Physiological responses of African elephant (*Loxodonta africana*) immobilised with a thiafentanil-azaperone combination

By

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Summary

Physiological responses of African elephant (*Loxodonta africana*) immobilised with a thiafentanil-azaperone combination

Objective To determine the cardiopulmonary and blood gas status of elephants during chemical capture (immobilisation) with a thiafentanil-azaperone drug combination kept in lateral recumbency.

Study design Prospective descriptive study.

Animal population Ten free-ranging adult African elephant bulls (estimated weight range 3000 to 6000 kg).

Methods Elephants were immobilised using a thiafentanil (15-18 mg) and azaperone (75-90 mg) by darting from a helicopter. Once recumbent, the tidal volume, minute volume, end-tidal carbon dioxide, arterial blood pressure and pulse rate were recorded immediately after instrumentation and at five-minute intervals until T20. Arterial and venous blood gases were analysed at the time of initial instrumentation and at 20 minutes. On completion of the data collection, the thiafentanil was antagonised using naltrexone (10 mg mg^{-1} thiafentanil). A stopwatch was used to record time to recumbency (dart placement to recumbency) and time to recovery (administering antagonist to standing). Data was checked for normality and was found to be parametric. Data were compared using a one-way analysis of variance and reported as mean (± SD).

Results All elephants were successfully immobilised and all physiological variables remained constant with minimal non-significant variation over time. Average time to recumbency was 12.5 minutes. The estimated expiratory tidal volume was 21 (± 6) L $breath^{-1}$ or 4.8 ± 0.8 mL kg^{-1}, and the measured minute volume was 103 (± 31) L $minute^{-1}$. The heart and respiratory

rates were 49 (±6) beats and 5 (± 1) breaths minute^{-1}, respectively. The mean arterial blood pressure was 153 (± 31) mmHg. The elephants were acidaemic (pH 7.18 ±0.06; bicarbonate ion 20 ±4 mmol L^{-1}; lactate 11 ± 4 mmol L^{-1}), mildly hypoxemic (PaO_2 68 ± 15 mmHg) and mildly hypercapnic ($PaCO_2$ 52 ± 7 mmHg). Average time to recovery was 2.2 minutes.

Conclusion and clinical relevance African elephant bulls can be successfully immobilised using thiafentanil-azaperone. Recumbency was rapid, the cardiopulmonary variables were stable and within acceptable ranges, and recovery was rapid and complete. Mild hypoxaemia and hypercapnia were evident, but does not necessarily require oxygen supplementation.

Key words: azaperone, elephant, *Loxodonta Africana*, naltrexone, thiafentanil, tidal volume

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Photo 3 The 150L Douglas bag being emptied between data collection periods.

List of abbreviations

$P(A\text{-}a)O_2$	alveolar-arterial oxygen gradient
$PaCO_2$	arterial carbon dioxide partial pressure
PaO_2	arterial oxygen partial pressure
$P(a\text{-}E')CO_2$	arterial to end-tidal carbon dioxide difference
Patm	atmospheric pressure
BE-ecf	base access of extracellular fluid
BGA	blood gas analysis
HCO_3^-	bicarbonate ion
SO_2	haemoglobin oxygen saturation
CO_2	carbon dioxide
PCO_2	carbon dioxide partial pressure
H_2CO_3	carbonic acid
°C	degrees Celsius
E	east
$PE'CO_2$	end-tidal carbon dioxide partial pressure
=	equal to
V_T	expiratory tidal volume
FiO_2	fraction of inspired oxygen
>	greater than
Hb	haemoglobin
hr	hour
H^+	hydrogen ion
kg	kilogram(s)
km	kilometre(s)
Lac	lactate
<	less than
p	level of marginal significance within a statistical hypothesis test
L	litre(s)
m	metre(s)

mg	milligram(s)
mL	millilitre(s)
mm	millimetre(s)
mmHg	millimetres Mercury
mmol	millimole(s)
V_E	expired minute volume
pH	negative log of hydrogen ion concentration
O_2	oxygen
PO_2	oxygen partial pressure
PH_2O	saturated vapour pressure of water
%	percentage
SpO_2	peripheral oxygen haemoglobin saturation
±	plus minus
PEEP	Positive End-Expiratory Pressure
V/Q	pulmonary ventilation and perfusion ratio
RQ	respiratory quotient
n	sample size
S	south
SD	standard deviation
T	time period
TCO_2	total carbon dioxide
H_2O	water

Literature review

Immobilisation of elephants

The immobilisation of elephants is fundamental in their management within captive (zoological gardens and sanctuaries) and free-ranging (reserves) environments. It allows for their translocation and release, population census, clinical examinations, surgical procedures and physiology studies (Stegmann et al. 2014). The varying habitat settings result in different modalities of drug delivery and use of attending crew to ensure safe immobilisation for the elephants and crew. In free-ranging environments, the use of helicopter and vehicle tracking produces the safest and quickest times of immobilising an elephant (Stegmann et al. 2014). This capture technique is usually coupled with radio tracking. Intramuscular drug delivery is remote using a rifle dart projector during helicopter darting; and restraint and mechanical equipment is needed to reposition the elephant into an ideal recumbent position. The ideal position of elephant is in lateral recumbency on a flat dry surface with the truck extended and patent (Burroughs et al. 2014a). Lateral recumbency, as opposed to the sternal recumbency, prevents reduction of lung volume due to the pressure placed on the diaphragm by the abdominal organs as well as the animal's weight on the sternum (Burroughs et al. 2014a). The appropriate medical equipment and materials should be availed in a clean and working condition in accordance to the procedure(s) to be carried out once the elephant is in recumbency and stable. The crew involved in the system should be familiar with their roles and responsibilities for the capture procedure, equipment and materials used (Horne & Loomis 2007) with direction taken from the veterinarian heading the capture. Observing these capture technique etiquettes allows for rapid completion of the procedure(s) and the shortest possible immobilisation time spent in recumbency which minimises the risks of capture related injuries like myopathies or neuropathies.

Immobilisation drugs of interest to this study (thiafentanil and azaperone)

The use of potent opioids in combination with sedatives has been the norm for free-ranging African elephant immobilisation. The drug combination is thought to result in a synergy whereby each drug interacts with different receptors to produce a reliable state of immobilisation with minimal overall physiological effect. Opioids have various G-protein coupled receptors namely *mu*, *delta*, *kappa* and NOP (nociceptin opioid peptide) within the body and each elicits certain physiological responses (Grimm & Lamont 2007; Al-Hasani & Bruchas 2011). All receptors activate inhibitory G-proteins (Hasani & Bruchas 2011).

The *mu* receptor elicits effects such as analgesia, sedation, euphoria, respiratory depression and reduced gastrointestinal motility. The *delta* receptor has a lesser effect of analgesia but may at times modify *mu* receptor-mediated analgesia as well as mediate opioid receptor "cross-talk". The *kappa* receptor has an analgesic effect, although difficult to distinguish from that of the mu receptor; but known to have less of a somatic analgesic effect in most species. The physiological effects of opioid agonist-antagonist, of which the most common for veterinary use is butorphanol, displays best the duality of these receptors. Butorphanol has shorter analgesic effect due to its *mu* antagonism and minimal influence on the cardiopulmonary system as a *kappa* agonist (Grimm & Lamont 2007). The NOP receptor is similar to other three opioid receptors in that opioids (particularly partial opioid agonist such as buprenorphine) can act upon the receptor to disrupt calcium and potassium ion channels inhibiting signal transduction (Al-Hasani & Bruchas 2011; McDonald & Lambert 2005). However, unlike other receptors, the NOP receptor produces anti-opioid effects by dynamic homeostatic mechanisms (Grimm & Lamont 2007; Al-Hasani & Bruchas 2011; Feng et al. 2012); which results in a pronociceptive and anti-analgesic effect at a supraspinal level (McDonald & Lambert 2005)

Thiafentanil is a synthetic fentanyl derivative belonging to the anilidopiperidine chemical opioid class that was introduced into the market in the early 1990s (Vardanyan & Hruby 2014). Thiafentanil, similar to etorphine, is a quick-acting potent pure opioid receptor agonist and

binds more specifically to the mu-opioid receptors to induce its clinical effects, compared to etorphine, which binds to all opioid receptors (Lance & Kenny 2012). Etorphine-azaperone has been the go-to combination for immobilisation of mega-herbivores (Harthoorn & Bligh 1965); however, the substitution of etorphine with thiafetanil has come into play of recent (Lance 2008). This has improved induction time and quality of immobilisation in elephants (Lance 2008). Furthermore, elephants immobilised with thiafentanil take up the preferred lateral recumbency orientation upon the initial induction; unlike etorphine where elephants become sternal recumbent and require assistance for repositioning (Horne & Loomis 2007; Lance 2008). Given thiafentanil's potency, it is mandatory that a reversal drug such as Naltrexone, a pure opioid antagonist, is made available for its administration (Grimm & Lamont 2007).

The use of neuroleptics has been mostly confined to use in psychotic patients as an anxiolytic but two of its classes, phenothiazines and butyrophenones, have gained use since the 1980s in wildlife immobilisation (Grimm & Lamont 2007; Wilde et al 2008). They have an anti-dopamine effect by blocking D2-dopamine transmitter (Grimm & Lamont 2007), which causes the patient to be in a tranquil detached state of mind (Murray et al. 2008); this state aids in combating stress and anxiety during immobilisation. Phenothiazines are regarded as long-acting neuroleptics and butyrophenones are regarded as short-acting neuroleptics, making them more ideal for wildlife immobilisation (Burroughs et al. 2014b).

The combined use of azaperone, a neuroleptic butyrophenone, with a potent opioid hastens onset of sedation and aids in muscle relaxation and reduces hypertension (Grimm & Lamont 2007; Wolfe et al. 2008, Pohlin et al. 2019). Its *alpha*-1 adrenoceptor antagonist effects cause peripheral vasodilation consequently reducing the afterload and systemic arterial blood pressure (Meyer et al. 2008). Azaperone has minimal effects on the respiratory system (Lees & Serrano 1976) making it an ideal pairing for potent opioids which readily cause severe respiratory suppression in mega-herbivores (Lance 2008). A study by Hattingh et al. (1994) revealed that the combined use of azaperone with carfentanil or etorphine helped reduced

incidents of fatal pulmonary oedema, in immobilised elephants as opposed to when the opioids were used alone. They recommended that azaperone be considered in the immobilisation drug mixture for elephants which are herded prior to darting. However, Horne and Loomis (2007) found its addition unnecessary and believe that hypertension in elephants is not directly opiate drug induced. Horne and Loomis (2007) found that during immobilisation of two elephants with an etorphine constant infusion; there was negligible cardiovascular effect; and this was considered a general property of opiates in all species. Azaperone has been used on its own for standing sedation in elephants, with anecdotal reports of excitement (Horne & Loomis 2007).

