albumin (g/L) | 6 | 13.099 | <.001 | 1 | 33.206 | <.001 |
| Globulin (g/L) | 6 | 10.396 | <.001 | 1 | 6.793 | .012 |
| Albumin/Globulin ratio | 6 | 13.080 | <.001 | 1 | 1.241 | .271 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CK (IU/L @ 37C) | 6 | 24.425 | <.001 | 1 | 16.241 | <.001 |
| Phosphate (P04-) (mmol/L) | 6 | 12.768 | <.001 | 1 | 1.755 | .192 |

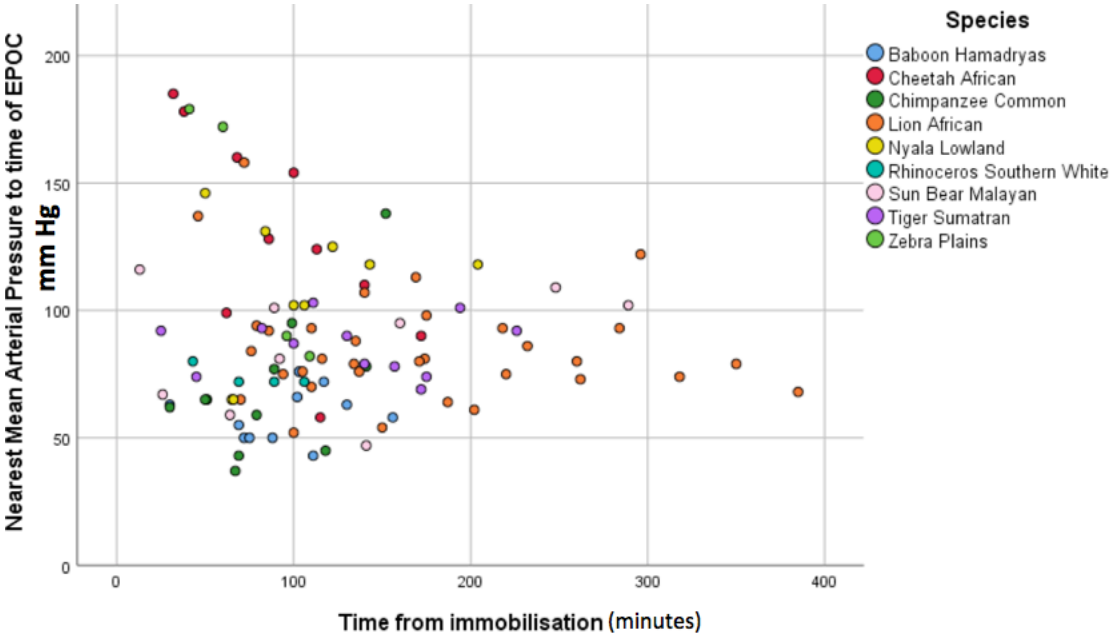


***Figure 2.1a:*** *Physiological variables: Scatter plot showing effect of species and time from immobilisation on Mean Arterial Pressure (in mm Hg) of zoo mammals under anaesthesia.*

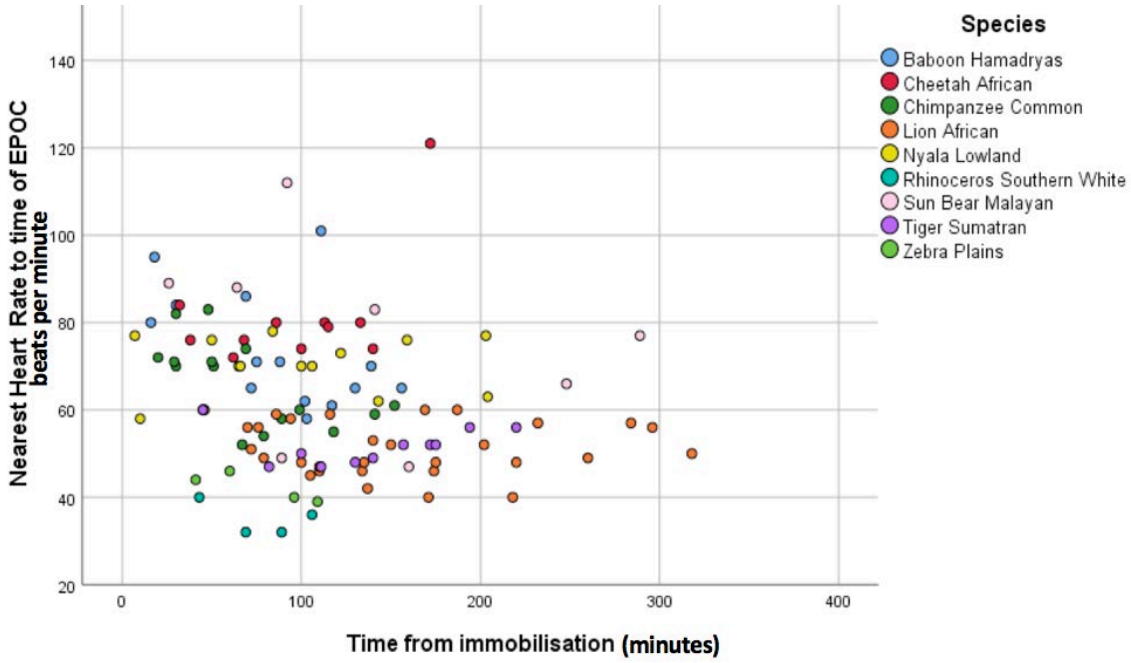


***Figure 2.1b:*** *Physiological variables: Scatter plot showing effect of species and time from immobilisation on Heart Rate (in beats per minute) of zoo mammals under anaesthesia.*

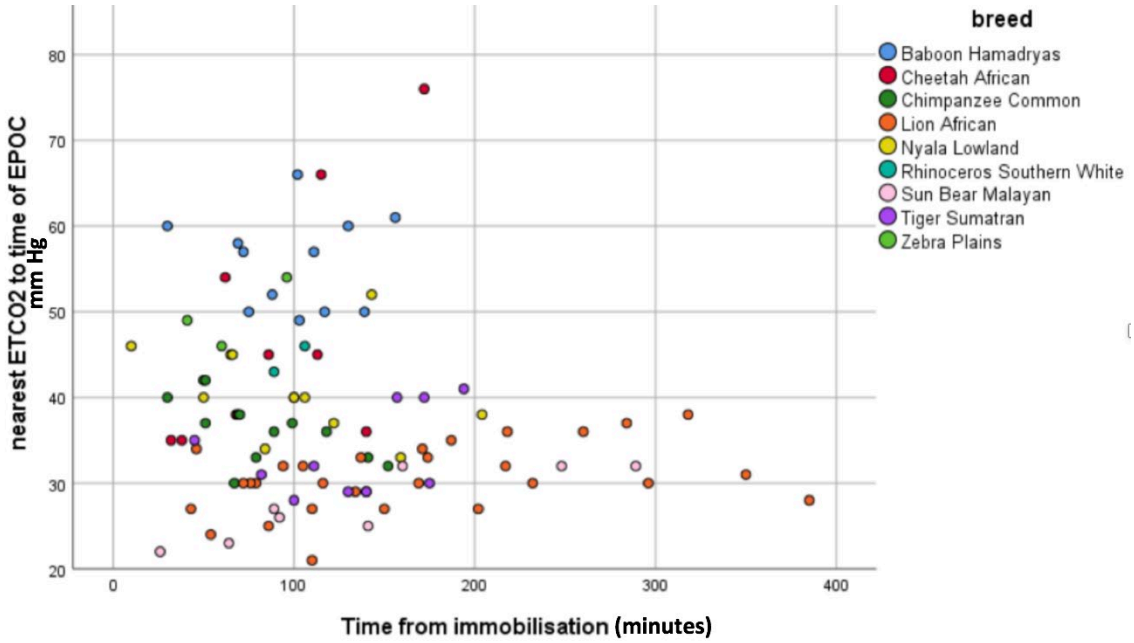


***Figure 2.1c:*** *Physiological variables: Scatter plot showing effect of species and time from immobilisation on End Tidal Carbon Dioxide (measured in mm Hg) of zoo mammals under anaesthesia.*

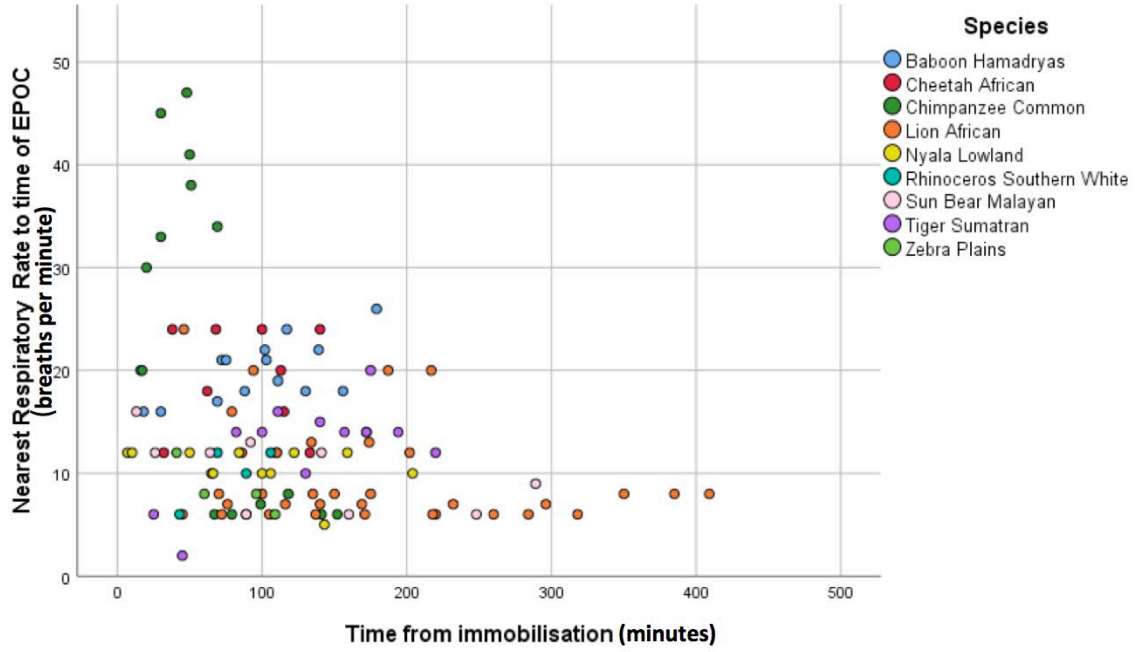


***Figure 2.1d:*** *Physiological variables: Scatter plot showing effect of species and time from immobilisation on Respiratory Rate (in breaths per minute) of zoo mammals under anaesthesia.*

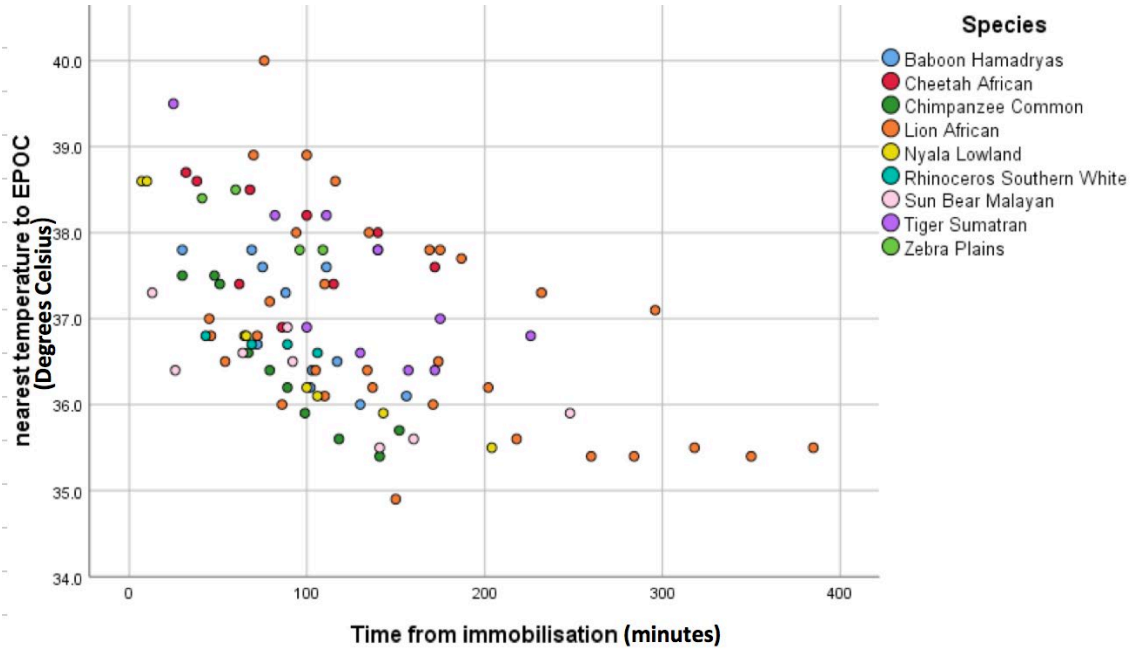


***Figure 2.1e:*** *Physiological variables: Scatter plots showing effect of species and time from immobilisation on Body Temperature (in degrees Celsius) of zoo mammals under anaesthesia.*

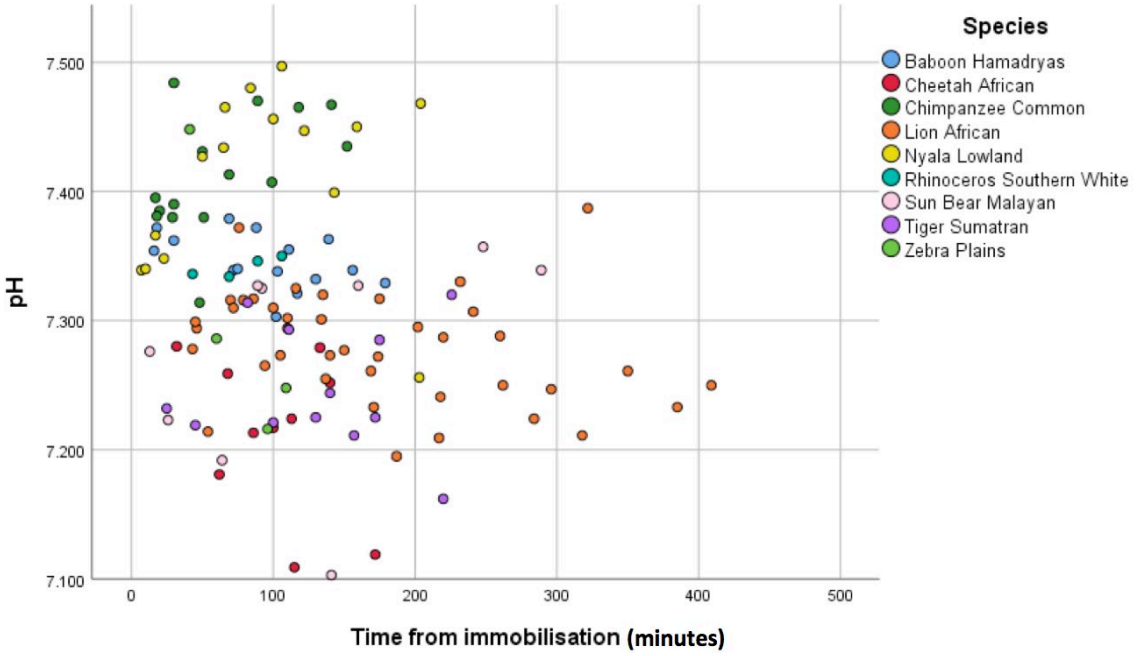


***Figure 2.2a:*** *Blood gas variables: Scatter plots showing the effect of species and time from immobilisation on pH of zoo mammals under anaesthesia.*

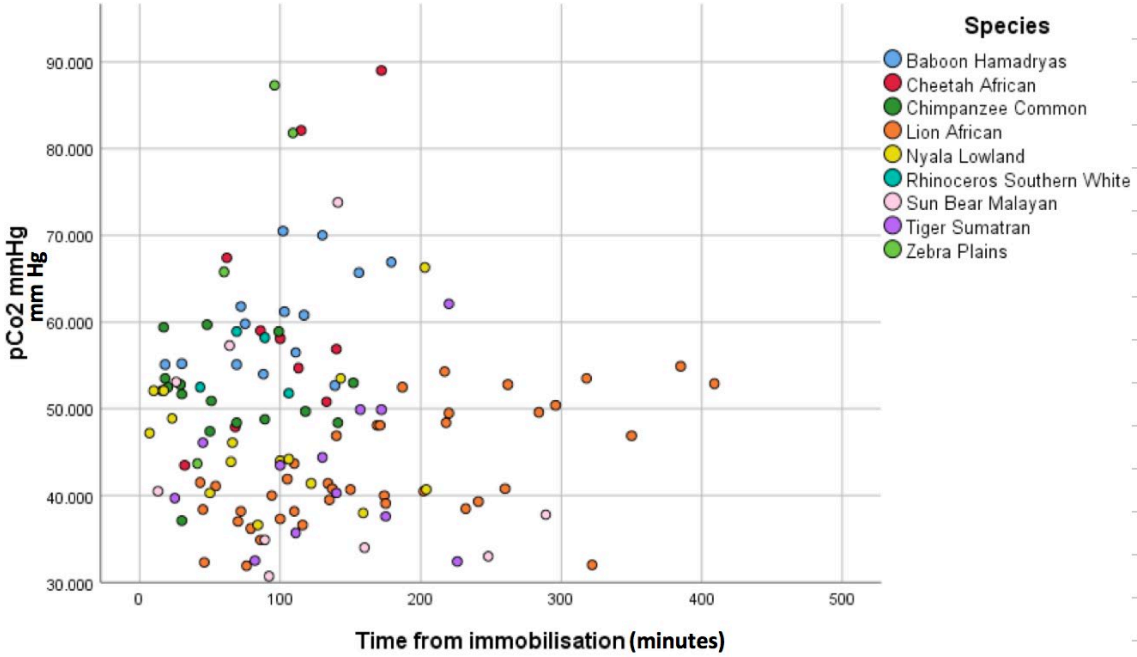


***Figure 2.2b****: Blood gas variables: Scatter plots showing the effect of species and time from immobilisation on pCO2 (measured in mm Hg) of zoo mammals under anaesthesia.*

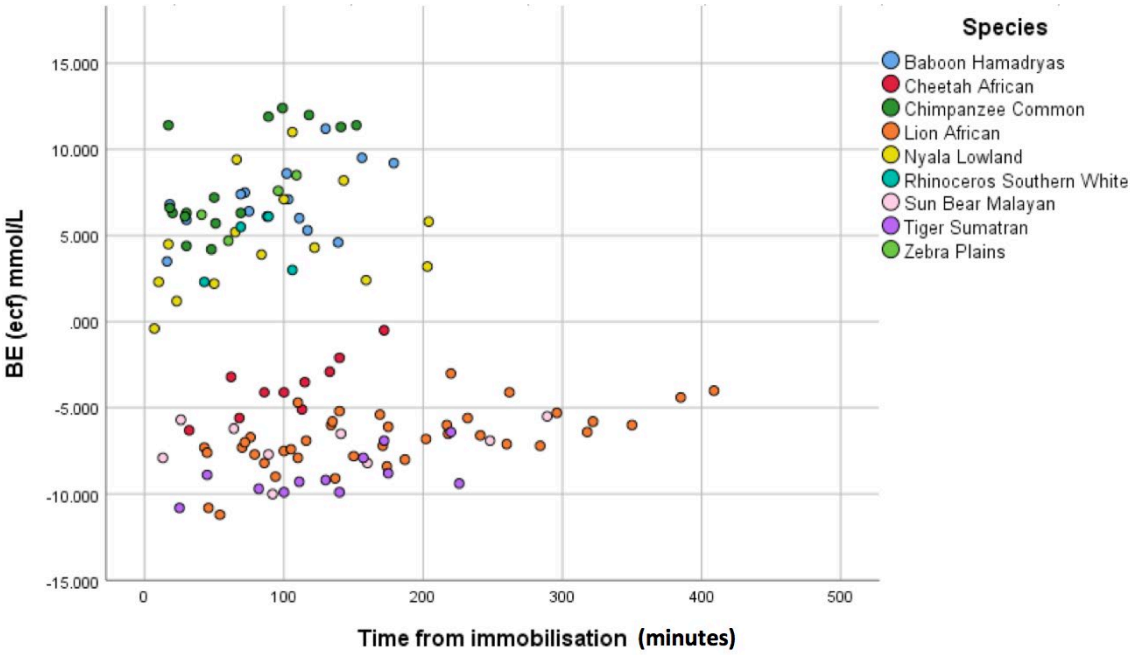


***Figure 2.2c:*** *Blood gas variables: Scatter plots showing the effect of species and time from immobilisation on Extracellular Base Excess (measured in Mmol/L) of zoo mammals under anaesthesia.*

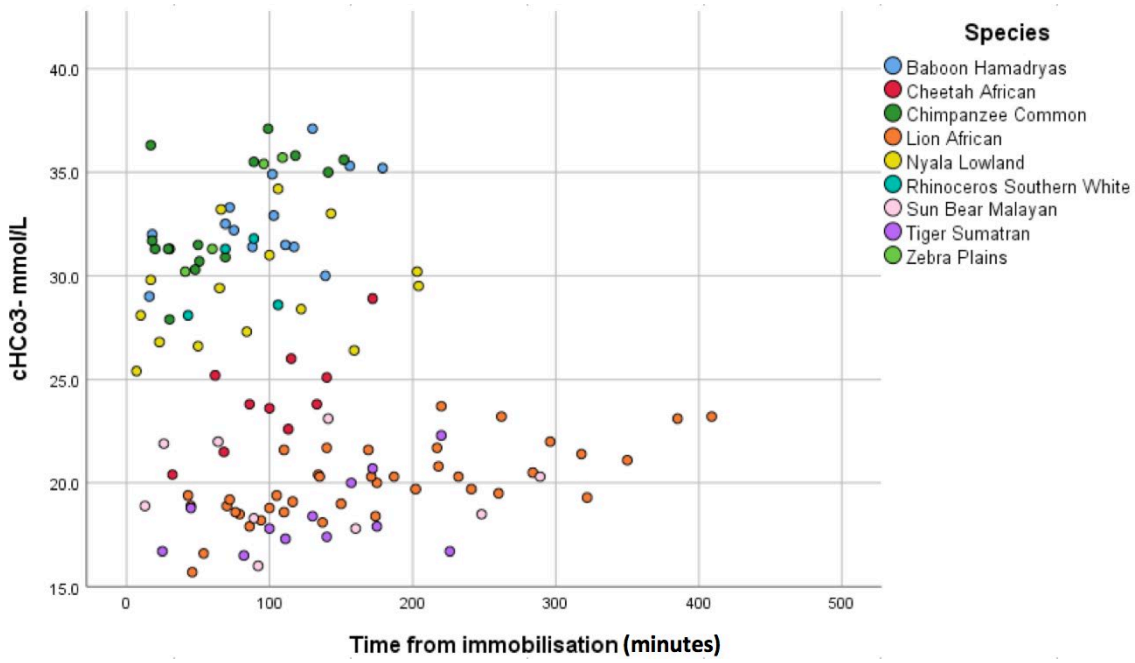


***Figure 2.2d:*** *Blood gas variables: Scatter plots showing the effect of species and time from immobilisation on Bicarbonate (measured in Mmol/L) of zoo mammals under anaesthesia.*

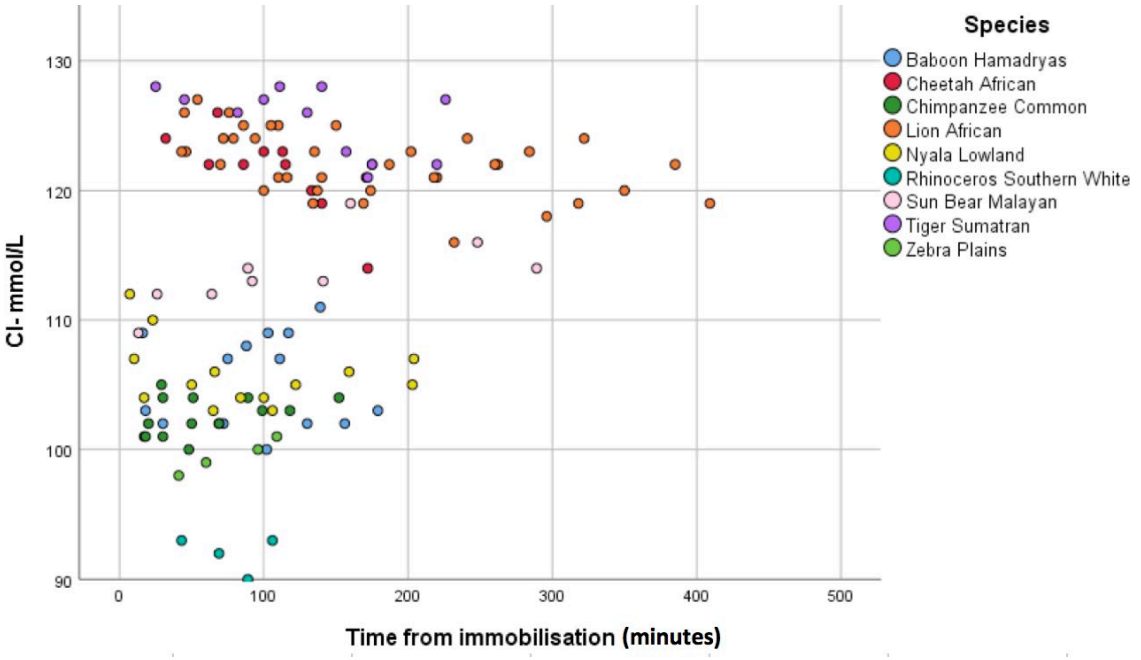


***Figure 2.3a:*** *Electrolyte Parameters: Scatter plots showing the effect of species and time from immobilisation on Chloride (measured in Mmol/L) of zoo mammals under anaesthesia.*

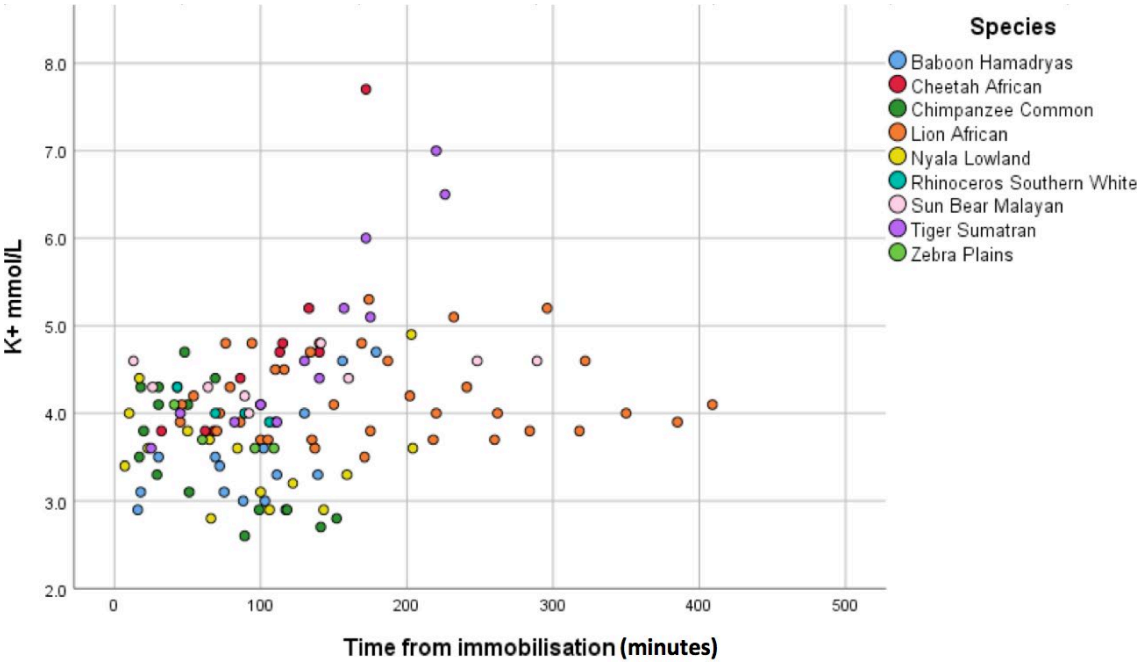


***Figure 2.3b:*** *Electrolyte Parameters: Scatter plots showing the effect of species and time from immobilisation on Potassium (measured in Mmol/L) of zoo mammals under anaesthesia.*

