Physiological parameters of farmed Nile crocodiles (*Crocodylus niloticus*) captured manually and by electrical immobilisation

by

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DECLARATION

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

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other University.

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LIST OF ABBREVIATIONS

µL	Microliters
AC	Alternating current
ACTH	Adrenocorticotropin hormone
ADP	Adenosine Di phosphate
ALT	Alanine aminotransferase
ASP	Alkaline phosphatase
AST	Aspartate aminotransferase
ATP	Adenosine Tri phosphate
CK	Creatinine kinase
cm	Centimetres
CRF	Corticotropin releasing factor
CWS	Chui Wildlife Services
D1	Research trial day 1, 19 January 2012
D2	Research trial day 2, 2 February 2012
DC	Direct current
EEG	Electroencephalogram
ECOG	Electrocorticogram
EIA	Enzyme immunoassay
H/L ratio	Heterophil/lymphocyte ratio
HPA	Hypothalamic-pituitary-adrenal axis
Hz	Hertz
L	Litres
LDH	Lactate dehydrogenase
m	Metre
MCHC	Mean cell haemoglobin concentration
MDH	Malate dehydrogenase
mL	Millilitres
mm	Millimetre
mmol	Millimol
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide

	Phosphate
ng	Nanograms
RIA	Radio immunoassay
rpm	Rounds per minute
s	Seconds
SACFA	South African Crocodile Farmers Association
SANS	South African National Standard
T0	Time interval from beginning of capture of a crocodile to first blood collection
T1	Blood collection four hours later
TL	Total length
U/L	International units per litre
V	Volts

ABSTRACT

During the past 15 years crocodile farming has become more important and sophisticated all over the world. In South Africa there are currently an estimated one million Nile crocodiles (*Crocodylus niloticus*) on commercial farms, mostly for leather production. The management, especially of crocodiles that are close to slaughter, is very intensive as the skins of these animals have to be in immaculate condition to achieve good prices on the international markets. In this regard, the electric stunner is often used on a daily basis on most farms in South Africa to safely handle crocodiles. However, this technique (electrical immobilisation) has only been scientifically evaluated in the Australian saltwater crocodile (*C. porosus*). As crocodilian species might react differently to the electrical immobilisation procedure, the aim of the project was to compare certain physiological parameters of Nile crocodiles captured by either electrical immobilisation (stunning) or captured manually by noosing. This study was conducted during the summer of 2012 on a commercial crocodile farm near Pongola, South Africa.

In total 45 crocodiles were used of which 23 crocodiles were captured by electrical immobilisation and 22 by means of noosing. Physiological parameters chosen for monitoring were serum corticosterone, blood lactate, blood glucose, as well as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatinine kinase (CK). The concentrations and activities of these parameters were determined in blood samples collected immediately after capture by the two methods. Animals were then tied and blind-folded and kept in a quiet place. Four hours later blood samples were collected again from each animal to monitor changes in concentrations and activities of these parameters. In all cases the time was recorded that it took to capture each animal. In addition, total handling time until blood collection was also recorded on an individual basis. Our results indicate that although corticosterone increased greatly within the four hour interval in both groups, there was no difference ($p > 0.05$) between the two methods of capture. Lactate did not increase significantly within the four hour period in both groups, but was higher when animals were noosed. Glucose concentrations rose within four hours, but no significant differences could be detected between the two capture methods. While ALT and ALP did not show any clear trend, increased activities were detected for AST and CK in the four hour period after capture. Both, AST and CK levels were higher in noosed animals.

Noosing a crocodile took longer to restrain the animal when compared to the stunning method. On average stunning took 118 seconds from start of capture until an animal was under control while noosing took 186 seconds per animal. As a consequence the noosed animals struggle for a longer time, which most probably caused exhaustion and muscle damage; explaining the higher levels of blood lactate, AST and CK. One helper was injured (bite wound) trying to control a crocodiles using the noose method. Electrical immobilisation is therefore considered to be the better option for commercial farms, from a physiological perspective, as well as an animal welfare and human safety viewpoint.

1. INTRODUCTION

During the past 15 years crocodile farming has become more important, and sophisticated all over the world. In South Africa there are currently an estimated 1 000 000 Nile crocodiles (*Crocodylus niloticus*) on commercial farms mostly for skin production. These animals are handled intensively on an everyday basis (Blake 2005; personal communication Robert Reader, SACFA 2011). An average South African commercial crocodile farm accommodates between 2 000 and 10 000 crocodiles which are kept in ponds of 200 to 1 000 individuals, graded according to their size (personal communication Robert Reader, SACFA 2011; Huchzermeyer 2003). If the crocodiles are not used for breeding, animals are usually slaughtered for their skins between two to four years of age. The management, especially of crocodiles that are nearly ready for slaughter, is intensive as the skins of these animals have to be in immaculate condition to achieve a good price on the international market (Davis 2001).

Nowadays, immobilisation of crocodiles for management purposes is usually carried out by means of an electric stunner, a technique firstly introduced in Australia during 2000 (Davis *et al.* 2000). The use of the stunner has led to a logistical improvement in the handling of crocodiles worldwide, because with this technique as many as 60 animals may be captured within an hour (Franklin *et al.* 2003). Previously, crocodiles had to be shot in their ponds or otherwise physically restrained, and skins evaluated only after the animals had been culled. With the use of the stunner only animals with excellent skin quality will be culled, while the others are left behind to be slaughtered at a later stage once skin quality has improved (Davis *et al.* 2000).

The electric stunner has been approved to handle farmed crocodiles in South Africa (National Standard on Crocodiles in captivity; SANS 631: 2009). However, this method has only been scientifically evaluated in *C. porosus* (Davis *et al.* 2000; Franklin *et al.* 2003). As crocodilian species might react differently to the stunning procedure, it was suggested by SACFA, that the electrical immobilisation technique, as used on South African crocodile farms, should be evaluated in terms of its stress inducing potential. This is important to justify the use of the stunner technique from an animal welfare point.

This project was conducted in order to scientifically determine the physiological effects of electrical immobilisation by comparing it to manual capture. Special emphasis was put on physiological stress parameters by measuring serum corticosterone, blood glucose and lactate concentrations. Changes in blood enzyme concentrations of alanine aminotransferase ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatinine kinase (CK) were also examined as an indication of which organs might be affected by the different capture methods.

2. LITERATURE REVIEW

2.1. Methods to capture and restrain crocodiles on commercial farms

The previously common method of shooting of crocodiles directly out of their ponds without prior skin inspection is dangerous and stressful to animals and handlers because of the firearms involved. It also does not lead to the harvest of only high quality skins. Therefore different methods have to be applied to handle and inspect crocodiles before slaughter on crocodile farms (Davis *et al.* 2000). Further, animals are not just handled for slaughter but often have to be handled also to be regrouped according to size or for live sales. Therefore, it is important to investigate practical capture and restraint methods.

2.1.1. Electrical immobilisation

The electric stunner for crocodiles was first described in 2000 by Davis *et al.* who investigated it on *C. porosus*. The conclusion was that not only has the use of the electric stunner made crocodile handling less hazardous (Davis *et al.* 2000), it has also lead to a reduction of handling stress for the crocodiles (Franklin *et al.* 2003).

The stunner consists of a pair of electrodes at the end of a cleft wand. The electrodes are connected to a modified 400 Hz inverter which allows a choice of different voltages. The stunning equipment uses a combination of high amps and low voltage to reduce the risk of damaging electrical shock to the animal (Davis *et al.* 2000). In South Africa most stunners work on a commercial 120 Watt, 50 Hz DC-AC inverter. While some stunners use electricity from the mains, other stunners run on a 12V battery and can be conveniently carried around in a backpack. An electric charge of between 80 and 160 V is delivered to a crocodile for three to 11 seconds to the back of the neck and leads to immobilisation and presumably causes unconsciousness of crocodiles for about five to ten minutes, long enough for routine procedures such as, skin evaluation, capture for sale or regrouping of animals (Franklin *et al.* 2003). The stunning position, on the neck just behind the head, was chosen as it is very close to the crocodile's brain and has proven to be most effective and safe for the animals

The electrical immobilisation technique developed for crocodiles is the same as the electric stunners used to render domestic animals insensible before slaughter. For domestic animals Grandin (1997) recommended that alternating current (AC) should be used for stunning to

achieve satisfactory results. Grandin (1997) points out that most stunners for domestic animals in the United States and Europe operate on 50 to 60 Hz alternating current (AC). Lower (less than 25 Hz) or higher frequencies (more than 500 Hz) are less likely to produce unconsciousness. High frequency stunners can cause pain without producing unconsciousness due to the fact that the electricity of high frequencies seems to stay on the surface of the animal (Grandin 1997). Sensitivity can be affected by weight, fat thickness, hydration, wetness of skin, contact of the animal with the ground and many other factors. If the electrodes of the stunner touch the ground, current might go into the ground and voltage might therefore not be high enough to achieve the stunning effect (Grandin 1997). The effectiveness of stunning can be assessed by observing spontaneous physical behaviour and the return of reflexes such as the rhythmic breathing, corneal reflex and pain reflex. In addition an electroencephalogram (EEG) or electrocorticogram (ECOG) can be used to monitor epileptiforme activity of the brain (Anil *et al*. 2000; McKinstry & Anil 2004). Based on experience in humans, it is assumed that a grand mal type epileptiform activity in the brain is indicative of unconsciousness (Gregory 1994; Anil *et al*. 2000). This was also accepted by the European Union which accepts electrical stunning as a method to render animals insensible before slaughter in the council regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing.

The length of time that animals remain unconscious differs and is a lot shorter in domestic animals compared to crocodiles that are presumed insensible for five to ten minutes (Davis *et al.* 2000). After electric head stunning, sheep will stay unconscious for 18 to 42 seconds, cattle for up to 60 seconds – depending on age, and chickens are insensible for 30 to 60 seconds (Grandin 1997).

Davis *et al.* (2000) reported that the carcases of *C. porosus* that had been stunned before slaughtering did not show any obvious adverse effects related to the stunning procedure. Likewise, on examination of the joints and organs of animals after stunning, no adverse effects could be detected (Davis *et al.* 2000). Stress levels in stunned crocodiles (*C. porosus*) seem to be a lot lower compared to manually noosed animals according to Franklin *et al.* (2003) who analysed and compared values such as plasma glucose, lactate, corticosterone, haematocrit and haemoglobin concentration and mean cell haemoglobin concentration (MCHC).