The immobilisation of free-ranging elephants comes with the limitation of not being able to accurately measure the animal's body weight prior to darting and therefore unable to deliver the precise drug dosage. The elephant's adult body weight varies from 3000 to 6000 kg, implying that one runs the risk of delivering an inappropriate drug dosage resulting in significant, undesirable, physiological effects (Stegmann et al. 2014). To minimise this risk the veterinarian is required to estimate the body weight of the elephant based on previous experience, published dose ranges for the species; and by comparison of the elephant's body size in relation to surrounding objects (trees, rocks, pathways and other animals) during the herding phase of the capture.

Monitoring the cardiovascular and respiratory systems

It is important to continuously monitor their cardiopulmonary function as means of determining the immobilised elephant's physiological stability, regardless of the remote geographic setting of free-ranging elephants (Horne & Loomis 2007). The most common monitored cardiovascular parameters are heart rate and systemic arterial blood pressure (Stegmann et al. 2014). The most common monitored respiratory parameters are respiratory rate and end-tidal carbon dioxide pressure ($P_E'CO_2$) measurements (using capnography). More advanced

respiratory system monitoring includes spirometry which is designed to measure expiratory tidal volume (V_T) and minute volume (V_E). These measurements can be achieved automatically via the use of an electronic spirometer or V_E can be manually measured by collection of expired gases using a Douglas bag method. Arterial blood gas analysis is used to measure blood oxygenation and the adequacy of ventilation by measuring the PaO_2 and $PaCO_2$, respectively. Furthermore, arterial blood gas analysis (BGA) can be used as a tool to monitor the cardiopulmonary function during immobilisation (Horne & Loomis 2007).

The indoor clinical setting allows for the use of various powered monitoring devices as the environment is controlled with a fixed power source. The modification of monitoring devices becomes imperative in an outdoor setting. The benefit of using monitoring machines is that monitoring is continuous with a warning system in place, thus the team can divert attention to the clinical procedures at hand. However, machines do have species limitations and would, at times, need to be calibrated accordingly. There also comes a need for equipment to be mobile and self-powered or rechargeable (Horne & Loomis 2007). The importance of using one's own sensory cognisance is important to assure that the readings of the equipment is reliable, be it an indoor or outdoor setting (Heard 2007).

Heart rate and systemic arterial blood pressure monitoring methods

The heart rate and rhythm can be monitored by means of a compact and rechargeable electrocardiograph in elephants immobilised in remote areas (Horne & Loomis 2007). A non-machinery dependent method would be to feel of the pulse on one of the auricular arteries. It is recommended that this method be used together with the electrocardiograph to assess the accuracy the electrocardiograph readings. Blood pressure can be measured directly by means of an arterial line connected to a monitor; or by indirect methods. Of the indirect methods (oscillometric and Doppler), automated oscillotonometry has been used successfully in the field for elephant immobilisation (Heard 2007; Horne & Loomis 2007). The direct measure of blood pressure is used primarily for large mammals due to ease of access to the auricular

artery (Heard 2007). This makes it ideal for use in elephants. The direct blood pressure measurement gives a continuous reading; and the added benefit that the catheter can be used for intermittent arterial blood sampling for gas analysis (Heard 2007).

Capnography

Capnography is a monitoring method used primarily during anaesthesia as it primarily gives the anaesthetist an idea of the adequacy of ventilation during supressed physiological function. However, its use outside of the operating room has been explored over the last couple of decades (Kodali 2013). This out of hospital capability constitutes a place for capnography in field work. A capnometer is used to monitor the amount of exhaled carbon dioxide ($P_E'CO_2$). Under physiologic circumstances The exhaled CO_2, during normal physiological function, is closely related to the arterial partial pressure of carbon dioxide ($PaCO_2$); and therefore, this parameter can be used to determine the adequacy of minute ventilation (Ve). Most clinically used devices make use of infrared technology to measure the concentration of CO_2 in respiratory gases (Kodali 2013). The device measures the $P_E'CO_2$, which is the CO_2 partial pressure at the end of an expiratory phase of a breath. A capnometer can be either diverting (side-stream) or non-diverting (mainstream); with the difference being the clinical measure of CO_2 in the monitor distant from the sample site with side-stream capnography, as opposed to measuring CO_2 at the sample site with mainstream capnography (Jaffe 2002). Mainstream capnography provides more immediate readings compared to that of side-stream capnography, especially in small animals. Ideally the mainstream sensor must be connected to the proximal end of an endotracheal tube.

Spriometry

Spirometry is the process measuring the V_T (the amount of gas exhaled during the expiratory phase of breathing), along with other respiratory variables such as V_E, of the patient. These measurements offer vital pulmonary mechanic information during spontaneous and

mechanical ventilation (Moens et al. 2009). A spirometer is the device measuring the V_T during spirometry, of which there are several technologies available. Recently a Pitot tube-based flow sensor (D-Lite flow sensor) has been adapted to measure large animal V_T (Moens et al. 2009, Moens 2010). The shortfall of the human D-Lite Pitot tube is that it is not adapted to the diameter of the endotracheal tube used in large animals. As such the Pitot tube has to be remodelled to a larger size for large animals (Moens 2010). For a study such as this one, investigating respiratory volumes of a mega-herbivore, the use of modified LA Pitot tube would be fitting. This flow sensor can also be integrated with a respiratory gas sample port (to measure partial pressures of respiratory gases) which is coupled up to a dedicated host multiplier monitor.

The Pitot tube is designed with a fixed internal diameter tube where the volume within the tube is consistent and the resistance to air flow is calculated as a constant. The flow of the respiratory gases moving through the Pitot tube during the respiratory cycle could be turbulent or laminar. The Pitot tube is designed to measure a differential pressure change over time during inhalation and exhalation phases of the respiratory cycle. The construct of the Pitot tube has two pressure sensing ports within the centre of the tube, aimed in opposite directions. The pressure sensors can then detect a change in pressure of air that moves across the tube, where the upstream sensor detects an increase in pressure as respiratory gases moves towards the port, and the opposite downstream sensor detects the baseline pressure within the tube. The change in pressure between the positive upstream pressure and the baseline downstream sensors is plotted against time thus the flow and V_Ts are calculated using Ohm's Law equations that have been adapted to calculate gas motion (Moens et al. 2009).

Spirometry offers a quantifiable measurement of V_T whereas in routine practice this parameter is often estimated by observation (thoracic movements during the respiratory cycle or evaluating the rebreathing bag movement when the animal is connected to an anaesthetic machine). The dated way of estimating the V_T is by direct measurement of V_E by collecting expired gases in a Douglas bag (an inflatable polyvinyl chloride bag with electrically welded

seams); One then takes heed of other respiratory rate, time taken to fill the bag and the maximum bag volume (if there are no other ways of determining the volume expired after 1 minute) to calculate the V_E and the estimate other spirometry variables such as V_T. Concerns regarding this method is the reliability of trapping all the expired gas and the diffusion thereof through the interior lining (Shepherd 1955). Taking these factors into account Hopker et al. (2012) found the Douglas bag technique to be highly reliable provided one was collecting large expired gas samples. Therefore, for the purposes of this study, we will make use of the Douglas bag technique because the large animal Pitot tube has not been validated for tidal volumes greater than 15 L per breath (Moens et al. 2009).

Blood gas analysis

Blood gas analysis is used to assess the patient's gas exchange and acid-base status, as well as provide information about electrolytes at the time of testing (Singh et al. 2013). The BGA machine either be described as bench top or point of care. The bench top machines analyse serum and are mostly confined to laboratory spaces (Jain et al. 2009). The point of care machines are handheld, portable and able to analyse a small whole blood volume with accuracy, suitable for field work (Heard 2007, Jain et al. 2009).

For this study a point of care machine, i-STAT blood gas analyser, will be used. Its use has been successful in the field for elephant immobilisation in forest-savannahs and rainforests; it has a rechargeable battery and relies on an operational temperate of 16-30˚C (Heard 2007). The procedure involved collecting arterial or venous blood in pre-heparinised syringes which is then loaded on to a cartridge (i-STAT CG4+ cartridge) which was placed inside an i-STAT blood gas analyser to measure several parameters [negative log of hydrogen ion concentration (pH), oxygen partial pressure (PO_2), carbon dioxide partial pressure (PCO_2), basal access of extracellular fluid (BE-ecf), bicarbonate ion (HCO_3^-), total carbon dioxide (TCO_2), blood oxygen saturation (SO_2) and lactate (Lac)]. Any deviation from the normal

values are indicative of either respiratory/metabolic acidosis or respiratory/metabolic alkalosis, as indicated in Table 1 (Singh et al. 2013).

Table 1 Classification of metabolic and respiratory acidosis, and respiratory and metabolic acidosis according to physiological variables.

Variable	Acidosis		Alkalosis	
	Respiratory	Metabolic	Respiratory	Metabolic
pH	< 7.35	< 7.35	> 7.45	> 7.45
$PaCO_2$	> 45 mmHg	< 35 mmol (respiratory compensation)	< 35 mmHg	> 45 mmHg (respiratory compensation)
BE-ecf	< -2 mmol L^{-1}	< -2 mmol L^{-1}	> 2 mmol L^{-1}	> 2 mmol L^{-1}
HCO_3^-	> 24 mmol L^{-1} (renal compensation)	< 22 mmol L^{-1}	< 22 mmol L^{-1} (renal compensation)	> 24 mmol L^{-1}

pH: negative log of hydrogen ion concentration; ($PaCO_2$) arterial partial pressure of carbon dioxide; BE-ecf: basal access of extracellular fluid; HCO_3^-: bicarbonate ion.

Elephant respiratory adaptation

Given their large frame, elephants have developed unique respiratory structures to accommodate the effect of gravity. The typical mammalian thoracic cavity has a fluid lined negatively pressured anatomical space called the pleural space. The negative pressure within the pleural spaces allows the lung to be “sucked” against the inside of the thoracic cage; which allows expansion of the lungs during the inspiration phase of the breathing cycle (Jurgen & Gros 2002). The elephant’s pleural space is unique to other mammals in that the pleural space is filled with a distensible network of collagen fibres and pockets of surfactant. These fibres extend into the lung parenchyma which is segmented into approximately $1cm^3$ units, thus connecting the lung capsule loosely to the chest wall (Brown et al. 1997). This structural modification of an elastic pleural space of connective tissue with pockets of surfactant allows the elephant to maintain pleural structural integrity and compliance when breathing despite the gravitational stress on their large lungs. However, breathing is comprised when in

recumbence due to the pressure exerted on the lungs and diaphragm by the abdominal organs (Brown et al. 1997).