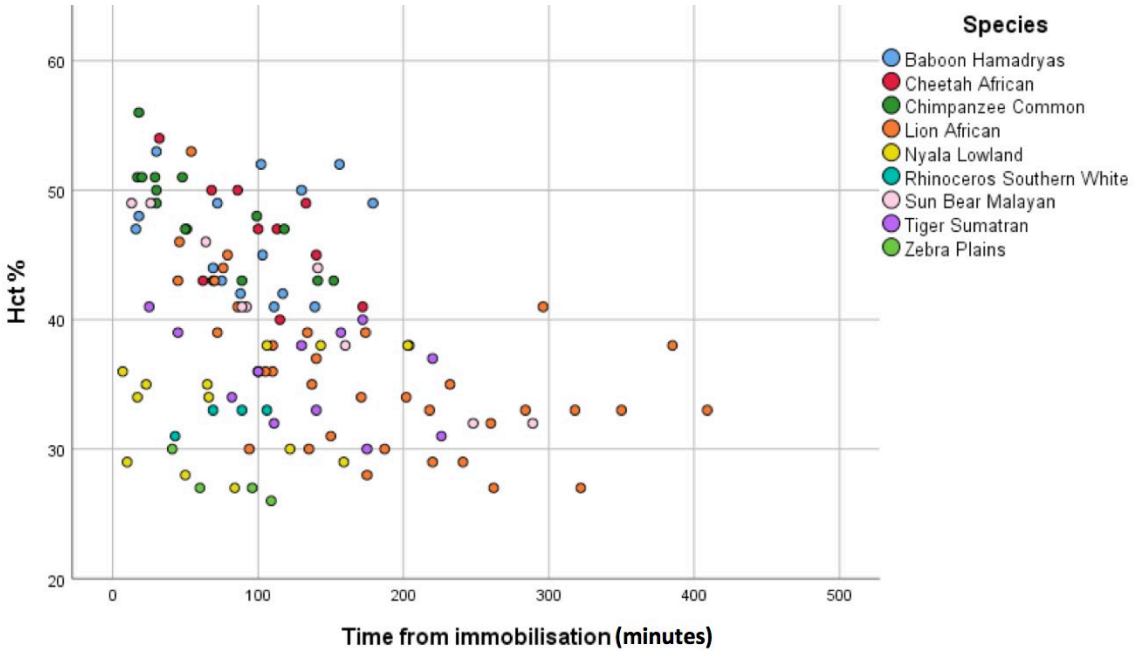


***Figure 2.4a:*** *Clinical pathology parameters related to oxygen carrying capacity and serum protein levels: Scatter plots showing the effect of species and time from immobilisation on Haematocrit (in percentage) of zoo mammals under anaesthesia.*

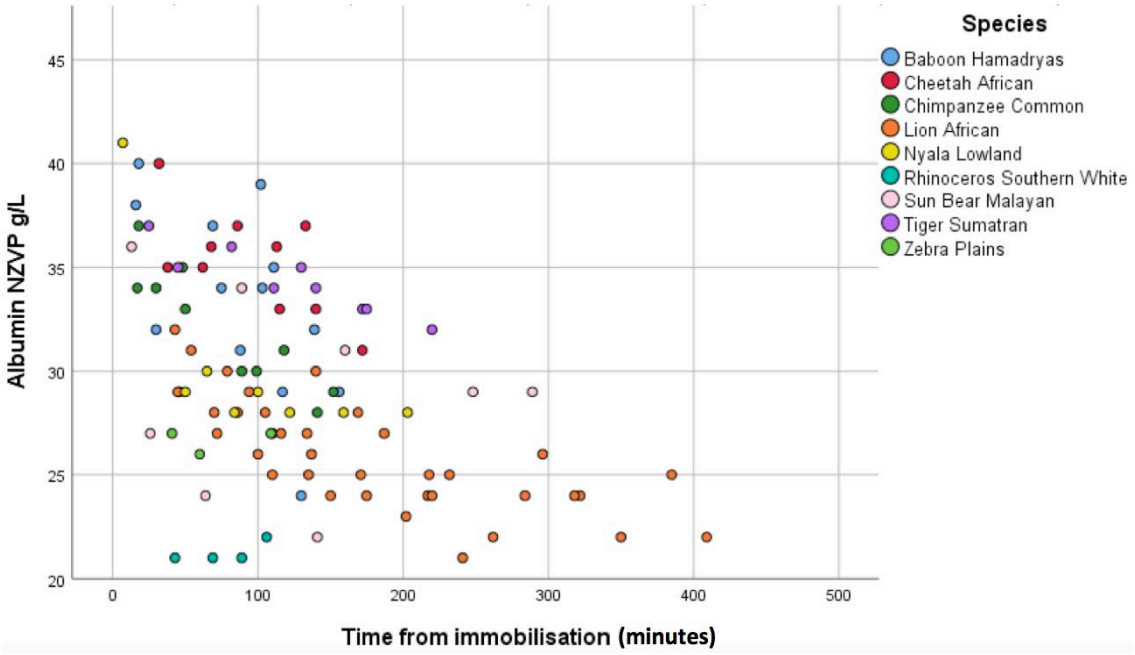


***Figure 2.4b:*** *Clinical pathology parameters related to oxygen carrying capacity and serum protein levels: Scatter plots showing the effect of species and time from immobilisation on Serum Albumin (measured in g/L) of zoo mammals under anaesthesia.*

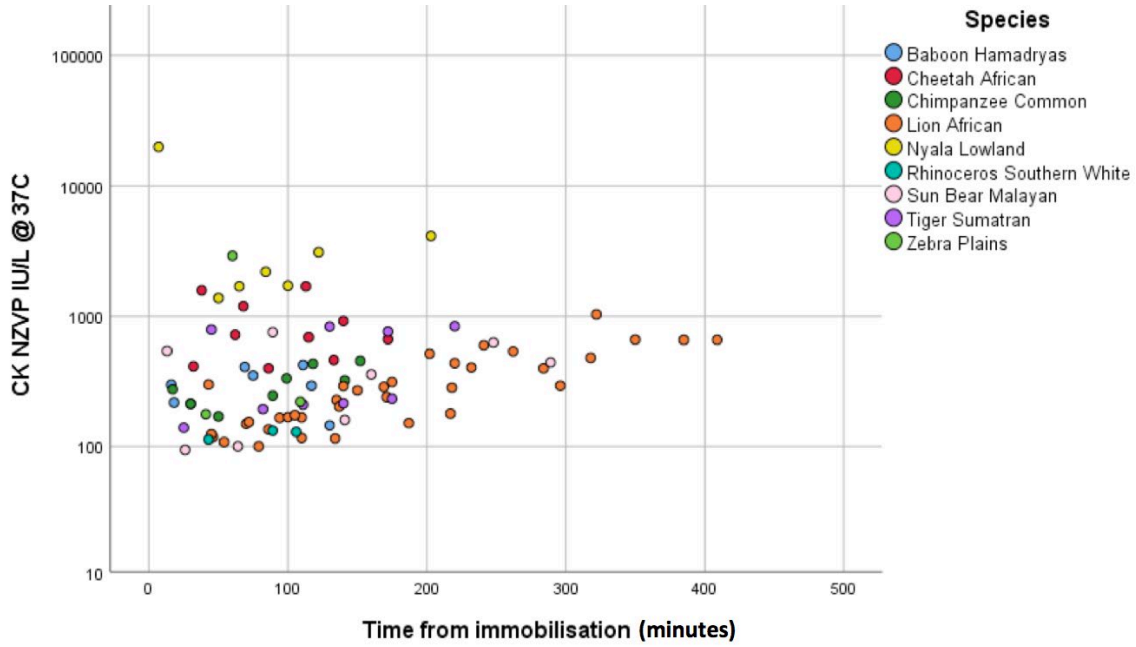


***Figure 2.5:*** *Clinical pathology parameters related to cellular damage: Scatter plot showing the effect of species and time from immobilisation on logarithm of Creatine Kinase (measured in IU/L at 37 degrees Celsius) of zoo mammals under anaesthesia.*

Regarding laboratory clinical pathology parameters, there was insufficient evidence for an effect of time from immobilisation on any of the markers of extents of azotaemia (BUN, SDMA and creatinine). Apparent inter-assay variation between EPOC and laboratory creatinine measurements was noteworthy (*data sheet supplied in Appendix A1).*

As measures of plasma protein and capacities for blood oxygen carriage, there was very strong evidence for time effects involving albumin, globulin and HCT, all of which generally demonstrated reductions over the duration of anaesthesia (Table 2.2, Figures 2.4b and 2.4a). Interestingly, the rhino and zebra cases provided exceptions to these generally reducing trends over time. Of the electrolyte and clinical pathology measures with the potential to become elevated under circumstances of gross cellular injury (Vanholder et al 2000, Breed et al 2019), CK levels showed strong evidence of the effects of time since immobilisation, although relative variations in CK levels were generally small (Figure 2.5). As the primary intracellular cation which may accumulate in the extracellular fluid in response to acidosis-induced cellular injury (Breed et al 2019) and/or reduced excretion (Schaer 1995), there was insufficient evidence for any effects of time since immobilisation on the plasma concentrations of potassium.

Pertinent to overall evaluation of patient support were the frequency (19%) of MAP recordings of 65mmHg or less which is consistent with clinically significant hypotension (occurring relatively early during anaesthesia in primates; Figure 2.1a). Likewise, of potential concern was the frequency (26%) of pCO2 recordings exceeding 55mm Hg, consistent with clinically significant hypercapnia (occurring early into anaesthesia in primates and later in felids; Figure 2.2b). Alveolar-venous CO2 gradients exceeded 15mmHg in 23% of cases, consistent with possible instances of inappropriate alveolar dead space (Hardman and Aitkenhead 1999, McSwain et al 2010, Mosing et al 2018). Certain outlier values are noteworthy for their clinical significance for the individual animals. These included cases of hyperkalaemia (>5.5 mmol/l, two of which were >=7mmol/l) in three felids (cheetah, two tigers), profound acidaemia (pH< 7.11) in two carnivores (cheetah, sun bear), relative elevation in plasma concentration of CK (19,000 IU/L, nyala), concerning hypoglycaemia (<2mmol/l, chimpanzee) and marked hypoxaemia (venous pO2, 24mm Hg, nyala).

***Table 2.3:*** *Species differences in physiological measures and analytes for zoo mammals under anaesthesia as assessed by post-hoc analysis of a GLM. Empty areas represent species and parameters for which insufficient data was available to permit comparison. '0' denotes insufficient statistical evidence (p>0.1) for the species and parameter differing from the grouped species mean. '>' and '<' indicate there is statistical strength of evidence for which the species mean for the parameter is respectively greater or less than the grouped species mean. p <0.001 (red arrows) indicates very strong evidence; p<0.01 (green arrows) indicates strong evidence; p<0.05 (blue arrows) indicates moderate evidence.*

| Species | Baboon | Cheetah | Chimpanzee | Lion | Nyala | Rhino | Sun Bear | Tiger |
|---|---|---|---|---|---|---|---|---|
| **Physiological Parameters** | | | | | | | | |
| Temperature | 0 | > | < | > | < | 0 | < | > |
| Respiratory Rate | > | > | 0 | 0 | 0 | 0 | 0 | 0 |
| Heart Rate | > | > | 0 | < | > | < | > | < |
| Mean Arterial Pressure | < | > | < | 0 | 0 | 0 | 0 | 0 |
| End tidal CO2 | > | > | < | < | 0 | 0 | < | < |
| **EPOC Blood Gas/ Electrolyte Parameters** | | | | | | | | |
| pH | > | < | | 0 | > | | | |
| pCo2 mmHg | > | > | | < | < | | | |
| pO2 mm hg | 0 | 0 | | 0 | > | | | |
| cHCo3- mmol/L | > | > | | < | > | | | |
| BE (ecf) mmol/L | > | 0 | | < | > | | | |
| cSO2 % | 0 | < | | 0 | 0 | | | |
| Na+ mmol/L | < | > | | > | < | | | |
| K+ mmol/L | < | > | | 0 | < | | | |
| Ca++ mmol/L | < | 0 | | > | < | | | |
| Cl- mmol/L | < | > | | > | < | | | |

***Table 2.3*** *(Continued): Species differences in physiological measures and analytes for zoo mammals under anaesthesia as assessed by post-hoc analysis of a GLM. Denotations are as per the legend for the first part of Table 2.3 (above).*

| Species | Baboon | Cheetah | Chimpanzee | Lion | Nyala | Rhino | Sun Bear | Tiger |
|---|---|---|---|---|---|---|---|---|
| **EPOC Blood Gas/ Electrolyte Parameters (Continued)** | | | | | | | | |
| A Gap K mmol/L | < | > | | 0 | 0 | | | |
| Hct % | > | > | | 0 | < | | | |
| cHgb g/dL | > | > | | 0 | < | | | |
| Glu mmol/L | 0 | > | | < | 0 | | | |
| Lac mmol/L | < | < | | 0 | > | | | |
| EPOC Crea micromol/L | < | > | | > | < | | | |
| **Laboratory Clinical Pathology Parameters** | | | | | | | | |
| SDMA micrograms/dL | < | > | 0 | > | < | | 0 | |
| CK IU/L @ 37C | < | 0 | 0 | < | > | | < | |
| TP g/L | < | > | > | > | > | | < | |
| ALB g/L | > | > | 0 | < | 0 | | < | |
| Glob g/L | < | 0 | > | > | > | | < | |
| A/G ratio | > | 0 | 0 | < | < | | 0 | |
| BUN mmol/L | < | > | 0 | > | < | | > | |
| Crea micromol/L | < | > | < | > | < | | > | |
| PO4 mmol/L | < | > | < | < | > | | > | |

## 2.4 Discussion:

To the best of our knowledge, this is the first report documenting broad, intensive serial monitoring in a range of mammalian species to characterise homeostatic alterations occurring in everyday zoo anaesthesia practice. The results suggest many progressive

changes to physiological homeostasis are occurring, often with species differences, warranting further research to determine the external validity of the findings for zoo anaesthetic practice. Patient homeostasis sometimes demonstrated significant compromise, based on the premise that different mammalian species likely share generally similar homeostatic tolerances.

The marked species' differences evident in virtually all of the homeostatic parameters measured is an expected finding, attributable largely to the uniqueness of each species' physiology. Nevertheless, within the broad species ranges that were apparent in the homeostatic parameters measured, many unexpected findings were manifest in the way that different species and/or different conspecific individuals responded to anaesthesia, highlighting the individuality of each anaesthetic event and likely reflecting the importance of many other factors besides the anaesthetic regimens adopted in influencing the course of homeostasis under anaesthesia. This emphasises that no single protocol can be defined as ideal for a particular species, and provides a reminder that the kind of homeostatic disturbances observed in this study may be expected even for protocols which have been identified by anaesthetists as reflecting current best practice.

Mild hypothermia was evident in most of the patients, despite body temperature being actively managed to prevent its progression, thus mitigating serious risks of potentiating hypotension and cardiac arrhythmias (Ko and Krimins 2014). Otherwise, evidence of mild hypoventilation was the most widely manifest disturbance recorded in this study, with somewhat unexpected species representations in terms of ventilatory performance. For instance, rhinoceroses normally hypoventilate under anaesthesia, even with ventilation support, and ventilation-perfusion mismatches are often associated with the difficulties in supplying the required tidal volumes without simultaneously prolonging pulmonary vascular compression. Yet the pCO2 concentrations sometimes attained during the rhino procedure in this study compared favourably against those in other studies following the administration of potent opioids (Hattingh et al 1994, Fahlman et al 2016, Buss et al 2016), even matching pCO2 levels in non-sedated rhinos (Citino and Bush 2007). While comprising a dataset from only a single anaesthetic episode, such performance suggests ventilation benefits may accrue from the substitution of potent opioids with a combination of butorphanol, midazolam,

medetomidine and ketamine in these animals under zoo conditions. That pCO2 concentrations were lower in the rhinoceros relative to many smaller species (baboons, chimpanzees, cheetahs) in this study draws attention to the often inadequate ventilation of the latter species under anaesthesia, although most of the pCO2 manifestations may be considered generally mild under high inspired oxygen fractions if blood pH reductions are small (Mama 2019). The relatively normal (35-45mm Hg) pCO2 concentrations often recorded in the lions in this study may have reflected the predominant use of mechanical ventilation throughout anaesthesia in this species and advances have permitted the safer application of this technology even in some larger megafauna (Horne et al 2001, Citino et al 2007). Such support deserves much more extensive physiological evaluation, especially for its possibly wide application in zoological settings. Of interest, an arterial pO2 level of 706mmHg under 100% oxygen was achieved for a nyala receiving mechanical ventilation while being maintained under propofol anaesthesia, likely attained through optimisation of ventilation-perfusion matching.

Measures of cardiovascular performance showed insufficient evidence of showing time effects, however it is worth noting that the cardiovascular system is actively managed by anaesthetists which may have masked the effect of time from immobilisation. Important trends in cardiovascular performance were nevertheless evident. Given the insensitivity of MAP as a measure of mild to moderate hypo-perfusion (Boag and Hughes 2005) and the possible difficulties associated with re-establishing microcirculatory perfusion under such circumstances, the extent and occasionally sustained nature of MAP recordings below 65mmg Hg observed in this study was concerning, particularly as concurrent heart rates (as a means to augment cardiac output) often fell below those expected from extrapolations based on allometric scaling of heart rate for bodyweight (Sedgewick 1991). Furthermore, the instances of venous pCO2-ETCO2 gradients exceeding 15mm Hg may crudely reflect reduced pulmonary blood flow as a cause of inappropriate alveolar dead space (Hardman and Aitkenhead 1999, McSwain et al 2010, Mosing et al 2018). Intra-procedure ventilation varied widely and ETCO2 values may also have under-represented true values if endotracheal seals were not fully tight. Furthermore, variable venous gradients in this range are often observed in larger

mammals (Haskins 2015, Mama 2019). While lactate elevations were rarely even considered moderate (as per Kovacic 2009, defined as beyond 2mmol/l) and never appeared sustained, this marker may be insensitive for detecting regional hypo-perfusion (Gillespie et al 2017).

Although the pathophysiology of peri-anaesthetic hypotension is multifactorial, the depressive effects of injectable and inhalational anaesthetics on cardiovascular system performance are well recognised (Hall and Clarke 1991, Lees 1991, Mazzafero and Wagner 2001, Noel-Morgan and Muir 2018), with excessive anaesthetic depth being implicated as an important mechanism for haemodynamic compromise particularly in human geriatric anaesthesia (Wickham et al 2016). The possibility this may frequently be the case under zoo anaesthesia conditions warrants serious consideration and demands further research. Despite the aforementioned challenges associated with permitting valid comparisons of anaesthetic depth, parameters besides cardiovascular measures for further characterising depth were rarely recorded, possibly reflecting significant paucity of their comprehensive documentation in wider zoo anaesthesia practice.

Base excess provides another useful general measure of homeostasis at the cellular environment level and patterns in this study suggest either that many patients presented with pre-anaesthetic metabolic compromise, and/or that metabolic challenges to patients (reflected by extents of relative metabolic acidosis) become particularly manifest late in the immobilisation phase or early during the initiation of anaesthetic maintenance. Given the marked and biphasic effects of medetomidine on blood pressure (Lamont et al 2001, Saleh et al 2005), its potential influence on blood flow should be evaluated in more detail as a possible contributory differential for the metabolic acidosis often recorded in this early period.

The extensive time effect on chloride levels and the variation in chloride and anion gap evident during anaesthesia in some individuals indicates that aetiologies which respectively induce chloride and anion gap alterations (Kellum 2005, Corey 2005) are occurring in the patients monitored in this study and these may in turn reflect homeostatic alterations involving a variety of body processes and influences from

various interventions. Further discrimination of the causative anions is thus an important first step in narrowing aetiological differentials (Kellum 2005), but anion gap may be an insensitive indicator of unmeasured ion levels in altered weak acid states (Lloyd 2004). Furthermore, per the reasoning of Sano et al (2005) and Damen et al (2016), the significant and widespread declines in HCT, albumin and globulin occurring during anaesthesia provide evidence for plasma dilution, through volume expansion, occurring as anaesthesia progressed in many patients in this study. This trend needs to be accommodated for when interpreting other blood analyte results and reasons for these changes deserve further investigation. Derivation of the strong ion gap and control for free water effects are required to account for the associated effects of changing weak acids and electrolyte concentrations as contributors to changes in base excess (Lloyd 2004, Anstey 2010).

The three instances of felids demonstrating marked hyperkalaemia in this study were of particular concern for the health of the individuals involved and progressive potassium elevations in anaesthetised large felids are well documented in the literature (Gunkel and Lafortune 2007, Reilly et al 2014, McEntire et al 2020). In one extensive study of lion and tiger anaesthesia, these elevations were universal, independent of creatinine, always associated with progressively elevating glucose levels and more apparent with longer procedures in which medetomidine was used as the sedation agent (Reilly et al 2014).

In a more recent retrospective study of lions and tigers (with a quarter of the anaesthetic episodes involving those included in Reilly et al's 2014 study) in which medetomidine or dexmedetomidine were part of the induction protocols, a quarter of the anaesthetic episodes recorded potassium elevations exceeding 5.5mmol/L and time under anaesthesia and early administration of atipamezole were respectively found to predict increasing and decreasing potassium concentrations. Furthermore, lions developed significantly greater potassium concentrations than tigers over equivalent anaesthetic times (McEntire et al 2020).

In the present study, not all felids demonstrated glucose elevations and none of the lions developed potassium elevation above 5 mmol/L. Interestingly, anaesthetic

interventions involving the latter were occasionally prolonged to five or six hours' duration. Such prolonged anaesthetic episodes also involved geriatric lions, maintained on propofol and for which approximately a third of the total atipamezole dose was given within a hundred minutes of medetomidine delivery. In cases which did develop potassium elevations over time, potassium rises were independent of creatinine (and creatinine to sodium ratio), consistent with the findings of Reilly et al (2014). Reductions in pH were marked only in two (both predominantly respiratory) of the three cases showing marked potassium elevations (=>6.5mmol/l), while phosphate and CK levels were often much higher in non-effected felids, implying that tissue damage and extreme acidosis were not good predictors of hyperkalaemia. Falsification of ionised potassium readings by low pH still needs to be ruled out (Mama 2019). Aldosterone and insulin assays were not undertaken in this study and while medetomidine probably plays a major role in the development of progressive potassium rises in large felids through its effect on insulin secretion as suggested by Reilly et al (2014), the results of the present study re-emphasize these authors' views that other poorly determined factors are required to precipitate marked potassium elevations.

The most likely reasons for markedly prolonged recoveries sometimes observed in this study, particularly following continuous propofol infusion in felids, could not be established. In the felids, they may have been related to age-related impairments in propofol metabolism, although prolonged recoveries have also been previously documented with propofol use in non-geriatric cheetahs (Buck et al 2017a). It is notable, though, that alterations in some of the parameters encountered in this study, such as marked changes in blood pressure and changes in albumin concentrations, may also affect propofol pharmacokinetics through altered protein binding (Mazoit and Samii 1999) and through the potential for altered hepatic and extrahepatic flow to influence drug metabolism (Gelman 1987, Sahinovic et al 2018).

Inability to perform pre-induction evaluations rendered it difficult to differentiate homeostatic disturbances attributable to pre-anaesthetic morbidity from those attributable to the effects of anaesthesia. This is a common feature of zoo animal anaesthesia. Patient training to permit such evaluation will be the only way to discriminate them, but will still leave uncertainty about how expected physiological

variables in the anaesthetic (and surgical) context should be adjusted to allow for an animal's physiological response to such context. The observational study design and intermittence of monitoring imposed further interpretative limitations. Significant time trends and unexpected species differences for many of the parameters may partly reflect recruitment bias or uneven individual weighting in anaesthesia of different durations. Significant homeostatic changes over time may also remain undetected with general linear modelling, which does not provide specific characterisation of their pattern.

Particularly pertinent in this regard are the fluctuations observed in EPOC creatinine levels in many of the species. Although interpretation of repeated creatinine measurement is usually predicated on attainment of equilibrium states across body compartments, it is possible in human medicine to make inferences from creatinine fluctuations about functions such as glomerular filtration rates (Khayat et al 2019). A hypothesis deserving further evaluation in the zoo context is that creatinine should not fluctuate markedly under anaesthetic conditions where the haemodynamic status of the patient is stable, particularly in herbivores (V Walsh pers comm). It is uncertain what the biological significance of the creatinine fluctuations observed in this study were and given the discrepancies between the two analytical techniques used, further validation studies are required. Profoundly high creatinine levels (with both assays) in all the aged felids nevertheless suggest they presented in a state of renal insufficiency, possibly rendering them more sensitive to the renal and pre-renal effects which anaesthesia may potentially have on renal function (Mercatello 1990, Longley 2012, Greene et al 2014, McKinlay et al 2018).

Generation of larger, more systematically gathered datasets using the methodology described may permit better assessment of how different procedural approaches influence patient safety. Such analytical capabilities may also be enhanced by closer examination of homeostatic parameters in relation to particular procedural changes occurring during the course of anaesthetic events. For example, further analysis of homeostatic changes in relation to more specific quantification of drug delivery (see, for example, McEntire et al 2020), particular phases of the anaesthesia protocol (see, for example, Napier et al 2013 and Rodriguez et al 2018), defined timeframes since

immobilisation (see, for example, Zeiler et al 2015) or combinations of these (see, for example, Siegal-Willott et al 2019) may also help to identify changes associated with the nature of specific interventions undertaken at different times.

## 2.5 Conclusions:

This study identified several physiological disturbances to homeostasis occurring during zoo anaesthetic procedures using established and frequently applied anaesthetic protocols. The external validity of the findings as a reflection of wider zoo anaesthetic practice remains to be determined. The clinical implications or pathological consequences of the homeostatic alterations identified are also uncertain, with such alterations including blood pressure changes, changes to ventilation, possible ventilation-perfusion mismatches, moderate to marked alterations in acid-base status and occasional but concerning hypoglycaemia, hypoxaemia and hyperkalaemia. Some of these would remain undetected or are unlikely to be anticipated if only routine physiological parameters are monitored, emphasising the value of applying strategic, serial point of care blood analysis even in cases which do not appear at elevated risk.

Within species, markedly different individual responses to anaesthesia were apparent, sometimes despite use of relatively similar induction protocols, highlighting the importance of other procedure-related considerations, pre-anaesthetic states and different individual physiological responses to drugs in determining the course of anaesthesia. Protocols and other procedure-related management methods are widely disseminated and may quickly become established into institutional routine, yet they should not be considered as generic best practice for all occasions and if they are used, the kind of homeostatic disturbances identified in this study should be anticipated for better risk management. Findings from this study support a need to continuously seek procedure refinements and to closely tailor anaesthesia to individual patient responses.

With a systematic approach and generation of a much broader database, the methodology described in this study should provide a means to promote a better mechanistic understanding of how and why homeostatic disturbances are occurring under anaesthesia, particularly when covariate parameters can be related to each other

in fuller context in order to provide more information about physiological functioning. However, the marked inter-species differences in these results suggest the comparative method used here would be better replaced by a tighter focus on more closely related species (Chapter 3). As such a dataset is built, the approach may also become better able to inform about the relevance of particular responses to anaesthesia and whether or not they are associated with particular post-anaesthetic outcomes.

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**Chapter Three: Metabolic acid-base changes in large felids under anaesthesia: An observational study assessing the role of unmeasured anions:**

## 3.1 Introduction:

Important objectives of anaesthetic monitoring are to assess whether blood flow is likely to be preserved to vital organs and whether oxygen delivery is likely to meet tissue demands (Ozeki and Caulkett 2014). In zoo anaesthesia practice, various haemodynamic parameters are used in conjunction with one another to make assessments about tissue perfusion (Ozeki and Caulkett 2014). Unfortunately, such assessment is not easy (Lonjaret et al 2014, Ackland et al 2019), especially since blood pressure may not always provide an accurate gauge of cardiac output and therefore of blood flow (Edner 2005, Wagner 2005, Lawson and Hutton 2012). Mucous membrane colour, refill time and refill vigour retain some utility as general gauges of perfusion under immobilisation and anaesthesia, but they may not necessarily reflect critical organ blood flow (Haskins 2015).

Some organs may be more liable to hypoxia than others as they are afforded lower circulatory priority in low flow states (Bonanno 2011, Post and Vincent 2018). Renal medullary thick ascending tubular cells are particularly sensitive to hypovolaemia-induced ischaemia (Kellen et al 1994, Goren and Matot 2015, Layton 2016, Meersch et al 2017, Salmasi et al 2017). In domestic felids, anaesthesia has been identified as a risk factor for chronic kidney disease (Greene et al 2014). Similar risks may apply to non-domestic *Felidae*, particularly with the potential cardiopulmonary impact of high dosages of immobilising drugs (Forsyth et al 1999) and the depths at which anaesthesia may be maintained in *Pantherae* for the safety of human operators. There are challenges with assessing blood pressure in conscious states in these dangerous wild animals, which leads to uncertainty about how this parameter scales to bodyweight in mammals (Poulsen et al 2018), while scaling of perfusion pressure still needs to be examined (White and Seymour 2013). Overall, these factors compound uncertainty about the ranges and combinations of blood pressure and heart rates likely to sustain renal perfusion in large felids under anaesthesia.

Techniques which allow haemodynamic and pulmonary monitoring to detect compromises to cellular respiration may aid in protecting organs afforded lower circulatory priority. Serial blood lactate measurement is used to monitor haemodynamic resuscitative efforts, although lactate elevation may not occur until hypo-perfusion becomes moderate to severe (Haldane 2015) and it may be an insensitive marker for regional hypo-perfusion (Gillespie et al 2017). In high-risk human anaesthesia, sophisticated means for monitoring peripheral haemodynamics, microcirculation and tissue oxidation have been developed (Kipnis et al 2012, Kipnis and Vallet 2016). For zoo animal anaesthesia, where time and safe access to the patient are limited, more practical means of alerting anaesthetists to deteriorating states of cellular respiration will be required.