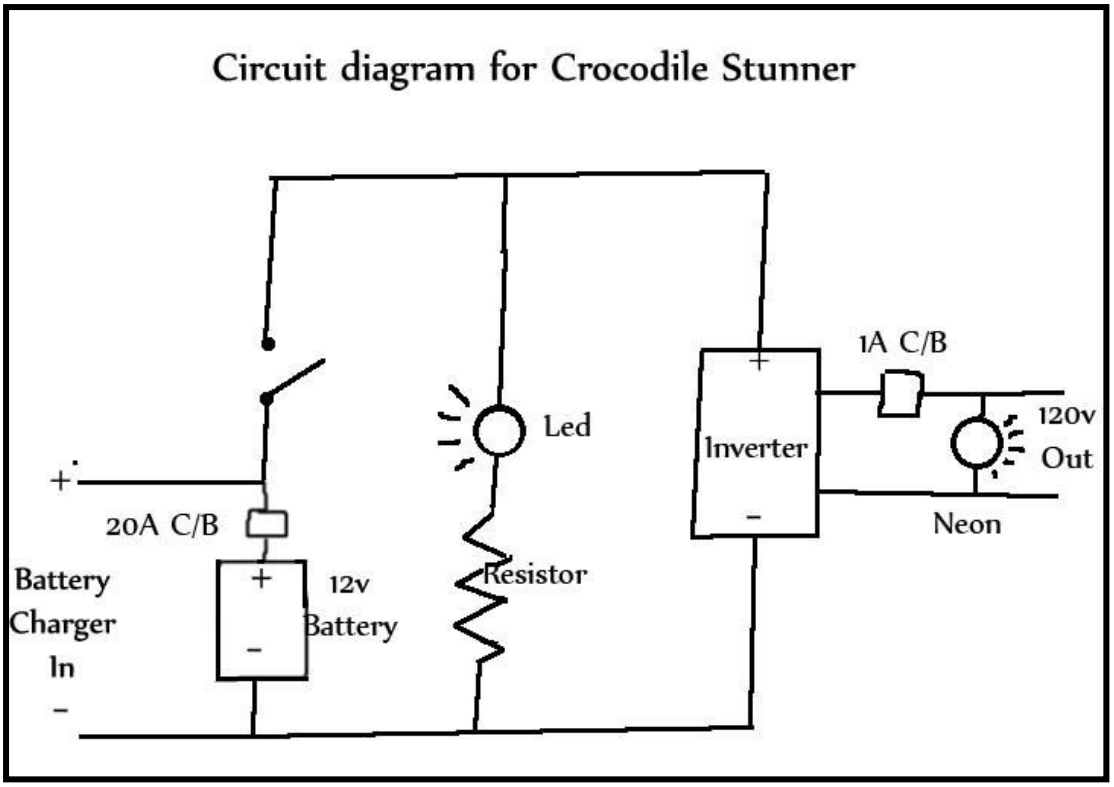


Figure 1: Circuit diagram of the electric stunner

2.1.2. Manual capture of crocodiles

Manual capture of crocodiles is traditionally carried out with the help of a noose that is positioned around the neck of the crocodile. The crocodile is then pulled onto land and restrained by hand. Crocodiles that are captured with the electric stunner, physically struggle for the short period of three to 11 seconds that it takes until they are immobilized (Franklin *et al.* 2003), while animals that are manually captured often struggle vigorously and thrash their bodies and tail and sometimes continue to struggle during handling until they are exhausted (Franklin *et al.* 2003). As a result of this handling, which can be assumed to be stressful, manually captured crocodiles often refuse to eat for several days and their growth rate and immune system might be affected (Huchzermeyer 2003).

As crocodiles are fully conscious during the entire period of manual capture, the procedure takes longer compared to stunning (Franklin *et al.* 2003) and handlers can get seriously hurt during the process (Davis *et al.* 2000; personal experience Silke Pfitzer 2011).

2.1.3. Other capture methods for crocodiles

As an alternative to manual capture and electrical stunning, immobilisation of crocodiles with various drugs has been used for the past 25 years. Crocodiles are usually injected with immobilising agents shortly before or sometimes even after physical capture (Loveridge & Blake 1987). The most popular drugs in use still is the peripheral muscle relaxant gallamine triethiodide (Flaxedil®) which could be reversed with neostigmine methylsulphate. As a competitive neuromuscular blocker, gallamine raises the threshold for depolarization of the motor endplates by acetylcholine, therefore leading to flaccid paralysis. As a result, the crocodile is immobile, but not unconscious. Gallamine should, therefore, never be used for any painful procedures. The drug has a wide safety margin and has been successfully used in Nile crocodiles ranging from 1.75 to 423 kg (Loveridge & Blake 1987; Flamand *et al.* 1992; Blake 1993).

In Australia, Bates *et al*. (1987) tested the drug pancuronium bromide for the capture of crocodiles. Pancuronium bromide is also a non-depolarising neuromuscular blocking agent and therefore can also be reversed with neostigmine methylsulphate. Olsson & Phalen (2012a & 2012b) found that the α2 agonist, medetomidine, injected into the forelimb could achieve reliable immobilisation of small and larger estuarine (*C. porosus*) and freshwater crocodiles (*C. johnstoni*) from three to 350 kg which lasted at least 40 minutes. This drug can be reversed with atipamezole injected intra-muscularly which usually causes recovery within five minutes.

An obvious disadvantage that arises with the chemical capture technique is the fact that animals have to be darted or injected with a pole syringe. This is difficult with an animal that is in the water. In addition, osteoderms along the back and tail area of the crocodile prevent the penetration of the needle (Flamand *et al.* 1992). Furthermore, the injected individuals will invariably drown, if they decide to stay or go into water after injection (Loveridge & Blake 1987).

Gallamine, neostigmine as well as medetomidine are scheduled four and five drugs in South Africa (Medicines and related substances act, act 101 of 1965) and can only be prescribed to a crocodile farmer by a veterinarian for individual crocodiles under his/her care. Therefore, while chemical capture is an option to treat large individual animals, the chemical capture of crocodiles for routine procedures on farms would be impractical and prohibitively expensive.

Further, the withdrawal period of drugs would be a problem if the crocodile meat is intended for human consumption as most handling procedures of farmed crocodiles take place within the last six months before slaughter when animals are evaluated for their skin quality, treated with disinfectant and regrouped regularly to improve skin quality. For these reasons, the use of pharmaceuticals for the routine handling of crocodiles is not a practical solution for a commercial crocodile farmer.

2.2. Stress in crocodiles

Acute stress in crocodiles has many features in common with stress in homeotherms (Lance *et al.* 2001). Multiple systems are activated within the central nervous system when an animal is stressed and in crocodilians lead to the release of catecholamines, an increase in plasma lactate, a rise in blood glucose for several hours and secretion of glucocorticoids (Lance *et al.* 2001). In addition, a rise in plasma calcium (Lance & Elsey 1999a), a change of haemoglobin and haematocrit, as well as the heterocyte / lymphocyte (H/L) ratio were reported to be indicators of a stress response in crocodilians (Lance & Elsey 1999a; Franklin *et al*. 2003).

Limited information exists about catecholamines in crocodilians and it seems that their secretion can be very variable in different species and is influenced by different factors. The release of catecholamines has been investigated in the American alligator (*Alligator mississippiensis*) by Lance & Elsey (1999a & 1999b) who measured catecholamines in juvenile alligators that were exposed to restraint stress and cold shock respectively. Plasma norepinephrine, epinephrine and dopamine were monitored using high-pressure liquid chromatography. Pre-treatment levels of norepinephrine and epinephrine were on average 4 ng/mL in alligators that were going to be exposed to cold shock and both catecholamines increased to 40ng/mL (norepinephrine) and 7 ng/mL (epinephrine) one hour post treatment. Mean plasma dopamine levels averaged at 0.7 ng/mL at the initial bleed and rose to 10 ng/mL post treatment, although values were too variable to show statistical significance (Lance & Elsey 1999b). In restrained alligators initial plasma concentrations of epinephrine and norepinephrine averaged also at 4 ng/mL but epinephrine declined steadily thereafter while norepinephrine rose to 8 ng/mL after one hour post-treatment and declined thereafter followed by another increase after 48 hours. Plasma dopamine was low at the initial bleed, rose to 8 ng/mL after one hour and declined thereafter to below pre-treatment levels (Lance & Elsey 1999a).

The initial epinephrine values for alligators are twice as high as those measured for aquatic turtles and for the lizard *Dipsosaurus dorsalis* (Lance & Elsey 1999a). In contrast, another lizard – *Urosaurus ornatus* – had epinephrine values that were twice as high as those measured in alligators (Lance & Elsey 1999a). These species/study-specific differences might be an indication of the fact that it is hard to take blood without disturbing and therefore stressing an animal. While it seems that in the alligator, stimuli that lead to the release of noradrenalin also lead to release of dopamine, in mammals dopamine release is usually associated with haemorrhage (Lance & Elsey 1999a).

Corticosterone is the main glucocorticoid secreted by reptiles and birds in response to stress (Lance *et al.* 2001). This is in contrast to mammals and fish, which mostly release cortisol (Romero 2004). Corticosterone release is influenced by various stressors which act on the hypothalamic-pituitary-adrenal (HPA) axis. The cortex of the brain reacts to an acute stressor such as capture by sending signals to the hypothalamus. The hypothalamus sends a hormonal signal - corticotropin releasing factor (CRF) to the pituitary which then releases adrenocorticotropin hormone (ACTH). ACTH release into the bloodstream leads to the release of glucocorticoids such as corticosterone from the adrenal cortex of mammals and from the interrenal tissue of reptiles and birds (Lance *et al.* 2001; Romero 2004). In mammals and birds it takes about three to five minutes until the release of glucocorticoids (Romero 2004). A negative feedback acts on the hypothalamus and glucocorticoid release ceases. However, if a stressor persists, this negative feedback stops functioning and glucocorticoids are excreted chronically (Romero 2004).

With the development of radio- (RIA) and enzyme immunoassays (EIA), it is now possible to determine steroid concentrations in very small volumes, which often allows multiple sampling even from smaller animals. Therefore, also temporal alterations in plasma glucocorticoid secretion on an individual basis can be monitored. In the past, the RIA and EIA techniques have been used for measuring corticosterone levels in plasma of alligators and *C. porosus* (Lance *et al.* 2001; Franklin *et al.* 2003) and by using a non-invasive approach also in faecal samples of Nile crocodiles (Ganswindt 2012).

Species-specific differences in baseline plasma glucocorticoid levels are described in the literature (Lance *et al.* 2001), although an impact of the potentially different capture as well

as analysis techniques used in these studies cannot be excluded (Lance *et al.* 2001). Baseline plasma corticosterone concentrations for caiman (*Caiman crocodylus*) were found to be around 20 ng/mL (Gist & Kaplan 1976), while baseline plasma corticosterone values for American alligators were reported lower than 2 ng/mL (Guillette *et al.* 1997). Plasma corticosterone concentrations in *C. johnstoni* averaged at around 4 ng/mL (Jessup *et al.* 2003) and for *C. niloticus* at around 6 ng/mL (Balment & Loveridge 1989). Baseline plasma corticosterone values for *C. porosus* as determined by Franklin *et al.* (2003) were around 1 ng/mL and increased only slightly (two fold) when animals were manually restrained. This is in contrast to the reaction in a manually restrained American alligator where the plasma corticosterone values increased dramatically (30-fold) after a two hour restraint period (Guillette *et al.* 1997). In a case study, plasma corticosterone levels in a Nile crocodile that had been caught in a noose trap and struggled for several hours increase to 100 ng/mL (Lance *et al.* 2001).

It was also found that the corticosterone secretion in American alligators had a biphasic pattern, with one peak after four hours and another peak after 48 hours (Lance & Elsey 1986; Elsey *et al.* 1991; Lance & Elsey 1999a). This is in contrast to *C. porosus*, where only one peak after 30 minutes was observed by Franklin *et al.* (2003). However, while the alligators were restrained for 48 hours, Franklin *et al.* (2003) released the estuarine crocodiles after capture and only immobilized them again to take blood samples at a later stage, indicating that the biphasic pattern in corticosterone secretion found for American alligators might be rather a result of the continuous restraint. It should be further mentioned that as a result of the rise in plasma corticosterone, testosterone secretion in male alligators can be completely inhibited and suppressed for the next 24 hours (Lance & Elsey 1986).

Plasma lactate increases immediately in crocodiles that are subjected to acute stress (Coulson & Hernandez 1983). Reptiles depend heavily on anaerobic metabolism as the energy demand during exercise often greatly exceeds the capacity of the cardiovascular system to supply oxygen. Therefore, during strenuous exercise, muscles of reptiles operate anaerobically and produce large amounts of lactic acid which enters the systemic circulation, causing metabolic acidosis with detrimental effect to the entire body (Bennett *et al.* 1985).

Bennett *et al.* (1985) and Seymour *et al.* (1987) described the death of large estuarine crocodiles (*C. porosus*) in Australia after manual capture and suspected that death was due to

lactic acid build up after a severe and long struggle during capture by means of ropes and harpoons. To prove this hypothesis, Bennett *et al.* (1985) Seymour *et al.* (1987) measured blood lactate values of crocodiles under laboratory conditions after forced exercise. Lactate values peaked within 10 to 20 minutes after exhaustion. In field trials, the authors further established that large crocodiles could be exercised for much longer (up to 50 minutes) compared to smaller crocodiles. Crocodiles of less than 10 kg bodyweight were exhausted after five to ten minutes. However, the lactate levels found in exhausted large crocodiles went up to 50 mmol/L. The authors reported that these were the highest lactate acid values reported in any animal as result of activity (Bennett *et al.* 1985).