Respiratory derangements

The mega-herbivores (elephant, rhino, giraffe) are known to be very sensitive to potent opioids, and their immobilisation is challenging due to the difficultly of anaesthesia management and physical manipulation (Lance 2008). Recumbency in elephants results in hypoventilation which has been associated with hypoxaemia and hypercapnia (Stegmann et al. 2014). The decreased minute volume, hypoventilation, is a result of reduced lung functional volume. Opioids have a compounding effect in that they cause rigidity of the intercostal muscles (Horne et al. 2002). Hypoxaemia is defined as the below normal blood PO_2, and for elephants clinically relevant hypoxaemia is assumed to be below 60 mmHg. Hypercapnia is defined as the above normal blood PCO_2, and for elephants this is in the range of 50-70 mmHg (Stegmann et al. 2014). The use of potent opioids has been linked to lung oedema due to its systemic hypertensive effect causing pulmonary hypertension and blood being shunted within the lung parenchyma to provide relieve (Horne et al. 2002). This results in V/Q mismatch whereby there is poor diffusion of O_2 from the alveoli into the circulation at the alveoli-capillary junction, as areas of adequate blood flow are not in conjunction with areas of adequate ventilation. Thus, hypoxaemia results from one or a combination of the above abnormalities (hypoventilation, intrapulmonary shunting, V/Q mismatch, alveolar-capillary diffusion impairment, and/or decreased cardiac output), (Horne et al. 2002). Furthermore, CO_2, which is readily diffusible from the circulation into the alveoli, is not expired as effectively due to the supersession of the respiratory centre by opioids, to elevated blood PCO_2 resulting in hypercapnia (Burroughs et al. 2014b).

With regards to respiratory control of blood pH, the build-up of blood PCO_2 which makes the animal acidaemic as there is an increase in H^+, from the formation of carbonic acid (H_2CO_3; equation below shifts right). The opposite ensues when the respiratory response to elevated

CO_2 increases Ve, resulting in expulsion of CO_2; which makes the animal alkalaemic as there is a decrease in H^+ (equation below shifts left) This can be explained by the following chemical reaction (Singh et al. 2013):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

The blood pH buffering system is reliant on ionic balance with the most influential being the respiratory regulation of CO_2 and the metabolic regulation of HCO_3^- by the kidneys; each resulting in either respiratory/metabolic acidosis or respiratory/metabolic alkalosis (Table 1), (Singh et al. 2013).

There is a question of whether these derangements are predominantly a drug effect or more related to the species physical design and physiology. A study by Haw et al. (2015) showed that white rhinoceroses immobilised with etorphine-azaperone had no significant difference in the degree of hypoxaemia experienced between free-ranging and captive rhinoceroses. However, the state of hypercapnia and acidaemia was more severe in those that were free-ranging than those captives. Boesch et al. (2018) found severe hypoxaemia in a study looking only at etorphine-azaperone immobilised boma habituated white rhinoceroses who were administered an additional intravenous bolus of etorphine. This alludes to the notion that pre capture stress alone is not an aggressor of respiratory derangements in immobilised mega-herbivores. Immobilised elephants could be at risk from drug and animal related derangements that could compromise their cardiovascular and respiratory physiology. Still et al. (1996) reported that juvenile elephants, weighing less than 600 kg, captured using etorphine-azaperone, had worse cardiopulmonary derangements, especially hypoxia, compared to heavier elephants. Adult elephants captured using etorphine alone had peripheral oxygen-haemoglobin saturation (SpO_2) ranging from 70% and 96%, which suggests some elephants were hypoxic (Osofsky 1997). Although these derangements have been reported in elephants, no formal investigation has been conducted to determine the underlying aetiology of the hypoxia. Horne et al. (2002) states that the cause of hypoxaemia

in etorphine-immobilised elephants is unknown, but probably a combination of a drug mediated respiratory depression, decreased thoracic wall compliance, and recumbency promoting redistribution of blood flow within the lungs. It can be assumed that the same conditions factor for hypercapnia.

The spirometry respiratory volumes (V_T and V_E) can be compared with that of other terrestrial mammals using allometric scaling to see if they fall within the normal mammalian range (Bide et al. 2000). Spirometry can be used to assess lung function during immobilisation (Schramel et al. 2014), by determining respiratory volumes and if whether an impairment is obstructive, restrictive or a combination of both (Morris 1976).

The oxygen and carbon dioxide partial pressure gradients can be determined to improve our understanding of how well the alveoli are being perfused. The alveolar-arterial oxygen gradient, $P(A\text{-}a)O_2$, and the arterial to end-tidal carbon dioxide difference, $P(a\text{-}E')CO_2$ are the two most common gradients that are calculated. The $P(A\text{-}a)O_2$ measures the PO_2 potential difference that should exist among the alveoli and the capillary to allow for adequate diffusion of oxygen (O_2) from the alveoli with a higher PO_2 (>99 mmHg) to the capillary network with a lower PO_2 (80-100 mmHg), (Singh et al. 2013, Wagner 2015). The normal mammalian $P(A\text{-}a)O_2$ range is 5 to 10 mmHg in young healthy individuals (Wagner 2015); implying that the alveolar PO_2 needs to be higher than that of the capillaries. The $P(a\text{-}E')CO_2$ measures the PCO_2 potential difference that should exist among the capillary network to allow for adequate diffusion of CO_2 from the capillary network with a higher PCO_2 (45 mmHg) to the alveoli with a lower PCO_2 (40 mmHg), (Singh et al. 2013, Wagner 2015). The normal mammalian $P(a\text{-}E')CO_2$ range varies from 2 to 5 mmHg; implying that the capillary PCO_2 needs to be higher than that of the alveoli. Deviations from this give an indication of ventilation perfusion (V/Q) mismatch.

Introduction

There has been momentum gained in wildlife anaesthesiology of mega-herbivores over the past two decades with increased formal accounts of elephant immobilisation. This study investigates and documents the efficacy of thiafentanil-azaperone immobilisation in free-ranging African elephants. To date, studies have not related the gas derangements (state of hypoxia and hypercapnia) in immobilised African elephants kept in lateral recumbence to the opioid drug effects, hypoventilation or other physiological disturbances. To quantify ventilation, spirometry is used; and to quantify the alveolar-capillary integrity gas partial pressures gradients are calculated. Furthermore, most of the elephants were never weighed and all of the current data is interpreted on estimated body weights. Therefore, we wanted to also investigate if morphometric data (such as body length for example) could be a useful tool to compare physiological data like tidal volumes, minute volumes and systemic arterial blood pressure to the size of the elephant.

Aim

We aimed to investigate and describe the efficacy and cardiopulmonary physiological effects of thiafentanil-azaperone immobilised free-ranging trunk breathing African elephants kept in lateral recumbency. Furthermore, we aimed to investigate if there were morphometric parameters that could be used as a proxy for animal mass to predict tidal volumes and systemic arterial blood pressures.

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Objectives and hypotheses

Drug cardiopulmonary effects

Objective: determine the state of the cardiopulmonary effects of immobilised elephants using thiafentanil-azaperone kept in lateral recumbency.

Hypothesis: The cardiopulmonary variables of elephants during immobilisation will be stable and within known reference intervals for elephants during thiafentanil-azaperone immobilisation.

Morphometrics

Objective: determine if there is a relationship among the cardiopulmonary parameters measured and the body length of the elephants.

Hypothesis: There will be a significant correlation of the cardiopulmonary parameters measured and the body length of the elephants.

Benefits arising from the study

From this study, we gained a further understanding of the efficacy and physiological response of African elephants immobilised with a thiafentanil-azaperone combination. More specifically, we investigated more advanced cardiovascular and respiratory system variables compared to previous reports, with the aim of defining possible causes of hypoxaemia and hypercapnia during immobilisation. Furthermore, the linear correlation relationship among the respiratory volumes (estimated V_T and measured V_E) and blood pressure (SAP and MAP) with the body length were determined. This information has increased our understanding of elephant immobilisation and we can make recommendations to improve the management of these animals while immobilised.

Materials and methods

Animals and study design

Ten free-ranging African elephant bulls of an estimated age and weight range of 25 to 40 years and 3000 kg to 5000 kg, respectively, were opportunistically used for the prospective, descriptive study. The total number of elephants was determined by the availability of the helicopter used during capture and the study budget. The present study was approved by the ethics committees of the University of Pretoria (V058-18) and South African National Parks (#006/18).

Capture procedure and immobilisation

The capture site was the south-western area of the Kruger National Park (approximately 25°03′50.1″S by 31°23′33.4″E; 300 meters above sea level). The mean barometric pressure was 737 mmHg, estimated using local meteorological data obtained from the Skukuza airport. The elephants were captured from 05h30 to 12h30 while ambient temperatures ranged from 17°C to 27°C. The distance covered by an elephant was not measured but estimated by the experienced pilot using a scaled electronic navigational map.

Elephants were darted from a helicopter using an air-pressurised dart (3 mL Dan-Inject dart; Dan-Inject; 60 mm collared needle) propelled by a CO_2 powered rifle (Dan-Inject JM-special rifle; Dan-Inject). Darts were loaded with a 1 to 5 ratio of thiafentanil (Thianil; Wildlife Pharmaceuticals) to azaperone (Zapanil; Wildlife Pharmaceuticals) combination, with dose ranges based on subjective weight and age estimation by the same veterinarian. Elephants (*n* = 5*)* estimated to weigh at least 5000 kg received 18 and 90 mg animal^{-1} of thiafentanil and azaperone, respectively. Elephants (*n* = 4) estimated to weigh 4000 kg received 16 and 80 mg animal^{-1} of thiafentanil and azaperone, respectively. One elephant estimated to weigh 3000 kg received 15 thiafentanil and 75 mg animal^{-1} of azaperone. Once the elephants were

recumbent, the ground crew approached and assisted the elephants into lateral recumbency, if required (**Photo 1**).

Photo 1 Repositioning of elephant into lateral recumbency following helicopter darting.