To this end, the body's pH homeostasis is defended as vigorously as that of oxygen transport and perfusion pressure (Rubash 2001) and while there is much to be learned about the identity and significance of unmeasured anions (UA) in different disease processes (Lemann et al 2003, Vanholder et al 2003, Forni et al 2006, Bruegger et al 2007, Annecke et al 2012), changes to blood flow may result in the accumulation of anionic metabolites favouring hydronium ion generation which are produced during compromised states of cellular respiration. In hypoxic states, many UA have been found to be intermediary products in energy substrate metabolism, products of incomplete oxidation (Kellum 2000, Bruegger et al 2007) or the products of endothelial glycocalyx shedding (Annecke et al 2012), the former two often being quantitatively (and in valence) far more significant than lactate in such circumstances (Forni et al 2006). Theoretically, UA increases may reflect tissue hypo-perfusion, highlighting suboptimal blood flow in patients under anaesthesia. In clinical zoo anaesthesia where point of care electrolyte measurement is easily deployed, UA increases may be reflected by exacerbations in metabolic acidosis and increases in the anion gap and/or by exacerbations in base deficit that cannot be explained by changes in strong ion difference (SID) (Story et al 2002).

While the metabolic component of acid-base status reflects the cellular performance of many organs (Reddi 2018), reduced blood flow and oxygen provision to organs such as

the liver and kidneys may alter their effectiveness to participate in its homeostasis. Unmeasured ion elevation has been documented in states of renal and hepatic dysfunction in critically ill patients (Moviat et al 2008, Abramowitz et al 2012, Zingg et al 2018). Besides a role in elimination of many fixed acids (Vanholder et al 2003), the hepato-renal system works in unison to mitigate acidosis by permitting increases in SID (Vanholder et al 2003, Hamm et al 2015). Decreased renal perfusion and urine output limit urine acidification mechanisms and cause metabolic acidosis (Seifter and Chang 2017). Altered renal performance, including for pre-renal reasons, may also be reflected in UA accumulation (Wesson et al 2011) and possibly also in SID reduction.

Approaches to better understanding the possible causes of peri-operative metabolic acidosis have been applied to human anaesthesia (for example, see Lawton et al 2019) but are somewhat foundational in the zoo anaesthesia context. This study describes serial changes to acid-base status occurring in captive large felids under anaesthesia, focussing on the metabolic component, the involvement of UA and whether UA values are associated with mean arterial blood pressures in the anaesthetic period preceding blood sampling events.

The chapter includes some base data (first procedures on each animal) that has been included in the previous comparative anaesthesia chapter (Chapter Two).

## 3.2 Materials and Methods:

### 3.2.1 Study Design, Inclusion Criteria and Patient Recruitment:

Serial acid-base status of large felids during anaesthetic procedures was monitored at two New Zealand zoos between November 2017 and June 2019. Patient recruitment was opportunistic, governed by zoo veterinary staff decisions on which animals required anaesthetic interventions. The extent and timing of monitoring varied depending on safety considerations and accessibility to the animals at the time. Monitoring commenced as soon as safely feasible following intramuscular delivery of immobilising drugs, continuing until final medetomidine reversal.

### 3.2.2 Blood Sampling, Analytes Measured and Physiological Monitoring:

Peripheral venous samples (n=83) were drawn into 3mL non-heparinised syringes via 20G needles or intravenous catheters, discarding the first 1-2mLs of blood. Samples filled 600 microlitre lithium heparin/ PST gel BD microtainers®, which were then carefully agitated. The remainder was placed into silicon coated serum BD microtainers® (BD Diagnostics, Franklin Lakes, NJ, USA). Venous heparinised samples were analysed as soon as feasible with the same EPOC device (EPOC®, Siemens Healthineers, Erlangen, Germany) using EPOC® cartridges (EPOC® BGEM, Epocal Inc, Ottowa, ON, Canada) to directly determine or calculate (prefix 'c') acid-base parameters pH, pCO2, cHCO3, cBE(ecf) (hereinafter base deficit or BD), cAG(K), Na, Cl, K, Ca and lactate. The silicon-coated microtainer samples were chilled to 2°C, left to clot for 30 min then centrifuged at ca.1000G for 10 min. Pipetted serum was frozen to -81°C and later analysed in two batches by wet biochemistry at a commercial laboratory (IDEXX – New Zealand Veterinary Pathology, Palmerston North, New Zealand) for phosphate (PO4) and albumin (Alb) determination. Time from sample collection to analysis varied from one to eighteen months. Arterial samples (n=17) were taken from pre-catheterised dorsal pedal arteries, discarding the first 2mLs of blood. Samples were analysed immediately from capped, unpressurised 3mL non-heparinised syringes with the EPOC device, with the remaining sample managed as per venous blood.

A Surgivet® Advisor® Monitor (Smiths Medical, Dublin, OH 43017, USA) or Tafonius® Large Animal Monitor (Vetronics®, Abberkerswell, UK) were used (n=8, n=3 respectively) to estimate mean arterial pressure (MAP) non-invasively, at intervals as close as feasible to 5 minutes, using recommended blood pressure cuffs with a width measuring 30-40% of appendage circumference (Haskins 2015). When accessed, invasive methods to continually monitor MAP corrected to atrial level were preferentially used. MAP readings over a period of 30 minutes prior to each blood sampling were then averaged (hereinafter termed 'tMAP').

### 3.2.3: Acid Base Interpretations:

Per criteria proposed by Hopper and Epstein (2012) for domestic felids, metabolic acidosis was defined as a base deficit exceeding -7mmol/L. A quantitative acid-base approach (Stewart 1983) was used to differentiate strong ion and non-volatile buffer ion (A-) contributions to extracellular base deficit (BD). Charge differences after summation of strong ions and non-volatile buffer ions were considered to represent "unmeasured" ions. Referred to as the strong ion gap (SIG), this measure contributes to the non-respiratory component of body fluid pH, together with A- and apparent strong ion difference (SIDa).

The calculated SIDa included lactate and calcium per methods of Figge et al (1992), but excluded magnesium (see Box 1). Quantification of the anionic effects of albumin and phosphate (summated as A-) and bicarbonate (altogether summated as effective strong ion difference, or SIDe) were based on human derivations (Figge et al 1992). Justification for this was based on similarity between domestic felid-specific and human total plasma concentrations and dissociation constants of non-volatile buffers (McCullough and Constable 2003). The SIG was calculated as SIDa –SIDe (per Kellum et al 1995), such that positive values indicate UA 'excesses'. Derivations are summarised in Box 1.

*Box 1: Summary of Stewart Acid-Base Derivations.*

| |
|---|
| SIDa = (Na+ + K+ + Ca2+) - (Cl- + Lac-) |
| A- = [Albumin] × (0.123 × pH - 0.631) + [Phosphate] × (0.309 × pH - 0.469). |
| SIDe = 1000 × 2.46 × 10exp-11 × (pCO2/10exp-pH) + A- |
| SIG = SIDa –SIDe. |

### 3.2.4 Statistical Analysis:

All statistical analysis was carried out using SPSS v25 (IBM, Armonk, NY, USA). A general linear model (GLM) was carried out to assess for differences between species in the dependent parameters SIDa, SIG and A-. Time from immobilisation, BD and tMAP were

included as covariates, with patient identity as a random factor. Where strong effects of a predictor variable were considered to obscure effects of other contributory parameters on BD as a covariate, forward stepwise automatic linear modelling was undertaken to identify those parameters which were the best predictors of BD.

## 3.3 Results:

Eleven large felid anaesthetic procedures (6 x African lion *Panthera leo subsp. melanochaita*, 3 x African cheetah *Acinonyx jubatus subsp. jubatus*, 2 x Sumatran tiger *Panthera tigris subsp. sumatrae*) were monitored. Anaesthetic duration from initial immobilisation varied (140 to 415 minutes), as did immobilisation, anaesthetic induction and maintenance regimens used (*data sheet supplied in Appendix A2).* Animals ranged from two to nineteen years of age, with many exceeding fifteen years and classifiable as geriatric (Table 3.1).

***Table 3.1:*** *Age breakdown by species for the felids included in the study.*

| ***Number of individuals of each species by age*** | ***Age in Years*** | | | |
|---|---|---|---|---|
| ***Species*** | ***0-5*** | ***6-10*** | ***11-15*** | ***16-20*** |
| **Lion** *Panthera leo subsp. melanochaita* | *0* | *0* | *1* | *5* |
| **Tiger** *Panthera tigris subsp. sumatrae* | *0* | *2* | *0* | *0* |
| **Cheetah** *Acinonyx jubatus subsp. jubatus* | *2* | *1* | *0* | *0* |

Increments and timing of medetomidine antagonism as well as more specific anaesthetic interventions to normalise blood pressure and support lung ventilation also varied between procedures. All individuals received intravenous fluids throughout the procedures (most often lactated ringers at between 5-10 ml/kg/hr) but despite this support, eleven relatively hypotensive events of MAP of 60mm Hg or less were recorded, involving five different individuals (Figure 3.1) and ranging in cumulative duration from to 5 to 30 minutes per procedure. In most of these cases, vasopressors

were administered to restore MAP (dopamine at 2-5Ug/kg/min, or noradrenaline at 0.02-0.05Ug/kg/min). In two cheetahs, prolonged, relative hypertension was apparent, involving MAP greater than 150mm Hg for circa 60 minutes' cumulative duration. All lions and tigers were mechanically ventilated, while ventilation in all cheetahs was principally spontaneous, with occasional manual assistance (*data sheet supplied in Appendix A2*). In one cheetah, marked hypercarbia was apparent (*data sheet supplied in Appendix A2*).

All patients survived the procedures and none developed clinically overt post-anaesthetic complications, although post-anaesthetic recoveries were reported in the Species360® Zoo Information Management System (ZIMS) as being notably protracted in the geriatric lions in which propofol was used as the primary anaesthetic maintenance agent.

On average, 5.5 EPOC measurements were taken per procedure (range: 3-12, SD: 2.61). SIDa and SIG could be calculated for 95% (59/62) and 83% (52/62) of EPOC measurements respectively, with an average of 5.5 SIDa measurements (range: 2-12; SD: 2.73) and 4.9 SIG measurements (range: 2-11; SD: 2.6) per procedure. Very strong evidence ($p<0.001$) was apparent for a species effect on SIDa, SIG and A- (Table 3.2). In all felids combined, there was moderate evidence ($p<0.05$) for an effect of SIG on BD, but not for SIDa or A-, and strong evidence ($p<0.01$) for effects of time and tMAP on A- (Table 3.2). However, the best predictors of BD were found to vary between species. For lions, these included SIG and to a lesser extent SIDa (Figure 3.2), while for tigers and cheetahs respectively, SIDa and time from immobilisation were better predictors (Figures 3.3 and 3.4). No single parameter provided extremely strong prediction of BD either between or within species. There was an absence of evidence for an effect of tMAP on SIG (Table 3.2).

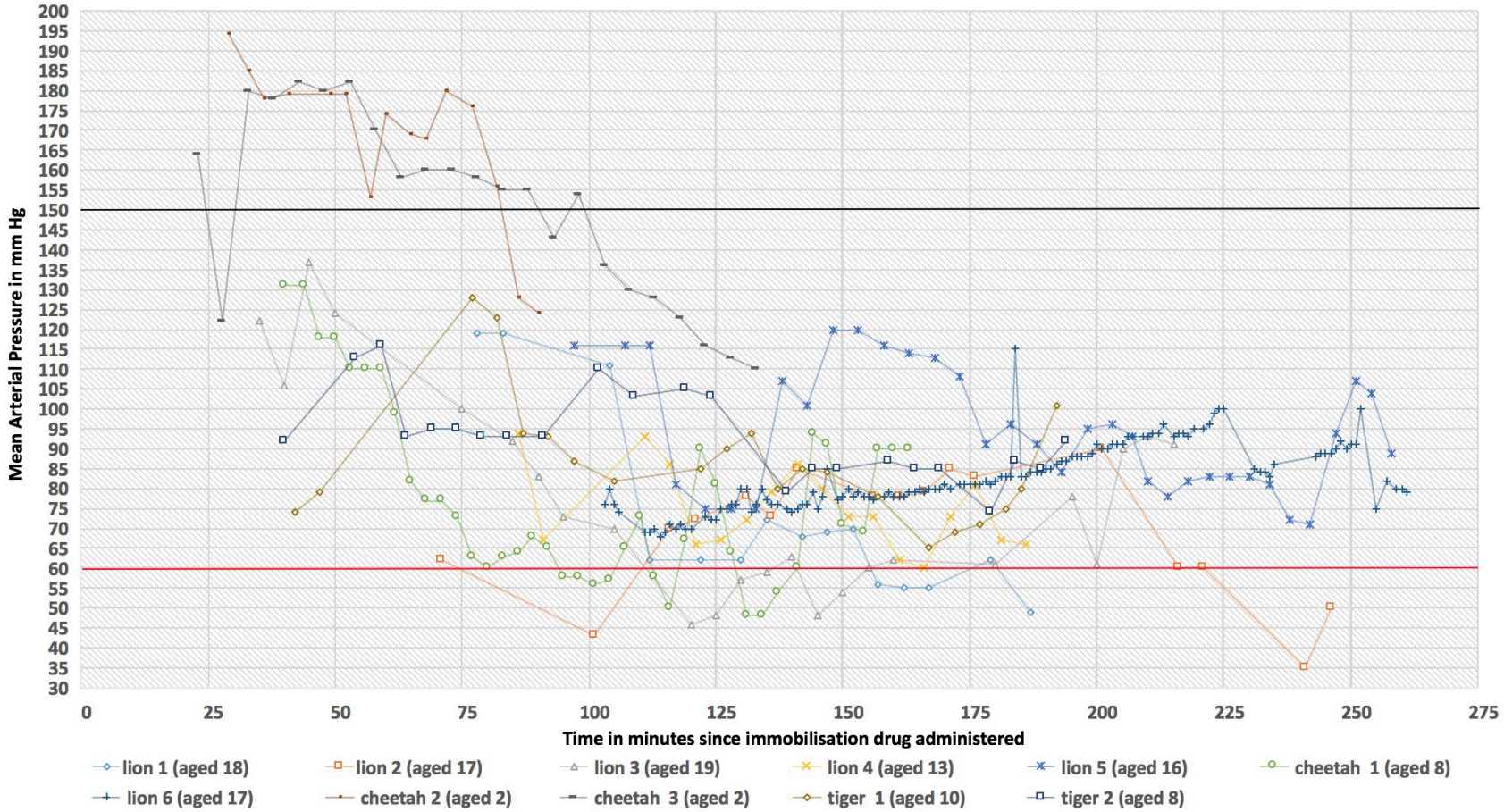


***Figure 3.1:*** *Measured blood pressure changes over time during anaesthesia for each of the felids in the study. Horizontal red line demarcates relative hypotension of MAP 60 mm Hg. Horizontal black line demarcates relative hypertension of MAP 150 mm Hg.*

***Table* 3.2*:*** *The results of a GLM assessing the effects of different factors on the parameters SIDa, SIG and A- of zoo felids under anaesthesia. Interpretation of p-values in terms of evidence for effect: p <0.001 indicates very strong evidence; p<0.01 strong evidence; p<0.05 moderate evidence, p<0.1 weak evidence; p>0.1 insufficient evidence.*

| Independent Variable and Covariates | Dependent Variable | DF | F - value | p-value |
|---|---|---|---|---|
| Time from Immobilisation | SIDa | 1 | .711 | .404 |
| | A- | 1 | 9.455 | .004 |
| | SIG | 1 | .981 | .328 |
| tMAP | SIDa | 1 | 1.395 | .245 |
| | A- | 1 | 10.382 | .003 |
| | SIG | 1 | 1.815 | .186 |
| Base Deficit (mmol/L) | SIDa | 1 | .376 | .544 |
| | A- | 1 | .013 | .911 |
| | SIG | 1 | 5.076 | .030 |
| Species | SIDa | 2 | 11.674 | <0.001 |
| | A- | 2 | 46.884 | <0.001 |
| | SIG | 2 | 17.391 | <0.001 |

The metabolic acid-base component (BD) generally contributed less acidity over time during the monitored period of anaesthesia (Figure3.5), but no meaningful patterns over time were observed for SIG and SIDa (Figures 3.6 and 3.7). BD occasionally exceeded -10.5 mmol/L (Figure 3.5) and many of these instances of metabolic acidosis were apparent prior to the administration of intravenous fluids, at a time when MAP of most of the individuals concerned were relatively high (Figure 3.1). SIG levels exceeded 5mmol/L in five individuals, two of which displayed these relatively high SIG levels during the early monitoring period, while in the other three cases, such levels developed as anaesthesia progressed (Figure 3.6). There was a wide apparent variation in SIDa, both within and between procedures (Figure 3.7).

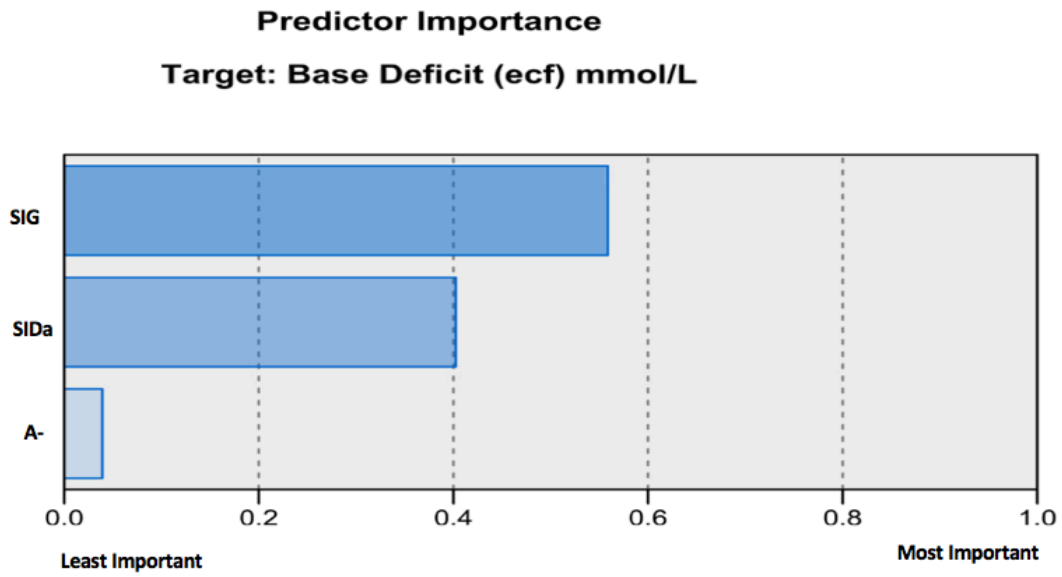


***Figure 3.2:*** *Results of Automatic Linear Modelling identifying factors that are the best predictors of Extracellular Base Deficit for lions under anaesthesia in the study, to accommodate for the obscuring effects of species as a predictor of this parameter.*

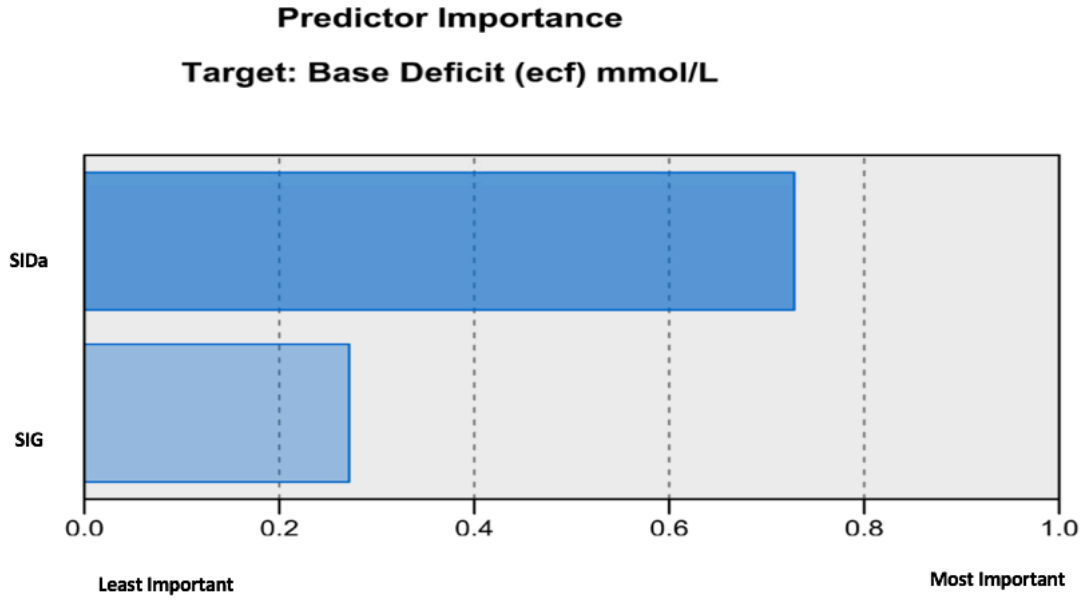


***Figure 3.3:*** *Results of Automatic Linear Modelling identifying factors that are the best predictors of Extracellular Base Deficit for tigers under anaesthesia in the study, to accommodate for the obscuring effects of species as a predictor of this parameter.*

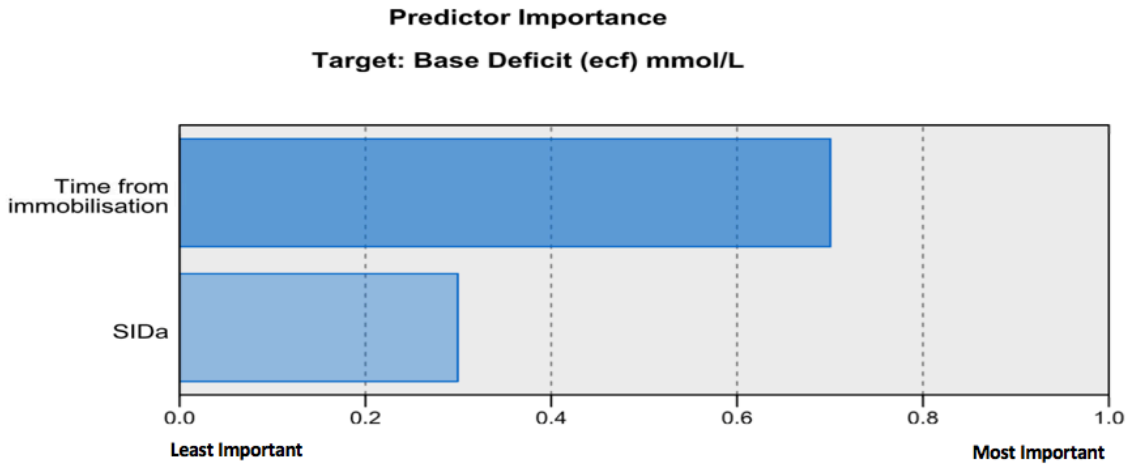


***Figure 3.4*** *Results of Automatic Linear Modelling identifying factors that are the best predictors of Extracellular Base Deficit for cheetahs under anaesthesia in the study, to accommodate for the obscuring effects of species as a predictor of this parameter.*

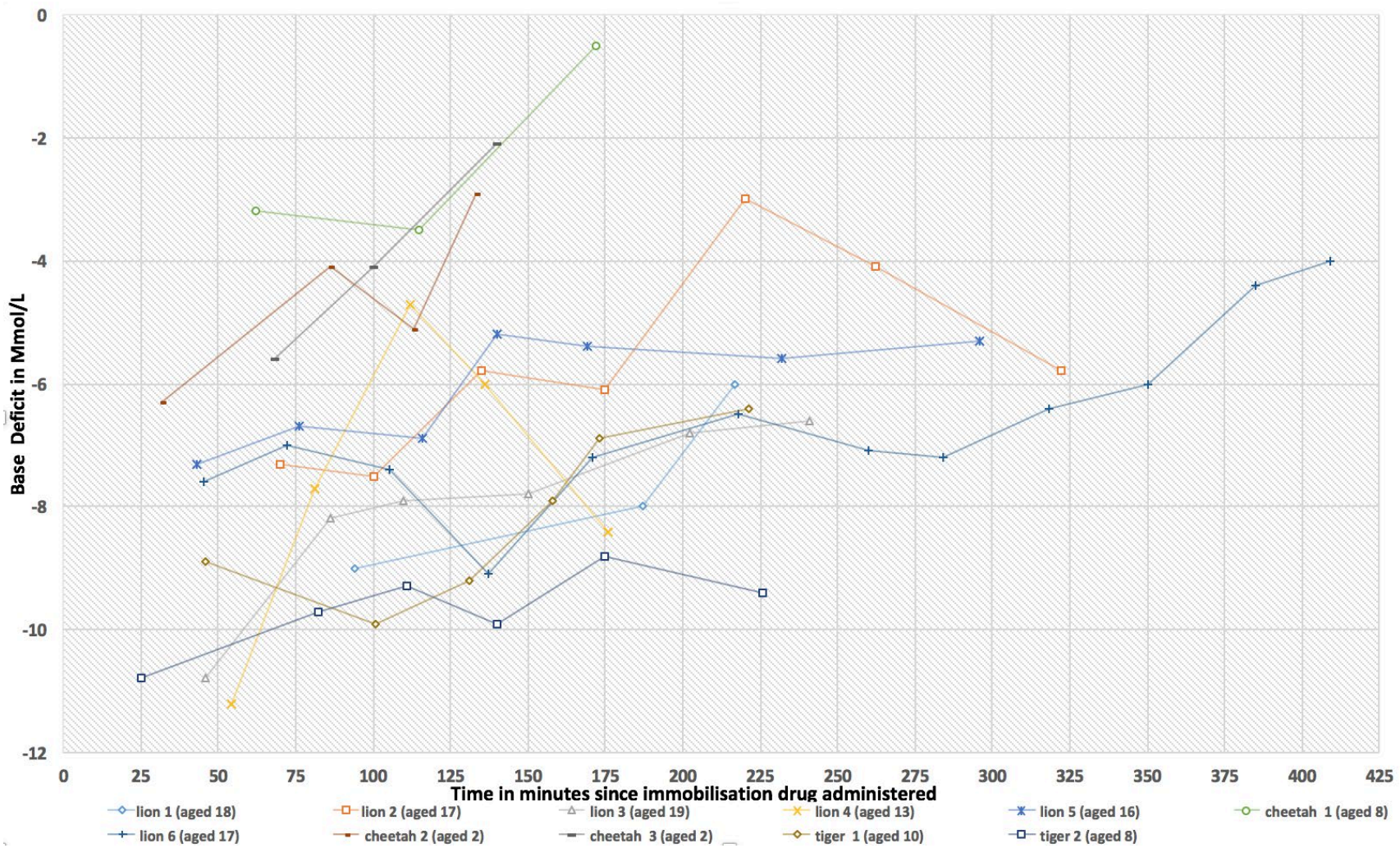


***Figure 3.5:*** *Extracellular Base Deficit (BD) changes over time during anaesthesia for each of the felids in the study.*