While lactate increased, the blood pH dropped as low as 6.6 in the above described experiments (Bennet *et al.* 1985; Seymour *et al.* 1987). Recovery from exhaustion was slow in these large crocodiles and took as long as 30 hours. Similar observations were made by Coulson & Hernandez (1983) who observed an immediate rise in plasma lactate in alligators that were subjected to two minutes of handling stress. Franklin *et al.* (2003) reported an immediate rise in plasma lactate in *C. porosus*, which was much more pronounced in manually captured animals if compared to electrically immobilised animals. In immobilised animals, lactate levels peaked only after 30 minutes at 10.7 mmol/L, whereas in manually captured animals, that were struggling for up to 15.5 minutes lactate concentrations increased up to 21 mmol/L and peaked one hour after restraint and remained elevated for four to eight hours. These findings clearly indicate that the amount of lactate that is produced during capture has serious implications on the recovery of the animal (Seymour *et al.* 1987; Franklin *et al.* 2003) and might be therefore an important indicator for the capture technique used.

Glucose levels increase in systemic circulation as a result of catecholamine secretion in stressful situations (Lance & Elsey 1999b). Blood glucose increases and rises for several hours when crocodiles are stressed and will remain elevated for days as a result of handling of the animals (Franklin *et al.* 2003; Lance & Elsey 1999a). Coulson & Hernandez (1983) reported plasma glucose levels in hand reared juvenile alligators of around 5 to 6 mmol/L. In captive fasted Mugger crocodiles (*C. palustris*), Stacey & Whitaker (2008) determined an average blood glucose level of 4.26 mmol/L, while the glucose levels of wild Nile crocodiles were around 1.8 to 11.45 mmol/L with a mean of 3.8 to 5.68 mmol/L, depending on the study (Swanepoel *et al.* 2000; Lovely *et al.* 2007; Botha 2010).

Alligators restrained for 48 hours showed a marked rise in plasma glucose at 24 hours of the procedure and glucose remained elevated for 48 hours (Lance & Elsey, 1999a). In contrast, alligators subjected to cold shock showed no significant rise in glucose levels despite the presence of catecholamines in these animals. In this regard, Lance & Elsey (1999b) speculated that catecholamine receptors might not have been able to respond at these cold temperatures.

Franklin *et al*. (2003) examined *C. porosus* for signs of stress while restrained during ambient temperatures of around 20 ^{o}C, which is lower than the optimum temperature range for crocodiles (25 to 35 ^{o}C) (Lang 1987; Lance *et al.* 2001). Despite suboptimal conditions for this study, glucose concentration increased significantly by 160% to 8.25 mmol/L, in restrained animals and glucose levels remained elevated for eight hours. The authors also describe a significant difference in glucose levels between manually restrained animals and animals that were immobilised by the electric stunner, with glucose levels being lower in stunned animals.

Further biochemical changes induced by acute stress are an increase in plasma calcium levels as observed in juvenile alligators subjected to 48 hour of restraint. Plasma calcium concentrations in the animals increased from 3.1 mmol/L to 10.3 mmol/L after two hours of restraint, and slowly returned to baseline concentrations within the next 48 hours. Calcium is possibly mobilised from skeletal bones during acute stress and serves to prevent lactic acidosis by forming complexes with lactate ions that were released as a result of muscle action and anaerobic glycolysis (Jackson & Heisler 1982; Jackson 2004). The average blood calcium concentrations in wild Nile crocodiles from Botswana and South Africa is 2.73 mmol/L and 3.35 mmol/L, respectively (Swanepoel *et al*. 2000; Lovely *et al*. 2007), and the revealed reduction in circulating calcium levels during the occurrence of stressors might be a metabolic disorder in captive Nile crocodiles, which in the long run may lead to decalcified teeth and osteoporosis (Huchzermeyer 2002). Biochemistry values of stressed crocodiles differed further in that chronically stressed animal suffered from hyponatraemia, hyperkalaemia, low osmolality, low cholesterol and low triglycerides (Watson 1990). During this study, lactate levels were also low and serum alkaline phosphatase concentrations increased (Watson 1990).

Franklin *et al.* (2003) observed a rise in haematocrit and haemoglobin values of *C. porosus* within one hour after capture. In contrast to these observations, Lance & Elsey (1999a) reported a decrease of the haematocrit of stressed juvenile alligators from 18 to less than ten within 48 hours. In addition, while the total white blood cell count remained unchanged, the differential cell count changed visibly. Throughout the 48 hour period heterophils increased and reached 60% after 48 hours while other white blood cells, especially lymphocytes decreased by 87%, giving an heterophil/lymphocyte ratio (H/L ratio) of 4.7 (Lance & Elsey 1999a). Haematological changes observed in chronically stressed Nile crocodiles by Watson (1990) comprised of a decrease in the white blood cell count, haemoglobin concentration and haematocrit value, if compared to unstressed animals.

A change of the H/L ratio as described in stressed juvenile alligators (Lance & Elsey 1999a) can also be observed in stressed chickens (Gross & Siegel 1983). However, the H/L ratio was considered to be a better measure of long term changes in the environment, while corticosterone concentrations in blood are a better measure of short term changes (Gross & Siegel 1983).

2.3. Enzymes indicating organ and muscle pathology

By measuring blood concentrations of certain enzymes, which are released from tissues, the pathological changes to organs can be deducted. Lovely *et al* (2007); Millan *et al*. (1997) and Stacey & Whitaker (2000) examined wild Nile crocodiles, Estuarine crocodiles and Mugger crocodiles, respectively, for physiological biochemistry values. Enzymes measured were alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Millan *et al.* (1997) point out that in *C. porosus* very often biochemical values of sick animals are still within the reference ranges.

ALT is predominantly found in liver tissue and to a lesser degree in kidney, heart and skeletal muscle as well as in red blood cells. ALT could therefore be a good indicator for liver disease. In dogs and cats an increase over two to three times the normal values can indicate hepatocellular injury. Levels of ALT can also be influenced by certain drugs. Large animals do not have significant amounts of ALT in their liver (Last *et al.* 2010). ALT concentrations were measured in Nile crocodiles from Botswana and averaged at 43.9 U/L; ranging from 15.0 to 63.0 U/L (Lovely *et al.* 2007). In Nile crocodiles in South Africa, ALT measurements

ranged from 13.0 to 30.0 U/L (Botha 2010). Biochemical values of *C. porosus* were similar to *C. niloticus* and reference ranges of 11.0 to 51.0 U/L were determined by Millan *et al.* (1997). An adult mugger crocodile (*C. palustris*) had lower ALT values (45.29 U/L) than younger animals (55.19 U/L) (Stacey & Whitaker 2000).

ALP, as a membrane bound enzyme, is also found in many tissues. It is used in dogs as a reliable indicator of cholestasis. In cats it can be elevated due to hepatic lipidosis, cholangiohepatitis, hyperthyroidism and diabetes mellitus (Last *et al.* 2010). ALP was measured at a mean of about 21.2 U/L in *C. niloticus* ranging from 3.0 to 72.0 U/L (Lovely *et al*. 2007). Watson (1990) reported values of 64,2 U/L for healthy captive Nile crocodiles and 437 U/L in chronically stressed Nile crocodiles. Botha (2010) measured a mean of 9.81 U/L in Nile crocodiles in various locations of South Africa. The reference range is 31.0 to 180 U/L in *C. porosus* (Millan *et al.*; 1997) and 52.75 U/L in *C. palustris* (Stacey & Whitaker 2000).

AST is found in almost all cells but shows a high activity in the liver and striated muscles. In large mammals AST is often used to measure liver necrosis. AST is also elevated with skeletal muscle and myocardial disease. AST can be tested in conjunction with CK to assess if the elevation of CK is of skeletal or cardiac origin. The enzyme half-life is 77 minutes in cats, five to 12 hours in dogs and one to two days in larger animals such as cattle and horses. Haemolysis can cause false increases of AST (Last *et al.* 2010). AST was measured at a mean of 66.5 U/L in Nile crocodiles in the Okavango Delta, Botswana (Lovely *et al.* 2007). In addition, Lovely and colleagues showed a significant differences between AST measured in yearlings which averaged at 36.5 U/L compared to subadults where the enzyme was measured at a mean of 135 U/L (Lovely *et al*. 2007). Botha (2010) found AST values in wild Nile crocodiles in various locations of South Africa to average from 24.0 to 47.2 U/L. Foggin (1987) points out that on crocodile farms AST as well as ALT values are about two fold higher in runts compared to normally growing crocodiles. In *C. porosus* reference values are given as 23.0 to 157 U/L (Millan *et al.* 1997). In the Mugger crocodile there was a difference between adults and subadults, with adults having a lower AST level (41.0 U/L) compared to subadults (50.94 U/L) and juveniles (52.13 U/L) (Stacey & Whitaker 2000), which coincide with values of domestic animals that range from 0 to 60.0 U/L in dogs to 259 to 595 U/L in horses (Last *et al*. 2010).

Creatinine kinase (CK) is an enzyme that is present in high concentrations in the cytoplasm of myocytes. When myocytes are injured or if cellular permeability is altered, CK escapes into the bloodstream and reaches peak concentrations by six to 12 hours. CK is organ specific in mammals and commonly used to diagnose neurological or muscular disorders (Vassella *et al.* 1965). CK has a short half-life of 60 to 90 minutes and after a single injuring event, concentrations return back to normal within 24 to 48 hours, with young animals usually having higher values (Last *et al*. 2010). In general, injections may cause an increase of two to three times the basic CK value (Last *et al*. 2010). Recumbent and transported animals may also have higher CK concentrations, so do anorectic and ill cats. Reference values for CK in dogs are 40.0 to 255 U/L. In horses a CK concentration of 430 U/L is still considered normal, but may vary with different assay methods (Last *et al.* 2010).

Watson (1990) measured CK concentrations in chronically stressed captive bred Nile crocodiles and compared the values to presumably non-stressed Nile crocodiles. CK concentrations of the control group were 211 U/L, while in chronically stressed animals the CK values increased to levels as high as 9 187 U/L. This is well above levels observed in mammals and it coincides with levels in birds where CK concentrations of 12 035 U/L could be found after stressful handling (Dabbert & Powell 1993). Stacey & Whitaker (2000) examined CK levels in captive bred mugger crocodiles, reporting concentrations of 7.0 to 10.0 U/L in all age groups, indicating that distinct species- and environmental-specific differences in CK values might be present.

Changes in CK levels could also be used as an indicator of capture myopathy. Capture myopathy is described as "*an acute degeneration of muscles resulting from intense muscular exertion and trauma caused by restraint and transport*" (Hullard 1985 cited by Dabbert & Powell 1993). As a consequence of muscle activity, extreme metabolic acidosis results from lactic acid build up in muscles. Clinical signs are muscle stiffness, paralysis, weakness and locomotive abnormalities. Due to subsequent cell lysis, intracellular enzymes such as CK and AST are released (Dabbert & Powell 1993). Muscles affected by capture myopathy usually look pale and dull with a soft friable texture. Histopathology usually reveals multiple foci of myofibre fragmentation, loss of striation and necrosis (Marco *et al*. 2006).

2.4. The influence of ambient temperature on specific parameters

Physiological responses in crocodiles are in most of the cases only apparent at their preferred housing temperature range which is 25 to 35 ^{o}C (Lang 1987; Lance *et al.* 2001). Any attempt to assess physiological responses in crocodilians must therefore take the ambient temperature into account. A stressor that elicits a vigorous response in crocodilians at 30 ^{o}C might have little or no response at 20 ^{o}C (Lance *et al*. 2001). Crocodiles kept only 4 ^{o}C above or below their optimum temperature of 30 to 32 ^{o}C show signs of severe stress that would influence any research results (Turton *et al.* 1997; Lance & Elsey 1999b; Lance *et al.* 2001).