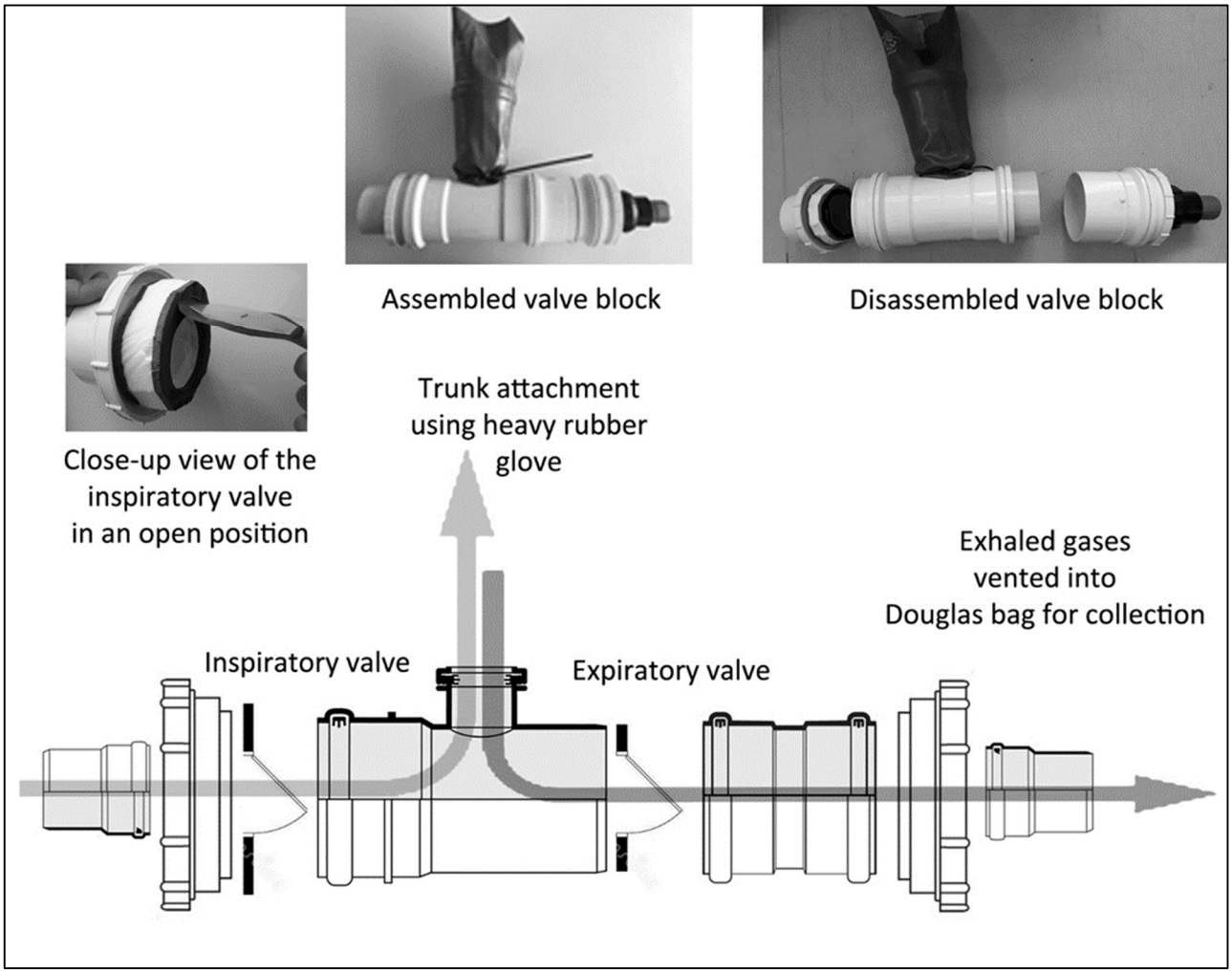
Instrumentation

Once in lateral recumbency, the elephant's head and trunk were extended to ensure airway patency, and a stick was placed in the end to keep the nostrils open (**Photo 2**). Indwelling cannulas (18 gauge; Jelco; Smith Medical) were aseptically placed into an auricular vein and artery of the caudal surface of the non-dependant ear to allow intermittent blood sampling and invasive blood pressure monitoring (via the arterial cannula). An electronic strain gauge (BD DTX; Becton & Dickson Medical) was zeroed to atmospheric (Patm) air pressure and positioned at the level of the manubrium of the sternum. Side-stream capnography was used. A gas sampling line was inserted approximately 150 mm deep into the non-dependent nostril of the trunk to measure $PE'CO_2$ using a multiparameter monitor (BM5 VET monitor; Bionet Co., Ltd). A purpose-built valve block designed to capture the expired gases was hand-made for the project using commercially available plumbing pipes (**Figure 1**; SE 400 Polyvinyl chloride 110 mm T-pipe; Marley). A non-porous heavy rubber glove was fitted tightly to the T-piece (55 mm internal diameter) and the proximal trunk was placed inside the glove to allow the unidirectional flow of inspiratory and expiratory respiratory gases to flow though the valve block. A 150 L Douglas bag (Harvard Apparatus, Holliston) was connected to the expiratory valve end via a 50 mm internal diameter pipe (Kreepy Krauly male to female hose; Pantair) to collect all expired gases. Once recumbent, a 5-minute interval was

allowed for patient positioning and instrumentation before the commencement of data collection.

Photo 2 Immobilised elephant in left lateral recumbency. The trunk was placed on a groundsheet, extended and with the lips kept patent with a stick. The non-dependent ear was reflected rostrally such that it covers the eye.

Figure 1 Purpose-built valve block used to collect exhaled gases via the trunk in spontaneously breathing African elephant bulls immobilised with thiafentanil-azaperone. Image insert is the actual built valve block. An exploded view of the construct is presented using actual schematic drawings of the components used to build the valve block (https://www.marleyplumbinganddrainage.com). Notice the position of the non-return inspiratory and expiratory valves (18 grams weight; cushioned with high density foam). During measurements, the valve block was coupled to a 150L Douglas bag to collect expiratory gases.



Data collection

Photo 3 Douglas bag being emptied between 5-minute data

Once instrumentation was complete, physiological data were recorded, including heart rate, respiratory rate, invasive blood pressure and $PE'CO_2$ measurements. The valve block was connected to the trunk to collect the expired gases and the elephant was allowed to breathe through the device until the Douglas bag was considered full (based on the distention of the bag and a palpable change in compliance; **Photo 3**). A stopwatch was used to record the time taken to fill the bag completely and the inspiratory time. The number of breaths taken to fill the bag was also recorded. These parameters were recorded at the start of data capture (T0) then at 5-minute intervals until 20 minutes (T20). Venous and arterial blood samples were collected at T0 and T20, from the respective intravascular cannulas into pre-heparinised syringes. The blood samples were analysed using a hand-held blood gas analyser (i-STAT CG4+ cartridges and i-STAT blood gas analyser; i-STAT Corporation). After the final data collection for the present study, the elephant underwent additional sampling procedures that took a further 15 minutes. Thiafentanil was antagonised by administering naltrexone (10 mg mg^{-1} thiafentanil; Trexanil;

Wildlife Pharmaceuticals) intravenously. The elephant was monitored from a distance throughout the recovery phase until it walked away from the area.

Morphometric data were collected for each elephant once in lateral recumbency. Measurements were of the shoulder height (measured from the base of the front foot to the cranial angle of the scapula), hip height (measured from the base of the hind foot to the iliac crest), back length (measured from the base of the neck, along the back, to the iliac crest) and body length (measured from the cranial most part of the neck, along the back, to the tail root). Measurements were done unilaterally on the non-dependent side of the body.

Times related to drug effect were recorded. The ataxia time was the time from dart placement to when the elephants began to show ataxia during the helicopter herding. The time to recumbency was the time from dart placement to when the elephant went into lateral recumbency (assisted or unassisted). The recovery time was the time taken by the elephant to rise to a standing position after administration of the reversal drug. The duration of immobilisation was the period from recumbency to standing.

Data analysis

The $P(a- E')CO_2$ was calculated by subtracting the $PE'CO_2$ from the $PaCO_2$. The $P(A-a)O_2$ was calculated using the following formula:

$$P(A-a)O_2 = [FiO_2(Patm – PH_2O) – PaCO_2/RQ] – PaO_2$$

where the fraction of inspired oxygen (FiO_2) was 0.21; the Patm was 737 mmHg; the saturated vapour pressure (PH_2O) was 47 mmHg; and the respiratory quotient (RQ) was 1.0 (Wagner 2015; Buss et al. 2016). The $PaCO_2$ and arterial oxygen partial pressure (PaO_2) were obtained from the arterial blood gas analysis. Gradients were calculated at T0 and T20 for each elephant and then averaged per elephant.

Data were assessed for normality and distribution by plotting histograms and evaluating descriptive statistics and the Anderson-Darling test for normality. Means and standard

deviation (SD) were calculated for all variables. One-way analysis of variance (ANOVA) was used to compare cardiopulmonary and blood gas variables over time, using post-hoc multiple comparisons using Dunnett's method (baseline values used as the control). The correlation between cardiopulmonary variables (heart rate, respiratory rate, V_T, V_E, systolic and mean arterial blood pressures), averaged over time, per elephant and body length were evaluated using Pearson's correlation; represented visually as scatter plots. The V_T was an estimation considering that the respiratory volumes of each breathing cycle could not be determined using a Douglas bag: the assumption is that V_T of each elephant was fairly constant because of the stable respiratory rates. The estimated body weight was used to calculate the estimated V_T and measured V_E on a per kg basis. Data were analysed using commercially available software (MiniTab version 18.1; MiniTab Inc.) and the level of significance was set to $p < 0.05$.

Results

Capture, immobilisation and recovery

Elephant immobilisations using thiafentanil-azaperone administration resulted in successful immobilisation of all ten elephants. The mean time from dart to ataxia was 5 minutes 32 seconds (± 55 seconds). The distance covered by an elephant during this stage was approximately 650 meters, whilst amendable to helicopter herding into an open flat area. All elephants experienced a state of standing sedation with ear flapping and flaccidity of the trunk and tail before going down. Most elephants (*n* = 8) went into lateral recumbency without aid. Two elephants that were in sternal recumbency were repositioned into lateral recumbency. Five elephants were in left recumbency and the other five were in right recumbency. The mean time to recumbency was 12 minutes and 29 seconds (± 3 minutes and 52 seconds). The smallest elephant (3000 kg) received an additional 2 mg thiafentanil intravenously after data collection to complete morphometric procedures because there was trunk curling indicating it was not adequately immobilised. All other elephants remained in lateral recumbency without signs of arousal. All elephants rose unassisted; and once standing, the elephants would stand still, with mild ataxic rocking and after a few minutes began to walk away without ataxia or signs of neuromuscular pathology (myopathy or neuropathy). The duration of immobilisation was 64 minutes and 8 seconds (± 6 minutes and 32 seconds). The mean recovery time was 2 minutes 10 seconds (± 30 seconds) after antagonist administration.