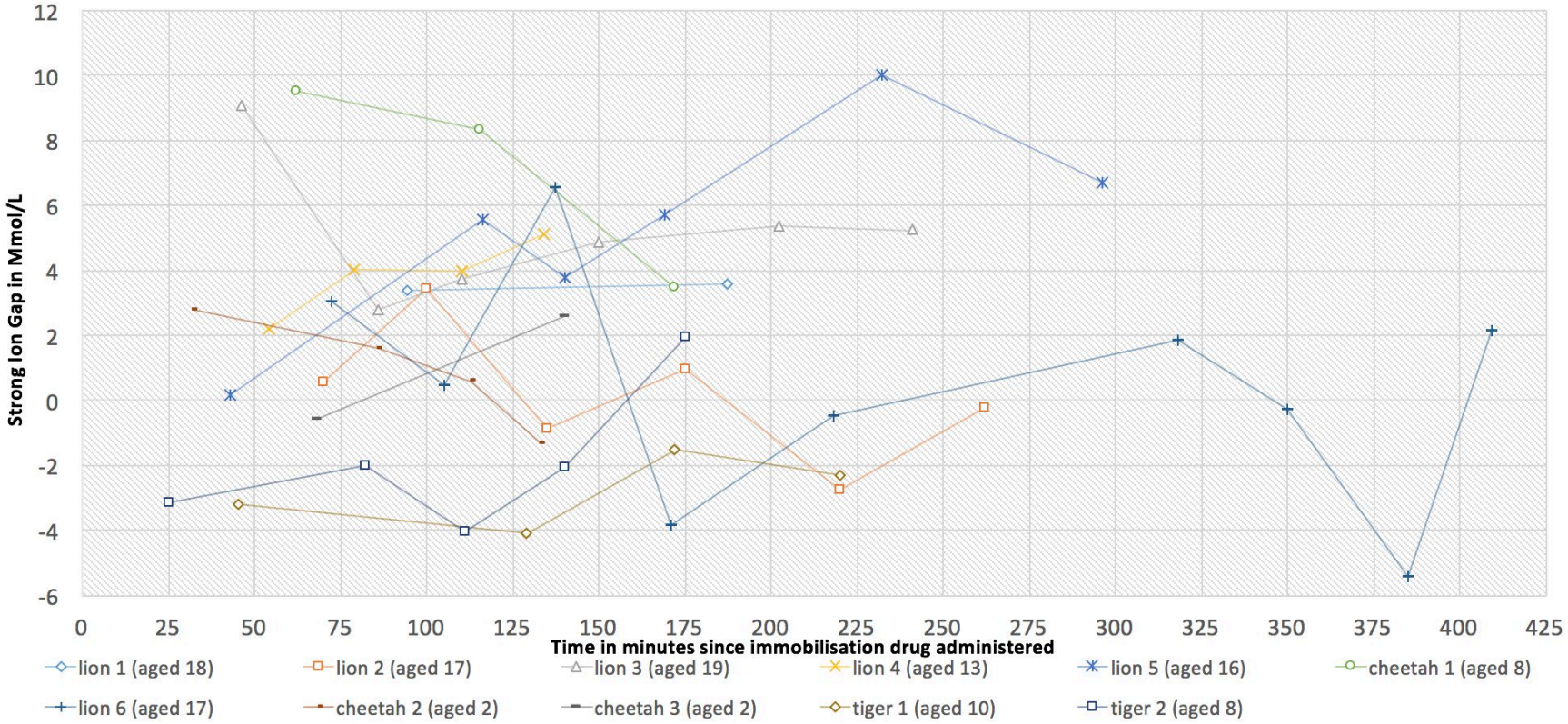


***Figure 3.6:*** *Strong Ion Gap (SIG) changes over time during anaesthesia for each of the felids in the study.*

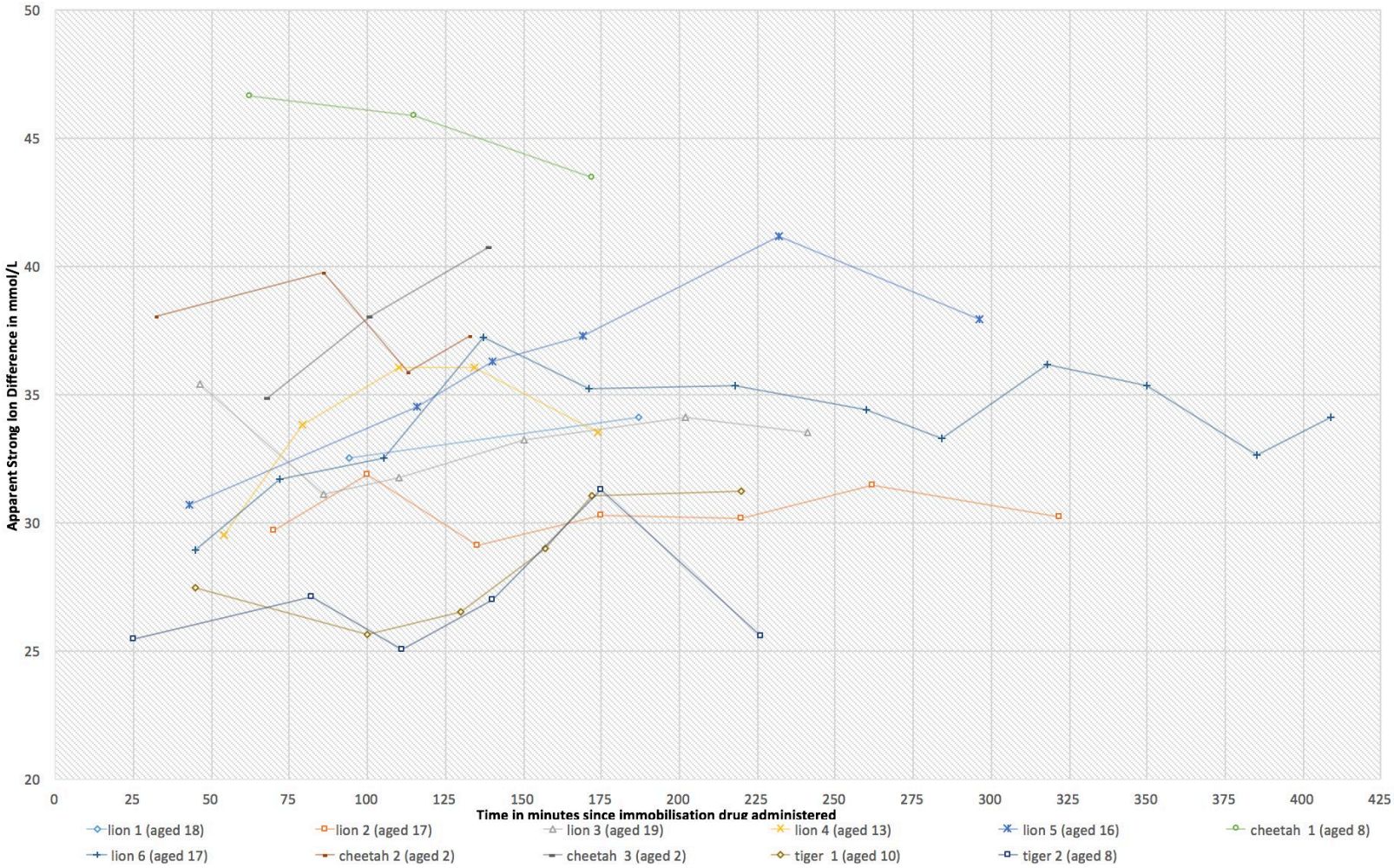


***Figure 3.7:*** *Graph showing Apparent Strong Ion Difference (SIDa) changes over time during anaesthesia for each of the felids in the study.*

## 3.4 Discussion:

This study aimed to characterise changes to metabolic acid-base status and particularly the UA contribution to such changes in captive large felids under anaesthesia. Its purpose was to assess whether metabolic acidosis and the UA contribution towards this condition are apparent enough to warrant further investigation of their causes. One application of such further research would be to assess whether serial UA measurement could augment haemodynamic monitoring as a means of identifying circumstances associated with clinically significant deteriorations in tissue blood flow under anaesthesia. Early identification of these changes allows the greatest chance of intervention during anaesthesia to limit or prevent tissue damage.

Metabolic acidosis was recorded frequently, per Hopper and Epstein's (2012) proposed definition of the state in domestic felids. BD in some of the study animals occasionally exceeded respective species mean and median venous whole blood values reported in the Species360® Global ZIMS database (accessed March 2020) for chemically restrained lions (ZIMS mean -8.7, median -9) and tigers (ZIMS mean -10, median -10), but not for cheetahs (ZIMS mean -6.7, median -7). Without recent pre-anaesthetic blood work, the effect of pre-anaesthetic health and physiological status on metabolic acid-base status during anaesthesia was unknown and training patients for conscious phlebotomy is strongly encouraged to assist in establishing pre-anaesthetic baselines for comparison.

The key finding of the study was that even within the group of large felids examined, there were marked species differences in acid-base status under anaesthesia, and further analysis showed that the drivers of the changes also varied between species. This finding is important to clinical zoo mammal anaesthesia because it seems likely that inter-species extrapolations of acid-base physiology will be flawed. While this does not diminish the value of applying acid-base monitoring to improve the safety of zoo animal anaesthesia, it does indicate that there is a strong need for readily accessible species-specific reference ranges.

We erroneously expected species within the family *Felidae* to share very similar acid-base physiology and respond similarly to the same challenges facing acid-base

homeostasis posed by anaesthesia. Very strong evidence for differences between species in all the contributors to BD may, in part, have reflected physiological differences between the species and thus different intrinsic responses to similar anaesthetic challenges. If so, differing species ecology and phylogenetic distance may underlie such differences, which are likely to be more pronounced between *Panthera* and *Acinonyx* genera than within the *Panthera* genus.

Some care is needed in evaluating this conclusion. The distribution of data pertaining to both tigers and cheetahs were discontinuous and not normally distributed, which is probably attributable to the much more limited representations of these species in the study. Unlike lions, data from these species conformed poorly to assumptions underpinning the validity of automatic linear modelling. Species differences may equally have reflected recruitment biases (in factors such as animal age and pre-anaesthetic clinical status) and differences in approach to the anaesthetic management of each species (including species bias in the qualification and experience of attending anaesthetists). Therefore, while there was strong evidence for our inferences from this study about species differences in acid-base physiology and responses to given anaesthetic challenges, further work should be carried out to compensate for the low power of our analysis.

Given the moderate strength of SIG as a predictor of BD in lions and the proportionately far greater representation of lions in the study, evidence for an effect of SIG on BD and absence of evidence of an effect of SIDa in the GLM may reflect such representational differences and obscuring species effects. It is also possible that the predictive strength of SIG for BD in lions may have reflected geriatric age bias in the lions recruited to the study. That neither SIG nor SIDa ever provided overwhelming strength in predicting BD imply that these parameters varied in direction of change and in proportionate contribution to metabolic acid-base status, especially since overall SID (and therefore BD) is largely a function of these two parameters (Kellum 2005a, Kellum 2005b).

Evidence suggesting UA accumulations did occur and that mixed metabolic states were apparent is supported by a few findings and observations in this study. Besides evidence for an overall effect of SIG on BD, these included the occasional inability of SIDa changes

to explain the direction of BD changes over time (see Figures 3.5 to 3.7), the instances in which metabolic acidosis were explained by relative increases in SIG and the number of cases displaying relatively marked elevation in SIG in spite of relative metabolic alkalosis. Interestingly, absolute SIG values or sudden SIG fluctuations exceeding 5mmol/L in this study are comparable in magnitude with those documented in hypoxic canine patients using similar assumptions and methods for calculating SIG (Bruegger et al 2007). Similarly, such magnitudes compare with absolute SIG values commonly documented in critically ill human patients (Story 2008). However, since SIG in states of health may vary between species and since ranges of pre-intervention anion gap (and/or SIG) values are rarely known for individual patients (Kraut and Madias 2007), it is difficult to interpret the significance of absolute values of anion gap or SIG for each individual (Wheeler and Kovacic 2017).

What may have been responsible for determining the SIDa in each of the felids was also difficult to ascertain from this study. As with SIG, acid-base determinations do not provide any mechanistic information about the processes determining the quantities of measured ions contributing to SIDa (Wooten 1999, Wooten 2003, Wooten 2004, Figge et al 2018). Furthermore, to better represent acid-base changes, more complex acid-base models than the plasma-based model used in this study may be required to control for fluid and electrolyte shifts occurring between body fluid compartments (Wolf and de Land 2011) and to ensure that extracellular base excess and SIDa are more invariant to carbon dioxide (Anstey 2010, Morgan 2011). Trans-erythrocytic chloride shifts in response to changing erythrocytic bicarbonate concentrations are important determinants of SIDa (de Morais and Leisewitz 2012, Johnson and de Morais 2012, de Caro Carella and de Morais 2017) and it is also likely that a combination of other causes of corrected chloride changes (including the possibility that SIDa is not independent of SIG) and free water changes also influenced SIDa at different times and to varying extents in different individuals. It is unknown to what extent, if any, differential rates of excretion of measured strong ions contributed to SIDa changes. While there was strong evidence for BD and time effects on A-, it is unlikely that A- was an important determinant of changes in BD, given the minor effects of weak acids on the dissociation of water (Stewart 1983, Kellum 2005a, Kellum 2005b) and given that weak acids varied

little in molar terms in comparison with SIDa and SIG variations (*data sheet supplied in Appendix A2*).

A number of explanations may be offered for absence of evidence for an effect of tMAP on SIG but without specific assessments of tissue blood flow, these remain speculative. Besides hypoxic states and/or uraemic waste accumulations, there are also many other causes of elevations in anion gap (Kraut and Madias 2007, Venkatesh and Morgan 2008, Artero 2017) and no attempt was made to inform of actual anion identity in this study. If other factors (including endogenous processes and/or the administration of exogenous substances) were important determinants of SIG, then SIG itself may have lacked specificity for the detection of hypoxic and/or uraemic states if they were occurring. Alternatively, SIG may not have borne any relation to perfusion-related capacities for maintaining acid-base homeostasis, tMAP may have provided a poor proxy for tissue blood flow, or no such compromises to blood flow were manifest in the study animals.

Limitations imposed by the observational nature of the study design may also have masked a possible association between SIG and tMAP. Wide SIG variations between individuals at initial EPOC monitoring, reflecting states of homeostasis prior to immobilisation and during early anaesthetic periods, will subsequently have influenced absolute SIG values during the ensuing monitoring period. If states of perfusion and renal function were playing a role in determining SIG over periods preceding particular measurements, it is possible that their effects on SIG may have endured or became manifest later in time. As such, association between tMAP and SIG may have been obscured by asynchrony between periods of major tMAP fluctuation and subsequent UA kinetics. A hypothesis deserving of testing in future single-species studies is that if both SIG and tMAP do bear relation to tissue perfusion, corresponding directions of tMAP fluctuations and SIG fluctuations would be expected to differ according to the particular blood pressure spectrum across which tMAP fluctuations are taking place, since alterations in tissue perfusion should be expected to become most manifest towards extremes of blood pressure.

If reduced blood flow does play a role in explaining rising SIG levels, the sensitivity of SIG

changes for identifying such altered states of blood flow may be hypothesised to improve in geriatric felids. Chronic renal disease is a significant cause of morbidity in aged captive felids (Wack 2008) and older animals are more likely than their younger counterparts to present with pre-anaesthetic hypertension and other pathologies associated with degenerative renal disease (Longley 2012). Already volume contracted patients may be much more susceptible to the vasodilatory effects of anaesthetics (Lawson and Hutton 2012, Barak et al 2015), rendering them more sensitive to hypotensive influences. It is also plausible that for geriatric patients, reduced renal abilities to excrete fixed acids (and possibly also to elicit increases in SIDa) may also arise for given reductions in blood flow, compared with younger animals which are more likely to possess less renal degenerative changes and more functioning nephrons.

While SIG was a better predictor of BD in lions (most of which were geriatric) and although geriatric animals appear to have displayed greater fluctuations and elevations in SIG than their younger counterparts (Figure 3.6), this cannot be taken as evidence in support of the proposed hypothesis, particularly given the low case number and significant representational bias in the species and age of animals recruited to the study. Interestingly, though, an eight-year old cheetah was the 'younger' exception displaying relatively marked SIG values and as a species, cheetah display demonstrably shorter longevity in captivity than lions and tigers (Species360® Global ZIMS data, accessed April 2020). Furthermore, in captive cheetah, Mitchell et al (2018) identified age and renal medullary fibrosis as primary factors influencing the pathogenesis of chronic renal disease, while Bolton and Munson (1999) found the severity of glomerulosclerosis to increase with age, occurring mainly in captive cheetahs exceeding seven years of age. Given their already high prevalence of renal disease (Munson 1993, Munson et al 1999), captive cheetah may benefit from and provide an informative focus for future studies investigating the determinants of SIG in large felids under anaesthesia.

While the goal of the study was to identify changes occurring under clinical anaesthesia management, it is not known whether (and if so, to what extent) the various efforts to restore blood pressure to more normotensive states may subsequently have influenced the progression of acid-base homeostasis. It is possible that such progression may have been very different had such interventions not occurred and without an experimental

study design, the influence of the restorative interventions themselves cannot be controlled for in assessing the relationship between blood pressure changes and acid-base homeostasis.

It should be borne in mind that the possibility of analyte measurement error may have generated varying degrees of imprecision in classifying contributory ions, leading to possible erroneous interpretation of the metabolic acid–base disorder (Gunnerson 2005). Bias and imprecisions associated with each extra analyte mean confidence intervals around SIG determination may be quite wide (Morgan 2004) and in this study, there was no opportunity to establish measurement-based variance in contributory parameters. Furthermore, SIG determination may also have been influenced by use of lithium-coated containers (Morgan 2004) and immediate point of care testing from non-heparinised syringes would be strongly recommended for future studies of this nature.

Drugs commonly used for immobilisation, induction and maintenance of anaesthesia of large felids are acknowledged for eliciting profound, dose and phase-dependent effects on various haemodynamic parameters (Scheinin et al 1989, Grimm et al 2001, Sinclair 2003, Saleh et al 2005). Anticipating how they interact to effect blood flow to lower priority organs such as the kidneys is not straightforward and finding a sensitive means to monitor and identify concerning changes in blood flow will help to associate these changes with anaesthetic events and identify better ways to manage anaesthesia of these animals. The possible impact of different anaesthetic regimens on renal blood flow has been studied specifically in domestic felids (Mitchell et al 1998) and large felids (Stagegaard et al 2017) and although the ultrasound-based approaches that were used are particularly valuable to research into the effects of anaesthetic interventions on renal perfusion, they probably hold little practical value as a routine means of monitoring in clinical zoo anaesthesia.

If domestic *Felidae* serve as a valid comparison, BD recorded in the Species360® ZIMS database support findings from this study to suggest that metabolic acidosis commonly occurs in chemically restrained or anaesthetised *Pantherae*. The prevalence of metabolic acidosis in conscious, captive large felids is unknown but results of this study suggest that its clinical significance under anaesthesia is deserving of further enquiry

and that further work to reveal the identity of normally unmeasured anions is warranted to help establish possible causes. More research is required to assess the value of serial SIG monitoring for the purpose proposed, with much transferable knowledge likely obtainable from anaesthesic studies involving species amenable to more robust study design and comprehensive data collection. Greater traction may, nevertheless, be gained from the application of other practical perfusion assessment techniques such as strategic oxygen extraction and arterio-venous carbon dioxide gradient determinations (Zhang and Vincent 1993, Kipnis et al 2012, Kipnis and Vallet 2016), to aid in matching anaesthetic events to perfusion endpoints. Such reference bases should then provide further means to assess quality of anaesthetic delivery and to refine anaesthetic techniques for improved patient safety.

## 3.5 Conclusions:

This study revealed marked species differences in the acid-base status of large felids under anaesthesia, with drivers of changes varying between species. This implies that inter-species extrapolations of acid-base physiology may be flawed, highlighting the need for readily accessible species-specific reference ranges to realise the value of acid-base monitoring as a means of improving the safety of zoo animal anaesthesia.

Metabolic acidosis was found to be common in the *Panthera* genus, if proposed definitions of the condition for domestic *Felidae* are also valid for *Pantherae*. Increased metabolic acidity could occasionally be attributable to relative increases in SIG, suggesting occasional UA accumulation, an inference supported by evidence for an effect of SIG on BD. There was an absence of evidence for any association between tMAP, as a crude proxy for blood flow, and SIG. However, many design limitations related to the study's observational nature, the limited number of animals recruited to the study and the marked species differences apparent in metabolic acid-base status limited the ability to make inferences about factors associated both with absolute SIG values and SIG fluctuations.

Contributors to SIG and determinants of anaesthesia-induced morbidity/mortality will both need to be better understood on a species-specific basis. Only with knowledge

relating determinants of SIG to such endpoints will it be possible to evaluate if unmeasured anion monitoring offers utility as a means to assess risks of patients developing anaesthesia-induced pathology.

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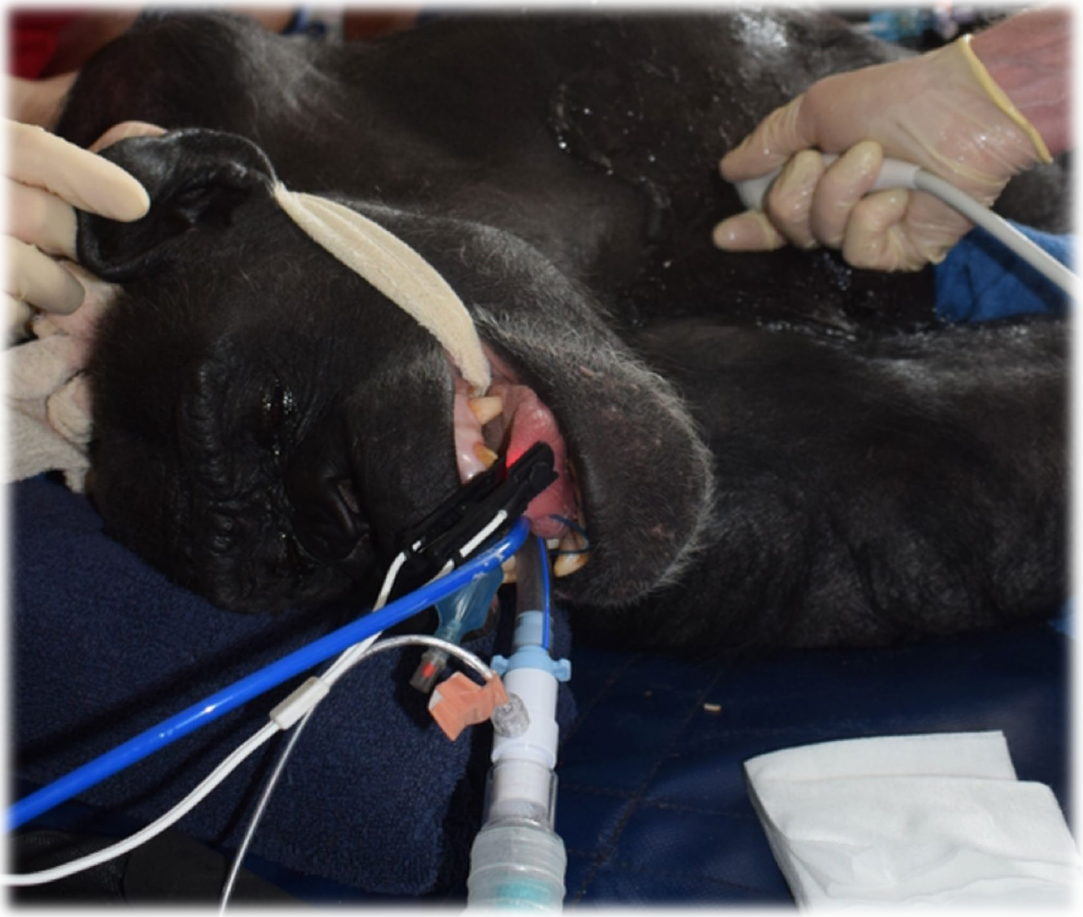
**Chapter Four:  General Discussion:**



## 1.1 Background:

Anaesthesia is not without risk. For dangerous zoo animals, additional risks to patients are associated with the precautions required to assure human safety. Evaluating the safety of anaesthetic approaches is not straightforward, given limitations associated with the measures used to assess physiological homeostasis, the range of potential parameters involved and the difficulties associated with relating disturbances to morbidity endpoints, particularly where subclinical injury is involved. Without recourse to experimental studies and meta-analyses to contribute to an evidence base, there are also particular challenges associated with attributing alterations in physiological parameters to particular interventions in zoo anaesthesia. Nevertheless, in zoo animal anaesthesia, much knowledge will necessarily have to be derived from observational studies in order to identify procedure-related refinements which enhance patient safety.

Safety-related evaluations have often been based on intermittent monitoring of a restricted range of physiological parameters, with choice usually predicated upon those most immediately pertinent to patient survival. This leaves uncertainty about the wider range of disturbances to homeostasis that patients may be experiencing. The broader the range of parameters which are serially monitored and related to each other, the more detailed will be the representation of physiological events occurring during anaesthesia and the better the prospect of being able to explain and mitigate the changes observed.

Consideration of the risk factors associated with alterations to physiological homeostasis is an equally important part of this explanatory process and besides the choice of immobilisation regimen, risk mitigation requires a better understanding of the factors most determinant of risk as anaesthesia is delivered. Few studies in the zoo medical literature have assessed patient support in zoo anaesthetic practice, highlighting the need to address how well anaesthesia is both monitored and delivered, and the safety implications of major procedure-related decisions made during the course of its delivery.

## 4.2 Reminder of Aims and Objectives:

The aims of the research presented in this thesis were:

- to identify homeostatic disturbances occurring in larger species of mammals under anaesthesia in zoo practice, in order to advance understanding about the safety of anaesthetic practices for zoo patients.

- to gain further insight into how and why such disturbances occur.

The practical application of this research is that a better understanding of the causes and mechanisms associated with the physiological disturbances identified should allow more effective measures to mitigate them, through refinements in both monitoring and procedural protocols.

These research aims were addressed by pursuing the following objectives:

1. To apply readily accessible techniques to serially monitor and describe the prevalence and extent of physiological disturbances occurring in large mammals during zoo anaesthesia practice;
2. To initiate examination of risk factors associated with these disturbances;
3. To describe and characterise serial alterations in metabolic acid-base and unmeasured ion status occurring in large felids during anaesthesia;
4. To assess whether unmeasured anion values are associated with mean arterial blood pressures in the anaesthetic period preceding blood sampling events;

## 4.3 Summary of Major Findings:

One of the key findings from the first part of this study was the strong evidence for the effects of time since immobilisation on a range of measures of physiological homeostasis that were monitored (Chapter Two). There were also marked individual or species differences evident in many of these parameters, representing important differences in species physiology that limits cross-species extrapolations when monitoring anaesthesia of this diverse group of animals. In some instances, involving closely related species,

strong evidence for time and species effects on parameters were apparent in spite of relatively minor variations in the immobilisation protocols used, as was the case within the *Panthera* species in the study.

In the second part of the study, which focussed on contributors to metabolic acid-base changes in anaesthetised large felids *(*Chapter Three*)*, a quarter of all blood samples showed a base deficit exceeding -7 mmol/L, with all such recordings involving the *Panthera* species (lions and tigers). If the definition proposed for this condition in domestic felids serves as a valid comparison to the *Panthera* species, this would suggest that metabolic acidosis is common in anaesthetised *Panthera*. Moderate evidence was established for an effect of strong ion gap (SIG) on base deficit (BD), while there was no evidence for an effect of mean arterial pressure (tMAP) on SIG. The key finding of this study was that even within the small group of large felids examined, there were marked species differences in acid-base status under anaesthesia, and further analysis showed that the drivers of the changes also varied between species. Unmeasured anion (UA) accumulations appeared to be occurring: SIG values above 5 mmol/L were recorded, sometimes in spite of concurrent base deficits being relatively alkalotic, and fluctuations of 5mmol/L or more occasionally occurred between successive EPOC measurements. Cautionary caveats should, nevertheless, be framed around inferences drawn from these study findings, given marked species differences and the possibility that representational bias and measurement-related errors may have influenced them.

While more an observation than a finding, the paucity of monitoring undertaken and documented even for routine physiological measures was notable for the zoo mammal anaesthetic procedures included in the study, relative to the monitoring undertaken during anaesthesia in many domestic animal settings. Such paucity was represented by the often protracted times taken to initiate the monitoring of many measures, the intermittence and briefness of their subsequent monitoring, or the fact that certain measures may not have been either monitored or recorded at all. Limitations to the extent of monitoring were attributable to the logistical constraints associated with the anaesthesia of dangerous zoo animals, together with mindfulness about procedure duration.

## 4.4 Implications of The Study Findings and Recommendations for Future Research:

Evidence for species and time effects on many of the measured parameters of homeostasis likely reflect a mix of influences, arising from individual and species differences in physiology, variations in pre-anaesthetic health status and differences in the way individuals were managed over each anaesthetic episode. These time effects and unexpected species effects were nevertheless evident following the use of carefully considered choices of immobilisation and induction protocols and sometimes profound disturbances to homeostasis were associated with their use. Such anaesthesia protocols have been applied widely and refined by multi-institutional experience and as such, strong empirical support has often been garnered for their purported safe use. This raises important questions about further scope for refinement of these regimens, the extents to which effects may primarily be attributable to the subsequent anaesthetic management of different individuals, and extents to which the homeostatic disturbances observed may be preventable.

With the exception of a repeat procedure on a baboon, which was euthanased for becoming and remaining comatose (and which was excluded from the overall analysis due to inclusion restrictions to initial procedures), no overtly apparent complications arose as a result of anaesthesia other than some protracted recoveries. Otherwise, it was not possible to ascertain the pathological significance, if any, of the alterations to homeostasis documented in this study. The dearth of reference intervals (as defined by Friedrichs et al 2012) also precluded examination of how much the parameters measured may have differed from healthy populations of comparable individuals. Nevertheless, if different mammals share similar tolerances to disturbances to homeostasis of similar magnitude, there are reasonable grounds to suggest that if progressive, some disturbances would have the potential to induce pathology and occasionally pose a risk to life. These disturbances included sustained relative hypotension below 60mm Hg, sustained relative hypertension above 150mm Hg, sustained relative hypercarbia above 65mm Hg and episodic but marked acidaemia, hypoglycaemia, hypoxaemia and hyperkalaemia. Identification of extreme oesophageal

lumen acidity (of pH 2) in a chimpanzee (*Pan troglodytes*) and difficulties with clearing regurgitated ingesta at the laryngeal entrance of a Hamadryas baboon (*Papio hamadryas*) at intubation provide illustration that despite the wide range of parameters that were measured, some potential disturbances to homeostasis and anaesthetic complications were not formally investigated or may not necessarily have been detected.

Amongst the most prevalent of the homeostatic alterations were instances of sustained relative hypercarbia, which were often associated with relative elevations in end tidal carbon dioxide and therefore likely attributable primarily to hypoventilation in such cases. While recognising the worth and the sometimes obligatory requirement to mechanically support ventilation, the haemodynamic impact of positive pressure ventilation may be potentially significant, further reducing cardiac output if hypovolaemic conditions are precipitated by anaesthesia and not duly recognised (Poor 2018). Further research comparing different approaches to mechanical ventilation in large mammal species is strongly warranted in a zoo context, particularly in terms of optimising the trade-off between the benefits of these techniques and the risks of unwanted consequences associated with their application.