In caimans, the auditory fibres cease firing below 11 ^{o}C (Smolders & Klink 1984) and hatching caimans were unable to produce a distress call at temperatures below 10 ^{o}C (Garrick & Garrick 1978). It was suggested by Lance & Elsey (1999b) that catecholamine receptors failed to respond when juvenile alligators were exposed to cold temperature (Lance & Elsey 1999b). When Turton *et al.* (1997) changed the water temperature of captive bred saltwater crocodiles (*C. porosus*) from the optimum of 32 ^{o}C to higher (36 ^{o}C) or lower (28 ^{o}C) temperatures, respectively, an increase in plasma corticosterone levels from 19.3 to 27 nmol/L was found in the group exposed to higher temperatures and the heterophil count increased while a decrease of corticosterone concentrations from 17.3 to 14.9 nmol/L and a decrease in total white blood cells was found in the group that was exposed to lower temperatures.

2.5. Objectives of this study

The overall aim of this project was to evaluate and compare physiological parameters in farmed Nile crocodiles (*Crocodylus niloticus*) captured either manually or by using an electric stunner.

Specific objectives are as follows:

- To determine capture technique related changes in serum corticosterone levels.
- To determine capture technique related changes in glucose and lactate concentrations.
- To determine capture technique related changes in blood enzyme levels (ALT, ALP, AST and CK).

The study was conducted in order to obtain the degree M Med Vet (Fer), according to the general regulations and stipulations of the University of Pretoria, study code WSK 890.

3. MATERIALS AND METHODS

3.1. Field site and animals

3.1.1. Field site

Nile crocodiles owned by Chui Wildlife Services (CWS), situated near Pongola in northern KwaZulu-Natal were used for this project. The small crocodile farm (GPS coordinates: S 27° 34.251' and E 031° 36.665') was situated in a suitably warm climate so that crocodiles could be farmed in outside ponds without any extra heating (Figure 2).

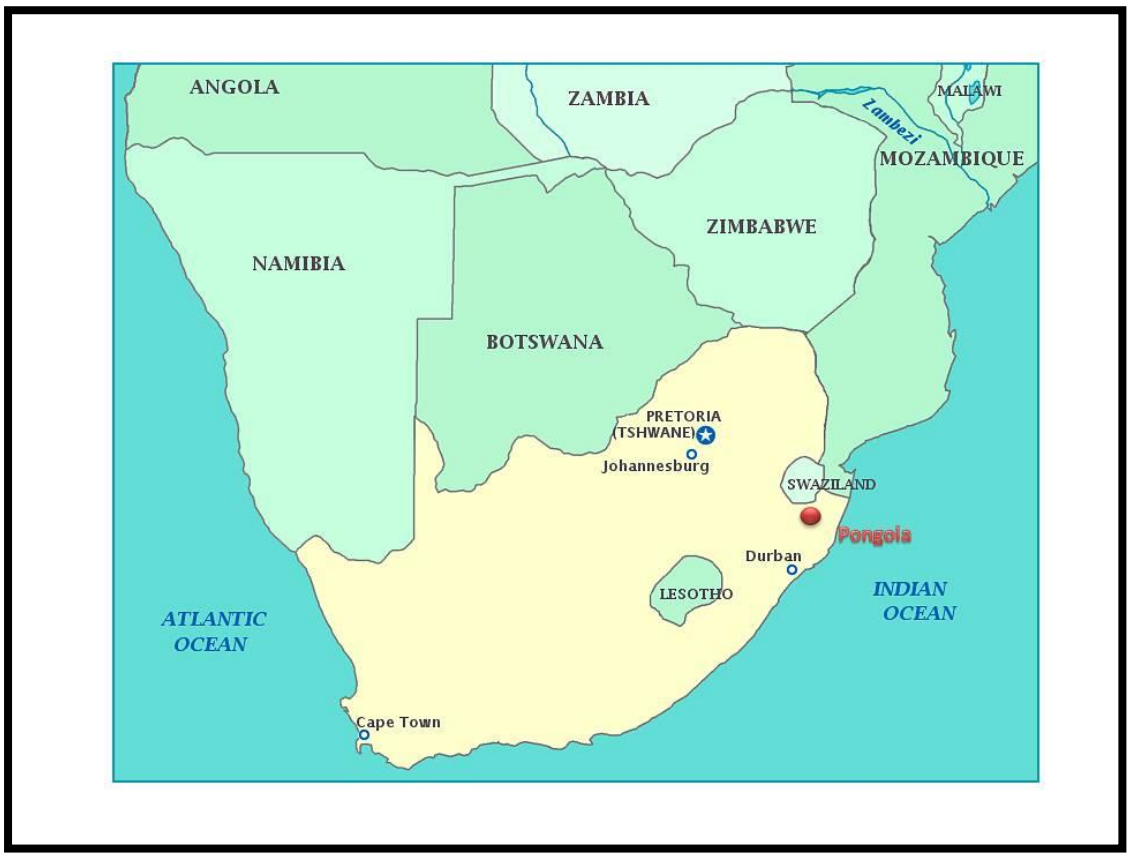


Figure 2: Map of South Africa indicating where Pongola is.

3.1.2. Study animals

CWS kept about 365 crocodiles of both sexes for skin production in an outside enclosure. The enclosure consisted of a large land area with two connected freshwater ponds in the middle. The total enclosure dimensions were 32 m x 28 m, the ponds took up about half of the enclosure. Crocodiles were fed every second day but food was withdrawn for four days prior to the respective research trials (Days 1 and 2).

Forty-five randomly chosen captive bred Nile crocodiles from this population were utilized for the study. The animals were around four years of age with a total length (TL) of 160 to 210 cm.

3.2. Experimental design

Before capturing, ponds were drained to 25% of the usual water depth.

During the study, blood was collected twice from each of the 45 study animals. Individual sampling took place on two days that were two weeks apart to insure independency of the respective data sets. During the first sampling day (D1), 19 January 2012, twelve animals were electrically immobilised and thereafter eleven animals were physically captured with a noose. On the second sampling day (D2), 2 February 2012, eleven animals were physically captured and thereafter eleven animals were electrically immobilised. This alternate design (flip-over) was chosen in order to account for external presumably stress - inducing factors, like prolonged presence of handlers during the capture operation. After restrain, the first blood sample was collected from each crocodile (T0) as quickly as possible Thereafter, animals were sexed and TL was determined. The crocodiles were then immediately moved to a quiet climate controlled house (± 30^{o}C), to prevent further exposure to stressors where they were kept tied up and blind-folded. After 3.5 to 4 hours another blood sample was collected (T1) from each crocodile. Thereafter crocodiles were tagged with different colour tags according to the capture techniques and dates. This was done to prevent capture of the same crocodiles on day two of the project; it also facilitated the post-trial monitoring of the affected animals. After the procedure, crocodiles were released back into their ponds.

3.3. Experimental procedures

3.3.1. Electrical immobilisation / stunning of crocodiles

Electrical immobilisation was carried out by Mr. Boksa Nkosi, an experienced crocodile handler who has carried out electrical immobilisation on many thousands of crocodiles. An electric charge of 135 V was delivered to each crocodile for five to 11 seconds to the back of the neck (Picture 1). This caused immobilisation with presumed unconsciousness for about five minutes (Gregory 1994; Anil *et al*. 2000). Straight after stunning the snout and eyes were

closed with insulation tape and crocodiles were taken to the examination table for immediate sample collection and further examination.

The stunner consisted of a pair of electrodes at the end of a forked, isolated aluminium wand. The electrodes were connected to a modified 120 Watts, 50 Hz DC-AC inverter, which ran on a 12V battery and allowed a choice of different voltages (D7 Electronics, Pongola) (Picture 2).

Picture 1: A crocodile being electrically immobilised during the grand-mal seizure just before it becomes unconscious and relaxed.

Picture 2: The crocodile stunner consisting of a forked wand and the inverter with battery in the rucksack.

3.3.2. Manual capturing by noosing

The manual capture was carried out by Dr. Hannes Botha by gently moving a standard self-locking 3S-72" Thompson steel snare over the head of the crocodile and positioning it over the neck area (Picture 3). The steel snare was pulled tight with the help of a 15 mm heavy duty braided rope attached by a steel coupling. The snare was prepared for the catching procedure by loosely attaching it to the end of a 5 m aluminium pole. Animals were pulled out of the water and subsequently, the animals were restrained, the snout and eyes were closed with insulation tape and they were carried to the examination table for immediate sample collection and further examination.

Picture 3: Manual capturing of a Nile crocodile using a steel snare.

3.3.3. Blood collection

Blood was collected by Dr. Jan Myburgh using the technique reported by Myburgh *et al.* (2013). In brief, blood was collected in serum tubes from the post-occipital spinal venous sinus with a 20 Gauge 1.5" needle and a 5 mL syringe. Each blood sample collected (± 10 mL) was divided into two 5 mL serum tubes; one for hormone analysis and the other one for further biochemistry analyses. In addition, drops of blood were collected for the handheld glucose and lactate meter. The serum tubes were kept in the shade for one hour in order for the blood to clot. Serum was subsequently centrifuged for ten minutes at 2000 rpm. Afterwards the serum was stored in Cryotubes, and stored frozen in liquid nitrogen until further analysis.

3.4. Additional data recording

Daily ambient temperatures were recorded throughout the study period and it was taken note of whether there were any other disturbances such as drastic weather changes, loud airplanes, trucks, etc. during the days of sampling.

Each crocodile was sexed and total body length was measured and recorded. The time of day when the trial started and when blood was taken was recorded for each crocodile. In addition, the time it took from of capture to blood collection for each individual was recorded.

3.5. Sample analysis

For serum corticosterone concentration determination, the Coat-a-count rat corticosterone radio immunoassay (RIA) (Diagnostic Products, Randburg) was used. This test has been validated for the use in Nile crocodiles in a previous study (Ganswindt 2012). In brief, 50 µL standards, controls, and samples were transferred in duplicates into coated tubes, respectively. 1 mL ^{125}I corticosterone solution was added, and the tubes were incubated for two hours at room temperature. Subsequently, all the liquid was removed; the tubes patted dry and immediately counted for one minute in a gamma counter (Wallac Wizzard^2, Perkin Elmer) using MULTICALC software. Sensitivity of the assay was 5.7 ng/mL and major cross-reactivities, as given in the manufacturer's pamphlet, were corticosterone, 100%; 11-deoxycorticosterone, 2.86%; progesterone, 0.83%; and cortisol, 0.35%. The analysis was done in the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria.

Blood biochemistry parameters, such as ALT, ALP, AST and CK, were determined by the Clinical Pathology Section of the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria. This was achieved via spectrophotometry using a Cobas Integra 400 plus.

ALT catalyses the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalysed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of the NADH oxidation is directly proportional to the catalytic ALT

activity. It is determined by measuring the decrease in absorbance at 340 nm. The measuring range for ALT is 2 to 700 U/L (Roche Diagnostics 2008).

ALP cleaves p-nitrophenyl phosphate into phosphate and p-nitrophenol in the presence of magnesium and zinc ions. The p-nitrophenol release is directly proportional to the catalytic ALP activity and is determined by measuring the increase in absorbance at 409 nm. The measuring range for ALP is 3.0 to 1200 U/L (Roche Diagnostics 2008).

AST catalyses the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH in the presence of malate dehydrogenase (MDH) to form NAD+. The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm. The measuring range for AST is 2 to 700 U/L (Roche Diagnostics 2008).

CK catalyses the formation of Adenosine Tri phosphate (ATP) from Creatinine phosphate and Adenosine Di phosphate (ADP). ATP and D-glucose with the help of HK (yeast) react to become ADP and G6P. G6P and NADP+ form NADPH D-6-phosphogluconate. The rate of the NADH formation is directly proportional to the catalytic CK activity. It is determined by measuring the increase in absorbance at 340 nm. The measuring range for CK is 7 to 2000 U/L (Roche Diagnostics 2008).

The Cobas Integra 400 analyser calculates automatically the activity for each sample in U/L and was calibrated with deionized water as zero calibrator.