Cardiopulmonary and blood gas variables

The cardiopulmonary and blood gas variables showed no significant changes over the monitoring period (**Table 2 & 3**). Overall mean respiratory rate was 5 ± 1 breaths minute^{-1}. The breathing pattern was regular with a mean inspiratory time of 3.8 ± 0.9 seconds and inspiratory to expiratory ratio of 1:2. The largest estimated V_T measured was 37 L from an elephant with an estimated weight of least 5000 kg. The smallest estimated V_T was 11 L from

an elephant with an estimated weight of 4000 kg. The average estimated V_T was 21 L per breath, or 4.8 ± 0.8 mL kg^{-1} per estimated body weight. The mean PaO_2 was 68 ±12 mmHg and mean $PaCO_2$ was 51 ±6 mmHg. The overall averaged $P(A\text{-}a)O_2$ and $P(a\text{-}E')CO_2$ was 7± 14 mmHg and 10 ± 4 mmHg, respectively. The mean heart rate was 49 ± 6 beats $minute^{-1}$ and arterial blood pressure was 153 ± 31 mmHg. The elephants were acidaemic (overall pH: 7.18 ± 0.06; $PaCO_2$: 51 ± 6 mmHg and HCO_3^-: 19 ± 3 mmol L^{-1}). The blood lactate concentration was 12 ± 4 mmol L^{-1}.

Table 2 Respiratory and cardiovascular variables of free-ranging African elephant bulls immobilised using thiafentanil-azaperone.

Variable	**Unit**	**Time**											
		0 minute		5 minute		10 minute		15 minute		20 minute		Average	
		Mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
Respiratory rate	Breaths $minute^{-1}$	5	±1	5	±0	5	±1	5	±1	5	±1	5	±1
V_T	L	21	±7	21	±7	21	±6	22	±8	22	±7	21	±6
V_E	L	105	±25	106	±34	105	±35	101	±34	99	±29	103	±31
$PE'CO_2$	mmHg	43	±7	42	±7	42	±7	42	±8	43	±8	42	±7
Heart rate	Beats $minute^{-1}$	51	±5	49	±5	49	±6	49	±7	49	±7	49	±6
SAP	mmHg	196	±39	185	±40	181	±42	185	± 41	177	±34	185	±38
DAP	mmHg	139	±27	135	±30	135	±36	137	± 46	125	±21	134	±32
MAP	mmHg	159	±31	153	±34	151	±37	150	± 35	150	±21	153	±31

V_T: estimated expiratory tidal volume; V_E: measured minute volume; VT per kg: expiratory tidal volume per body weight; PE'CO2: end-tidal carbon dioxide partial pressure; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure.

Table 3 Blood gas variables of free-ranging African elephant bulls immobilised using thiafentanil-azaperone.

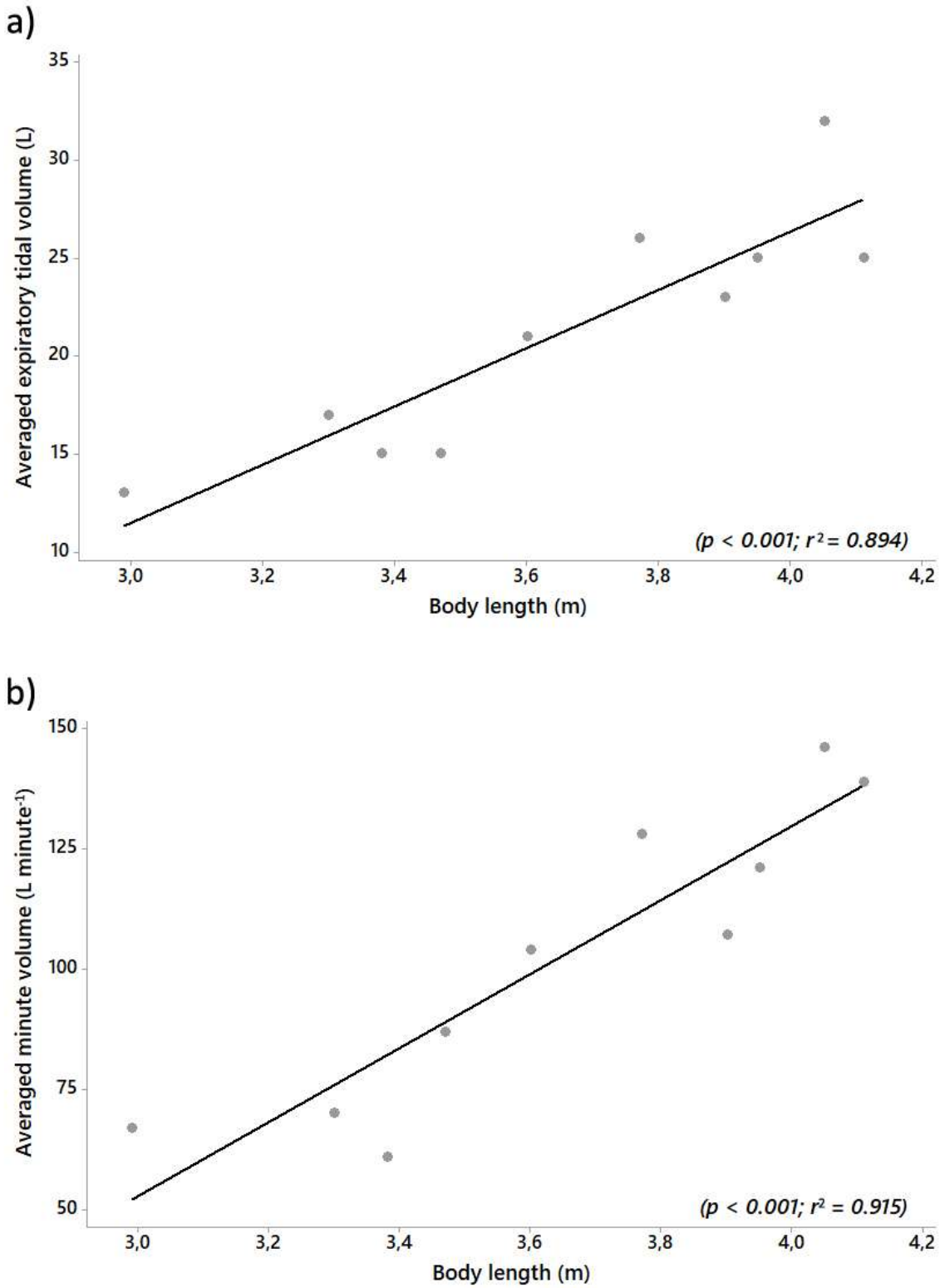
Variable	Unit	Time											
		0 minute				20 minute				Average			
		Arterial		Venous		Arterial		Venous		Arterial		Venous	
pH	pH	7.16	±0.06	7.16	±0.06	7.18	±0.05	7.187	±0.05	7.18	±0.06	7.18	±0.06
PCO_2	mmHg	54	±7	53	±7	52	±5	55	±7	51	±6	52	±7
PO_2	mmHg	71	±11	70	±13	74	±14	75	±21	68	±12	68	±15
BE–ecf	mmol L^{-1}	-9	±5	-10	±5	-9	±4	-7	±5	-9	±4	-9	±5
HCO_3^-	mmol L^{-1}	19	±4	19	±4	20	±3	21	±4	19	±3	20	±4
TCO_2	mmol L^{-1}	21	±4	21	±4	21	±3	23	±4	21	±4	21	±4
SO_2	%	87	±5	86	±5	89	±6	88	±7	88	±5	87	±6
Lac	mmol L^{-1}	13	±4	12	±4	11	±4	10	±4	12	±4	11	±4

PCO_2: partial pressure of carbon dioxide; PO_2: partial pressure of oxygen partial pressure; BE-ecf: basal access of extracellular fluid; HCO_3^-: bicarbonate ion; TCO_2: total carbon dioxide; SO_2: haemoglobin oxygen saturation; Lac: lactate.

Morphometrics and cardiopulmonary variables

The estimated V_T ($p < 0.001$; $r^2 = 0.894$; **Figure 2a**), V_E ($p < 0.001$; $r^2 = 0.915$; **Figure 2b**), systolic ($p = 0.01$; $r^2 = 0.762$) and mean arterial blood pressure ($p = 0.017$; $r^2 = 0.730$) had positive correlations with the body length. The respiratory rate ($p = 0.239$; $r^2 = 0.410$) and heart rate ($p = 0.431$; $r^2 = -0.281$) did not correlate to the body length.

Figure 2 The averaged estimated V_T (a) and averaged measured V_E (b) of African elephant bulls immobilised using thiafentanil-azaperone correlated to the body length (measured from the cranial most neck, along the back until the tail root).



Discussion

Thiafentanil-azaperone administration induced a reliable and rapid state of immobilisation in free-ranging African elephants. The monitored cardiopulmonary variables were stable and mostly better than expected compared to white rhinoceros immobilised with a potent opioid and sedative combination (Haw et al. 2015). The estimated V_T and measured V_E were similar to normal mammal volumes on a per kilogram basis (Bide et al. 2000). The data obtained from the calculated gradients suggest good alveolar-capillary gas exchange.

The behaviour exhibited from darting to recumbency was the expected response of a potent-opioid based drug combination in African elephants. All elephants experienced a state of standing sedation with ear flapping and flaccidity of the trunk and tail before going down, similar to etorphine-azaperone immobilisations (Stegmann et al. 2014). Thiafentanil-azaperone administration resulted in a time to recumbency of between 5 to 15 minutes for the study. Elephants showed mild excitement with ataxia, followed by standing sedation with ear flapping and flaccidity of the trunk and tail before moving into recumbency (Burroughs et al. 2014a).

The cardiovascular parameters were mostly within acceptable ranges, with the exception of evidence of systemic arterial hypertension. It is common for mega-herbivores, like white rhinoceros, to have arterial hypertension after being immobilised using a potent-opioid drug combination (LeBlanc et al. 1987; Buss et al. 2016). The proposed mechanism is that there is an increase in sympathetic tone, either by direct potent-opioid action at peripheral adrenoceptor sites; or through indirect mechanisms whereby common secondary messenger pathways (specifically g-protein coupled receptor pathways) are activated (LeBlanc et al. 1987). The vasodilatory effects of azaperone, through alpha1-adrenoceptor antagonism, did not prevent the systemic arterial hypertension in the elephants, contrary to what has been previously described in white rhinoceros and elephants immobilised using etorphine-azaperone (Stegmann et al. 2014; Buss et al. 2016). However, the values were similar to awake elephants which could suggest that the elephants have a higher baseline blood

pressure and were actually normotensive throughout the monitoring period (Honeyman et al. 1992). Therefore, species specific criteria should be used to define systemic arterial hypertension.