Instances of mean arterial blood pressures falling below 65 mmHg were also not uncommon (and were sometimes sustained) among the disturbances documented. The best way to characterise hypotension is still debated even in human medicine (Salmasi et al 2017), but important guidelines have nevertheless been established from studies of cardiac and renal injury associated with it in humans and some domestic animals. While such guidelines may be more applicable to critical patients undergoing higher risk procedures (Meersch et al 2017), sustained hypotension of less than 55mm Hg is nevertheless considered by Clarke and Trim (2014) to be life threatening, although the veterinary context is not specified. Elevated risk of acute kidney injury has been associated in retrospective human studies with MAP less than 60mm Hg for more than 20 minutes and MAP less than 55mm Hg for more than 10 minutes (Meersch et al 2017). Salmasi et al (2017) even concluded that in human operative cases, associations exist between myocardial and renal injuries and sustained MAP falling under 65mm Hg,

without any important interaction with preoperative blood pressure. It is unknown what minimum acceptable MAP ranges may apply to different species of large zoo mammals under anaesthesia and given the critical importance of age as a risk factor for the development of perioperative hypotension in human anaesthesia (Cheung et al 2015), widely different guidelines may apply to aged or geriatric individuals.

Irrespective of the appropriate guidelines associated with their classification, the prevalence of relative hypotension and hypoventilation evident in this study suggests the need for much more critical scrutiny of the circumstances leading to these states. Although the sometimes intermittent nature and limited breadth of monitoring records were particularly apparent for many of the combined measures usually used to refine estimation of anaesthetic planes, such states probably resulted from the inclination to maintain anaesthesia towards deeper planes, the prolonged effects of medetomidine, or a combination of these factors.

Better mitigation of the risks associated with the development of these states therefore likely rests in large part with the ability to be confident with the accuracy of depth of anaesthesia estimation. The ability to provide more standardised measures of depth would also assist with comparing different approaches to anaesthetic management. Realisation of these objectives is challenging because of the different physiological and autonomic responses of different species to the same drugs, the different depths at which these responses may be manifest in different species (Clarke, Trim and Hall 2014) and because of the unique way that different drugs alter cardinal signs both in a particular species and among conspecific individuals (Taylor and Clark 2007, Hubbell and Muir 2009).

To address these issues in the zoo anaesthesia context, measures which relate plasma concentrations of anaesthetic drugs to pharmacodynamic effects will have important research value. Certain measures of electro-encephalographic activity may, with much further research, offer some clinical potential (Hatt and Jurado 2012), but they are currently unlikely to serve any immediate practical value. However, better assessment of anaesthetic depth in zoo mammals will depend on more frequently measuring and

recording of the various somatic and autonomic measures of anaesthetic depth and relating these to injectable drug dosing history and end tidal anaesthetic concentrations.

In this respect, heart rate and blood pressure monitoring are usually considered to provide an insensitive and unreliable indicator of anaesthetic depth. In human anaesthesia, for example, haemodynamic measures have been found to provide a poor indicator of the hypnotic-anaesthetic status of the patient (Struys et al 2002). In the veterinary context, cardiopulmonary effects are determined by the combination of pre-anaesthetic and anaesthetic agents used and their dose rates, making heart rates and blood pressure unreliable guides to depth of anaesthesia (Popilskis et al 2008, Trim et al 2014). Haskins (2015) also cautions that autonomic parameters may not change until after the anaesthesia level suddenly becomes too light and in piglets, for example, changes in heart rate and blood pressure were not considered as a good measure of depth of anaesthesia when evaluated immediately after application of a noxious stimulus (Jaber et al 2015). Similarly, in horses, heart rate and arterial blood pressure are considered by Trim (1998) to provide an unreliable guide to the depth of anaesthesia.

For the anaesthesia of dangerous zoo animals, with inclination to maintain patients at relatively greater anaesthetic depths for reasons of human safety (Hatt and Jurado 2012), the onus of monitoring may lean more towards assessing for impending responsiveness rather than on mitigating risks of emerging awareness. At these greater anaesthetic depths, the accurate, real-time and trend information provided by invasive arterial blood pressure monitoring may offer more usefulness as a means to monitor, respond to, and re-evaluate changes in anaesthetic depth. This is particularly the case when such measures are interpreted in the clinical context of the haemodynamic status of the patient and when they are related to likely levels of nociception or other forms of stimulation, such as patient repositioning. Typically, the lighter the anaesthetic plane, the higher the sympathetic activity (Ozeki and Caulkett 2014) and with the inclination to make large changes to anaesthetic drug delivery in response to perceived lightening of anaesthetic planes in these dangerous animals, such real-time, trend information may also reduce tendencies towards over-reactive responses by the anaesthetist,

contributing to overall management of anaesthesia at less physiologically challenging depths. Since physical signs such as movement and protective reflex responses also tend to become more depressed and less reliable during prolonged anaesthetic procedures (Hubbell and Muir 2009), the time taken to establish invasive blood pressure monitoring should become justifiable from an anaesthetic depth monitoring point of view when such circumstances are anticipated.

During emergence from general anaesthesia in humans, responsiveness to noxious stimuli precedes awareness (Ghoneim 2000) and return of brainstem function and associated reflexes which involve breathing, heart rate, blood pressure and return of muscle tone respectively follow an approximate caudal-cranial progression from the medulla through the pons to the midbrain (Reshef et al 2019). Similar sequential events should apply to zoological species and provided that these various brainstem-mediated reflexes are all carefully monitored in integrated fashion, subtle lightening of anaesthetic depth may be achievable and animals should not suddenly awaken from general anaesthesia to the point of posing immediate risk to personnel.

Another important emphasis of future research will be to identify and explore dose-sparing options for reducing required quantities of anaesthetics such as isoflurane and propofol, which have varying inhibitory effects on cardiorespiratory performance. Continuous rate infusions of phenyl-piperidine opioid derivatives such as remifentanil have been shown to have strong MAC-sparing effects in humans (Lang et al 1996), while the nonspecific plasma and tissue esterase metabolism, anti-nociceptive and non-cumulative effects of remifentanil make it a potentially useful adjunct to anaesthesia involving painful procedures in felids (Ferreira et al 2015). Nevertheless, its use in large felids would need to be carefully considered, given the potential for opioids to induce CNS stimulation in this taxon (Lamont and Grimm 2014).

Besides possible difficulties associated with re-establishing blood flow following microcirculatory collapse (Moore et al 2015), the dominant contribution made by venous vasodilation to the anaesthesia-driven condition of relative hypovolaemia is another important reason for the pre-emption and avoidance of hypotension. In human

anaesthesia, venous vasodilation may be relatively unresponsive to the use of vasopressors (Noel-Morgan and Muir 2018) and this may be equally relevant to zoo animal anaesthesia. As important, therefore, as the need to find ways to pre-empt hypotension are the initiatives directed towards its early recognition and treatment. Given the safety and other practical constraints associated with assuring early initiation and continuity of monitoring, its observed paucity and often protracted nature in this study is likely to reflect extents of monitoring in wider zoo anaesthesia practice. Anecdotally, primates in this study displayed early tendencies towards hypotension, with relatively slow responses evident with the initiation of vasopressor support. Wherever possible, more timely initiation of monitoring would be strongly advised.

Sustained mean arterial pressures at the higher end of the blood pressure spectrum were also documented in this study (sometimes exceeding 170mm Hg), but the relevance of this finding as a possible influence on tissue perfusion is uncertain. More hypertensive blood pressures may not necessarily translate into normal perfusion when alpha-two agonists are responsible for the higher blood pressure. Although the effects of medetomidine on renal arterial blood flow in dogs have been shown to be much less pronounced than in other abdominal organs (Miño et al 2008), variations in its effects on renal blood flow and glomerular filtration rate have been found in different studies (Kushiro-Banker et al 2013). These variations may reflect the different drug regimens used, with the dosage and route of administration of medetomidine influencing its biphasic effects on blood pressure (Saleh et al 2005). Furthermore, glomerular blood flow may not equate with distal renal parenchymal flow, particularly as the effects of alpha-2 agonist vasoconstriction in the kidneys are mediated primarily through efferent arterioles (Kushiro-Banker et al 2013). As such, it is possible that increases in glomerular blood flow and glomerular filtration rates in states of hypertension may be associated with concomitant reductions in renal medullary blood flow.

This study highlighted a need to investigate practical means of improving the detection of hypo-perfusion in the zoo anaesthesia context. In this regard, assuming measurement artefact is ruled out, explanations are still required for the apparent accumulations of unmeasured anions documented for many of the felids in this study, particularly those

cases which developed this accumulation subsequent to the first monitoring period. While apparent high levels recorded at first monitoring may still reflect physiological states prior to immobilisation and while measures of homeostasis should always be interpreted to allow for the appropriate physiological response to the anaesthetic and surgical context (Hall and Desborough 1996, Khokhlova et al 2017), those accumulations developing subsequently have more likelihood of being directly attributable to the immobilisation and anaesthetic events themselves.

Although this study found no evidence of an association between tMAP and SIG, much further work will be required to evaluate whether or not particular relationships exist between the direction of fluctuation of SIG levels and those of MAP fluctuations, depending on the particular blood pressure spectrum across which MAP fluctuations are taking place. Much more accurate representations of perfusion states than those provided by blood pressure may be required to evaluate whether or not any association exists between changes in SIG levels and corresponding states of blood flow. In the zoo context, it is recommended that future safety-oriented research focuses on the strategic measurement of parameters such as arterio-venous carbon dioxide gradients (as proposed in Chapter Three), as these may provide clinically more practical and superior means to gauge the adequacy of perfusion than the sole or combined monitoring of various haemodynamic measures and unmeasured anion levels.

Until these more accurate representations of underlying perfusion states are combined with research to unravel the identity of unmeasured ions and characterise the underlying biochemical mechanisms associated with them, it will not be possible to draw firm conclusions about the biological significance of the apparent unmeasured anion accumulations in this study. In the interim, there is a need to rule out possible underlying perfusion-related causes in circumstances where the direction of apparent strong ion difference changes do not explain major base deficit exacerbations. Confounding influences (species, time and age effects) precluded evaluation of the predictive value of the anion gap and the sodium: chloride ratio as clinically accessible and practical means to identify more marked elevations in SIG at given base deficits. Our results show that further evaluation of these parameters in zoo animals will need to be

carried out on individual species under more controlled conditions than this observational study allowed.

Limited address was given to factors which may have been associated with apparent strong ion difference (SIDa) fluctuations in this study, though it is acknowledged that differential excretion of measured strong ions provides a principal means by which the kidneys and liver in particular moderate acid-base homeostasis. As such, if changes to SIDa do in any way reflect acute changes in the function of regulatory organs, this would need to be better accommodated for in future studies, particularly as there is emerging evidence to suggest that the kidneys may begin to alter ammonium kinetics and effect SIDa changes within minutes (Weiner and Verlander 2017). Fractional urinary excretion studies would be required to assess for acute alterations in their function (Maciel et al 2013) and the techniques required to achieve this in real-time are very unlikely to be applied in zoo animals.

Evidence for the effect of time under anaesthesia on many of the parameters measured in the grouped species analysis was difficult to interpret without corresponding information about the pre-anaesthetic physiological status of each animal to provide some indication of the overall effects of interventions. Furthermore, the stress imposed on an animal during interventions including from restraint, anaesthesia and surgery may have an effect on clinical parameters under study (Reichard 2008), particularly as anesthetic agents can directly alter tissue metabolism (Khokhlova et al 2017) and neuro-endocrine stress responses to surgical interventions (Hall and Desborough 1996). Given these well described physiological responses to the contexts of anaesthesia and surgery, there is a lack of knowledge in zoo species about the magnitude of physiological variables that may be considered appropriate to the clinical circumstances of anaesthesia, rendering it more difficult to determine the point at which measured clinical parameters become inappropriate.

It was also difficult to match changes to the chronology of interventions and possible underlying processes, making it problematic to assess which of the immobilisation, induction, maintenance and recovery components of the anaesthetic process may have

generated the greatest challenges to homeostasis. Parameters of physiological homeostasis would nevertheless be expected to remain relatively unchanged (or improve) over the subsequent course of anaesthesia under circumstances where delivery either supports homeostasis or poses little physiological challenge. As such, if the variety of parameters measured in this study provide timely and sensitive representation of the physiological conditions immediately preceding them, they should collectively provide some representation of the stability of anaesthesia delivered.

Anecdotally, certain individuals did appear to display markedly more stable parameters under anaesthesia than others and comparison of the circumstances involved may provide insight into possible causes. A standout example of a relatively stable anaesthesia involved a thirteen year-old Malayan sun-bear (*Helarctos malayanus*), for which relatively normotensive blood pressure ranges were maintained without any need for vasopressor support, in spite of the long duration the animal had been placed under anaesthesia (*data sheet supplied in Appendix A1*). Relative normocarbia (or slight hypocarbia) was consistently achieved and relatively less base deficit variation and much more minor changes in creatinine were also apparent, compared with other animals in the study (*data sheet supplied in Appendix A1*). It should, however, be noted that in one instance, blood pH varied very little in the sun-bear concerned, in spite of an episodic, marked increase in sodium to chloride ratio. Since pCO2 and weak acid concentrations remained stable at the time, this may suggest that relatively significant unmeasured ion accumulations may nevertheless still have been occurring (*data sheet supplied in Appendix A2*). In this patient, a ventilator was used very promptly after the initial approach, while response to lightening planes of anaesthesia involved strategic propofol use and local nerve blocks to reduce the amount of isoflurane delivered.

One of the practical applications of the research in this study was towards identification of safer ways to manage anaesthesia. Many management questions or clinical conundrums require addressing, with some pressing examples being proposed in the second chapter. The ability to address these questions in zoo animal anaesthesia departs greatly from the kind of clinical trials and meta-analyses that are able to support evidence based decision making in human anaesthesia. In the latter case, while major

areas of uncertainty are still strongly apparent, it is nevertheless still possible to specify clinical contexts where available evidence favours one approach over another (Fleischer 2013).

In zoo animal anaesthesia, it is rarely possible to match morbidity endpoints to specific anaesthetic actions. The sample size of this current study was far too small to permit investigation of the many risk factors that were considered *a priori* for their potential role in influencing homeostasis over the course of anaesthesia. Nevertheless, it was still possible to review the small dataset and begin the process of comparative assessment which may yield important retrospective information about risk factors most deserving of further enquiry. In contrast to the relatively stable sun-bear procedure that was referred to previously, certain procedures stood out for the diversity of changes to measures of physiological homeostasis. These cases displayed varying constellations of elevated pCO2, elevated alveolar to arterial (or venous) carbon dioxide gradients, prolonged hypotension, elevations in blood lactate, anion gap, potassium and creatinine, and declines in blood pH and base excess (*data sheet supplied in Appendix A1*).

Although many factors other than the quality of anaesthetic delivery may have been responsible for these inter-procedural differences, such factors are less likely to be as convincing at explaining why particular groups of disturbances were more or less apparent (or absent altogether) in different procedures involving the same individuals. More systematic collection of information about homeostasis and application of the investigatory methods to a large scale database may retrospectively permit improvements in anaesthetic quality and outcome. As such a database is built, it may also become possible to compare and relate anaesthetic progression in large zoo mammals with progression in domestic animals which share similar size-related anaesthetic challenges. In this regard, equine anaesthesia may serve as a useful comparative model for many zoo animals, as there is more scope to anaesthetise horses well in comparison with zoo animals which are of wild and more often of dangerous disposition. Furthermore, relationships between the progression of homeostatic

parameters and outcome in horses are better understood, permitting risk categories for specific outcomes to be identified that may have relevance for species held in zoos.

## 4.5 Conclusions:

While the particular protocols which were chosen by anaesthetists in this study may represent current best practices that have been refined through multi-institutional experience, the findings of the study emphasise the need to appreciate the very unique response each individual is likely to have to any particular immobilisation and anaesthetic induction protocol. There is a need to be careful with preconceptions about the expected progression of anaesthesia for any given protocol and patient risk category. The question then follows of how much variability is attributable to the patient and how much relates to the manner in which anaesthesia is delivered. This study illustrates that even with the protocols widely considered to represent current best practice, some clinically important physiological disturbances were occurring in zoo animal anaesthesia that were considered to have successful post-anaesthetic outcomes. None of the disturbances documented in the data chapters were associated with mortality or any overt morbidity and thus no conclusions can be drawn from the data presented in them about zoo anaesthesia safety. Nevertheless, if we accept that our goal should be to refine and improve zoo animal anaesthesia practice towards minimising risk of patient harm, then these disturbances should not be accepted as routine and much benefit is likely to be derived from better understanding of the reasons for their occurrence.

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**Appendices:**

**Appendix A1:**

***Data sheet showing physiological, blood gas (EPOC) and laboratory clinical pathology parameters for all procedures covered under Massey University MUAEC Permit 17/50 and special permit for non-human hominids granted by the Director General of the New Zealand Ministry of Primary Industries. Only first procedures are included in the analysis (Chapter Two).***

| Animal ID | Procedure number | age | weight Kg | Induction Agents | Time from immobilisation | Iso or TIVA or etorphine? | nearest temperature to EPOC | Nearest Respiratory Rate to time of EPOC | Nearest Heart Rate to time of EPOC | Nearest MAP to time of EPOC | nearest ETCO2 to time of EPOC | Arterial (A) or venous (V) | pH | pCo2 mmHg | pO2 mm hg | cHCo3- mmol/L | BE (ecf) mmol/L | cSO2 % | Na+ mmol/L | K+ mmol/L | Ca++ mmol/L | Cl- mmol/L | cTCO2 mmol/L | Anion Gap K mmol/L | Hct % | cHgb g/dL | Glucose mmol/L | Lactate mmol/L | Creatinine micromol/L | SDMA NZVP micrograemmes/dL | CK NZVP IU/L @ 37C | Total Protein NZVP g/L | Albumin NZVP g/L | Globulin NZVP g/L | BUN (Urea) NZVP mmol/L | Creatinine NZVP micromol/L | Phosphate (PO4-) NZVP mmol/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lion 1 | 1 | 18.5 | 136 | MeK | 94 | Iso | 38 | 20 | 58 | 75 | 32 | V | 7.265 | 40 | 45 | 18.2 | -9 | 74 | 151 | 4.8 | 1.32 | 124 | 18 | 15 | 30 | 10.2 | 2.1 | 0.63 | 309 | 24 | 166 | 80 | 29 | 51 | 18.9 | 339 | 1.91 |
| lion 1 | 1 | 18.5 | 136 | MeK | 187 | Iso | 37.7 | 20 | 60 | 64 | 35 | V | 7.195 | 52.5 | 37 | 20.3 | -8 | 57 | 151 | 4.6 | 1.32 | 122 | 22 | 13 | 30 | 10.2 | 3.9 | 0.79 | 282 | 24 | 151 | 76 | 27 | 49 | 18.5 | 345 | 1.96 |
| lion 1 | 1 | 18.5 | 136 | MeK | 217 | Iso |  | 20 |  |  | 32 | V | 7.209 | 54.3 | 53 | 21.7 | -6 | 79 |  |  |  |  |  |  |  |  |  |  |  | 26 | 179 | 69 | 24 | 45 | 18.4 | 350 | 2.15 |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 40 | TIVA | 34.7 | 12 |  |  |  | V | 7.299 | 60.5 | 129.1 | 29.7 | 3.3 | 98.5 | 135 | 4 | 1.34 | 95 | 31.6 | 14 | 31 | 10.6 | 7.7 | 0.78 | 106 |  |  |  | 24 |  |  |  |  |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 56 | TIVA | 34.4 | 10 | 28 | 88 |  | V | 7.324 | 53.2 | 142.2 | 27.7 | 1.7 | 98.9 | 134 | 3.8 | 1.37 | 95 | 29.3 | 15 | 30 | 10.2 | 8.8 | 0.8 | 107 |  | 167 |  | 24 |  |  | 140 | 1.73 |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 81 | TIVA | 34 | 10 | 28 |  |  | V | 7.314 | 62.7 | 138.6 | 31.9 | 5.7 | 98.8 | 134 | 3.7 | 1.38 | 92 | 33.8 | 14 | 30 | 10.3 | 10.4 | 0.75 | 108 |  | 175 |  | 23 |  |  | 149 |  |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 112 | TIVA | 34.2 | 12 | 28 |  |  | V | 7.328 | 62.5 | 96 | 32.8 | 6.8 | 96.6 | 135 | 3.8 | 1.34 | 94 | 34.7 | 12 | 31 | 10.5 | 10.6 | 0.89 | 108 |  |  |  | 25 |  |  |  |  |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 127 | TIVA | 34.1 | 12 | 28 |  |  | V | 7.326 | 61.8 | 85.5 | 32.3 | 6.3 | 95.2 | 135 | 3.7 | 1.32 | 88 | 34.2 | 18 | 31 | 10.4 | 11.1 | 0.97 | 114 |  |  |  | 26 |  |  | 137 |  |
| rhino 1 | 3 | 18.0 | 1915 | DMiBK | 139 | TIVA | 34.1 | 12 | 28 |  |  | V | 7.353 | 59.2 | 89.4 | 32.9 | 7.4 | 96.1 | 135 | 3.8 | 1.24 | 93 | 34.7 | 13 | 31 | 10.5 | 9.6 | 0.95 | 112 |  | 173 |  |  |  | 5.1 |  |  |
| lion 4 | 1 | 13.8 | 192 | MeK | 54 | Iso | 36.5 |  |  |  | 24 | V | 7.214 | 41.1 | 64.3 | 16.6 | -11.2 | 87.4 | 153 | 4.2 | 1.32 | 127 | 17.9 | 14 | 53 | 17.9 | 4.9 | 2.04 | 185 |  | 108 | 79 | 31 | 48 | 12.8 | 189 | 1.58 |
| lion 4 | 1 | 13.8 | 192 | MeK | 79 | Iso | 37.2 | 16 | 49 | 94 | 30 | V | 7.316 | 36.2 | 212.7 | 18.5 | -7.7 | 99.7 | 153 | 4.3 | 1.28 | 124 | 19.6 | 15 | 45 | 15.4 | 9.4 | 0.8 | 181 |  | 100 | 74 | 30 | 44 | 13 | 183 | 1.82 |
| lion 4 | 1 | 13.8 | 192 | MeK | 110 | Iso | 37.4 | 12 | 46 | 93 | 27 | A | 7.302 | 43.7 | 583.7 | 21.6 | -4.7 | 100 | 152 | 4.5 | 1.33 | 121 | 23 | 14 | 38 | 12.8 | 12.6 | 0.77 | 202 |  | 116 | 69 | 27 | 42 | 13 | 187 | 1.85 |
| lion 4 | 1 | 13.8 | 192 | MeK | 134 | Iso | 36.4 | 13 | 46 | 79 | 29 | A | 7.301 | 41.4 | 550 | 20.4 | -6 | 100 | 150 | 4.7 | 1.21 | 119 | 21.7 | 15 | 39 | 13.4 | 16.8 | 0.85 | 219 |  | 115 | 66 | 27 | 39 | 12.7 | 185 | 1.87 |
| lion 4 | 1 | 13.8 | 192 | MeK | 174 | Iso | 36.5 | 13 | 46 | 81 | 33 | A | 7.272 | 40 | 548 | 18.4 | -8.4 | 100 | 148 | 5.3 | 1.26 | 120 | 19.7 | 15 | 39 | 13.4 | 20.9 | 1.06 | 191 |  |  |  |  |  |  |  |  |
| baboon 1 | 1 | 15.9 | 39 | MeK | 30 | Iso | 37.8 | 16 | 84 | 63 | 60 | V | 7.362 | 55.2 | 51.1 | 31.3 | 5.9 | 83.5 | 143 | 3.5 | 1.24 | 102 | 33 | 13 | 53 | 17.9 | 11.4 | 1.83 | 77 |  | 213 |  | 32 |  |  | 96 |  |
| baboon 1 | 1 | 15.9 | 39 | MeK | 72 | Iso | 36.7 | 21 | 65 | 50 | 57 | V | 7.339 | 61.8 | 34.8 | 33.3 | 7.5 | 61 | 143 | 3.4 | 1.23 | 102 | 35.2 | 11 | 49 | 16.7 | 12.1 | 1.08 | 83 |  |  |  |  |  |  |  |  |
| baboon 1 | 1 | 15.9 | 39 | MeK | 102 | Iso | 36.2 | 22 | 62 | 66 | 66 | V | 7.303 | 70.5 | 93 | 34.9 | 8.6 | 95.9 | 143 | 3.6 | 1.22 | 100 | 37.1 | 12 | 52 | 17.7 | 12.8 | 1.18 | 91 |  |  |  | 39 |  |  |  |  |
| baboon 1 | 1 | 15.9 | 39 | MeK | 130 | Iso | 36 | 18 | 65 | 63 | 60 | V | 7.332 | 70 | 58.1 | 37.1 | 11.2 | 86.4 | 143 | 4 | 1.2 | 102 | 39.2 | 8 | 50 | 17.1 | 12.1 | 1.07 | 93 | 10 | 145 | 59 | 24 | 35 | 6 | 65 | 1.55 |
| baboon 1 | 1 | 15.9 | 39 | MeK | 156 | Iso | 36.1 | 18 | 65 | 58 | 61 | V | 7.339 | 65.7 | 62.7 | 35.3 | 9.5 | 89.1 | 143 | 4.6 | 1.23 | 102 | 37.3 | 10 | 52 | 17.7 | 10.5 | 1.2 | 87 |  |  |  | 29 |  | 7.4 | 74 | 1.94 |
| baboon 1 | 1 | 15.9 | 39 | MeK | 179 | Iso |  | 26 |  |  |  | V | 7.329 | 66.9 | 43.1 | 35.2 | 9.2 | 73.1 | 143 | 4.7 | 1.22 | 103 | 37.2 | 9 | 49 | 16.5 | 10.5 | 1.03 | 103 |  |  |  |  |  |  |  |  |
| lion3 | 1 | 19.0 | 117 | MeK | 46 | Iso | 36.8 | 24 | 60 | 137 | 34 | V | 7.294 | 32.3 | 51.5 | 15.7 | -10.8 | 82.7 | 154 | 4.1 | 1.39 | 123 | 16.7 | 19 | 46 | 15.8 | 6.8 | 1.11 | 272 | 20 | 118 | 84 | 29 | 55 | 12.7 | 277 | 1.66 |
| lion3 | 1 | 19.0 | 117 | MeK | 86 | Iso | 36 | 12 | 59 | 92 | 25 | V | 7.317 | 34.9 | 60.6 | 17.9 | -8.2 | 89 | 152 | 3.9 | 1.38 | 125 | 19 | 13 | 41 | 14 | 7 | 1.19 | 249 | 19 | 135 | 84 | 28 | 56 | 13.2 | 261 | 1.66 |
| lion3 | 1 | 19.0 | 117 | MeK | 110 | Iso | 36.1 | 12 | 47 | 70 | 21 | V | 7.294 | 38.2 | 55.2 | 18.6 | -7.9 | 85.1 | 153 | 3.9 | 1.37 | 125 | 19.8 | 13 | 36 | 12.3 | 6.9 | 1.51 | 243 | 17 | 167 | 76 | 25 | 51 | 12.7 | 262 | 1.62 |
| lion3 | 1 | 19.0 | 117 | MeK | 150 | Iso | 34.9 | 8 | 52 | 54 | 27 | V | 7.277 | 40.7 | 87.9 | 19 | -7.8 | 95.4 | 154 | 4.1 | 1.35 | 125 | 20.2 | 14 | 31 | 10.7 | 6.9 | 1.23 | 328 | 20 | 270 | 72 | 24 | 48 | 12.9 | 270 | 1.71 |
| lion3 | 1 | 19.0 | 117 | MeK | 202 | Iso | 36.2 | 12 | 52 | 61 | 27 | V | 7.295 | 40.5 | 73 | 19.7 | -6.8 | 92.7 | 153 | 4.2 | 1.35 | 123 | 21 | 15 | 34 | 11.6 | 6.5 | 1.43 | 288 | 19 | 516 | 68 | 23 | 45 | 11.7 | 261 | 1.67 |
| lion3 | 1 | 19.0 | 117 | MeK | 241 | Iso |  |  |  |  |  | V | 7.307 | 39.3 | 67.5 | 19.7 | -6.6 | 91.3 | 153 | 4.3 | 1.43 | 124 | 20.9 | 14 | 29 | 9.8 | 6.8 | 1.2 | 238 |  | 599 |  | 21 |  |  | 273 | 1.71 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 17 | Iso |  | 20 |  |  |  | V | 7.395 | 59.4 | 55.3 | 36.3 | 11.4 | 87.1 | 143 | 3.5 | 1.06 | 101 | 38.1 | 9 | 51 | 17.5 | 9 | 1.59 | 126 |  | 275 |  | 34 |  |  |  | 1.54 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 67 | Iso | 36.6 | 6 | 52 | 37 | 30 | V |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 79 | Iso | 36.4 | 6 | 54 | 59 | 33 | A |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 89 | Iso | 36.2 | 6 | 58 | 77 | 36 | A | 7.47 | 48.8 | 499.8 | 35.5 | 11.9 | 100 | 148 | 2.6 | 1.09 | 104 | 37 | 11 | 43 | 14.8 | 7.2 | 1.28 | 108 | 12 | 246 | 78 | 30 | 48 | 9.4 | 118 | 1.42 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 99 | Iso | 35.9 | 7 | 60 | 95 | 37 | V | 7.407 | 58.9 | 51.8 | 37.1 | 12.4 | 85.2 | 146 | 2.9 | 1.11 | 103 | 38.9 | 9 | 48 | 16.2 | 6.3 | 1.73 | 131 | 13 | 334 |  | 30 |  | 11.1 | 111 | 1.64 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 118 | Iso | 35.6 | 8 | 55 | 45 | 36 | A | 7.465 | 49.7 | 503.8 | 35.8 | 12 | 100 | 148 | 2.9 | 1.16 | 103 | 37.3 | 12 | 47 | 15.8 | 3 | 1.33 | 132 | 15 | 432 |  | 31 |  | 12 | 114 | 1.72 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 141 | Iso | 35.4 | 6 | 59 | 78 | 33 | A | 7.467 | 48.4 | 529.6 | 35 | 11.3 | 100 | 150 | 2.7 | 1.13 |  | 36.5 |  | 43 | 14.8 | 1.8 | 1.29 | 111 | 12 | 322 | 70 | 28 | 42 | 8.2 | 105 | 1.15 |
| chimp 1 | 1 | 39.6 | 86 | MeKMi | 152 | Iso | 35.7 | 6 | 61 | 138 | 32 | V | 7.435 | 53 | 57.2 | 35.6 | 11.4 | 89.5 | 148 | 2.8 | 1.11 | 104 | 37.2 | 11 | 43 | 14.8 | 2.4 | 1.58 | 133 |  | 455 |  | 29 |  | 11.3 | 114 | 1.63 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 43 | Iso |  |  |  |  | 27 | V | 7.278 | 41.5 | 71.5 | 19.4 | -7.3 |  | 151 | 4.3 | 1.1 | 123 | 20.7 | 13 |  |  | 7 | 2.71 | 305 | 21 | 299 | 77 | 32 | 46 | 10.3 | 307 | 1.52 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 76 | Iso | 40 | 7 | 56 | 84 | 30 | A | 7.372 | 31.9 | 457.6 | 18.6 | -6.7 | 100 | 152 | 4.8 | 1.2 | 126 | 19.5 | 12 | 44 | 14.9 | 10.1 | 0.42 | 335 |  |  |  |  |  |  |  |  |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 116 | Iso | 38.6 | 7 | 59 | 81 | 30 | V | 7.325 | 36.6 | 294.5 | 19.1 | -6.9 |  | 152 | 4.5 | 1.29 | 121 | 20.2 | 16 |  |  | 9.7 | 2.28 | 273 | 19 |  | 66 | 27 | 39 | 10 | 312 | 1.47 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 140 | Iso | 37.8 | 7 | 53 | 107 | 29 | V | 7.273 | 46.9 | 50.2 | 21.7 | -5.2 | 79.8 | 152 | 4.8 | 1.25 | 121 | 23.1 | 14 | 37 | 12.6 | 10.6 | 0.8 | 288 | 19 | 291 | 73 | 30 | 43 | 11.2 | 347 | 1.64 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 169 | Iso | 37.8 | 7 | 60 | 113 | 30 | V | 7.261 | 48.1 | 42.2 | 21.6 | -5.4 |  | 151 | 4.8 | 1.3 | 119 | 23.1 | 15 |  |  | 11.4 | 0.84 | 319 | 21 | 287 | 67 | 28 | 39 | 9.8 | 310 | 1.47 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 232 | Iso | 37.3 | 7 | 57 | 86 | 30 | A | 7.33 | 38.5 | 640.2 | 20.3 | -5.6 | 100 | 151 | 5.1 | 1.34 | 116 | 21.5 | 20 | 35 | 12 | 14.1 | 0.3 | 271 |  | 405 |  | 25 |  | 15.5 | 278 | 2.3 |
| lion 5 | 1 | 16.3 | 144 | MeKMi | 296 | Iso | 37.1 | 7 | 56 | 122 | 30 | V | 7.247 | 50.4 | 51.3 | 22 | -5.3 | 79.4 | 150 | 5.2 | 1.28 | 118 | 23.5 | 15 | 41 | 13.9 | 13.7 | 0.58 | 340 | 21 | 293 | 77 | 26 | 51 | 11.7 | 292 | 1.43 |
| chimp 2 | 1 | 33.0 | 62 | MeKMi | 30 | Iso |  | 33 | 70 | 62 | 40 | V | 7.39 | 51.7 | 75 | 31.3 | 6.3 | 94.4 | 142 | 4.1 | 1.1 | 104 | 32.9 | 11 | 49 | 16.5 | 11 | 1.91 | 98 |  | 213 |  | 34 |  |  | 81 | 0.9 |