Blood glucose and blood lactate was measured on site straight after blood collection by means of a hand held glucose and lactate meter Cobas® (Accutrend® Plus, Roche Diagnostics). In brief, one drop of blood was put directly onto an Accutrend® glucose test strip (Roche Diagnostics, Germany) and a BM- Lactate test strip (Roche Diagnostics, Germany) respectively. The test strips were inserted into the hand held glucose and lactate meter according to the manual. At the beginning of each day and whenever a new batch of test-strips was opened the meter was coded and the control solutions Accutrend Control G1 and G2 and Accutrend Control Lactate were run to make sure that values fell inside the given limits. Glucose and lactate readings were given in mmol/L. For Accutrend® Glucose the

limits given in the instruction pamphlet were 1.1 to 33.3 mmol/L. For BM Lactate the respective limits were given as 0.8 to 22 mmol/L. This method was validated using Nile crocodiles from Le Croc commercial crocodile farm (Myburgh unpublished data).

3.6. Data analysis

Blood parameters, corticosterone, lactate, glucose, ALT, ALP, AST and CK, were determined and recorded for each crocodile.

To see whether two unpaired sets of data differ significantly (e.g. the difference between ALT concentrations in stunned and noosed crocodiles, or AST levels in stunned crocodiles determined on day 1 and day 2), respective blood parameter data sets were examined by using either t-test or Mann-Whitney rank sum test. To see whether two conditions in the same individual differ significantly (e.g. the difference between plasma corticosterone levels in samples collected straight after capture and again after four hours), respective blood parameter data sets were examined by using either Paired t-test or Wilcoxon signed-rank test. Data was tested for normality using Kolmogorov-Smirnov Test. All tests were two tailed, with the α level of significance set at 0.05. In this regard, a revealed P-value between 0.05 - 0.1 was rated as a trend. Further, respective physiological parameters were correlated to the time it took from capture to bleed as well as to overall trial duration. R values between 0.3 - 0.5 were rated as a weak correlation, and R values > 0.5 were rated as a strong correlation. Respective relationships were further examined descriptively.The computer programs Excel, version 2010, as well as Jandel Sigma Stat, version 2.0, was used for all statistical analyses.

4. RESULTS

4.1. General recorded data and observations

Our study was carried out on two sampling days, 19th January and 2nd February 2012, respectively. On the first sampling day we started at 6:38 and finished at 13:25. The weather conditions during this time were sunny and temperatures ranged between 25 - 30°C. The temperature in the holding area was 29°C on that day. On the second sampling day we started at 6:42 and finished at 13:10. The weather during sample collection was cloudy at first but then sunny with temperatures ranging between 24 - 31°C. The temperature in the holding area was 30°C. There were no distinct weather changes during the week before each sampling day. During our experiment, there were no major external disturbances such as e.g. aeroplanes flying low over the ponds which could have caused additional stress.

The range of total length (TL) of the crocodiles captured for this study was 158 to 210 cm with a mean of 183 cm (Table 1). All but one crocodile turned out to be females.

On research day two there were a lot of lipaemic serum samples (30 out of 44) and the blood did not clot as quickly as on day one. On the first sampling day, we had one incident where a crocodile bit and destroyed the gum boot of one of the capture team members. This happened during the noosing of the animal and could have caused severe injury to the handler if he would not have been able to retract his foot in time.

Table 1: Total length of crocodiles captured for this study.

Day 1

	Immobilised	**Noosed**
Mean (cm)	179	191
Range (cm)	163-192	168-210

Day 2

	Immobilised	**Noosed**
Mean (cm)	181	182
Range (cm)	158-207	168-210

4.2. Blood chemistry results

The results for the blood chemistry parameters determined for all samples collected on sampling day one and two are summarised in table 2 and 3, respectively.

Table 2: Blood chemistry results for sampling day one.

D1	Corticosterone (ng/mL)	Lactate (mmol/L)	Glucose (mmol/L)	ALT(U/L)	ALP (U/L)	AST (U/L)	CK(U/L)
Immobilised T0							
Median	41.7	3.95	2.70	45.0	47.5	35.0	460
Range	5.15-69.8	0.80-7.80	1.40-5.50	13.0-64.0	23.0-184	25.0-55.0	162-7075
Noosed T0							
Median	31.6	10.2	3.8	45.0	41.0	34.0	479
Range	9.14-54.3	7.00-15.4	3.00-4.20	34.0-90.0	31.0-56.0	27.0-41.0	215-1499
Immobilised T1							
Median	66.9	5.65	6.05	44.5	48.0	43.5	1051
Range	4.80-136	2.40-10.7	4.10-8.90	17.0-58.0	21.0-130	30.0-62.0	224-5222
Noosed T1							
Median	67.5	5.70	6.10	46.0	77.0	51.0	1116
Range	19.9-108.4	4.00-11.6	4.70-7.20	21.0-64.0	38.0-201	39.0-75.0	320-3331

Table 3: Blood chemistry results for sampling day two.

D2	Corticosterone (ng/mL)	Lactate (mmol/L)	Glucose (mmol/L)	ALT(U/L)	ALP (U/L)	AST (U/L)	CK(U/L)
Immobilised T0							
Median	40.3	3.80	3.80	36.0	36.0	26.0	190
Range	20.2-118	1.60-19.1	2.70-5.90	19.0-52.0	17.0-77.0	14.0-67.0	93-1634
Noosed T0							
Median	33.2	9.80	4.40	36.0	55.0	33.0	327
Range	10.7-87.2	7.30-17.7	3.30-7.90	7.00-54.0	26.0-81.0	17.0-48.0	184-1293
Immobilised T1							
Median	124	3.40	6.30	42.0	35.0	37.0	422
Range	31.2-201	2.20-8.30	4.90-7.60	18.0-61.0	16.0-56.0	24.0-59.0	136-1213
Noosed T1							
Median	96.3	8.20	5.50	27.0	49.0	43.0	1 012
Range	32.8-152	4.20-16.1	2.70-6.90	21.0-55.0	19.0-145	31.0-67.0	375-3156

4.2.1. Corticosterone

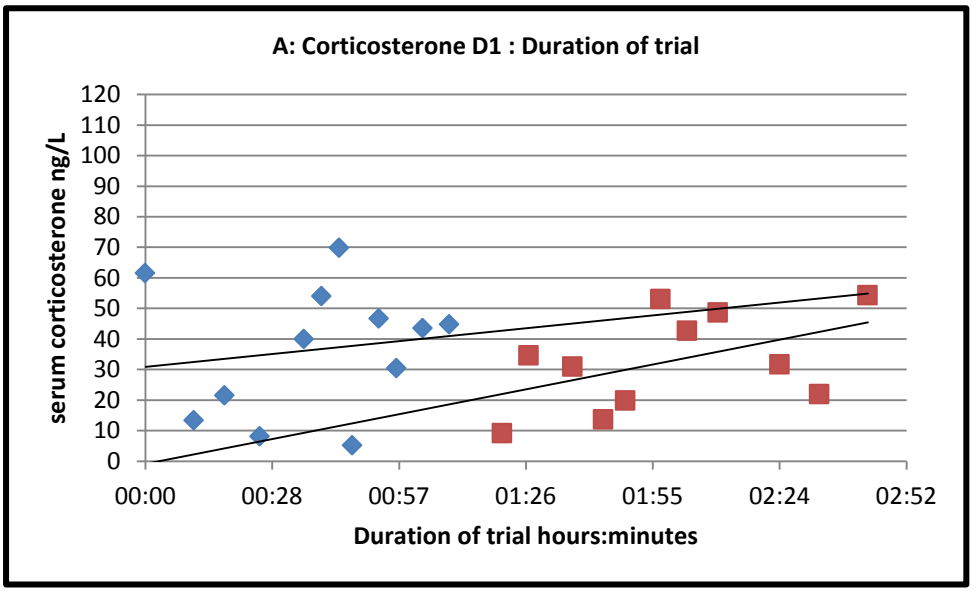
For samples collected directly after capture (T0), there was a trend (t = -1.766; P = 0.092) that stunned animals had lower corticosterone values on D1 compared to D2, which became significant (t = -2.278, P = 0.033) when comparing respective values for samples collected after four hours (T1) (Table 4).

On both sampling days, corticosterone levels significantly increased in noosed as well as electrically immobilised animals when comparing concentrations in samples taken at T0 with respective values for samples collected at T1 (t = -5.034 to -2.611; P = 0.024 – 0.001) (Table 5).

No statistical significant difference in corticosterone values were found when comparing the two capture methods (electrical immobilisation and noosing) (Table 6).

On D1, corticosterone concentrations in stunned animals were comparatively lower when the time from capture to bleed (T0) took longer (R = 0.625; Slope = -227.65) (Table 7). In contrast, respective corticosterone values in noosed crocodiles on D1 were comparatively higher when T0 increased (R = 0.544; Slope = 185.35) (Table 7).

There was also an overall positive relationship between corticosterone levels and the duration of the trial, as respective corticosterone values were comparatively higher in animals which experienced a longer disturbance due to the on-going trial (R = 0.341 to 0.872) (Table 8 and Figure 3).



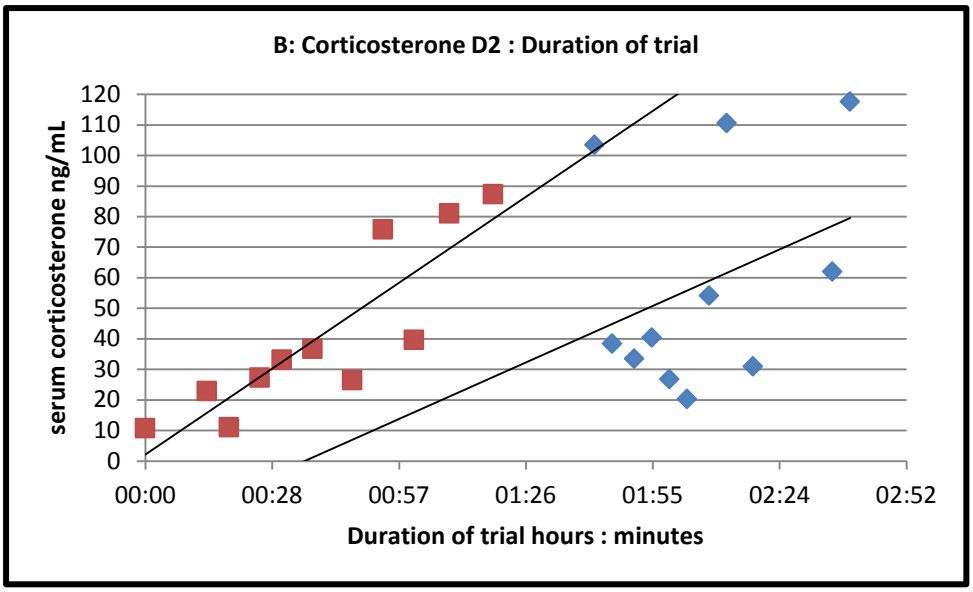


Figure 3 A & B: Individual serum corticosterone concentrations for electrically immobilised and for noosed animals in relation to the individually experienced duration of the trial on day 1 (A) and day 2 (B).

Immobilised animals; **Noosed animals**

4.2.2. Lactate and Glucose

Lactate:

Apart from a trend in immobilised animals where blood lactate was higher after four hours on D1 compared to D2 (t = 1.849; P = 0.079), lactate concentrations did not differ significantly between blood samples collected on D1 and D2 (Table 4).

Lactate concentrations did not differ between blood samples taken at T0 and T1 with one exception in stunned animals on D2 when lactate was significantly lower after four hours (t = 2.422; P = 0.036) (Table 5).

Blood lactate values were significantly higher in noosed animals compared to electrically immobilised animals on D1 at T0 (t = -6.187; P = < 0.0001), as well as on D2 at T0 (T = 81; P = 0.003) and on D2 at T1 (t = -3.533; P = 0.002) (Table 6).

There was a positive correlation with regards to lactate and the duration from capture to first blood collection (T0). In most instances lactate increased when the time from capture to blood collection increased. This correlation was strong in immobilised crocodiles (R = 0.747)

as well as in noosed animals on D1 (R = 0.769), and we found a weak correlation for noosed animals on D2 (R = 0.329) (Figure 4) (Table 7).