The pulmonary variables were also mostly unchanged from expected values; however, this is the first report of measured V_T and V_E in this species. An allometric scaling study by Villar and Kacmarek (2014) presented the formula for the calculation of the V_T in terrestrial mammals: VT = 0.0063(body weight in kg)$^{1.02}$. When this equation is applied to a 4000 kg elephant, the predicted V_T is 30 L (for an awake elephant). This amount is greater than the measured overall averaged estimated V_T of 21 (± 6) L (the average weight was 4400 kg) in the immobilised elephants. The estimated V_T per body weight, 4.8 (± 0.8) mL kg^{-1}, is within the, possible minimal, mammalian range of 4 to 8 ml kg^{-1} (Villar & Kacmarek 2014) regardless of the elephant being the largest terrestrial mammal. The V_T to body weight ratio remains within this range because the alveolar function of the mammalian lung remains the same regardless of size, with structural modifications as per body design (Sapoval et al. 2002). The density of mammals is similar regardless of body size; therefore, perfusion of O_2 per tissue mass per breath should be similar in the absence of a lung pathology (Villar & Kacmarek 2014).

Studies using allometric scaling of mammals have concluded that V_E of an unanaesthetised animal is 0.499(body weight in kg)$^{0.809}$ (Bide et al. 2000). Using this equation, the predicted V_E of a 4000 kg elephant is 450 L minute^{-1}. The measured overall averaged V_E was 103 (± 31) L minute^{-1}. When comparing the V_E of the awake (74 L minute^{-1}) and anaesthetised giraffe (61 L minute^{-1}) to the predicted V_E (84 L minute^{-1}) using the above allometric equation, it is noted that the difference among the V_E is not as large when comparing the elephant predicted and measured V_E (Bide et al. 2000). The average V_E per kilogram of immobilised elephants in the study was 23 mL kg^{-1} minute^{-1}, which is four times less than that of the giraffe (110 mL kg^{-1} minute^{-1}). This difference can be explained by the trend among terrestrial mammals where V_E to body weight ratio decreases as animal size increases (Bide et al. 2000). This decrease is based on the decrease in the basal metabolic rate (less energy and O_2 demand) as the surface

to volume ratio decreases with increasing body size (Da Silva et al. 2006). It can be expected that the V_E of elephants is higher when awake, with an unsuppressed basal metabolic rate, because they have a higher respiratory rate of 4 to12 breaths minute^{-1} (Schmidt 2003) and presumptively, a higher V_T.

The arterial and venous pH were less than 7.35, the arterial and venous HCO_3^- values were less than 24 mmol L^{-1}, the base excess was more negative than -5 mmol L^{-1}, and the lactate concentrations were greater than 4 mmol L^{-1}, which all suggest a primary metabolic acidosis. Furthermore, the arterial and venous PCO_2 were more than 45 mmHg which suggests no respiratory compensation for the metabolic acidosis. This scenario suggests a poor compensatory respiratory response to the metabolic acidosis, most likely due to drug effects, however, we cannot exclude the effects of lateral recumbency. Potent opioids decrease the sensitivity of the respiratory centre to CO_2 which then begins to accumulate, resulting in hypercapnia (Burroughs et al. 2014b). However, the $PaCO_2$ was not as elevated as expected for a mega-herbivore because in etorphine-immobilised white rhinoceros, this value often exceeds 65 mmHg (Buss et al. 2015).

The blood gas parameters measured from the arterial blood sample, when compared to the same parameters of the venous blood sample, did not vary and were stable throughout the immobilisation. The $P(a\text{- }E')CO_2$ of 10 ± 4 mmHg in the immobilised elephants, is similar to that of anaesthetised horses and well within clinically acceptable limits, which suggests that the mild hypercapnia was due to V/Q mismatch. Mosing et al. (2018) states that an increase in $PaCO_2$ in relation to $PE'CO_2$ is indicative of poor ventilation and venous admixture at the alveoli capillary network in anaesthetised spontaneously breathing adult horses. Potent opioids have been linked to increased metabolic rate, and therefore increased CO_2 production, which could also contribute to hypercapnia in the elephants (Buss et al. 2015). Furthermore, the hyperlactatemia is a common clinical finding in free-ranging mega-herbivore immobilisation and could be because of a combination of accelerated glycolysis and O_2-dependant metabolism during peri-darting activity (Gladden 2004; Haw et al. 2015). With a

$P(A\text{-}a)O_2$ of 7 ± 14 mmHg the hypoxemia was unexpected; this observation suggests an intact alveolar-capillary unit, unlike the white rhinoceros which had severe hypoxaemia and wide $P(A\text{-}a)O_2$ gradients exceeding 20 mmHg (Buss et al. 2015). Again, these findings suggest that the pulmonary ventilation and perfusion relationship of the elephants was normal. It has been suggested that elephants have a greater O_2 affinity to haemoglobin at lower PO_2 (Honeyman et al. 1992). Which could mean that despite an acceptable PaO_2, most of the O_2 remained bound to the haemoglobin and not transferred to the metabolising tissues. The PaO_2 of conscious elephants voluntarily lying in lateral recumbency was 84 mmHg which did not vastly different from the study's results, however, these elephants were smaller and were in a resting state before being placed into lateral recumbency (Honeyman et al. 1992).

This raises the question of whether the elephant's adaption of arrested breathing underwater, much like other marine mammals, implies better O_2 delivery mechanisms per respiratory effort than other mega-herbivores. Mammals are capable of inspiratory pause whereby the inspired breath is held for an extended period creating positive pressure with the acini before being expired. Elephants are observed to exercise this breathing mechanism to greater effect with a long inspiratory pause (an observed 1:2 inspiratory expiratory ratio). This in turn allows the diffusion of the inspired O_2 to the alveoli bed at the extreme acini, with a more extensive capillary bed, for an extended time making the respiratory exchange of O_2 more efficient (Sapoval et al. 2002). It is also considered that the narrower diameter of the valve block outlet connecting to the Douglass bag could have increased air resistance during expiration causing the elephant to have a Positive End-Expiratory Pressure (PEEP) with each breathing cycle. This would in turn promote O_2 diffusion at the alveoli capillary network. These mechanisms, however, have minimal influence on CO_2 expulsion as CO_2 it has 20 times the diffusion coefficient than that of O_2 meaning that its diffusion gradient throughout the upper and distal acini is efficient during the inspiratory pause and PEEP. Expulsion of CO_2 thus relies on the effectiveness of ventilation. Sapovol et al. (2002) states that the PCO_2 remains high in the deeper and proximal larger proximal parts of the acinus, during hypoventilation or apnoea,

and should be considered an important factor to in acid-base regulation. The unique structure of having an elastic pleural space connective tissue, with pockets of surfactant, allows the elephant to maintain pleural structural integrity under gravitational stress when in lateral recumbency better than other mega-herbivores, thus preventing alveolar collapse (Brown et al. 1997).

The relationship among the morphometrics and cardiopulmonary variables revealed positive linear correlations. The arterial hypertension was expected post potent-opioid administration, despite the vasodilatory effects of azaperone (Stegmann et al. 2014; Buss et al. 2016). But our values were similar to awake elephants (Honeyman et al. 1992). Honeyman et al. (1992) showed that clinically healthy adult African elephants, in lateral recumbency run a risk of developing significant hypoxemia and hypertension. The strongest relation of the SAP and MAP was with the shoulder and hip height (data not presented in this dissertations results); although of interest was the relation to body length as the elephants were in lateral recumbency.

The averaged estimated V_T and measured V_E had a strong positive linear correlation with morphological aspects (shoulder height, hip height, back length and body length). The strongest relation of the estimated V_T and V_E was with the back and body length, which can be inferred to the V_T and V_E increasing with increasing thoracic volume (comprising of the trachea and the lung parenchyma). The elephant's lung has been described as 'tall' with the diaphragm and is obliquely (nearly 45°) orientation to the spinal axis (and gravitation force); this varies to the more perpendicular diaphragm orientation of other terrestrial mammals (Brown et al. 1997). For elephants with a greater lung length, the volume would significantly increase. This becomes even more apparent when other thoracic and abdominal organs compressed the 'tall' lung during recumbency. It was noted that the elephant with the largest estimated V_T had the second longest body length and the elephant with the lowest estimated V_T had the shortest body length which could support this notion.

The main limitation of our study was that we only used free-ranging adult bull African elephants and therefore we cannot state with confidence that these results would be similar in juveniles and female elephants or in captive African elephants. The elephants responded to the presence of the helicopter, the darting and herding and thus the level of exertion, excitement and distance travelled before becoming recumbent could not be controlled for in this free-ranging population. The use of the Douglas bag method made it not possible to establish the true V_T thus the estimated V_T from the study is in fact a mean of the varied V_T used to fill up the bag. Furthermore, we only investigated thiafentanil-azaperone and cannot conclude that a similar result will be obtained when etorphine is used instead.

Conclusion

Free-ranging African elephant bulls were successfully immobilised using thiafentanil-azaperone, with no sustained injury to the elephants and crew throughout the immobilisation. Mild hypoxia and hypercapnia were evident and not life threatening, which was unexpected for immobilised mega-herbivores in lateral recumbency. Immobilised elephants have a estimated V_T of 4.8 ± 0.8 mL kg^{-1} and the estimated V_T and V_E positively correlate to the body length.