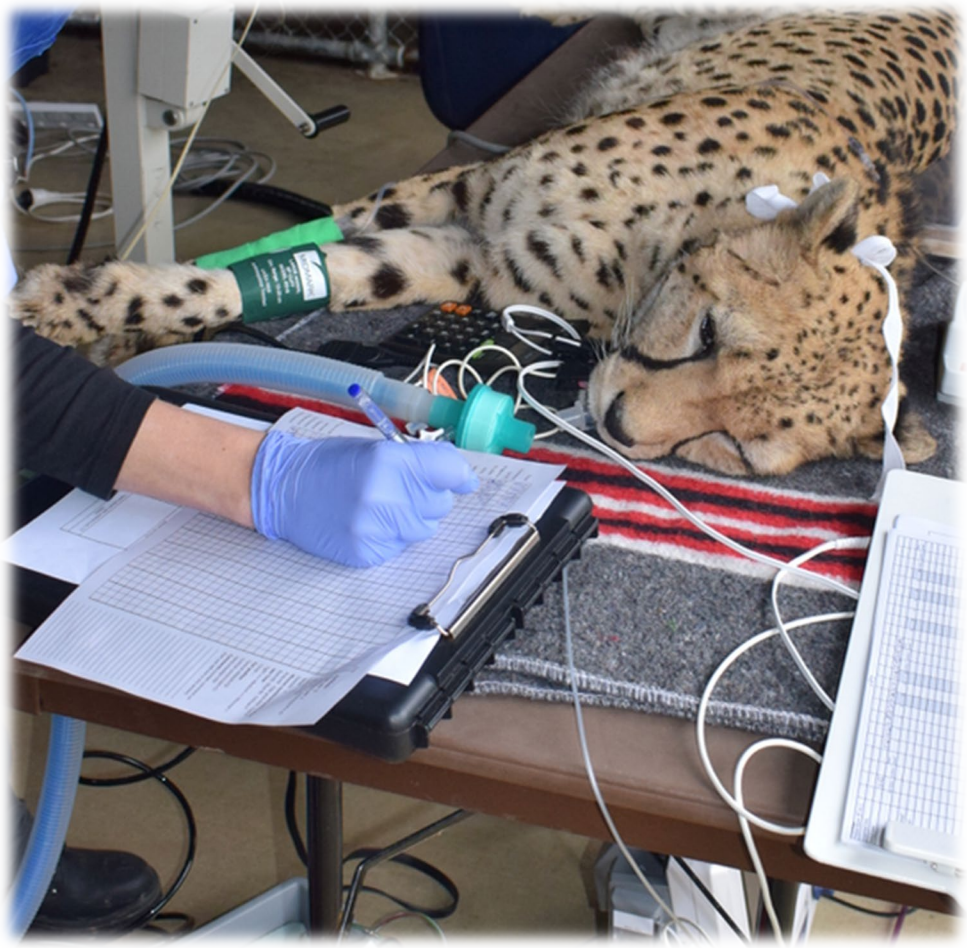
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chimp 2 | 1 | 33.0 | 62 | MeKMi | 69 | Iso | | 34 | 74 | 43 | 38 | V | 7.413 | 48.4 | 125.2 | 30.9 | 6.3 | 98.8 | 140 | 4.4 | 1.1 | 102 | 32.4 | 12 | 43 | 14.7 | 10.9 | 1.73 | 129 | | | | | | |
| chimp 3 | 1 | 36.0 | 61 | MeKMi | 29 | Iso | | | 71 | | | V | 7.38 | 52.8 | 102.9 | 31.3 | 6.1 | 97.7 | 143 | 3.3 | 1.05 | 105 | 32.9 | 10 | 51 | 17.5 | 11.4 | 1.21 | 84 | | | | | | |
| chimp 3 | 1 | 36.0 | 61 | MeKMi | 51 | Iso | 37.4 | 38 | 70 | 65 | 37 | A | 7.38 | 50.9 | 282 | 30.7 | 5.7 | 99.9 | 142 | 3.1 | 1.07 | 104 | 32.3 | 10 | 47 | 15.8 | 10.7 | 1.45 | 53 | | | | | | |
| cheetah 3 | 1 | 2.0 | 40 | MeK | 38 | Iso | 38.6 | 24 | 76 | 178 | 35 | V | | | | | | | | | | | | | | | | | 33 | 1582 | | 35 | | 19.4 | 277 | 2.89 |
| cheetah 3 | 1 | 2.0 | 40 | MeK | 68 | Iso | 38.5 | 24 | 76 | 160 | 38 | V | 7.259 | 47.9 | 91 | 21.5 | -5.6 | 95.5 | 157 | 3.8 | 1.32 | 126 | 22.9 | 13 | 50 | 17 | 10.4 | 1.24 | 212 | 20 | 1197 | 75 | 36 | 39 | 17.1 | 252 | 2.61 |
| cheetah 3 | 1 | 2.0 | 40 | MeK | 100 | Iso | 38.2 | 24 | 74 | 154 | 40 | V | 7.217 | 58.1 | 99.5 | 23.6 | -4.1 | 96 | 157 | 4.1 | 1.35 | 123 | 25.4 | 15 | 47 | 16 | 10.8 | 1.39 | 233 | | | | | | | |
| cheetah 3 | 1 | 2.0 | 40 | MeK | 140 | Iso | 38 | 24 | 74 | 110 | 36 | V | 7.252 | 56.9 | 104.1 | 25.1 | -2.1 | 96.8 | 155 | 4.7 | 1.26 | 119 | 26.9 | 16 | 45 | 15.2 | 10.1 | 1.24 | 234 | 19 | 921 | 65 | 33 | 32 | 16 | 206 | 2.54 |
| cheetah 2 | 1 | 2.0 | 39 | MeK | 32 | Iso | 38.7 | 12 | 84 | 185 | 35 | V | 7.28 | 43.5 | 59 | 20.4 | -6.3 | 86.6 | 158 | 3.8 | 1.32 | 124 | 21.8 | 17 | 54 | 18.4 | 10.9 | 1.06 | 224 | 17 | 413 | 79 | 40 | 39 | 18.7 | 244 | 2.41 |
| cheetah 2 | 1 | 2.0 | 39 | MeK | 86 | Iso | 36.9 | | 80 | 128 | 45 | V | 7.213 | 59 | 89.3 | 23.8 | -4.1 | 94.5 | 157 | 4.4 | 1.35 | 122 | 25.6 | 16 | 50 | 16.9 | 15.1 | 1.01 | 228 | | 399 | | 37 | | 21.3 | 275 | 2.82 |
| cheetah 2 | 1 | 2.0 | 39 | MeK | 113 | Iso | | 20 | 80 | 124 | 45 | V | 7.224 | 54.7 | 132 | 22.6 | -5.1 | 98.3 | 154 | 4.7 | 1.34 | 123 | 24.3 | 13 | 47 | 16 | 13.8 | 1.18 | | 16 | 1695 | | 36 | | 18.4 | 266 | 1.96 |
| cheetah 2 | 1 | 2.0 | 39 | MeK | 133 | Iso | | 12 | 80 | | | V | 7.279 | 50.8 | 84.1 | 23.8 | -2.9 | 94.6 | 152 | 5.2 | 1.27 | 120 | 25.4 | 13 | 49 | 16.6 | 14.8 | 1.2 | 211 | | 462 | | 37 | | | 282 | 2.84 |
| cheetah 1 | 1 | 8.5 | 55 | MeK | 62 | Iso | 37.4 | 18 | 72 | 99 | 54 | V | 7.181 | 67.4 | 85 | 25.2 | -3.2 | 92.9 | 164 | 3.8 | 1.35 | 122 | 27.3 | 21 | 43 | 14.6 | 11.3 | 0.51 | 250 | 16 | 722 | 67 | 35 | 32 | 16 | 218 | 1.8 |
| cheetah 1 | 1 | 8.5 | 55 | MeK | 115 | Iso | 37.4 | 16 | 79 | 58 | 66 | V | 7.109 | 82.1 | 58.2 | 26 | -3.5 | 77.5 | 162 | 4.8 | 1.35 | 122 | 28.6 | 19 | 40 | 13.7 | 13 | 0.3 | 270 | 21 | 691 | 75 | 33 | 42 | 15.6 | 233 | 2.05 |
| cheetah 1 | 1 | 8.5 | 55 | MeK | 172 | Iso | 37.6 | 14 | 121 | 90 | 76 | V | 7.119 | 89 | 41.6 | 28.9 | -0.5 | 57.7 | 149 | 7.7 | 1.15 | 114 | 31.6 | 14 | 41 | 13.9 | 30.5 | 0.38 | 325 | 21 | 667 | 75 | 31 | 44 | 15.8 | 256 | 2.09 |
| sun bear 1 | 1 | 13.3 | 100 | MeZoTi | 26 | Iso | 36.4 | 12 | 89 | 67 | 22 | V | 7.223 | 53.1 | 56.9 | 21.9 | -5.7 | 82.8 | 145 | 4.3 | 1.38 | 112 | 23.5 | 15 | 49 | 16.8 | 7.6 | 1.64 | 206 | 11 | 94 | 60 | 27 | 33 | 12.9 | 198 | 1.83 |
| sun bear 1 | 1 | 13.3 | 100 | MeZoTi | 64 | Iso | 36.6 | 12 | 88 | 59 | 23 | V | 7.192 | 57.3 | 47.7 | 22 | -6.2 | 72.4 | 145 | 4.3 | 1.45 | 112 | 23.7 | 15 | 46 | 15.6 | 8.7 | 1.72 | 194 | 11 | 100 | 54 | 24 | 30 | 13 | 198 | 1.99 |
| sun bear 1 | 1 | 13.3 | 100 | MeZoTi | 92 | Iso | 36.5 | 13 | 112 | 81 | 26 | V | 7.325 | 30.7 | 63.6 | 16 | -10 | 90.6 | 147 | 4 | 1.2 | 113 | 16.9 | 22 | 41 | 14 | 7.8 | 1.68 | 172 | | | | | | | |
| sun bear 1 | 1 | 13.3 | 100 | MeZoTi | 141 | Iso | 35.5 | 12 | 83 | 47 | 25 | V | 7.103 | 73.8 | 48.4 | 23.1 | -6.5 | 67.4 | 143 | 4.8 | 1.22 | 113 | 25.4 | 12 | 44 | 14.9 | 8.2 | 1.96 | 224 | 13 | 160 | 51 | 22 | 29 | 13.4 | 237 | 2.49 |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 16 | Iso | | 20 | 80 | | | V | 7.354 | 52.1 | 56.4 | 29 | 3.5 | 87 | 143 | 2.9 | 1.2 | 109 | 30.6 | 8 | 47 | 15.9 | 12.9 | 2.06 | 60 | 8 | 298 | 68 | 38 | 29 | 4.4 | 73 | 1.15 |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 75 | Iso | 37.6 | 21 | 71 | 50 | 50 | V | 7.34 | 59.8 | 53.5 | 32.2 | 6.4 | 84.2 | 144 | 3.1 | 1.18 | 107 | 34.1 | 8 | 43 | 14.6 | 12.6 | 0.84 | 67 | 12 | 351 | 60 | 34 | 26 | 4.5 | 72 | 1.73 |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 88 | Iso | 37.3 | 18 | 71 | 50 | 52 | V | 7.372 | 54 | 55.3 | 31.4 | 6.1 | 86.8 | 148 | 3 | 1.14 | 108 | 33 | 12 | 42 | 14.3 | 11.1 | 0.5 | 52 | | | | 31 | | | | |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 103 | Iso | 36.4 | 21 | 58 | 76 | 49 | V | 7.338 | 61.2 | 162.4 | 32.9 | 7.1 | 99.3 | 146 | 3 | 1.2 | 109 | 34.8 | 7 | 45 | 15.4 | 11.9 | 0.53 | 58 | | | | 34 | | | | |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 117 | Iso | 36.5 | 24 | 61 | 72 | 50 | V | 7.321 | 60.8 | 41.2 | 31.4 | 5.3 | 70.7 | 147 | 2.9 | 1.14 | 109 | 33.2 | 10 | 42 | 14.4 | 10.4 | 0.66 | 59 | 9 | 293 | 65 | 29 | 36 | 5 | 62 | 1.85 |
| baboon 2 | 1 | 12.8 | 38 | MeKMi | 139 | Iso | | 22 | 70 | | 50 | V | 7.363 | 52.7 | 137.4 | 30 | 4.6 | 99 | 145 | 3.3 | 1.14 | 111 | 31.6 | 7 | 41 | 14 | 10.6 | 1.03 | 55 | | | | 32 | | | 69 | |
| chimp 4 | 1 | 19.5 | 54 | MeKMi | 20 | Iso | | 30 | 72 | | | V | 7.385 | 52.5 | 107.8 | 31.3 | 6.3 | 98 | 141 | 3.8 | 1.09 | 102 | 33 | 12 | 51 | 17.2 | 8.4 | 1.35 | 86 | | | | | | | |
| chimp 4 | 1 | 19.5 | 54 | MeKMi | 50 | Iso | | 41 | 71 | 65 | 42 | V | 7.431 | 47.4 | 191.6 | 31.5 | 7.2 | 99.7 | 142 | 4.1 | 1.08 | 102 | 33 | 13 | 47 | 16 | 9.5 | | 81 | | 170 | | 33 | | | 95 | |
| rhino 1 | 2 | 17.9 | 1915 | DMiBK | 61 | TIVA | 36.2 | 14 | 32 | 144 | | V | 7.398 | 43 | 105.4 | 26.5 | 1.7 | 98 | 133 | 4 | 1.29 | 97 | 27.9 | 14 | 30 | 10.3 | 9.2 | 0.89 | 126 | | | | | | | |
| rhino 1 | 2 | 17.9 | 1915 | DMiBK | 81 | TIVA | | 10 | 32 | 151 | 36 | V | 7.331 | 51.1 | 53.1 | 27 | 1 | 84.2 | 132 | 3.9 | 1.26 | 96 | 28.5 | 13 | 28 | 9.6 | 10.9 | 0.85 | 128 | 8 | 125 | | 23 | | 4.5 | 158 | 1.2 |
| rhino 1 | 2 | 17.9 | 1915 | DMiBK | 95 | TIVA | 36 | 12 | 32 | 162 | 43 | V | 7.319 | 57.7 | 52.6 | 29.7 | 3.6 | 83 | 133 | 3.9 | 1.3 | 94 | 31.4 | 13 | 29 | 9.8 | 11.7 | 0.93 | 134 | 8 | 113 | | 22 | | | 154 | 1.29 |
| rhino 1 | 2 | 17.9 | 1915 | DMiBK | 111 | TIVA | 35.9 | 12 | 32 | 133 | 35 | V | 7.356 | 51.3 | 52.3 | 28.7 | 3.2 | 84.5 | 132 | 3.9 | 1.27 | 94 | 30.3 | 13 | 29 | 9.8 | 12.5 | 1.09 | 145 | 10 | 122 | | 22 | | 4.5 | 161 | 1.37 |
| rhino 1 | 2 | 17.9 | 1915 | DMiBK | 118 | TIVA | | 16 | | | | V | 7.346 | 52.6 | 44.2 | 28.8 | 3.1 | 76.4 | 133 | 4 | 1.3 | 95 | 30.4 | 13 | 30 | 10.2 | 12.1 | 1.19 | 135 | 7 | 111 | | 22 | | 4.5 | 164 | 1.4 |
| chimp 5 | 1 | 7.7 | 38 | MeKMi | 18 | Iso | | | | | | V | 7.381 | 53.5 | 52.4 | 31.7 | 6.6 | 85.2 | 138 | 4.3 | 1.23 | 101 | 33.4 | 10 | 56 | 19 | 10.4 | 1.79 | 80 | | | | 37 | | | 77 | |
| chimp 5 | 1 | 7.7 | 38 | MeKMi | 30 | Iso | 37.5 | 45 | 82 | | | V | 7.484 | 37.1 | 103.2 | 27.9 | 4.4 | 98.4 | 138 | 4.3 | 1.08 | 101 | 29 | 13 | 50 | 16.9 | 11.2 | 1.61 | 78 | | | | | | | |
| chimp 5 | 1 | 7.7 | 38 | MeKMi | 48 | Iso | 37.5 | 47 | 83 | | | V | 7.314 | 59.7 | 85.5 | 30.3 | 4.2 | 95.2 | 137 | 4.7 | 1.19 | 100 | 32.2 | 11 | 51 | 17.2 | 13.7 | 2.23 | 102 | | | | 35 | | | 90 | |
| baboon 3 | 1 | 7.3 | 25 | MeK | 18 | Iso | | 16 | 95 | | | V | 7.372 | 55.1 | 70.3 | 32 | 6.8 | 92.9 | 143 | 3.1 | 1.2 | 103 | 33.7 | 11 | 48 | 16.2 | 11.2 | 1.7 | 75 | 9 | 218 | 62 | 40 | 22 | 4.9 | 74 | 1.22 |
| baboon 3 | 1 | 7.3 | 25 | MeK | 69 | Iso | 37.8 | 17 | 86 | 55 | 58 | A | 7.379 | 55.1 | 455.1 | 32.5 | 7.4 | 100 | 142 | 3.5 | 1.15 | 102 | 34.2 | 11 | 44 | 15.1 | 11 | 0.38 | 67 | 11 | 409 | 62 | 37 | 25 | 5.9 | 79 | 1.66 |
| baboon 3 | 1 | 7.3 | 25 | MeK | 111 | Iso | 37.6 | 19 | 101 | 43 | 57 | A | 7.355 | 56.5 | 532 | 31.5 | 6 | 100 | 147 | 3.3 | 1.1 | 107 | 33.3 | 12 | 41 | 13.9 | 9.1 | 0.3 | 57 | 10 | 423 | 58 | 35 | 23 | 5.7 | 72 | 1.57 |
| rhino 1 | 1 | 17.5 | 1885 | DMiBK | 43 | TIVA | 36.8 | 6 | 40 | 80 | | V | 7.336 | 52.5 | 161.1 | 28.1 | 2.3 | 99.3 | 131 | 4.3 | 1.39 | 93 | 29.7 | 14 | 31 | 10.5 | 9.7 | 0.78 | 160 | | 113 | | 21 | | | 237 | 1.63 |
| rhino 1 | 1 | 17.5 | 1885 | DMiBK | 69 | TIVA | 36.7 | 12 | 32 | 72 | | V | 7.334 | 58.9 | 52.7 | 31.3 | 5.5 | 83.5 | 130 | 4 | 1.3 | 92 | 33.1 | 11 | 33 | 11.2 | 11.5 | 0.54 | 153 | | | | 21 | | | | |
| rhino 1 | 1 | 17.5 | 1885 | DMiBK | 89 | TIVA | 36.7 | 10 | 32 | 72 | 43 | V | 7.346 | 58.2 | 47.6 | 31.8 | 6.1 | 79.6 | 129 | 4 | 1.37 | 90 | 33.6 | 11 | 33 | 11.4 | 13.1 | 0.42 | 177 | | 132 | | 21 | | | 242 | 1.61 |
| rhino 1 | 1 | 17.5 | 1885 | DMiBK | 106 | TIVA | 36.6 | 12 | 36 | 72 | 46 | V | 7.35 | 51.8 | 94 | 28.6 | 3 | 96.7 | 130 | 3.9 | 1.33 | 93 | 30.2 | 12 | 33 | 11.3 | 12.3 | 1.02 | 159 | | 129 | | 22 | | | 209 | |
| lion 4 | 2 | 14.5 | 206 | MeK | 26 | Iso | | 10 | 60 | | | V | 7.352 | 29.8 | 54.5 | 16.6 | -9 | 87 | 154 | 4 | 1.21 | 126 | 17.5 | 15 | 44 | 14.8 | 6.8 | 1.17 | 203 | 8 | 106 | 73 | 29 | 44 | 14.2 | 230 | 1.39 |
| lion 4 | 2 | 14.5 | 206 | MeK | 49 | Iso | | 14 | 50 | | | V | 7.301 | 36.7 | 73.9 | 18.1 | -8.3 | 93.2 | 153 | 4 | 1.26 | 126 | 19.2 | 13 | 40 | 13.5 | 7.7 | 1.24 | 213 | | | | 28 | | | 289 | |
| nyala 1 | 1 | 2.2 | 100 | EtMe | 7 | Etor/Me | 38.6 | 12 | 77 | | | V | 7.339 | 47.2 | 45.7 | 25.4 | -0.4 | 78.2 | 145 | 3.4 | 1.15 | 112 | 26.9 | 11 | 36 | 12.2 | 7.3 | 1.98 | 105 | | 19884 | 84 | 41 | 43 | 7 | 60 | 1.15 |
| nyala 1 | 1 | 2.2 | 100 | EtMe | 23 | Etor/Me | | | | | | V | 7.348 | 48.9 | 220.3 | 26.8 | 1.2 | 99.7 | 146 | 3.6 | 1.15 | 110 | 28.3 | 13 | 35 | 11.8 | 9.6 | 1.15 | 91 | | | | | | | | |
| sun bear 1 | 2 | 22.0 | 111 | MeKMi | 30 | TIVA | | 20 | | | | V | 7.325 | 39.8 | 62.4 | 20.7 | -5.3 | 89.8 | 138 | 4 | 1.27 | 112 | 21.9 | 9 | 45 | 15.3 | 7.8 | 1.32 | 165 | | 76 | | 30 | | 14.1 | 177 | 2.45 |
| sun bear 1 | 2 | 22.0 | 111 | MeKMi | 68 | TIVA | 37.1 | 12 | 86 | 146 | 36 | V | 7.268 | 47.2 | 104 | 21.6 | -5.4 | 97.1 | 138 | 3.8 | 1.33 | 108 | 23 | 12 | 43 | 14.7 | 8.8 | 1.48 | 149 | 29 | 65 | 82 | 30 | 52 | 11.7 | 168 | 2.04 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sun bear 1 | 2 | 22.0 | 111 | MeKMi | 98 | TIVA | 37 | 8 | 85 | 139 | 38 | V | 7.261 | 46 | 80.6 | 20.7 | -6.3 | 93.8 | 139 | 3.8 | 1.28 | 112 | 22.1 | 10 | 43 | 14.7 | 8.8 | 1.49 | 141 | | | | | | |
| sun bear 1 | 2 | 22.0 | 111 | MeKMi | 128 | TIVA | 37.2 | 8 | 83 | 162 | 38 | V | 7.246 | 47.6 | 151.7 | 20.7 | -6.6 | 98.9 | 139 | 3.8 | 1.33 | 113 | 22.2 | 9 | 42 | 14.3 | 9.3 | 1.63 | 147 | 13 | 68 | 85 | 28 | 57 | 12.4 | 160 | 2.47 |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 17 | TIVA | | | | | | V | 7.366 | 52.1 | 107 | 29.8 | 4.5 | 97.8 | 140 | 4.4 | 1.14 | 104 | 31.4 | 11 | 34 | 11.7 | 4.9 | 1.58 | 90 | | | | | | | |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 65 | TIVA | 36.8 | 10 | 70 | 65 | 45 | V | 7.434 | 43.9 | 191.7 | 29.4 | 5.2 | 99.7 | 142 | 3.7 | 1.11 | 103 | 30.8 | 13 | 35 | 12 | 7.4 | 1.49 | 89 | 7 | 1695 | 82 | 30 | 52 | 6.2 | 66 | 1.96 |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 66 | TIVA | 36.8 | 10 | 70 | 65 | 45 | A | 7.465 | 46.1 | 548 | 33.2 | 9.4 | 100 | 146 | 2.8 | 1.15 | 106 | 34.6 | 10 | 34 | 11.6 | 8.4 | 0.64 | 69 | | | | | | | |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 100 | TIVA | 36.2 | 10 | 70 | 102 | 40 | V | 7.456 | 44 | 256 | 31 | 7.1 | 99.9 | 144 | 3.1 | 1.13 | 104 | 32.3 | 12 | 36 | 12.1 | 8 | 1.51 | 73 | 6 | 1714 | 82 | 29 | 53 | 6.2 | 65 | 1.96 |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 106 | TIVA | 36.1 | 10 | 70 | 102 | 40 | A | 7.497 | 44.2 | 706 | 34.2 | 11 | 100 | 147 | 2.9 | 1.19 | 103 | 35.6 | 13 | 38 | 12.8 | 8.2 | 0.65 | 88 | | | | | | | |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 143 | TIVA | 35.9 | 5 | 62 | 118 | 52 | V | 7.399 | 53.5 | 66.5 | 33 | 8.2 | 92.3 | 145 | 2.9 | 1.19 | | 34.7 | | 38 | 12.9 | 7.6 | 0.6 | 103 | | | | | | | |
| nyala 2 | 1 | 2.8 | 64 | MeKB | 204 | TIVA | 35.5 | 10 | 63 | 118 | 38 | V | 7.468 | 40.7 | 135.3 | 29.5 | 5.8 | 99.2 | 142 | 3.6 | 1.11 | 107 | 30.7 | 9 | 38 | 13 | 6.7 | 0.84 | 108 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 22 | Iso | | | 86 | 92 | 56 | V | 7.314 | 58 | 84.2 | 29.5 | 3.3 | 95 | 142 | 4.5 | 1.25 | 103 | 31.2 | 14 | 39 | 13.4 | 11.1 | 2.93 | 99 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 100 | Iso | | | 85 | 56 | 31 | V | 7.241 | 37.7 | 235.1 | 16.2 | -11.2 | 99.7 | 149 | 3.3 | 0.81 | 119 | 17.4 | 17 | 25 | 8.5 | 7.8 | 1.63 | 55 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 200 | Iso | | | | 89 | 46 | V | 7.09 | 97.8 | 76.1 | 29.8 | -0.1 | 87.2 | 143 | 6.2 | 1.18 | 104 | 32.8 | 15 | 50 | 16.9 | 8.9 | 7.05 | 183 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 320 | Iso | | | | | | V | 7.253 | 61.5 | 188 | 27.2 | 0 | 99.4 | 143 | 5.3 | 1.14 | 109 | 29 | 12 | 49 | 16.8 | 2.6 | 2.3 | 231 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 369 | Iso | | | | | | V | 7.354 | 52.3 | 179 | 29.1 | 3.6 | 99.5 | 146 | 6.1 | 1.2 | 108 | 30.7 | 15 | 36 | 12.3 | 1.7 | 2.15 | 183 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 420 | Iso | | | | | | V | 7.3 | 60.7 | 94.1 | 30.2 | 3.8 | 96.2 | 145 | 5.1 | 1.15 | 108 | 32 | 12 | 42 | 14.3 | 3.4 | 1.33 | 208 | | | | | | | |
| baboon 3 | 2 | 8.5 | 29 | MeKMi | 448 | Iso | | | | | | V | 7.33 | 54 | 199 | 29.1 | 3.3 | 99.6 | 147 | 4.4 | 1.11 | 110 | 30.7 | 12 | 37 | 12.6 | 3.9 | 0.93 | 176 | | | | | | | |
| lion 2 | 1 | 17.0 | 135 | MeK | 70 | TIVA | 38.9 | 8 | 56 | 65 | 32 | V | 7.316 | 37 | 45.4 | 18.9 | -7.3 | 77.6 | 148 | 3.8 | 1.28 | 122 | 20 | 11 | 43 | 14.7 | 8.5 | 1.38 | 232 | 12 | 149 | 72 | 28 | 44 | 17.2 | 241 | 1.53 |