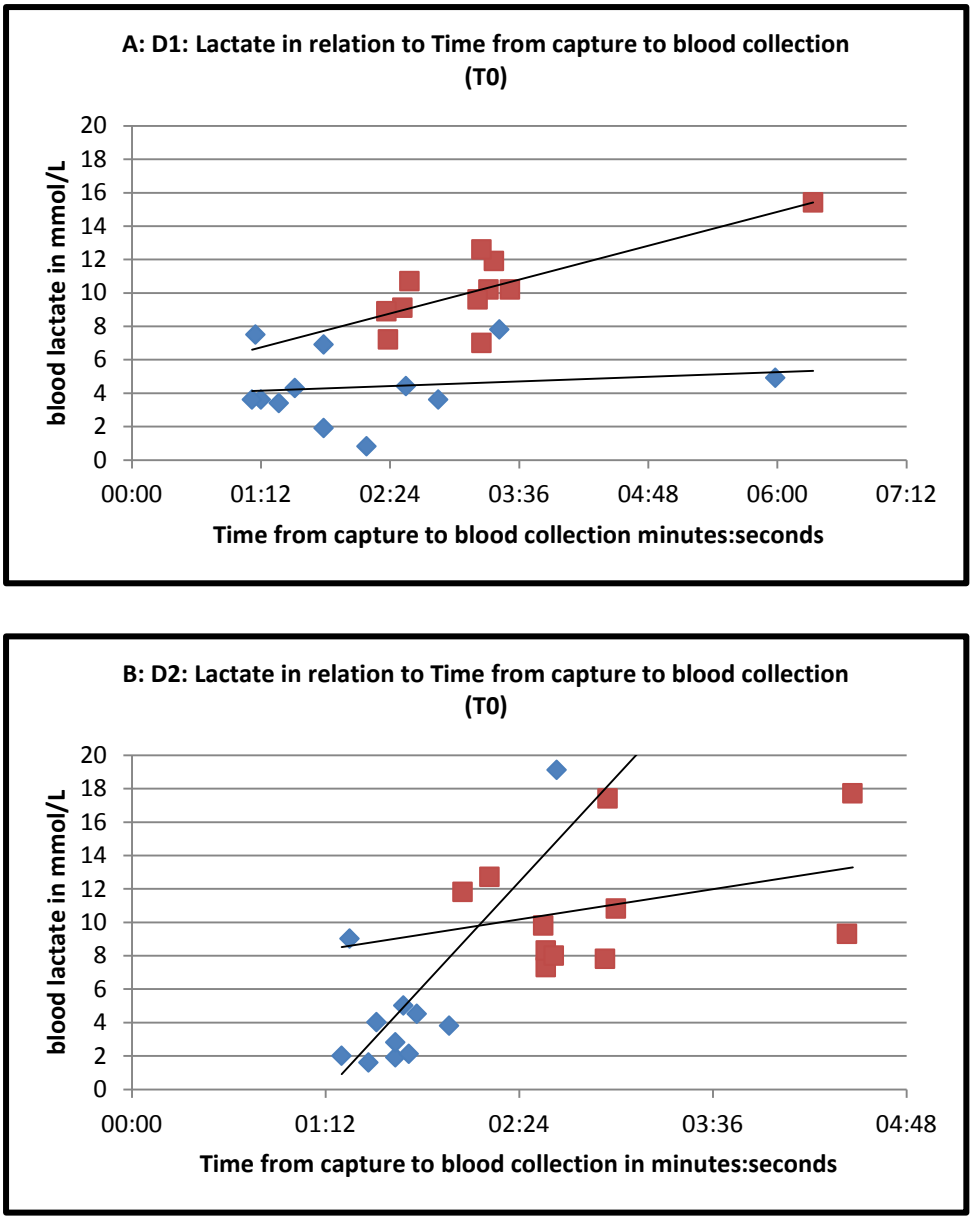


Figure 4 A & B: Individual lactate concentrations for immobilised and for noosed crocodiles on day one (A) and two (B) in relation to the individual time from capture to blood collection in minutes : seconds.

Immobilised animals; **Noosed animals**

On D1, lactate levels in stunned animals decreased if crocodiles experienced a longer disturbance due to the on-going trial (R = 0.321; Slope = 0.113), while the group that was noosed on D2 showed an increase in lactate levels with the on-going duration of the trial (R = 0.376; Slope = 80.575) (Table 8).

Glucose:

Glucose concentrations in blood samples taken on D1 at T0 were significantly lower in electrically immobilised (t = -2.842; P = 0.01) as well as in noosed animals (T = 95.5; P = 0.045) compared to respective values determined on D2. However, there was also a trend that glucose concentrations were higher on D1 compared to D2 in blood samples taken from noosed animals after four hours (t = 1.845; P = 0.08) (Table 4).

Compared to blood glucose concentrations determined in samples taken at T0, respective values were higher in samples taken after four hours waiting period (Table 5). This difference was significant in noosed and in immobilised crocodiles on D1 (t = -10.22 and -9.299; P = <0.0001) as well as in immobilised crocodiles on D2 (t = -2.728; P = 0.021) while this was just a trend in crocodiles noosed on D2 (t = -1.883; P = 0.099).

Blood glucose concentrations determined on D1 at T0 were significantly lower in electrically immobilised animals compared to noosed animals (t = -2.593; P = 0.017) (Table 6). In contrast, on D2 at T1 electrically immobilised crocodiles had significantly higher glucose levels than noosed crocodiles (t = 2.533; P = 0.02).

On D1, blood glucose concentrations in electrically immobilised animals decreased when the time interval from capture to first blood collection (T0) was longer (R = 0.406; Slope = -8.0311) (Table 7). In contrast, on D2, the blood glucose levels of electrically immobilised as well as in noosed animals increased when the time from capture to first blood collection (T0) was longer (R = 0.350 and 0.493; Slope = 21.932 and 19.592) (Table 7).

There was a strong correlation in noosed animals on D2 where blood glucose levels increase in animals which experienced a longer disturbance due to the on-going trial (R = 0.778; Slope = 60.446) (Table 8).

4.2.3. Enzymes: ALT, ALP, AST and CK

ALT:

ALT concentrations were significantly higher on D1 compared to D2 in noosed crocodiles at T0 as well as T1 (t = 2.451 and 2.398; P = 0.024 and 0.027). In addition, there was a trend in immobilised animals at T0 that ALT concentrations were higher on D1 compared to D2 (t = 1.836; P = 0.081) (Table 4).

No significant differences in ALT concentrations could be found between blood samples taken at T0 and samples taken at T1 (Table 5).

ALT concentrations did not differ significantly when comparing respective values from electrically immobilised and noosed animals (Table 6).

There was a weak positive correlation of ALT concentrations on D2 in electrically immobilised crocodiles regarding the duration from capture to first blood collection (T0) (R = 0.418; Slope = 312.93) (Table 7).

ALT concentrations determined in electrically immobilised animals on D2 positively but weakly correlated with the duration of the trial (R = 0.335; Slope = 288.26). In contrast, ALT concentrations determined in noosed crocodiles on D2 strongly but negatively correlated if animals experienced a longer disturbance due to the on-going trial (R = 0.517; Slope = -423.19) (Table 8).

ALP:

ALP concentrations showed a trend to be higher on D1 compared to D2 in noosed animals when blood was collected after four hours (t = 1.827; P = 0.082) (Table 4).

No significant differences could be found in the ALP concentrations between samples taken at T0 and samples taken at T1 with an exception of stunned crocodiles on D2 where ALP concentrations were significantly lower after four hours (W = 64; P = 0.002) (Table 5).

In most instances ALP concentration were higher when animals were noosed compared to when they were electrically immobilised. This difference was significant on D2 at T0 (t = 1.731; P = 0.049) and we found a trend for samples collected on D1 at T1 (t = -1.731; P = 0.098) and on D2 at T1 (t = -1.076; P = 0.062) (Table 6).

There was a strong positive correlation between ALP concentrations and the time from start of capture to the first blood collection of each crocodile in immobilised crocodiles on D1 (R = 0.681; Slope = 551.99) (Table 7). The latter finding might be driven by one particular outlier of 184 U/L as the median for this group was 47.5 U/L (Table 2). Once this outliner was removed, there was no correlation (R = 0.0574) (Figure 5).

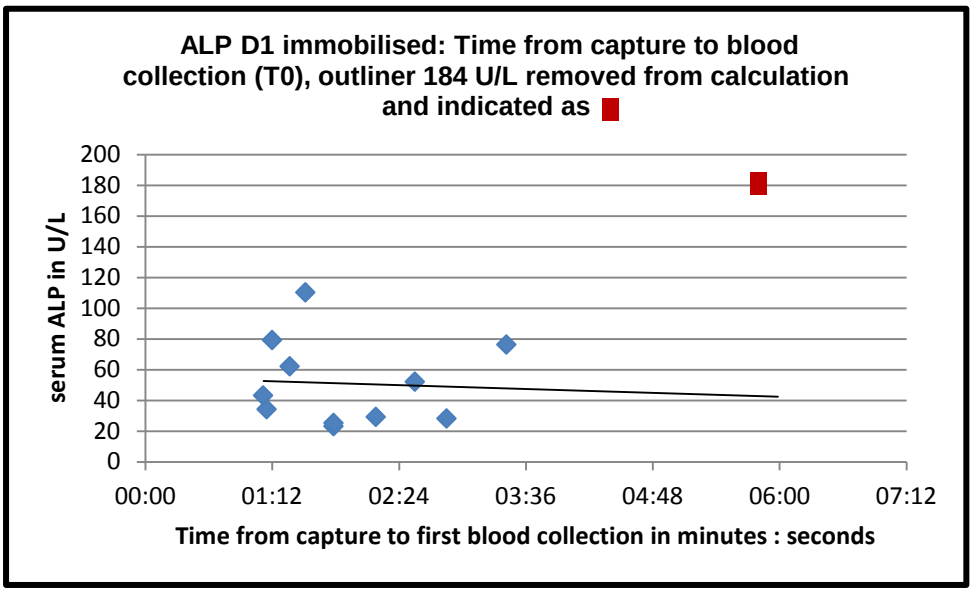


Figure 5: Individual ALP concentrations for immobilised crocodiles on day one in relation to the individual time from capture to blood collection in minutes : seconds, with outliner 184 U/L removed from calculation but indicated as red square.

On D1, ALP concentrations showed a strong negative correlation with the duration of the trial in noosed crocodiles (R = 0.658; Slope = -339.87) (Table 8).

AST:

AST concentrations were significantly lower on D1 compared to D2 in electrically immobilised animals at T0 (T = 94.5; P = 0.0228) (Table 4).

When comparing respective concentrations between T0 and T1, AST values were significantly higher after four hours in electrically immobilised animals on D1 and D2 (t = -2.487 and -5.748; P = 0.030 and >0.0001) and in noosed animals on D2 (t = -3.652; P = 0.004). In addition, there was a clear trend for AST concentrations to be higher after four hours in noosed crocodiles on D1 (W = 44; P = 0.054) (Table 5).

While there was no difference in AST concentrations between electrically immobilised and noosed animals at T0 of either day, there were significant differences in AST values after four hours, with higher AST levels found in noosed animals on D1 as well as on D2 (t = -2.197 and -2.387; P = 0.039 and 0.027) (Table 6).

AST concentrations weakly increased when the time from capture to first blood collection (T0) took longer on D1 in electrically immobilised animals (R = 0.460; Slope = 73.184). In contrast, AST concentrations weakly decreased on D1 in noosed animals (R = 0.470; Slope = -48.575) (Table 7).

There was a weak negative correlation between AST concentrations and the on-going duration of the trial in stunned animals on D1 (R = 0.476; Slope = -294.5), as well as in noosed animals on D2 (R = 0.414; Slope = -235.03); whilst respective AST values strongly increased with the on-going duration of the trial in electrically immobilised animals on D2 (R = 0.693; Slope = 768.58).

CK:

CK concentrations were significantly higher in electrically immobilised crocodiles on D1 in samples directly taken after restrain (T0) as well as after four hours (T1) compared to respective sample sets collected on D2 (T = 93; P = 0.018 for both instances) (Table 4).