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Addendum

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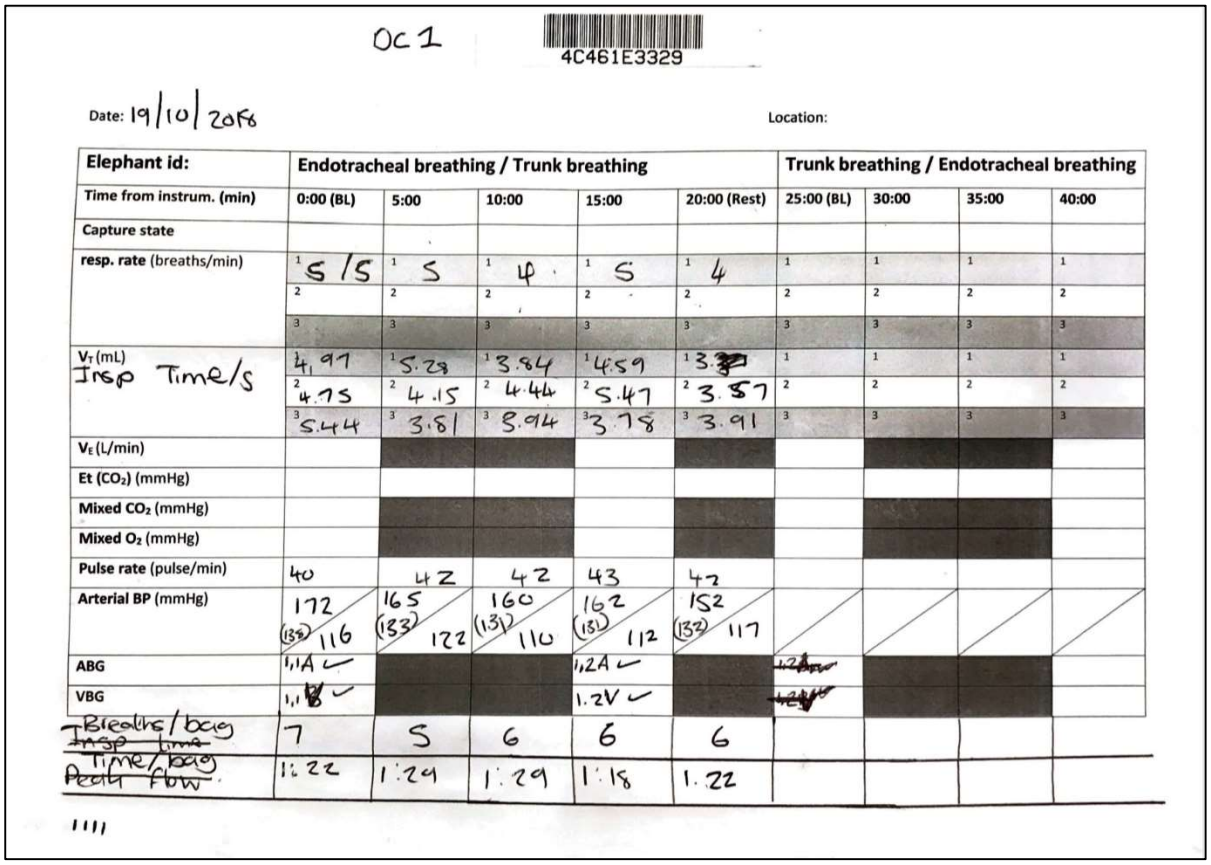
Data collection forms

Cardiopulmonary data collection sheet.



Oc 1

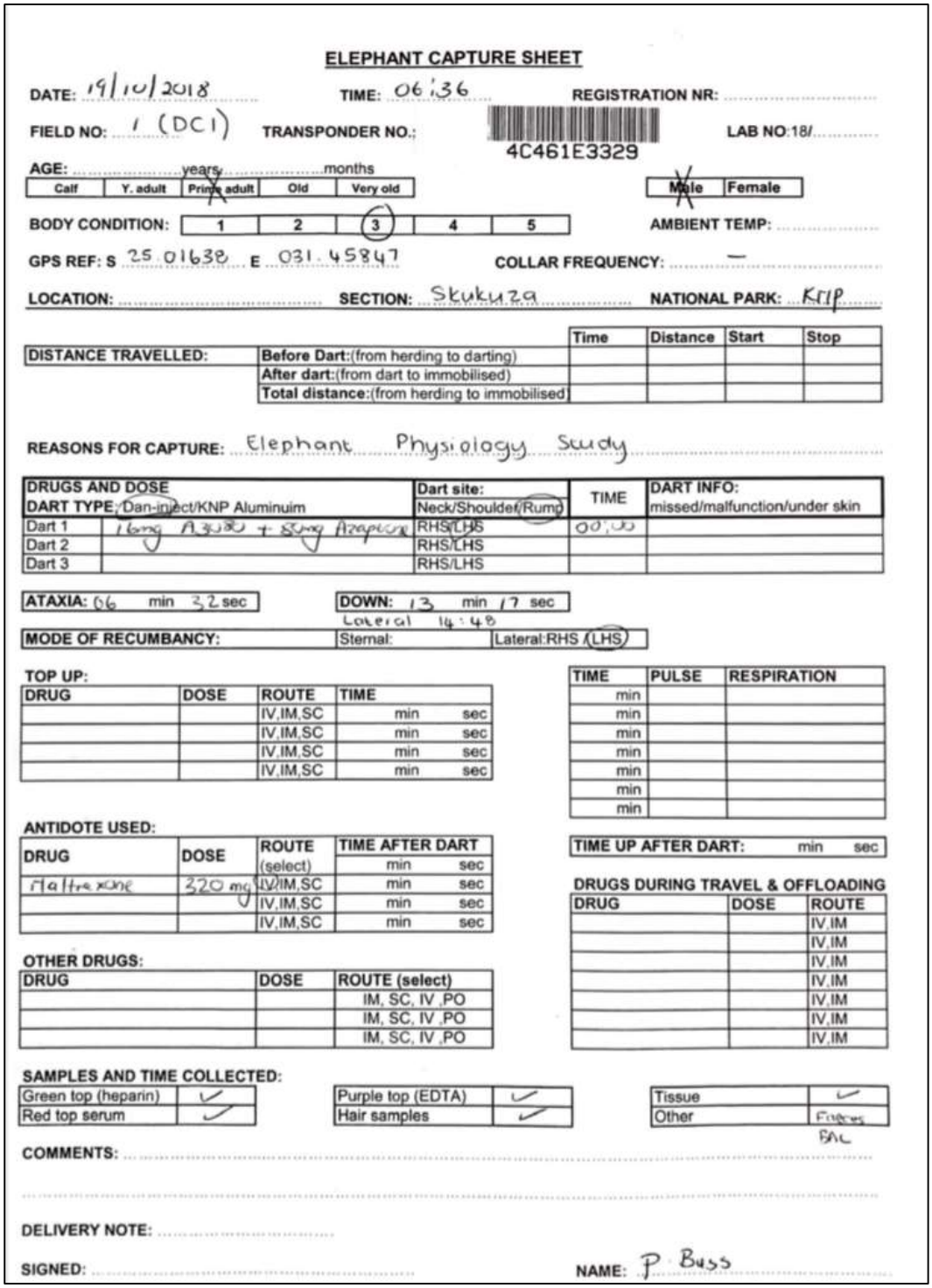
4C461E3329

Date: 19/10/2018 Location:

Elephant id:	Endotracheal breathing / Trunk breathing					Trunk breathing / Endotracheal breathing			
Time from instrum. (min)	0:00 (BL)	5:00	10:00	15:00	20:00 (Rest)	25:00 (BL)	30:00	35:00	40:00
Capture state									
resp. rate (breaths/min)	1 5/5	1 5	1 4	1 5	1 4	1	1	1	1
	2	2	2	2	2	2	2	2	2
	3	3	3	3	3	3	3	3	3
V_T (mL) Insp Time/s	1 4.97	1 5.28	1 3.84	1 4.59	1 3.37	1	1	1	1
	2 4.75	2 4.15	2 4.44	2 5.47	2 3.57	2	2	2	2
	3 5.44	3 3.81	3 3.94	3 3.78	3 3.91	3	3	3	3
V_E (L/min)									
Et (CO_2) (mmHg)									
Mixed CO_2 (mmHg)									
Mixed O_2 (mmHg)									
Pulse rate (pulse/min)	40	42	42	43	42				
Arterial BP (mmHg)	172 (139) 116	165 (133) 122	160 (131) 110	162 (131) 112	152 (132) 117				
ABG	1,1A			1,2A					
VBG	1,1			1.2V					
~~Insp time~~ Breaths/bag	7	5	6	6	6				
~~Peak flow~~ Time/bag	1:22	1:24	1:29	1:18	1:22				

IIII

Elephant capture sheet.



ELEPHANT CAPTURE SHEET

DATE: 19/10/2018 TIME: 06:36 REGISTRATION NR:

FIELD NO: 1 (DC1) TRANSPONDER NO.: 4C461E3329 LAB NO: 18/

AGE: ..... years ..... months

Calf	Y. adult	Prime adult	Old	Very old
		X		

Male	Female
X	

BODY CONDITION:

1	2	3	4	5
		○		

AMBIENT TEMP:

GPS REF: S 25.01638 E 031.45847 COLLAR FREQUENCY: —

LOCATION: SECTION: Skukuza NATIONAL PARK: KNP

DISTANCE TRAVELLED:		Time	Distance	Start	Stop
	Before Dart:(from herding to darting)				
	After dart:(from dart to immobilised)				
	Total distance:(from herding to immobilised				

REASONS FOR CAPTURE: Elephant Physiology Study

DRUGS AND DOSE DART TYPE: Dan-inject/KNP Aluminuim		Dart site: Neck/Shoulder/Rump	TIME	DART INFO: missed/malfunction/under skin
Dart 1	16mg M3050 + 80mg Azaperone	RHS/LHS	00:00	
Dart 2		RHS/LHS		
Dart 3		RHS/LHS		

ATAXIA: 06 min 32 sec

DOWN: 13 min 17 sec Lateral 14:48

MODE OF RECUMBANCY:

Sternal:	Lateral:RHS (LHS)

TOP UP:

DRUG	DOSE	ROUTE	TIME
		IV,IM,SC	min sec
		IV,IM,SC	min sec
		IV,IM,SC	min sec
		IV,IM,SC	min sec

TIME	PULSE	RESPIRATION
min		
min		
min		
min		
min		
min		
min		

ANTIDOTE USED:

DRUG	DOSE	ROUTE (select)	TIME AFTER DART
			min sec
Naltrexone	320 mg	IV,IM,SC	min sec
		IV,IM,SC	min sec
		IV,IM,SC	min sec

TIME UP AFTER DART: min sec

DRUGS DURING TRAVEL & OFFLOADING

DRUG	DOSE	ROUTE
		IV,IM
		IV,IM
		IV,IM
		IV,IM
		IV,IM
		IV,IM
		IV,IM

OTHER DRUGS:

DRUG	DOSE	ROUTE (select)
		IM, SC, IV ,PO
		IM, SC, IV ,PO
		IM, SC, IV ,PO

SAMPLES AND TIME COLLECTED:

Green top (heparin)	✓	Purple top (EDTA)	✓	Tissue	✓
Red top serum	✓	Hair samples	✓	Other	Faeces BAL

COMMENTS:

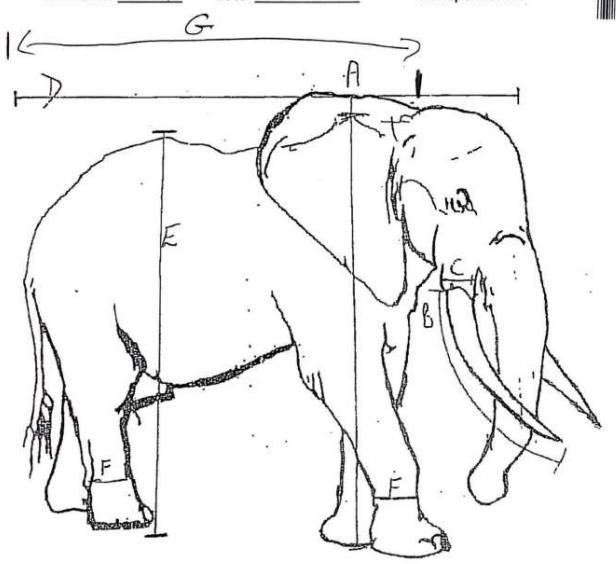
DELIVERY NOTE:

SIGNED: NAME: P. Buss

Elephant measurements sheet.