| lion 2 | 1 | 17.0 | 135 | MeK | 100 | TIVA | 38.9 | 8 | 48 | 52 | 34 | V | 7.31 | 37.3 | 68.5 | 18.8 | -7.5 | 91.8 | 148 | 3.7 | 1.3 | 120 | 19.9 | 13 | 36 | 12.2 | 8.5 | 1.15 | 294 | 13 | 168 | 68 | 26 | 42 | 16.8 | 233 | 1.51 |
| lion 2 | 1 | 17.0 | 135 | MeK | 135 | TIVA | 38 | 8 | 48 | 88 | 30 | V | 7.32 | 39.5 | 68.3 | 20.3 | -5.8 | 91.9 | 148 | 3.7 | 1.33 | 123 | 21.5 | 8 | 30 | 10.3 | 8.8 | 0.93 | 210 | 13 | 228 | 65 | 25 | 40 | 16.6 | 242 | 1.63 |
| lion 2 | 1 | 17.0 | 135 | MeK | 175 | TIVA | 37.8 | 8 | 48 | 98 | 31 | V | 7.317 | 39.1 | 89.2 | 20 | -6.1 | 96.1 | 148 | 3.8 | 1.32 | 122 | 21.2 | 10 | 28 | 9.6 | 8.6 | 0.85 | 205 | 14 | 313 | 62 | 24 | 38 | 16.1 | 241 | 1.62 |
| lion 2 | 1 | 17.0 | 135 | MeK | 220 | TIVA | | 6 | 48 | 75 | 38 | V | 7.287 | 49.5 | 54 | 23.7 | -3 | 83.3 | 147 | 4 | 1.34 | 121 | 25.2 | 6 | 29 | 10 | 7.8 | 1.2 | 186 | 14 | 437 | 65 | 24 | 41 | 15.4 | 241 | 1.66 |
| lion 2 | 1 | 17.0 | 135 | MeK | 262 | TIVA | | | | 73 | 40 | V | 7.25 | 52.8 | 48 | 23.2 | -4.1 | 76.1 | 149 | 4 | 1.37 | 122 | 24.8 | 8 | 27 | 9.3 | 7.3 | 0.91 | 239 | 13 | 538 | 58 | 22 | 36 | 14.4 | 230 | 1.6 |
| lion 2 | 1 | 17.0 | 135 | MeK | 322 | TIVA | | | | | | V | 7.387 | 32 | 99.2 | 19.3 | -5.8 | 97.7 | 149 | 4.6 | 1.22 | 124 | 20.2 | 10 | 27 | 9.2 | 7.4 | 0.59 | 231 | 16 | 1036 | 64 | 24 | 40 | 14.6 | 253 | 1.87 |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 10 | TIVA | 38.6 | 12 | 58 | | 46 | V | 7.34 | 52.1 | 24.2 | 28.1 | 2.3 | 38.5 | 140 | 4 | 1.21 | 107 | 29.7 | 9 | 29 | 10 | 6.4 | 1.76 | 109 | | | | | | | |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 50 | TIVA | | 12 | 76 | 146 | 40 | V | 7.427 | 40.3 | 42 | 26.6 | 2.2 | 78.7 | 137 | 3.8 | 1.2 | 105 | 27.8 | 9 | 28 | 9.6 | 12.5 | 1.61 | 114 | 4 | 1382 | 64 | 29 | 36 | 5.7 | 85 | 2.44 |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 84 | TIVA | | 12 | 78 | 131 | 34 | A | 7.48 | 36.6 | 639.2 | 27.3 | 3.9 | 100 | 139 | 3.6 | 1.16 | 104 | 28.5 | 11 | 27 | 9.3 | 12.9 | 1.27 | 113 | | 2199 | | 28 | | | 95 | |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 122 | TIVA | | 12 | 73 | 125 | 37 | A | 7.447 | 41.4 | 660.8 | 28.4 | 4.3 | 100 | 141 | 3.2 | 1.24 | 105 | 29.6 | 11 | 30 | 10.2 | 10.7 | 1.45 | 130 | | 3100 | | 28 | | | 89 | |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 159 | TIVA | | 12 | 76 | | 33 | V | 7.45 | 38 | 82.9 | 26.4 | 2.4 | 96.7 | 139 | 3.3 | 1.21 | 106 | 27.6 | 10 | 29 | 10 | 9.2 | 2.11 | 117 | | | | 28 | | | | |
| nyala 3 | 1 | 1.1 | 65 | MeKB | 203 | TIVA | | | | 77 | | V | 7.256 | 66.3 | 23.1 | 30.2 | 3.2 | 31.1 | 139 | 4.9 | 1.18 | 105 | 32.2 | 9 | 38 | 12.8 | 9.9 | 3.69 | 147 | 4 | 4121 | 69 | 28 | 41 | 6 | 103 | 2.77 |
| zebra 1 | 1 | 26.0 | 290 | EtDB | 41 | Etor/TIVA | 38.4 | 12 | 44 | 179 | 49 | V | 7.448 | 43.7 | 76.3 | 30.2 | 6.2 | 95.6 | 135 | 4.1 | 1.32 | 98 | 31.6 | 11 | 30 | 10.1 | 13.8 | 1.74 | 173 | 9 | 177 | 59 | 27 | 32 | 5.5 | 175 | 1.17 |
| zebra 1 | 1 | 26.0 | 290 | EtDB | 60 | Etor/TIVA | 38.5 | 8 | 46 | 172 | 46 | V | 7.286 | 65.8 | 51 | 31.3 | 4.7 | 79.8 | 137 | 3.7 | 1.32 | 99 | 33.4 | 10 | 27 | 9.1 | 14 | 2.25 | 164 | 10 | 2915 | | 26 | | 7.1 | 229 | 1.65 |
| zebra 1 | 1 | 26.0 | 290 | EtDB | 96 | Etor/TIVA | 37.8 | 8 | 40 | 90 | 54 | V | 7.216 | 87.3 | 100.7 | 35.4 | 7.6 | 95.7 | 140 | 3.6 | 1.25 | 100 | 38.1 | 8 | 27 | 9.2 | 13.8 | 1.9 | 159 | | | | | | | |
| zebra 1 | 1 | 26.0 | 290 | EtDB | 109 | Etor/TIVA | 37.8 | 6 | 39 | 82 | | V | 7.248 | 81.8 | 126.9 | 35.7 | 8.5 | 98 | 141 | 3.6 | 1.23 | 101 | 38.2 | 8 | 26 | 8.7 | | 1.75 | 167 | 10 | 221 | 63 | 27 | 36 | 5.7 | 172 | 1.67 |
| rhino 1 | 4 | 18.5 | 1915 | DMiBK | 41 | TIVA | | | | 168 | | V | 7.332 | 55.4 | 36 | 29.3 | 3 | 64 | 133 | 4.2 | 1.49 | | 31 | | 35 | 11.9 | 6.7 | | | 7 | 267 | 75 | 25 | 50 | 5.3 | 129 | 1.08 |
| rhino 1 | 4 | 18.5 | 1915 | DMiBK | 65 | TIVA | | 12 | 32 | | | V | 7.343 | 55.7 | 48 | 30.2 | 5 | 80 | 130 | 3.9 | 1.44 | | 32 | | 30 | 10.2 | 8.1 | | | 7 | 244 | 85 | 24 | 61 | 6 | 123 | 1.18 |
| rhino 1 | 4 | 18.5 | 1915 | DMiBK | 102 | TIVA | 36.7 | 12 | 36 | | | V | 7.369 | 50.7 | 46 | 29.3 | 4 | 79 | 130 | 4 | 1.41 | | 31 | | 29 | 9.9 | 9.8 | | | 7 | 262 | 75 | 24 | 51 | 5.1 | 123 | 1.1 |
| rhino 1 | 4 | 18.5 | 1915 | DMiBK | 112 | TIVA | | 12 | 36 | | | V | 7.394 | 46.5 | 169 | 28.4 | 4 | 99 | 130 | 4.1 | 1.32 | | 30 | | 28 | 9.5 | 9.7 | | | 6 | 200 | 74 | 24 | 50 | 4.9 | 116 | 1.05 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 45 | TIVA | 37 | 6 | 60 | | | V | 7.299 | 38.4 | 58 | 18.9 | -7.6 | 86.9 | 151 | 3.9 | 1.3 | 126 | 20 | 10 | 43 | 14.6 | 6.7 | 1.31 | 242 | 18 | 124 | 72 | 29 | 43 | 17.3 | 263 | 1.31 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 72 | TIVA | 36.8 | 6 | 51 | 158 | 30 | A | 7.31 | 38.2 | 291.9 | 19.2 | -7 | 94.9 | 151 | 4 | 1.39 | 124 | 20.4 | 12 | 39 | 13.3 | 12 | 0.69 | 234 | 17 | 154 | 68 | 27 | 41 | 16.5 | 251 | 1.25 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 105 | TIVA | 36.4 | 6 | 45 | 76 | 32 | A | 7.273 | 41.9 | 276.8 | 19.4 | -7.4 | 99.8 | 153 | 3.7 | 1.42 | 125 | 20.7 | 12 | 36 | 12.4 | 10.8 | 0.62 | 226 | 18 | 174 | 66 | 28 | 38 | 16.3 | 251 | 1.29 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 137 | TIVA | 36.2 | 6 | 42 | 76 | 33 | A | 7.255 | 40.8 | 280.5 | 18.1 | -9.1 | 99.8 | 153 | 3.6 | 1.42 | 120 | 19.3 | 19 | 35 | 11.8 | 9.8 | 0.81 | 218 | 16 | 203 | 63 | 26 | 37 | 15.7 | 249 | 1.3 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 171 | TIVA | 36 | 6 | 40 | 80 | 34 | A | 7.233 | 48.1 | 281.5 | 20.3 | -7.2 | 99.8 | 152 | 3.5 | 1.44 | 121 | 21.8 | 14 | 34 | 11.5 | 9.2 | 0.74 | 226 | 17 | 240 | 62 | 25 | 37 | 15.2 | 246 | 1.29 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 218 | TIVA | 35.6 | 6 | 40 | 93 | 36 | A | 7.241 | 48.4 | 271.2 | 20.8 | -6.5 | 99.8 | 152 | 3.7 | 1.45 | 121 | 22.3 | 14 | 33 | 11.2 | 8.7 | 0.82 | 280 | 16 | 283 | 62 | 25 | 37 | 14.5 | 246 | 1.32 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 260 | TIVA | 35.4 | 6 | 49 | 80 | 36 | A | 7.288 | 40.8 | 249.4 | 19.5 | -7.1 | 99.8 | 152 | 3.7 | 1.4 | 122 | 20.8 | 14 | 32 | 11 | 8.4 | 0.69 | 228 | | | | | | | |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 284 | TIVA | 35.4 | 6 | 57 | 93 | 37 | A | 7.224 | 49.6 | 225.9 | 20.5 | -7.2 | 99.7 | 152 | 3.8 | 1.44 | 123 | 22 | 12 | 33 | 11.1 | 8.4 | 0.99 | 223 | 18 | 399 | 62 | 24 | 38 | 14.2 | 244 | 1.39 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 318 | TIVA | 35.5 | 6 | 50 | 74 | 38 | A | 7.211 | 53.5 | 148.8 | 21.4 | -6.4 | 98.7 | 151 | 3.8 | 1.45 | 119 | 23.1 | 14 | 33 | 11.3 | 8.4 | 1.1 | 227 | 17 | 480 | 61 | 24 | 37 | 13.9 | 245 | 1.46 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 350 | TIVA | 35.4 | 8 | | 79 | 31 | A | 7.261 | 46.9 | 126.9 | 21.1 | -6 | 98.3 | 151 | 4 | 1.42 | 120 | 22.5 | 14 | 33 | 11.1 | 8.3 | 1.07 | 219 | 18 | 663 | 57 | 22 | 35 | 13.6 | 257 | 1.55 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 385 | TIVA | 35.5 | 8 | | 68 | 28 | V | 7.233 | 54.9 | 44.2 | 23.1 | -4.4 | 70.5 | 151 | 3.9 | 1.37 | 122 | 24.8 | 10 | 38 | 12.8 | 7.1 | 1.64 | 228 | 17 | 659 | 63 | 25 | 38 | 13.7 | 252 | 1.53 |
| lion 6 | 1 | 17.5 | 135 | MeKMi | 409 | TIVA | | 8 | | | | V | 7.25 | 52.9 | 44.9 | 23.2 | -4 | 72.5 | 149 | 4.1 | 1.37 | 119 | 24.8 | 11 | 33 | 11.1 | 7.3 | 1.35 | 252 | 18 | 660 | 56 | 22 | 34 | 13.8 | 258 | 1.53 |
| tiger 1 | 1 | 10.0 | 124 | MeK | 45 | Iso | | 2 | 60 | 74 | 35 | V | 7.219 | 46.1 | 69.1 | 18.8 | -8.9 | 89.5 | 150 | 4 | 1.37 | 127 | 20.3 | 8 | 39 | 13.3 | 11.1 | 0.92 | 173 | | 791 | 72 | 35 | 37 | 17 | 150 | 1.64 |
| tiger 1 | 1 | 10.0 | 124 | MeK | 100 | Iso | 36.9 | 14 | 50 | 87 | 28 | V | 7.221 | 43.5 | 50.2 | 17.8 | -9.9 | 77.6 | 149 | 4.1 | 1.27 | 127 | 19.2 | 8 | 36 | 12.1 | 8.6 | 1.73 | 167 | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tiger 1 | 1 | 10.0 | 124 | MeK | 130 | Iso | 36.6 | 10 | 48 | 90 | 29 | V | 7.225 | 44.4 | 71.7 | 18.4 | -9.2 | 90.7 | 148 | 4.6 | 1.31 | 126 | 19.8 | 8 | 38 | 12.9 | 10.8 | 1.38 | 171 | 834 | 70 | 35 | 35 | 16.8 | 154 | 1.85 |
| tiger 1 | 1 | 10.0 | 124 | MeK | 157 | Iso | 36.4 | 14 | 52 | 78 | 40 | V | 7.211 | 49.9 | 76.6 | 20 | -7.9 | 91.8 | 147 | 5.2 | 1.32 | 123 | 21.5 | 9 | 39 | 13.2 | 11.9 | 1.56 | 163 | | | | | | | |
| tiger 1 | 1 | 10.0 | 124 | MeK | 172 | Iso | 36.4 | 14 | 52 | 69 | 40 | V | 7.225 | 49.9 | 90.6 | 20.7 | -6.9 | 95 | 146 | 6 | 1.28 | 121 | 22.2 | 10 | 40 | 13.5 | 13.2 | 1.23 | 164 | 764 | 66 | 33 | 33 | 16.9 | 164 | 1.95 |
| tiger 1 | 1 | 10.0 | 124 | MeK | 194 | Iso | | 14 | 56 | 101 | 41 | V | | | | | | | | | | | | | | | | | | | | | | | | |
| tiger 1 | 1 | 10.0 | 124 | MeK | 220 | Iso | | 12 | 56 | | | V | 7.162 | 62.1 | 55.6 | 22.3 | -6.4 | 78.7 | 146 | 7 | 1.33 | 122 | 24.2 | 9 | 37 | 12.7 | 13.3 | 1.14 | 197 | 839 | 64 | 32 | 32 | 16.5 | 177 | 1.9 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 25 | Iso | 39.5 | 6 | | 92 | | V | 7.232 | 39.7 | 70.4 | 16.7 | 10.8 | 90.6 | 150 | 3.6 | 1.28 | 128 | 16.7 | 10 | 41 | 13.8 | 11.9 | 1.46 | 193 | 139 | 75 | 37 | 38 | 17.2 | 200 | 1.31 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 82 | Iso | 38.2 | 14 | 47 | 93 | 31 | V | 7.314 | 32.5 | 178.1 | 16.5 | -9.7 | 99.5 | 149 | 3.9 | 1.26 | 126 | 16.2 | 12 | 34 | 11.7 | 11.8 | 1.08 | 184 | 194 | 71 | 36 | 35 | 16.4 | 197 | 1.63 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 111 | Iso | 38.2 | 16 | 47 | 103 | 32 | V | 7.293 | 35.7 | 176.1 | 17.3 | -9.3 | 99.4 | 149 | 3.9 | 1.29 | 128 | 17.1 | 9 | 32 | 11 | 11.4 | 1.14 | 169 | 210 | 68 | 34 | 34 | 15.3 | 191 | 1.56 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 140 | Iso | 37.8 | 15 | 49 | 79 | 29 | V | 7.244 | 40.3 | 163.7 | 17.4 | -9.9 | 99.2 | 150 | 4.4 | 1.32 | 128 | 17.4 | 10 | 33 | 11.2 | 12 | 0.77 | 191 | 215 | 68 | 34 | 35 | 15.9 | 191 | 1.56 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 175 | Iso | 37 | 20 | 52 | 74 | 30 | V | 7.285 | 37.6 | 140.3 | 17.9 | -8.8 | 98.9 | 148 | 5.1 | 1.3 | 122 | 17.7 | 14 | 30 | 10.2 | 14 | 1.15 | 185 | 233 | 67 | 33 | 34 | 14.6 | 194 | 1.54 |
| tiger 2 | 1 | 8.8 | 93 | MeKMi | 226 | Iso | 36.8 | | | 92 | | V | 7.32 | 32.4 | 218.3 | 16.7 | -9.4 | 99.7 | 146 | 6.5 | 1.18 | 127 | 16.4 | 10 | 31 | 10.6 | 17.1 | 1.1 | 192 | | | | | | | |
| tiger 2 | 2 | 8.8 | 94 | MeKMi | 68 | Iso | 39.1 | 12 | 40 | 140 | | V | 7.268 | 34 | 113.1 | 15.5 | 11.4 | 97.8 | 153 | 3.1 | 1.25 | 121 | 16 | 20 | 40 | 13.5 | 15.1 | 2.03 | 239 | | | | | | | |
| tiger 2 | 2 | 8.8 | 94 | MeKMi | 82 | Iso | 39.1 | 12 | 40 | 129 | 37 | A | 7.318 | 38.4 | 500.1 | 19.7 | -6.4 | 100 | 155 | 3.3 | 1.34 | 124 | 20.2 | 15 | 35 | 11.8 | 14 | 0.61 | 211 | 221 | 72 | 35 | 37 | 21.1 | 190 | 1.55 |
| tiger 2 | 2 | 8.8 | 94 | MeKMi | 118 | Iso | 38.2 | 8 | 45 | 124 | 37 | A | 7.331 | 38.7 | 465.4 | 20.4 | -5.5 | 100 | 154 | 3.8 | 1.24 | 120 | 20.9 | 18 | 33 | 11.4 | 12.9 | 0.64 | 193 | 229 | 70 | 34 | 36 | 20 | 188 | 1.63 |
| tiger 2 | 2 | 8.8 | 94 | MeKMi | 148 | Iso | 37.8 | 8 | 50 | 114 | 40 | A | 7.298 | 43.9 | 515.1 | 21.5 | -5 | 100 | 155 | 3.8 | 1.36 | 123 | 22 | 15 | 30 | 10.2 | 13.9 | 0.77 | 203 | 259 | 67 | 33 | 34 | 19.2 | 188 | 1.64 |
| lion 5 | 2 | 17.8 | 120 | MeKMiB | 22 | Iso | 37.1 | 10 | 60 | | | V | 7.215 | 41.3 | 112 | 16.7 | 11.1 | 97.3 | 153 | 4.3 | 1.29 | 125 | 17.4 | 16 | 24 | 8 | 9.6 | 1.2 | 268 | | | | | | | |
| lion 5 | 2 | 17.8 | 120 | MeKMiB | 98 | Iso | 36.2 | 15 | 52 | 78 | 36 | V | 7.227 | 42.3 | 49.2 | 17.6 | -10 | 76.9 | 154 | 4.4 | 1.27 | 126 | 18.2 | 15 | 21 | 7.2 | 8.7 | 1.05 | 281 | | | | | | | |
| lion 5 | 2 | 17.8 | 120 | MeKMiB | 131 | Iso | 36.1 | 16 | 55 | 82 | 35 | V | 7.234 | 44.7 | 47.8 | 18.9 | -8.6 | 75.6 | 154 | 4.3 | 1.31 | 124 | 19.6 | 16 | 19 | 6.6 | 8 | 0.71 | 274 | | | | | | | |
| lion 5 | 2 | 17.8 | 120 | MeKMiB | 169 | Iso | 35.8 | 18 | 50 | 112 | 38 | V | 7.196 | 49.5 | 68.5 | 19.2 | -8.9 | 88.5 | 154 | 4.6 | 1.32 | 124 | 20 | 16 | 19 | 6.5 | 8.3 | 0.64 | 290 | | | | | | | |
| lion 5 | 2 | 17.8 | 120 | MeKMiB | 245 | Iso | 35.7 | | 50 | 100 | 32 | V | 7.294 | 38.4 | 51.5 | 18.6 | -7.9 | 82.3 | 154 | 4.8 | 1.24 | 125 | 19.1 | 16 | 19 | 6.5 | 7.4 | 0.57 | 290 | | | | | | | |
| tiger 2 | 3 | 8.9 | 95 | MeKMi | 58 | Iso | 38.4 | | 50 | 135 | 32 | V | 7.21 | 35.8 | 106.9 | 14.4 | 13.5 | 96.6 | 154 | 3.7 | 1.24 | 126 | 15 | 18 | 41 | 13.8 | 9.4 | 1.01 | 257 | | | | | | | |
| tiger 2 | 3 | 8.9 | 95 | MeKMi | 85 | Iso | 37.9 | 13 | 39 | 153 | 33 | V | 7.22 | 41.9 | 67.6 | 17.2 | 10.4 | 89.1 | 154 | 3.7 | 1.29 | 125 | 17.9 | 16 | 38 | 12.9 | 9.6 | 1.05 | 227 | | | | | | | |
| tiger 2 | 3 | 8.9 | 95 | MeKMi | 110 | Iso | 37 | 10 | 52 | 98 | 31 | V | 7.28 | 33 | 182.5 | 15.6 | 11.2 | 99.5 | 154 | 4.5 | 1.29 | 124 | 16 | 20 | 36 | 12.3 | 11 | 0.73 | 219 | | | | | | | |
| bear 2 sun | 1 | 13.0 | 92 | MeKMiB | 13 | Iso | 37.3 | 16 | | 116 | | V | 7.276 | 40.5 | 169.1 | 18.9 | -7.9 | 99.3 | 139 | 4.6 | 1.2 | 109 | 19.2 | 16 | 49 | 16.7 | 7.9 | 2.15 | 120 | 542 | 76 | 36 | 41 | 8.1 | 125 | 1.52 |
| bear 2 sun | 1 | 13.0 | 92 | MeKMiB | 89 | Iso | 36.9 | 6 | 49 | 101 | 27 | V | 7.327 | 34.9 | 172.1 | 18.3 | -7.7 | 99.5 | 143 | 4.2 | 1.26 | 114 | 18.5 | 16 | 41 | 13.8 | 7 | 1.49 | 115 | 755 | 73 | 34 | 39 | 8 | 129 | 1.69 |
| bear 2 sun | 1 | 13.0 | 92 | MeKMiB | 160 | Iso | 35.6 | 6 | 47 | 95 | 32 | V | 7.327 | 34 | 221.2 | 17.8 | -8.2 | 99.7 | 145 | 4.4 | 1.22 | 119 | 18 | 13 | 38 | 12.9 | 5.3 | 1.06 | 116 | 358 | 66 | 31 | 35 | 7.5 | 123 | 1.7 |
| bear 2 sun | 1 | 13.0 | 92 | MeKMiB | 248 | Iso | 35.9 | 6 | 66 | 109 | 32 | V | 7.357 | 33 | 191.8 | 18.5 | -6.9 | 99.6 | 146 | 4.6 | 1.19 | 116 | 18.8 | 17 | 32 | 11 | 5.6 | 1.64 | 114 | 630 | 63 | 29 | 34 | 7 | 120 | 1.95 |
| bear 2 | 1 | 13.0 | 92 | MeKMiB | 289 | Iso | | 9 | 77 | 102 | 32 | V | 7.339 | 37.8 | 200.6 | 20.3 | -5.5 | 99.7 | 145 | 4.6 | 1.22 | 114 | 20.6 | 16 | 32 | 11 | 5.9 | 1.66 | 122 | 443 | 62 | 29 | 33 | 6.8 | 122 | 1.95 |