There was a significant difference on D2 when CK concentrations were higher after four hours in stunned as well as in noosed crocodiles (t = -3.386 and -4.008; P = 0.007 and 0.002) while on D1 no difference could be detected between samples taken at T0 and samples taken at T1.

As for AST, CK concentrations showed no differences between electrically immobilised and noosed animals at T0 of either day. However, there were differences in CK concentrations between electrically immobilised and noosed animals after four hours. A trend to higher CK levels in noosed animals was visible on D1 (T = 99.5; P = 0.082) which became significant on D2 (T = 82; P = 0.004) (Table 6).

In electrically immobilised crocodiles on D2 there was a strong positive correlation of CK concentrations and the time it took from capture to blood collection (T0) (R = 0.902; Slope = 26689) (Table 7).

CK concentrations strongly decreased with the on-going duration of the trial in electrically immobilised animals on D1 (R = 0.684; Slope = -99365) as well as in noosed crocodiles on D2 (R = 0.517; Slope = -10006) (Table 8).

Table 4: Comparison of physiological blood parameters of crocodiles captured on day one with crocodiles captured on day two. Significant differences are marked bold and underlined. Trends are marked bold only. Where the Mann-Whitney rank sum test was applied, T-values are indicated in italics.

		immobilised (T0)	*noosed (T0)*	*immobilised (T1)*	*noosed (T1)*
Corticosterone	*t or T value*	-1.766	-0.87	-2.278	*112*
	P value	**0.092**	0.395	**<u>0.033</u>**	0.358
Lactate	*t or T value*	*127.5*	-0,557	1.849	1.221
	P value	0.806	0.584	**0.079**	0.236
Glucose	*t or T value*	-2.842	*95.5*	-0.879	1.845
	P value	**<u>0.01</u>**	**<u>0.045</u>**	0.389	**0.08**
ALT	*t or T value*	1.836	2.451	0.347	2.398
	P value	**0.081**	**<u>0.024</u>**	0.732	**<u>0.027</u>**
ALP	*t or T value*	1.53	-1.703	1.827	0.067
	P value	0.141	0.104	**0.082**	0.947
AST	*t or T value*	*94.5*	0.922	1.674	1.009
	P value	**<u>0.023</u>**	0.368	0.109	0.325
CK	*t or T value*	93	152	93	0.083
	P value	**<u>0.018</u>**	0.101	**<u>0.018</u>**	0.935

Table 5: Comparison of physiological blood parameters of crocodiles between blood samples taken straight after capture (T0) and four hours later (T1) from the same crocodile. Significant differences are marked bold and underlined. Trends are marked bold only. Where the Wilcoxon signed-rank test was applied, W values are indicated in italics.

		immobilised D1	*noosed D1*	*immobilised D2*	*noosed D2*
Corticosterone	*t or W value*	-2.611	-3.358	-5.034	-3.455
	P value	**0.024**	**0.007**	**0.001**	**0.006**
Lactate	*t or W value*	*33*	*6*	2.422	1.691
	P value	0.204	0.831	**0.036**	0.122
Glucose	*t or W value*	-10.22	-9.299	-2.728	-1.833
	P value	**>0.0001**	**>0.0001**	**0.021**	**0.099**
ALT	*t or W value*	0.542	0.768	*28*	0.426
	P value	0.599	0.460	0.240	0.679
ALP	*t or W value*	0.451	0.599	*64*	1.25
	P value	0.661	0.562	**0.002**	0.240
AST	*t or W value*	-2.487	*44*	-5.748	-3.652
	P value	**0.030**	**0.054**	**>0.0001**	**0.004**
CK	*t or W value*	-0.024	-0.835	-3.386	-4.008
	P value	0.981	0.423	**0.007**	**0.002**

Table 6: Comparison of physiological blood parameters of crocodiles captured by electric immobilisation with crocodiles captured by noosing. Significant differences are marked bold and underlined. Trends are marked bold only. Where the Mann-Whitney rank sum test was applied, T values are indicated in italics.

		D1, T0	*D1, T1*	*D2, T0*	*D2, T1*
Corticosterone	*t or T value*	0.485	0.191	1.24	0.877
	P value	0.632	0.850	?29	0.391
Lactate	*t or T value*	-6.187	-1.613	*81*	-3.533
	P value	**>0.0001**	0.122	**0.003**	**0.002**
Glucose	*t or T value*	-2.593	-0.229	*109*	2.533
	P value	**0.017**	0.821	0.264	**0.02**
ALT	*t or T value*	*139*	-0.95	0.104	1.111
	P value	0.689	0.353	0.918	0.28
ALP	*t or T value*	1.25	-1.731	-2.097	-1.976
	P value	0.225	**0.098**	**0.049**	**0.062**
AST	*t or T value*	0.333	-2.197	-0.703	-2.387
	P value	0.742	**0.039**	0.49	**0.027**
CK	*t or T value*	1.5552	*99.5*	0.622	*82*
	P value	0.135	**0.082**	0.541	**0.004**

Table 7: Physiological blood parameters of captured crocodiles correlated with the time taken from capture to blood collection (T0) of each individual crocodile. Strong correlations are marked bold and underlined, weak correlations are bold.

	Day	Cortisol	Lactate	Glucose	ALT	ALP	AST	CK
Immobilised R value	1	**0.625**	0.151	**0.406**	0.035	**0.681**	**0.460**	0.062
	2	0.126	**0.747**	**0.350**	**0.418**	0.071	0.062	**0.902**
Immobilised Slope	1	-227.65	5.5565	-8.0311	-6.8237	551.99	73.184	2284.2
	2	296.96	251.31	21.932	312.93	-75.163	-59.758	26689
Noosed R value	1	**0.544**	**0.769**	0.135	0.233	0.287	**0.470**	0.227
	2	0.129	**0.329**	**0.493**	0.045	0.123	0.192	0.128
Noosed Slope	1	185.35	40.571	-1.17	-77.514	-60.494	-48.575	2117
	2	106.26	36.098	19.592	-17.447	-65.237	-55.873	-1262.5

Table 8: Physiological blood parameters of captured crocodiles correlated with the on-going disturbance caused by the duration of the trial. Strong correlations are marked bold and underlined weak correlations are bold only.

	Day	Cortisol	Lactate	Glucose	ALT	ALP	AST	CK
Immobilised R value	1	0.148	**0.321**	0.125	0.017	0.020	**0.476**	**<u>0.684</u>**
	2	**0.341**	0.113	0.059	**0.335**	0.204	**<u>0.693</u>**	0.017
Immobilised Slope	1	210.11	-46.039	-9.6409	12.267	-20.892	-294.5	-99365
	2	925.17	-43.651	-4.287	288.26	-246.11	768.58	627.42
Noosed R value	1	**0.487**	0.074	0.091	**<u>0.517</u>**	**<u>0.658</u>**	0.251	0.002
	2	**<u>0.872</u>**	**0.376**	**<u>0.778</u>**	0.178	0.047	**0.414**	**<u>0.517</u>**
Noosed Slope	1	407.07	9.608	1.9336	-423.19	-339.87	63.787	56.213
	2	1406	80.575	60.446	137.37	-48.612	-235.03	-10006

4.3. Comparison of the two capture methods regarding the time taken from capture until blood sample collection

The time it took to capture each crocodile from the point of first touch until the crocodile was captured and restrained to take the first blood sample (T0) was longer in animals that were noosed compared to animals that were stunned (Table 9). In our project, T0 of electrically immobilised animals averaged 118 seconds (s) with the longest time being 359 s and the shortest time 67 s. Noosed animals averaged a time from first touch to blood collection of 186 s. The shortest time was measured to be 123 s in noosed animals while the longest time period was 380 s (Table 9).

Table 9: Time from beginning of capture to first blood collection for electrically immobilised and noosed crocodiles.

Day 1

T0	**Immobilised**	**Noosed**
Median (s)	107	195
Range (s)	67-359	142-380

Day 2

T0	**Immobilised**	**Noosed**
Median (s)	98	157
Range (s)	78-158	123-268

5. DISCUSSION

5.1. Blood samples

A total of 90 blood samples were collected and analysed from 45 crocodiles. One blood sample was collected immediately after capture at zero hours (T0) and the second blood sample was collected four hours later (T1) from the same crocodile. On sample day one (D1), 23 crocodiles were captured for this study while on sample day two (D2), 22 crocodiles were utilised. Blood lactate and blood glucose where measured at the study site. Serum ALT, ALP, AST and CK, as well as serum corticosterone were analysed by different laboratories of the University of Pretoria. Results were tested for normality. The results of samples collected from the same individual at T0 and at T1, as well as the two capture methods were compared. To make sure that our test results were not unduly influenced by external factors, results of D1 were also compared with results of D2. In addition, respective blood parameters were also correlated with the time it took to capture each individual until blood collection at T0, as well as with the overall duration of the trial.

The results of this investigation are compared in table 10 with reference ranges published by different authors for the Nile crocodile and in case of blood lactate for *C. porosus*. In general, most of our results compared favourably with those referred to in table 10.

Table 10: Comparing blood chemistry results of our study with results cited in literature: [a] = *C. niloticus*; [b] = *C. porosus*

Parameter	Range in this study	Mean in this study	Mean concentrations and ranges reported in literature	References
Serum corticosterone ng/mL	4.80 - 201	63.8	Range: 4.0 - 6.0	Balment & Loveridge 1989[a]
Blood lactate mmol/L	0.80 - 19.10	7.13	Mean: 21.0 in manually captured crocodiles and 10.7 in stunned crocodiles	Franklin *et al.* 2003[b]
Blood glucose mmol/L	1.40 - 8.90	4.86	Means: 3.87 - 5.68 Mean: 3.8; Range: 1.8 - 4.8 Mean: 5.68	Botha 2010[a]; Lovely *et al.* 2007[a]; Swanepoel *et al.* 2000[a]
ALT U/L	7.00 – 90.0	40.0	Means: 13.0 – 30.0 Mean: 43.9, Range:15.0 – 63.0 Mean: 13.1 Range: 9.0 - 20.4	Botha 2010[a] Lovely *et al.* 2007[a] Foggin 1987[a]
ALP U/L	16.0 – 263	57.1	Means: 9.18 – 28.0 , Mean: 21.1 Range: 3.0 – 72.0 Mean: 64.2 Mean of 437 when chronically stressed if healthy	Botha 2010[a] Lovely *et al.* 2007[a] Watson 1990[a]
AST U/L	14.0 -75.0	39.4	Means: 24.0 – 47.0 Mean: 66.5 Range: 14.0 – 211 Mean: 16.6 Range: 6.7 - 22.7	Botha 2010[a] Lovely *et al.* 2007[a] Foggin 1987[a]
CK U/L	93.0 – 7075	1 014	Mean: 211 Mean: 9 187 when chronically stressed	Watson 1990[a]

5.1.1. Corticosterone

Stress, especially chronic stress in crocodiles can have serious consequences such as inhibition of growth, inhibition of the reproductive system and suppression of the immune system (Lance 1990; Huchzermeyer 2002).

Although stress hormones are well-studied in humans and other mammals, this is not the case in crocodilians. While the endocrine response seems similar to that in mammals, qualitatively, the duration and magnitude of hormonal changes might be very different (Lance 2001). Corticosterone is the principle glucocorticoid secreted by reptiles and birds in response to stress (Lance *et al.* 2001). This is in contrast to mammals and fish, which mostly release cortisol (Romero 2004).

Corticosterone concentrations measured in different crocodilian species seemed a lot lower if compared to our results (Table 10). Serum corticosterone in this investigation ranged between 4.80 and 201 ng/mL with an overall mean of 63.8 ng/mL. This is much higher than values given for crocodilians in literature, which range from 6 ng/mL in Nile crocodiles (Balment & Loveridge 1989) to 2 ng/mL in American alligators (Guillette *et al.* 1997) and 1 ng/mL in saltwater crocodiles (Franklin *et al*. 2003). *C. johnstoni* baseline levels averaged 4.024 ng/mL (Jessop *et al.* 2003). The corticosterone levels of Caiman were comparatively high at 20 ng/mL (Gist & Kaplan 1976). However, this discrepancy most possibly has to do with different research models and test methods. Laboratory procedures as well as environmental factors could also have a lot of influence on absolute values in each study (Romero 2004). Our main interest for this study was in the difference between corticosterone concentration and in the change of corticosterone values over time.