Elephant Measurements Sheet

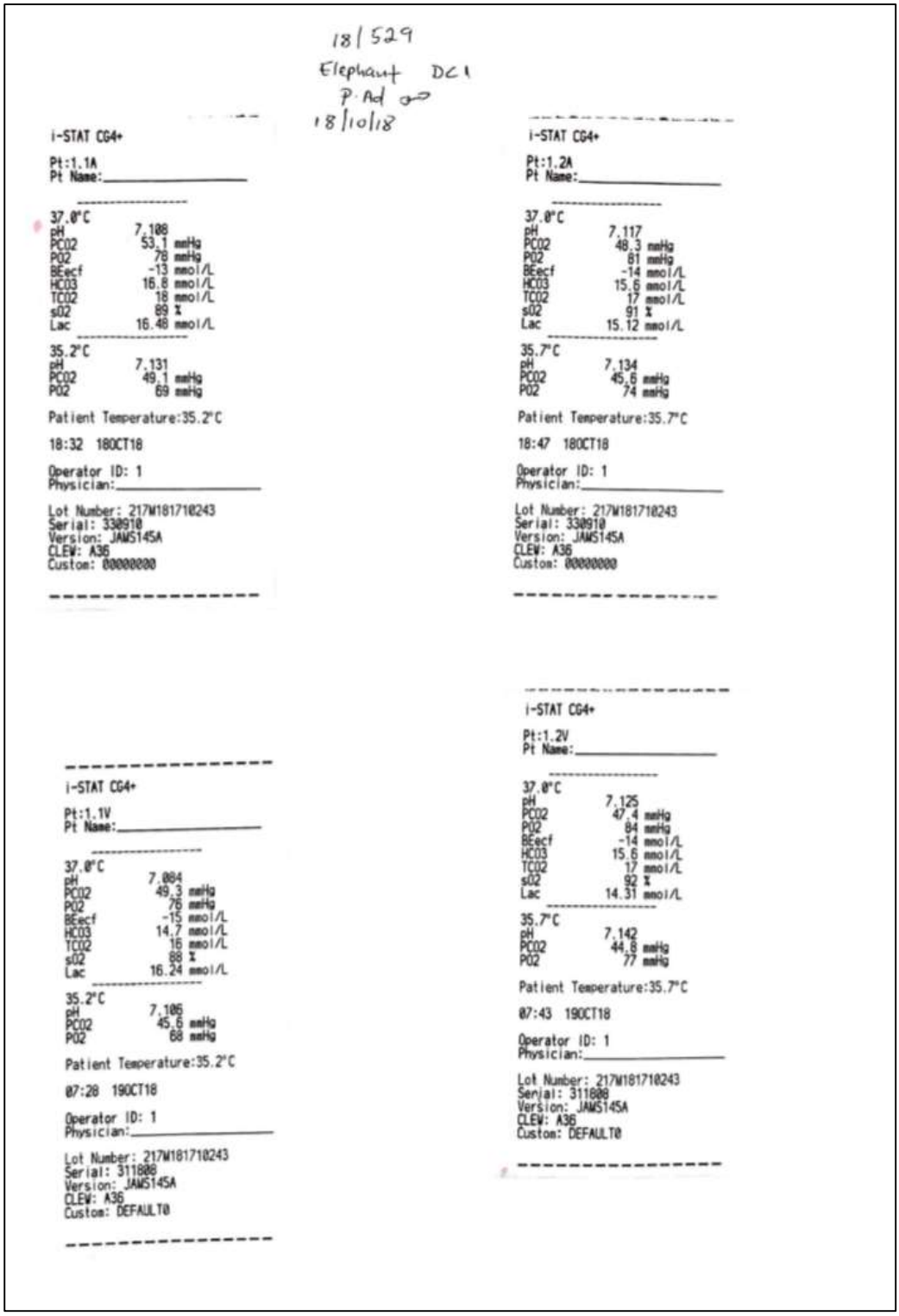
Animal ID: DC1 Date: 19/10/18 Transponder No 4C461E3329 Lab No.: _____



A: Shoulder Height	3.1m	cm
B: Tusk Length (R)	93cm	cm
Tusk length (L)	92 cm	cm
C: Tusk Circ. (R)	33 cm	cm
Tusk Circ. (L)	32 cm	cm
D: Body Length	3.9m	cm
E: Hip Height	2.8m	cm
F: Foot Circ. (Front)	~~132~~ 143cm	cm
Foot Circ. (Rear)	132 cm	cm

G Back length · 3m

Blood gas analysis results.



18/529
Elephant DC1
P. Ad
18/10/18

i-STAT CG4+

Pt:1.1A
Pt Name:_____

37.0°C
pH 7.108
PCO2 53.1 mmHg
PO2 76 mmHg
BEecf -13 mmol/L
HCO3 16.8 mmol/L
TCO2 18 mmol/L
sO2 89 %
Lac 16.46 mmol/L

35.2°C
pH 7.131
PCO2 49.1 mmHg
PO2 69 mmHg

Patient Temperature:35.2°C

18:32 18OCT18

Operator ID: 1
Physician:_____

Lot Number: 217W181710243
Serial: 330910
Version: JAWS145A
CLEW: A36
Custom: 00000000

i-STAT CG4+

Pt:1.2A
Pt Name:_____

37.0°C
pH 7.117
PCO2 48.3 mmHg
PO2 81 mmHg
BEecf -14 mmol/L
HCO3 15.6 mmol/L
TCO2 17 mmol/L
sO2 91 %
Lac 15.12 mmol/L

35.7°C
pH 7.134
PCO2 45.6 mmHg
PO2 74 mmHg

Patient Temperature:35.7°C

18:47 18OCT18

Operator ID: 1
Physician:_____

Lot Number: 217W181710243
Serial: 330910
Version: JAWS145A
CLEW: A36
Custom: 00000000

i-STAT CG4+

Pt:1.1V
Pt Name:_____

37.0°C
pH 7.084
PCO2 49.3 mmHg
PO2 76 mmHg
BEecf -15 mmol/L
HCO3 14.7 mmol/L
TCO2 16 mmol/L
sO2 88 %
Lac 16.24 mmol/L

35.2°C
pH 7.106
PCO2 45.6 mmHg
PO2 68 mmHg

Patient Temperature:35.2°C

07:28 19OCT18

Operator ID: 1
Physician:_____

Lot Number: 217W181710243
Serial: 311808
Version: JAWS145A
CLEW: A36
Custom: DEFAULT0

i-STAT CG4+

Pt:1.2V
Pt Name:_____

37.0°C
pH 7.125
PCO2 47.4 mmHg
PO2 84 mmHg
BEecf -14 mmol/L
HCO3 15.6 mmol/L
TCO2 17 mmol/L
sO2 92 %
Lac 14.31 mmol/L

35.7°C
pH 7.142
PCO2 44.8 mmHg
PO2 77 mmHg

Patient Temperature:35.7°C

07:43 19OCT18

Operator ID: 1
Physician:_____

Lot Number: 217W181710243
Serial: 311808
Version: JAWS145A
CLEW: A36
Custom: DEFAULT0

Presentations and publications arising from the study

South African Veterinary Association 2020 Wildlife Congress presentation.

Event	Venue	Date	Title	Type
SAVA Wildlife Congress 2020		2020	Physiological responses of free-ranging African elephant (*Loxodonta africana*) immobilised with a thiafentanil-azaperone combination	Abstract presentation

Veterinary Analgesia and Analgesia publication.

Ngwako D Chelopo, Peter E Buss, Michele A Millerc & Gareth E Zeilera Physiological responses of free-ranging African elephant (*Loxodonta africana*) immobilised with a thiafentanil-azaperone combination. *Veterinary Anaesthesia and Analgesia* (undergoing review)

Animal ethics approval certificates

University of Pretoria Animal Ethics Committee approval (V058-18).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Respiratory gas flow characteristics of trunk versus endotracheal tube spontaneous breathing in the African elephant *(Loxodanta africana)***
PROJECT NUMBER	**V058-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr ND Chelopo**

STUDENT NUMBER (where applicable)	**U_29119392**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES/SAMPLES	**African elephant *(Loxodanta africana)***
NUMBER OF ANIMALS	**18**
Approval period to use animals for research/testing purposes	**July 2018 – July 2019**
SUPERVISOR	**Prof. G Zeiler**

KINDLY NOTE:

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

APPROVED (With condition)	Date 28 August 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

Please provide Section 20 approval

S4285-15

South African National Parks Animal Use and Care Committee approval.

To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.

South African NATIONAL PARKS

ANIMAL USE AND CARE COMMITTEE: APPLICATION FOR APPROVAL

A. PROJECT DETAILS

Project Title	Respiratory gas flow characteristics of trunk versus tracheal intubated spontaneously breathing African elephants (*Loxodonta africana*).		
Researcher	Ngwako David Chelopo	SANParks Reference No.	006/18

B. SCIENTIFIC REVIEW STATEMENT

(Every application should be supported by a declaration that it has undergone prior scientific review through at least one of the SANParks Research Nodes.)

This research protocol has been reviewed by a Scientific Services Centre, SANParks, and has been judged to be of national importance, designed in accordance with accepted scientific practices and norms and is in the opinion of the reviewers likely to be successful in achieving its objective.

Name: H. Hendricks Designation: Snr GM: CSD Signature: [signature] Date: 23/7/2018

Ps. As per SANParks Application process

Note: In accordance with the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes", an animal is regarded as being "live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members from the Cephalopoda and Decapoda".

This form should be submitted with the SANParks standard Research Project Application, and (where relevant) the following supporting documents: CVs of practitioners in support of competence to handle or treat animals, notices of approval of other ethics committees, diagrams or references illustrating the equipment and/or techniques to be applied.

For Administrative Purposes			
Submission Date	06/07/2018	APPROVED	DISAPPROVED
AUCC approval / Disapproval Date	23/7/2018	Signature [signature]	
Reason for Decision	The study fully meets ethical standards		

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