**Appendix A2:**

***Data sheet for blood gas (EPOC), laboratory clinical pathology parameters, calculated Strong ion parameters and calculated Mean Arterial Pressure (tMAP) parameters for all individual felid procedures covered under Massey University MUAEC Permit 17/50. Only first procedures are included in the analysis (Chapter Three).***



| Animal ID | Procedure Number | Age | Time from immobilisation | Arterial (A) or Venous (V) | pH | pCO2 mmHg | pO2 mm hg | cHCo3- mmol/L | Base Deficit (ecf) mmol/L | cSO2 % | Na+ mmol/L | K+ mmol/L | Ca++ mmol/L | Cl- mmol/L | cTCO2 mmol/L | A Gap K mmol/L | Hct % | cHgb g/dL | Glu mmol/L | Lactate mmol/L | Creatinine micromol/L (EPOC) | SDMA NZVP | CK NZVP | Total Protein NZVP | albumin NZVP | globulin NZVP | A/G ratio NZVP | BUN (Urea) NZVP | Creatinine NZVP | Phosphate (PO4) NZVP | *SIDa* | *A-* | *SIDe* | *SIG* | *tMAP* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lion 4 | 1 | 13.8 | 54 | V | 7.214 | 41.1 | 64.3 | 16.6 | -11.2 | 87.4 | 153 | 4.2 | 1.32 | 127 | 17.9 | 14 | 53 | 17.9 | 4.9 | 2.04 | 185 | | 108 | 79 | 31 | 48 | 0.66 | 12.8 | 189 | 1.58 | 29.48 | 10.73 | 27.28 | 2.20 | |
| lion 4 | 1 | 13.8 | 79 | V | 7.316 | 36.2 | 212.7 | 18.5 | -7.7 | 99.7 | 153 | 4.3 | 1.28 | 124 | 19.6 | 15 | 45 | 15.4 | 9.4 | 0.8 | 181 | | 100 | 74 | 30 | 44 | 0.67 | 13 | 183 | 1.82 | 33.78 | 11.33 | 29.76 | 4.02 | |
| lion 4 | 1 | 13.8 | 110 | A | 7.302 | 43.7 | 583.7 | 21.6 | -4.7 | 100 | 152 | 4.5 | 1.33 | 121 | 23 | 14 | 38 | 12.8 | 12.6 | 0.77 | 202 | | 116 | 69 | 27 | 42 | 0.64 | 13 | 187 | 1.85 | 36.06 | 10.52 | 32.07 | 3.99 | 85 |
| lion 4 | 1 | 13.8 | 134 | A | 7.301 | 41.4 | 550 | 20.4 | -6 | 100 | 150 | 4.7 | 1.21 | 119 | 21.7 | 15 | 39 | 13.4 | 16.8 | 0.85 | 219 | | 115 | 66 | 27 | 39 | 0.68 | 12.7 | 185 | 1.87 | 36.06 | 10.55 | 30.92 | 5.14 | 77 |
| lion 4 | 1 | 13.8 | 174 | A | 7.272 | 40 | 548 | 18.4 | -8.4 | 100 | 148 | 5.3 | 1.26 | 120 | 19.7 | 15 | 39 | 13.4 | 20.9 | 1.06 | 191 | | | | | | | | | | 33.5 | | | | 70 |
| lion 3 | 1 | 19.0 | 46 | V | 7.294 | 32.3 | 51.5 | 15.7 | -10.8 | 82.7 | 154 | 4.1 | 1.39 | 123 | 16.7 | 19 | 46 | 15.8 | 6.8 | 1.11 | 272 | 20 | 118 | 84 | 29 | 55 | 0.53 | 12.7 | 277 | 1.66 | 35.38 | 10.68 | 26.32 | 9.06 | 122 |
| lion 3 | 1 | 19.0 | 86 | V | 7.317 | 34.9 | 60.6 | 17.9 | -8.2 | 89 | 152 | 3.9 | 1.38 | 125 | 19 | 13 | 41 | 14 | 7 | 1.19 | 249 | 19 | 135 | 84 | 28 | 56 | 0.5 | 13.2 | 261 | 1.66 | 31.09 | 10.51 | 28.32 | 2.77 | 96 |
| lion 3 | 1 | 19.0 | 110 | V | 7.294 | 38.2 | 55.2 | 18.6 | -7.9 | 85.1 | 153 | 3.9 | 1.37 | 125 | 19.8 | 13 | 36 | 12.3 | 6.9 | 1.51 | 243 | 17 | 167 | 76 | 25 | 51 | 0.49 | 12.7 | 262 | 1.62 | 31.76 | 9.55 | 28.04 | 3.72 | 80 |
| lion 3 | 1 | 19.0 | 150 | V | 7.277 | 40.7 | 87.9 | 19 | -7.8 | 95.4 | 154 | 4.1 | 1.35 | 125 | 20.2 | 14 | 31 | 10.7 | 6.9 | 1.23 | 328 | 20 | 270 | 72 | 24 | 48 | 0.5 | 12.9 | 270 | 1.71 | 33.22 | 9.38 | 28.33 | 4.89 | 55 |
| lion 3 | 1 | 19.0 | 202 | V | 7.295 | 40.5 | 73 | 19.7 | -6.8 | 92.7 | 153 | 4.2 | 1.35 | 123 | 21 | 15 | 34 | 11.6 | 6.5 | 1.43 | 288 | 19 | 516 | 68 | 23 | 45 | 0.51 | 11.7 | 261 | 1.67 | 34.12 | 9.11 | 28.76 | 5.36 | 67 |
| lion 3 | 1 | 19.0 | 241 | V | 7.307 | 39.3 | 67.5 | 19.7 | -6.6 | 91.3 | 153 | 4.3 | 1.43 | 124 | 20.9 | 14 | 29 | 9.8 | 6.8 | 1.2 | 238 | | 599 | | 21 | | | | 273 | 1.71 | 33.53 | 8.68 | 28.29 | 5.24 | 91 |
| lion 5 | 1 | 16.3 | 43 | V | 7.278 | 41.5 | 71.5 | 19.4 | -7.3 | | 151 | 4.3 | 1.1 | 123 | 20.7 | | | | 7 | 2.71 | 305 | 21 | 299 | 77 | 32 | 46 | 0.69 | 10.3 | 307 | 1.52 | 30.69 | 11.16 | 30.52 | 0.17 | |
| lion 5 | 1 | 16.3 | 76 | A | 7.372 | 31.9 | 457.6 | 18.6 | -6.7 | 100 | 152 | 4.8 | 1.2 | 126 | 19.5 | 12 | 44 | 14.9 | 10.1 | 0.42 | 335 | | | | | | | | | | 31.58 | | | | |
| lion 5 | 1 | 16.3 | 116 | V | 7.325 | 36.6 | 294.5 | 19.1 | -6.9 | | 152 | 4.5 | 1.29 | 121 | 20.2 | 16 | | | 9.7 | 2.28 | 273 | 19 | | 66 | 27 | 39 | 0.69 | 10 | 312 | 1.47 | 34.51 | 9.93 | 28.96 | 5.55 | 116 |
| lion 5 | 1 | 16.3 | 140 | V | 7.273 | 46.9 | 50.2 | 21.7 | -5.2 | 79.8 | 152 | 4.8 | 1.25 | 121 | 23.1 | 14 | 37 | 12.6 | 10.6 | 0.8 | 288 | 19 | 291 | 73 | 30 | 43 | 0.7 | 11.2 | 347 | 1.64 | 36.25 | 10.82 | 32.46 | 3.79 | 88 |
| lion 5 | 1 | 16.3 | 169 | V | 7.261 | 48.1 | 42.2 | 21.6 | -5.4 | | 151 | 4.8 | 1.3 | 119 | 23.1 | 15 | | | 11.4 | 0.84 | 319 | 21 | 287 | 67 | 28 | 39 | 0.72 | 9.8 | 310 | 1.47 | 37.26 | 9.95 | 31.53 | 5.73 | 114 |
| lion 5 | 1 | 16.3 | 232 | A | 7.33 | 38.5 | 640.2 | 20.3 | -5.6 | 100 | 151 | 5.1 | 1.34 | 116 | 21.5 | 20 | 35 | 12 | 14.1 | 0.3 | 271 | | 405 | | 25 | | | 15.5 | 278 | 2.3 | 41.14 | 10.90 | 31.14 | 10.00 | 88 |
| lion 5 | 1 | 16.3 | 296 | V | 7.247 | 50.4 | 51.3 | 22 | -5.3 | 79.4 | 150 | 5.2 | 1.28 | 118 | 23.5 | 15 | 41 | 13.9 | 13.7 | 0.58 | 340 | 21 | 293 | 77 | 26 | 51 | 0.51 | 11.7 | 292 | 1.43 | 37.9 | 9.30 | 31.20 | 6.70 | 97 |
| cheetah 3 | 1 | 2.0 | 38 | V | | | | | | | | | | | | | | | | | | 33 | | | 35 | | | 19.4 | 277 | 2.89 | | | | | 161 |
| cheetah 3 | 1 | 2.0 | 68 | V | 7.259 | 47.9 | 91 | 21.5 | -5.6 | 95.5 | 157 | 3.8 | 1.32 | 126 | 22.9 | 13 | 50 | 17 | 10.4 | 1.24 | 212 | 20 | 1197 | 75 | 36 | 39 | 0.92 | 17.1 | 252 | 2.61 | 34.88 | 14.06 | 35.45 | -0.57 | 172 |
| cheetah 3 | 1 | 2.0 | 100 | V | 7.217 | 58.1 | 99.5 | 23.6 | -4.1 | 96 | 157 | 4.1 | 1.35 | 123 | 25.4 | 15 | 47 | 16 | 10.8 | 1.39 | 233 | | | | | | | | | | 38.06 | | | | 154 |
| cheetah 3 | 1 | 2.0 | 140 | V | 7.252 | 56.9 | 104.1 | 25.1 | -2.1 | 96.8 | 155 | 4.7 | 1.26 | 119 | 26.9 | 16 | 45 | 15.2 | 10.1 | 1.24 | 234 | 19 | 921 | 65 | 33 | 32 | 1.03 | 16 | 206 | 2.54 | 40.72 | 13.11 | 38.12 | 2.60 | 118 |
| cheetah 2 | 1 | 2.0 | 32 | V | 7.28 | 43.5 | 59 | 20.4 | -6.3 | 86.6 | 158 | 3.8 | 1.32 | 124 | 21.8 | 17 | 54 | 18.4 | 10.9 | 1.06 | 224 | 17 | 413 | 79 | 40 | 39 | 1.03 | 18.7 | 244 | 2.41 | 38.06 | 14.87 | 35.26 | 2.80 | 194 |
| cheetah 2 | 1 | 2.0 | 86 | V | 7.213 | 59 | 89.3 | 23.8 | -4.1 | 94.5 | 157 | 4.4 | 1.35 | 122 | 25.6 | 16 | 50 | 16.9 | 15.1 | 1.01 | 228 | | 399 | | 37 | | | 21.3 | 275 | 2.82 | 39.74 | 14.44 | 38.14 | 1.60 | 163 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cheetah 2 | 1 | 2.0 | 113 | V | 7.224 | 54.7 | 132 | 22.6 | -5.1 | 98.3 | 154 | 4.7 | 1.34 | 123 | 24.3 | 13 | 47 | 16 | 13.8 | 1.18 | | 6 | 1695 | | 36 | | | 18.4 | 266 | 1.96 | 35.86 | 12.73 | 35.27 | 0.59 | 126 |
| cheetah 2 | 1 | 2.0 | 133 | V | 7.279 | 50.8 | 84.1 | 23.8 | -2.9 | 94.6 | 152 | 5.2 | 1.27 | 120 | 25.4 | 13 | 49 | 16.6 | 14.8 | 1.2 | 211 | | 462 | | 37 | | | | 282 | 2.84 | 37.27 | 14.84 | 38.59 | -1.32 | |
| cheetah 1 | 1 | 8.5 | 62 | V | 7.181 | 67.4 | 85 | 25.2 | -3.2 | 92.9 | 164 | 3.8 | 1.35 | 122 | 27.3 | 21 | 43 | 14.6 | 11.3 | 0.51 | 250 | 16 | 722 | 67 | 35 | 32 | 1.09 | 16 | 218 | 1.8 | 46.64 | 11.98 | 37.13 | 9.51 | 116 |
| cheetah 1 | 1 | 8.5 | 115 | V | 7.109 | 82.1 | 58.2 | 26 | -3.5 | 77.5 | 162 | 4.8 | 1.35 | 122 | 28.6 | 19 | 40 | 13.7 | 13 | 0.3 | 270 | 21 | 691 | 75 | 33 | 42 | 0.79 | 15.6 | 233 | 2.05 | 45.85 | 11.57 | 37.53 | 8.32 | 62 |
| cheetah 1 | 1 | 8.5 | 172 | V | 7.119 | 89 | 41.6 | 28.9 | -0.5 | 57.7 | 149 | 7.7 | 1.15 | 114 | 31.6 | 14 | 41 | 13.9 | 30.5 | 0.38 | 325 | 21 | 667 | 75 | 31 | 44 | 0.7 | 15.8 | 256 | 2.09 | 43.47 | 11.20 | 40.00 | 3.47 | 85 |
| lion 4 | 2 | 14.5 | 26 | V | 7.352 | 29.8 | 54.5 | 16.6 | -9 | 87 | 154 | 4 | 1.21 | 126 | 17.5 | 15 | 44 | 14.8 | 6.8 | 1.17 | 203 | 8 | 106 | 73 | 29 | 44 | 0.66 | 14.2 | 230 | 1.39 | 32.04 | 10.43 | 26.92 | 5.12 | |
| lion 4 | 2 | 14.5 | 49 | V | 7.301 | 36.7 | 73.9 | 18.1 | -8.3 | 93.2 | 153 | 4 | 1.26 | 126 | 19.2 | 13 | 40 | 13.5 | 7.7 | 1.24 | 213 | | | | 28 | | | | 289 | | 31.02 | | | | |
| lion 2 | 1 | 17.0 | 70 | V | 7.316 | 37 | 45.4 | 18.9 | -7.3 | 77.6 | 148 | 3.8 | 1.28 | 122 | 20 | 11 | 43 | 14.7 | 8.5 | 1.38 | 232 | 12 | 149 | 72 | 28 | 44 | 0.64 | 17.2 | 241 | 1.53 | 29.7 | 10.27 | 29.11 | 0.59 | 65 |
| lion 2 | 1 | 17.0 | 100 | V | 7.31 | 37.3 | 68.5 | 18.8 | -7.5 | 91.8 | 148 | 3.7 | 1.3 | 120 | 19.9 | 13 | 36 | 12.2 | 8.5 | 1.15 | 294 | 13 | 168 | 68 | 26 | 42 | 0.61 | 16.8 | 233 | 1.51 | 31.85 | 9.67 | 28.41 | 3.44 | 52 |
| lion 2 | 1 | 17.0 | 135 | V | 7.32 | 39.5 | 68.3 | 20.3 | -5.8 | 91.9 | 148 | 3.7 | 1.33 | 123 | 21.5 | 8 | 30 | 10.3 | 8.8 | 0.93 | 210 | 13 | 228 | 65 | 25 | 40 | 0.63 | 16.6 | 242 | 1.63 | 29.1 | 9.66 | 29.96 | -0.86 | 88 |
| lion 2 | 1 | 17.0 | 175 | V | 7.317 | 39.1 | 89.2 | 20 | -6.1 | 96.1 | 148 | 3.8 | 1.32 | 122 | 21.2 | 10 | 28 | 9.6 | 8.6 | 0.85 | 205 | 14 | 313 | 62 | 24 | 38 | 0.64 | 16.1 | 241 | 1.62 | 30.27 | 9.36 | 29.32 | 0.95 | 93 |
| lion 2 | 1 | 17.0 | 220 | V | 7.287 | 49.5 | 54 | 23.7 | -3 | 83.3 | 147 | 4 | 1.34 | 121 | 25.2 | 6 | 29 | 10 | 7.8 | 1.2 | 186 | 14 | 437 | 65 | 24 | 41 | 0.59 | 15.4 | 241 | 1.66 | 30.14 | 9.33 | 32.91 | -2.77 | 83 |
| lion 2 | 1 | 17.0 | 262 | V | 7.25 | 52.8 | 48 | 23.2 | -4.1 | 76.1 | 149 | 4 | 1.37 | 122 | 24.8 | 8 | 27 | 9.3 | 7.3 | 0.91 | 239 | 13 | 538 | 58 | 22 | 36 | 0.61 | 14.4 | 230 | 1.6 | 31.46 | 8.57 | 31.67 | -0.21 | 68 |
| lion 2 | 1 | 17.0 | 322 | V | 7.387 | 32 | 99.2 | 19.3 | -5.8 | 97.7 | 149 | 4.6 | 1.22 | 124 | 20.2 | 10 | 27 | 9.2 | 7.4 | 0.59 | 231 | 16 | 1036 | 64 | 24 | 40 | 0.6 | 14.6 | 253 | 1.87 | 30.23 | 10.05 | 29.24 | 0.99 | |
| lion 6 | 1 | 17.5 | 45 | V | 7.299 | 38.4 | 58 | 18.9 | -7.6 | 86.9 | 151 | 3.9 | 1.3 | 126 | 20 | 10 | 43 | 14.6 | 6.7 | 1.31 | 242 | 18 | 124 | 72 | 29 | 43 | 0.68 | 17.3 | 263 | 1.31 | 28.89 | 10.08 | 28.88 | 0.01 | |
| lion 6 | 1 | 17.5 | 72 | A | 7.31 | 38.2 | 291.9 | 19.2 | -7 | 94.9 | 151 | 4 | 1.39 | 124 | 20.4 | 12 | 39 | 13.3 | 12 | 0.69 | 234 | 17 | 154 | 68 | 27 | 41 | 0.65 | 16.5 | 251 | 1.25 | 31.7 | 9.48 | 28.66 | 3.04 | 158 |
| lion 6 | 1 | 17.5 | 105 | A | 7.273 | 41.9 | 276.8 | 19.4 | -7.4 | 99.8 | 153 | 3.7 | 1.42 | 125 | 20.7 | 12 | 36 | 12.4 | 10.8 | 0.62 | 226 | 18 | 174 | 66 | 28 | 38 | 0.72 | 16.3 | 251 | 1.29 | 32.5 | 9.67 | 29.00 | 3.50 | 112 |
| lion 6 | 1 | 17.5 | 137 | A | 7.255 | 40.8 | 280.5 | 18.1 | -9.1 | 99.8 | 153 | 3.6 | 1.42 | 120 | 19.3 | 19 | 35 | 11.8 | 9.8 | 0.81 | 218 | 16 | 203 | 63 | 26 | 37 | 0.69 | 15.7 | 249 | 1.3 | 37.21 | 9.10 | 27.16 | 10.05 | 73 |
| lion 6 | 1 | 17.5 | 171 | A | 7.233 | 48.1 | 281.5 | 20.3 | -7.2 | 99.8 | 152 | 3.5 | 1.44 | 121 | 21.8 | 14 | 34 | 11.5 | 9.2 | 0.74 | 226 | 17 | 240 | 62 | 25 | 37 | 0.67 | 15.2 | 246 | 1.29 | 35.2 | 8.74 | 28.98 | 6.22 | 79 |
| lion 6 | 1 | 17.5 | 218 | A | 7.241 | 48.4 | 271.2 | 20.8 | -6.5 | 99.8 | 152 | 3.7 | 1.45 | 121 | 22.3 | 14 | 33 | 11.2 | 8.7 | 0.82 | 280 | 16 | 283 | 62 | 25 | 37 | 0.66 | 14.5 | 246 | 1.32 | 35.33 | 8.83 | 29.56 | 5.77 | 90 |
| lion 6 | 1 | 17.5 | 260 | A | 7.288 | 40.8 | 249.4 | 19.5 | -7.1 | 99.8 | 152 | 3.7 | 1.4 | 122 | 20.8 | 14 | 32 | 11 | 8.4 | 0.69 | 228 | | | | | | | | | | 34.41 | | | | 87 |
| lion 6 | 1 | 17.5 | 284 | A | 7.224 | 49.6 | 225.9 | 20.5 | -7.2 | 99.7 | 152 | 3.8 | 1.44 | 123 | 22 | 12 | 33 | 11.1 | 8.4 | 0.99 | 223 | 18 | 399 | 62 | 24 | 38 | 0.64 | 14.2 | 244 | 1.39 | 33.25 | 8.63 | 29.07 | 4.18 | 83 |
| lion 6 | 1 | 17.5 | 318 | A | 7.211 | 53.5 | 148.8 | 21.4 | -6.4 | 98.7 | 151 | 3.8 | 1.45 | 119 | 23.1 | 14 | 33 | 11.3 | 8.4 | 1.1 | 227 | 17 | 480 | 61 | 24 | 37 | 0.65 | 13.9 | 245 | 1.46 | 36.15 | 8.71 | 30.11 | 6.04 | 87 |
| lion 6 | 1 | 17.5 | 350 | A | 7.261 | 46.9 | 126.9 | 21.1 | -6 | 98.3 | 151 | 4 | 1.42 | 120 | 22.5 | 14 | 33 | 11.1 | 8.3 | 1.07 | 219 | 18 | 663 | 57 | 22 | 35 | 0.64 | 13.6 | 257 | 1.55 | 35.35 | 8.52 | 29.56 | 5.79 | 81 |
| lion 6 | 1 | 17.5 | 385 | V | 7.233 | 54.9 | 44.2 | 23.1 | -4.4 | 70.5 | 151 | 3.9 | 1.37 | 122 | 24.8 | 10 | 38 | 12.8 | 7.1 | 1.64 | 228 | 17 | 659 | 63 | 25 | 38 | 0.64 | 13.7 | 252 | 1.53 | 32.63 | 9.17 | 32.26 | 0.37 | 75 |
| lion 6 | 1 | 17.5 | 409 | V | 7.25 | 52.9 | 44.9 | 23.2 | -4 | 72.5 | 149 | 4.1 | 1.37 | 119 | 24.8 | 11 | 33 | 11.1 | 7.3 | 1.35 | 252 | 18 | 660 | 56 | 22 | 34 | 0.65 | 13.8 | 258 | 1.53 | 34.12 | 8.45 | 31.59 | 2.53 | |
| tiger 1 | 1 | 10.0 | 45 | V | 7.219 | 46.1 | 69.1 | 18.8 | -8.9 | 89.5 | 150 | 4 | 1.37 | 127 | 20.3 | 8 | 39 | 13.3 | 11.1 | 0.92 | 173 | | 791 | 72 | 35 | 37 | 0.97 | 17 | 150 | 1.64 | 27.45 | 11.88 | 30.66 | -3.21 | 74 |
| tiger 1 | 1 | 10.0 | 100 | V | 7.221 | 43.5 | 50.2 | 17.8 | -9.9 | 77.6 | 149 | 4.1 | 1.27 | 127 | 19.2 | 8 | 36 | 12.1 | 8.6 | 1.73 | 167 | | | | | | | | | | 25.64 | | | | 105 |
| tiger 1 | 1 | 10.0 | 130 | V | 7.225 | 44.4 | 71.7 | 18.4 | -9.2 | 90.7 | 148 | 4.6 | 1.31 | 126 | 19.8 | 8 | 38 | 12.9 | 10.8 | 1.38 | 171 | | 834 | 70 | 35 | 35 | 1 | 16.8 | 154 | 1.85 | 26.53 | 12.28 | 30.62 | -4.09 | 86 |
| tiger 1 | 1 | 10.0 | 157 | V | 7.211 | 49.9 | 76.6 | 20 | -7.9 | 91.8 | 147 | 5.2 | 1.32 | 123 | 21.5 | 9 | 39 | 13.2 | 11.9 | 1.56 | 163 | | | | | | | | | | 28.96 | | | | 84 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tiger 1 | 1 | 10.0 | 172 | V | 7.225 | 49.9 | 90.6 | 20.7 | -6.9 | 95 | 146 | 6 | 1.28 | 121 | 22.2 | 10 | 40 | 13.5 | 13.2 | 1.23 | 164 | | 764 | 66 | 33 | 33 | 0.98 | 16.9 | 164 | 1.95 | 31.05 | 11.94 | 32.55 | -1.50 | 74 |
| tiger 1 | 1 | 10.0 | 194 | V | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 77 |
| tiger 1 | 1 | 10.0 | 220 | V | 7.162 | 62.1 | 55.6 | 22.3 | -6.4 | 78.7 | 146 | 7 | 1.33 | 122 | 24.2 | 9 | 37 | 12.7 | 13.3 | 1.14 | 197 | | 839 | 64 | 32 | 32 | 0.98 | 16.5 | 177 | 1.9 | 31.19 | 11.31 | 33.49 | -2.30 | 101 |
| lion 1 | 1 | 18.5 | 94 | V | 7.265 | 40 | 45 | 18.2 | -9 | 74 | 151 | 4.8 | 1.32 | 124 | 18 | 15 | 30 | 10.2 | 2.1 | 0.63 | 309 | 24 | 166 | 80 | 29 | 51 | 0.56 | 18.9 | 339 | 1.91 | 32.49 | 11.01 | 29.12 | 3.37 | |
| lion 1 | 1 | 18.5 | 187 | V | 7.195 | 52.5 | 37 | 20.3 | -8 | 57 | 151 | 4.6 | 1.32 | 122 | 22 | 13 | 30 | 10.2 | 3.9 | 0.79 | 282 | 24 | 151 | 76 | 27 | 49 | 0.55 | 18.5 | 345 | 1.96 | 34.13 | 10.30 | 30.53 | 3.60 | 68 |
| lion 1 | 1 | 18.5 | 217 | V | 7.209 | 54.3 | 53 | 21.7 | -6 | 79 | | | | | | | | | | | | 26 | 179 | 69 | 24 | 45 | 0.55 | 18.4 | 350 | 2.15 | | | | | |
| tiger 2 | 1 | 8.8 | 25 | V | 7.232 | 39.7 | 70.4 | 16.7 | -10.8 | 90.6 | 150 | 3.6 | 1.28 | 128 | 16.7 | 10 | 41 | 13.8 | 11.9 | 1.46 | 193 | | 139 | 75 | 37 | 38 | 0.98 | 17.2 | 200 | 1.31 | 25.42 | 11.88 | 28.54 | -3.12 | |
| tiger 2 | 1 | 8.8 | 82 | V | 7.314 | 32.5 | 178.1 | 16.5 | -9.7 | 99.5 | 149 | 3.9 | 1.26 | 126 | 16.2 | 12 | 34 | 11.7 | 11.8 | 1.08 | 184 | | 194 | 71 | 36 | 35 | 1.02 | 16.4 | 197 | 1.63 | 27.08 | 12.59 | 29.06 | -1.98 | 101 |
| tiger 2 | 1 | 8.8 | 111 | V | 7.293 | 35.7 | 176.1 | 17.3 | -9.3 | 99.4 | 149 | 3.9 | 1.29 | 128 | 17.1 | 9 | 32 | 11 | 11.4 | 1.14 | 169 | | 210 | 68 | 34 | 34 | 0.98 | 15.3 | 191 | 1.56 | 25.05 | 11.83 | 29.07 | -4.02 | 100 |
| tiger 2 | 1 | 8.8 | 140 | V | 7.244 | 40.3 | 163.7 | 17.4 | -9.9 | 99.2 | 150 | 4.4 | 1.32 | 128 | 17.4 | 10 | 33 | 11.2 | 12 | 0.77 | 191 | | 215 | 68 | 34 | 35 | 0.97 | 15.9 | 191 | 1.56 | 26.95 | 11.60 | 28.99 | -2.04 | 96 |
| tiger 2 | 1 | 8.8 | 175 | V | 7.285 | 37.6 | 140.3 | 17.9 | -8.8 | 98.9 | 148 | 5.1 | 1.3 | 122 | 17.7 | 14 | 30 | 10.2 | 14 | 1.15 | 185 | | 233 | 67 | 33 | 34 | 0.98 | 14.6 | 194 | 1.54 | 31.25 | 11.49 | 29.32 | 1.93 | 86 |
| tiger 2 | 1 | 8.8 | 226 | V | 7.32 | 32.4 | 218.3 | 16.7 | -9.4 | 99.7 | 146 | 6.5 | 1.18 | 127 | 16.4 | 10 | 31 | 10.6 | 17.1 | 1.1 | 192 | | | | | | | | | | 25.58 | | | | |
| tiger 2 | 2 | 8.8 | 68 | V | 7.268 | 34 | 113.1 | 15.5 | -11.4 | 97.8 | 153 | 3.1 | 1.25 | 121 | 16 | 20 | 40 | 13.5 | 15.1 | 2.03 | 239 | | | | | | | | | | 34.32 | 13.27 | 28.78 | 5.54 | 140 |
| tiger 2 | 2 | 8.8 | 82 | A | 7.318 | 38.4 | 500.1 | 19.7 | -6.4 | 100 | 155 | 3.3 | 1.34 | 124 | 20.2 | 15 | 35 | 11.8 | 14 | 0.61 | 211 | | 221 | 72 | 35 | 37 | 0.96 | 21.1 | 190 | 1.55 | 35.03 | 12.20 | 31.84 | 3.19 | 139 |
| tiger 2 | 2 | 8.8 | 118 | A | 7.331 | 38.7 | 465.4 | 20.4 | -5.5 | 100 | 154 | 3.8 | 1.24 | 120 | 20.9 | 18 | 33 | 11.4 | 12.9 | 0.64 | 193 | | 229 | 70 | 34 | 36 | 0.97 | 20 | 188 | 1.63 | 38.4 | 12.13 | 32.53 | 5.87 | 130 |
| tiger 2 | 2 | 8.8 | 148 | A | 7.298 | 43.9 | 515.1 | 21.5 | -5 | 100 | 155 | 3.8 | 1.36 | 123 | 22 | 15 | 30 | 10.2 | 13.9 | 0.77 | 203 | | 259 | 67 | 33 | 34 | 0.97 | 19.2 | 188 | 1.64 | 36.39 | 11.73 | 33.18 | 3.21 | 120 |
| lion 5 | 2 | 17.8 | 22 | V | 7.215 | 41.3 | 112 | 16.7 | -11.1 | 97.3 | 153 | 4.3 | 1.29 | 125 | 17.4 | 16 | 24 | 8 | 9.6 | 1.2 | 268 | | | | | | | | | | 32.39 | | | | |
| lion 5 | 2 | 17.8 | 98 | V | 7.227 | 42.3 | 49.2 | 17.6 | -10 | 76.9 | 154 | 4.4 | 1.27 | 126 | 18.2 | 15 | 21 | 7.2 | 8.7 | 1.05 | 281 | | | | | | | | | | 32.62 | | | | 72 |
| lion 5 | 2 | 17.8 | 131 | V | 7.234 | 44.7 | 47.8 | 18.9 | -8.6 | 75.6 | 154 | 4.3 | 1.31 | 124 | 19.6 | 16 | 19 | 6.6 | 8 | 0.71 | 274 | | | | | | | | | | 34.9 | | | | 81 |
| lion 5 | 2 | 17.8 | 169 | V | 7.196 | 49.5 | 68.5 | 19.2 | -8.9 | 88.5 | 154 | 4.6 | 1.32 | 124 | 20 | 16 | 19 | 6.5 | 8.3 | 0.64 | 290 | | | | | | | | | | 35.28 | | | | 104 |
| lion 5 | 2 | 17.8 | 245 | V | 7.294 | 38.4 | 51.5 | 18.6 | -7.9 | 82.3 | 154 | 4.8 | 1.24 | 125 | 19.1 | 16 | 19 | 6.5 | 7.4 | 0.57 | 290 | | | | | | | | | | 34.47 | | | | 93 |
| tiger 2 | 3 | 8.9 | 58 | V | 7.21 | 35.8 | 106.9 | 14.4 | -13.5 | 96.6 | 154 | 3.7 | 1.24 | 126 | 15 | 18 | 41 | 13.8 | 9.4 | 1.01 | 257 | | | | | | | | | | 31.93 | | | | 135 |
| tiger 2 | 3 | 8.9 | 85 | V | 7.22 | 41.9 | 67.6 | 17.2 | -10.4 | 89.1 | 154 | 3.7 | 1.29 | 125 | 17.9 | 16 | 38 | 12.9 | 9.6 | 1.05 | 227 | | | | | | | | | | 32.94 | | | | 154 |
| tiger 2 | 3 | 8.9 | 110 | V | 7.28 | 33 | 182.5 | 15.6 | -11.2 | 99.5 | 154 | 4.5 | 1.29 | 124 | 16 | 20 | 36 | 12.3 | 11 | 0.73 | 219 | | | | | | | | | | 35.06 | | | | 138 |