A trend is observed that electrically immobilised animals on T0 had lower corticosterone concentrations on D1 if compared to D2. This became even more obvious when it was compared to the T1 results (Table 4). It could be speculated that crocodiles remembered the capture event of D1 and therefore anticipated the disturbance, excreting corticosterone early on D2; possibly already when the research team approached the pens, long before capture. On D2, capture by means of electrical immobilisation was done after noosing, this could also have contributed that initial corticosterone values might have already been high before blood was collected. In contrast, stunning was carried out first on D1 and therefore maybe corticosterone concentrations were low for a long time on D1 as the crocodiles only started to

realise what was going on later during the trial. This theory is supported by the fact that cortisone values were rising with duration of trial (Figure 3).

Corticosterone mean values increased within four hours after capture in all groups to about double of what was measured at T0 (Table 2 and 3). This is in contrast to Franklin *et al.* (2003) who found that the two fold rise in corticosterone from 1 ng/mL to 2 ng/mL went back to normal levels within one hour after capture. However, the study set up by Franklin *et al.* (2003) was different from ours which might explain why crocodiles excreted less corticosterone. While our crocodiles were bled twice – once at T0 and then after a rest period of 4 hours during which they were blindfolded and tied up and therefore suffered some restraint stress; Franklin *et al.* (2003) used crocodiles that were in individual pens. These crocodiles were captured and released back into their individual pens straight away. Crocodiles were without any restraint in their pens until it was time to take the blood sample at different time intervals after the capture event.

Corticosterone in wild *C. johnstoni* increased with time during a ten hour capture stress exposure period (Jessop *et al.* 2003). It was also found that the corticosterone secretion in juvenile and adult American alligators had a biphasic pattern, with one peak after four hours and another peak after 48 hours (Lance & Elsey 1986; Elsey *et al.* 1991; Lance & Elsey 1999a). Guillette *et al.* (1997) determined a baseline level in wild Alligators of around 1 ng/ml. This increased 30 fold to around 30 ng/ml after two hours of restraint stress in a cloth bag on the boat.

Corticosterone in our study increased during the duration of the trial (Figure 3).This is an important find as it shows that the stress levels of crocodiles slowly increased during the entire period that crocodiles were captured and samples collected. Therefore, to avoid unnecessarily high corticosterone levels and the negative consequences, the work in ponds with large numbers of crocodiles should always be carried out as fast as possible.

In our study, no difference was found in corticosterone levels between the two capture methods. This also coincides with the results of Franklin *et al.* (2003) who compared acute stress response of estuarine crocodiles that were noosed with those that were electro-stunned.

5.1.2. Lactate and glucose

The rise of both, lactate and glucose concentrations in blood is a reaction to acute restraint stress of crocodilians (Lance *et al.* 2001). In addition to the HPA, the sympathetic and somatic nervous system also facilitates a rapid response to stress. It is believed that in alligators and estuarine crocodiles, the somatic nervous system facilitates the rapid mobilisation of muscle glycogen via anaerobic glycolysis and therefore the immediate rise in plasma lactate following handling stress (Seymour *et al.* 1987; de Roos *et al.* 1989). Lactate was not influenced by our research model as there were no statistically significant differences in values of D1 compared to D2 (Table 4). This is in contrast to corticosterone. This confirms that in Nile crocodiles, as in alligators and estuarine crocodiles, lactate is not so much influenced by corticosterone release but rather by reaction of the sympathetic and somatic nervous system (Coulson & Hernandez 1983; Seymour *et al.* 1987; de Roos *et al.* 1989). Lactate values in our study ranged between 0.80 to 19.1 mmol/L with a mean of 7.13 mmol/L (Table 10).

The immediate rise following handling stress might contribute to the fact that no difference was found between lactate measured at T0 and lactate measured at T1. Animals had to be handled again to take blood after four hours which might also have led to an additional rise in blood lactate. Alternatively, the four hour period might have been inadequate to see a change in lactate levels.

Lactate levels of *C. porosus* peaked one hour after capture and remained high for four to eight hours (Franklin *et al*. 2003). Bennet *et al.* (1985) and Seymour *et al*. (1985) reported a partial decrease in lactate levels of exhausted *C. porosus* within two hours. However, completely exhausted large estuarine crocodiles can develop lactate levels of over 50 mmol/L and take as long as 30 hours to get back to baseline levels (Bennet *et al*. 1985; Seymour *et al.* 1987).

In our study blood lactate values were significantly higher in noosed animals compared to electrically immobilised animals on D1 at T0 as well as on D2 at T0 and on D2 at T1 (Table 6). This coincides with the results of Franklin *et al*. (2003) who reported an immediate rise in plasma lactate in *C. porosus*, which was much more pronounced in manually captured animals if compared to immobilised animals. In stunned *C. porosus*, lactate levels peaked only after 30 minutes at about 10.7 mmol/L whereas in manually captured animals that were

struggling a lot the lactate concentrations went as high as 21 mmol/L and peaked one hour after restraint and remained elevated for four to eight hours. The amount of lactate that is produced during capture is directly correlated with the time from first touching the crocodile for capture until the crocodile is restrained enough to do the blood collection (Figure 4). This was also reported by Bennet *et al.* (1985) and Seymour *et al.* (1987). The process of noosing a crocodile and restraining it for blood collection in our study took on average 186 seconds – 68 seconds longer than electrical immobilisation. Franklin *et al*. (2003) took on average 1.2 minutes to restrain a crocodile by stunning and 2.4 minutes to manually restrain crocodiles. High lactate levels can have serious implications on the recovery of the animal (Seymour *et al.* 1987). Therefore, in this respect electrical immobilisation seems to give a definite advantage as it facilitates that the individual capture procedure is fast, animals struggle less and therefore less lactate is produced by the captured animals.

Glucose is mobilised during an acute stressful event such as capture in order to make energy available to facilitate a quick response (Lance *et al.* 2001; Jessop *et al*. 2003). Glucose baseline levels of different wild Nile crocodile populations vary between 3.8 mmol/L in the Okavango Delta (Lovely *et al.* 2007) and 5.68 mmol/L in South Africa (Botha 2010). Our values ranged from 1.40 to 8.90 mmol/L and were within these reference ranges (Table 10).

Following the capture and restraint of American alligators, glucose levels rise immediately at capture and stay elevated for 48 hours and more. This does not correlate with the biphasic corticosterone values that were found in American alligators and therefore it is speculated that glucose release might be a result of elevated catecholamines rather than related to corticosterone release (De Roos *et al.* 1989; Lance & Elsey 1999; Lance *et al.* 2001). In contrast, *C. johnstoni* had constantly rising glucose levels during a ten hour capture stress exposure which seemed linked to the continuously rising corticosterone levels (Jessop *et al* 2003).

In our study, the change of glucose levels and corticosterone levels coincided partially. Both values increased during the four hour period (T0 to T1). Glucose levels of stunned animals (first sample group) was lower at T0 on D1 compared to D2. The same happened to corticosterone. However, apart from these coincidences, corticosterone and glucose in this study did not seem to be directly linked further. As for corticosterone, it could be speculated that crocodiles started to be nervous on the second sampling day from the moment when

people started approaching the ponds and therefore some of the glucose baseline values were higher on D2.

Glucose levels in our study rose within the four hour period – this coincides with similar studies in *C. porosus*; *C. johnstoni* and *A. mississippiensis* (Lance & Elsey 1999a; Jessop *et al*. 2003; Franklin *et al.* 2003). Wild *C. johnstoni* were captured and subjected to restraint stress by Jessop *et al*. (2003). Blood was collected immediately after capture in some individuals and other were bled after time intervals of 0.5; 6 and 10 hours post capture. Each individual was only bled once and kept restrained for the necessary time period. Plasma glucose of these crocodiles started increasing within 30 minutes and rose constantly until the last samples were tested after ten hours.

Lance & Elsey 1999a used ten captive bred juvenile *A. mississippiensis* for their study. The animals were restrained in garbage bins for 48 hours. Blood samples were collected from all individuals at 0; 1; 2; 4; 8; 24 and 48 hours. Glucose values rose steadily until 24 hours of restraint. Glucose values after 48 hours were still elevated but decreased compared to the 24 hour measurements.

Franklin *et al.* (2003) captured farmed *C. porosus* either manually or by electrical immobilisation. After capture blood was taken immediately from some animals while others were left to rest in their pens without restraint. Blood was taken only once from each crocodile after different recovery periods of between 0.5 and 48 hours. In this study blood glucose concentrations reached their peaks within four hours after capture irrespective of the capture method.

Franklin *et al*. (2003) found a significant difference in glucose levels between manually restrained animals and animals that were electrically immobilised. Glucose levels were lower in immobilised animals. In contrast, we could not find any clear correlation between capture method and blood glucose release in our study.

When blood glucose levels were correlated to the time interval from capture to first blood collection, the results were very inconclusive. On D1 blood glucose concentrations in electrically immobilised animals decreased when the time interval from capture to first blood collection was longer (Table 7). In contrast, on D2 the blood glucose levels in electrically

immobilised as well as in noosed animals increased when this time interval was longer (Table 7).

It seems that the disturbance by the on-going duration of the trial did influence glucose values but only on one occasion (Table 8). The group that was manually captured on D2 (first group) had rising glucose levels with a longer overall duration of the trial. However, this correlation is not visible in the other groups. Therefore the meaning and significance is questionable.

In conclusion, while glucose was affected when crocodiles were captured and values rose within the four hour period (T0 to T1), no difference could be detected between capture methods.

5.1.3. Enzymes

Serum enzymes are commonly used as a tool to evaluate possible tissue or organ damage in animals and humans (Kaneko *et al.* 1997). When tissues get damaged, enzymes are released into the bloodstream by damaged cells. Depending on the tissue type, different enzymes or enzyme combinations would be more prominent in the serum as a result of the damage (Kaneko *et al* 1997).

Our main concerns were potential damage to internal organs by the electrical immobilisation process and neuromuscular damage which could be caused by either capture method. ALT and ALP were chosen as potential indicators of organ damage (Last *et al*. 2010) while a rise in CK and AST would be an indication for neuromuscular damage (Kaneko *et al.* 1997).

Reference values for Nile crocodiles were available for these chosen enzymes from previous studies of wild and captive bred crocodiles (Foggin 1986; Watson 1990; Lovely *et al.* 2007; Botha 2010) (Table 7).

ALT is predominantly found in liver tissue and to a lesser degree in kidney, heart and skeletal muscle as well as in red blood cells. ALT could therefore be a good indicator for hepatocellular injury (Last *et al*. 2010). This enzyme was chosen for our study to get an indication of potential organ damage as a result of the capture method. ALT baseline values for wild Nile crocodiles in Botswana have been determined by Lovely *et al.* (2007) to range

from 15.0 to 63.0 U/L. In South Africa ALT in wild Nile crocodiles in different populations ranged from 13.0 to 30.0 U/L (Botha 2010). Foggin (1987) mentions that ALT was elevated in runting juvenile Nile crocodiles on farms in Zimbabwe. In healthy hatchlings the mean ALT values were measured at 13.1 U/L while in runts the mean measured at 34.8 U/L.

ALT values in our study ranged from 7.00 to 90.0 U/L with a mean of 40.0 U/L. This is comparable with values given other literature (Table 10). ALT did not differ between samples taken immediately after capture and samples taken after four hours. Further there was no difference between noosed animals and electrically immobilised animals. Therefore we can conclude that ALT was not influenced by the capture method.

ALP as a membrane bound enzyme is also found in many tissues and was also chosen as a parameter to get an indication of any internal tissue damage that might have been caused by the capture method (Last *et al.* 2010). ALP was measured in wild *C. niloticus* ranging from 3.0 to 72.0 U/L in the Botswana Delta (Lovely *et al*. 2007) and 9.18 to 28.0 U/L in different wild Nile crocodile populations in South Africa (Botha 2010). Watson (1990) reported ALP values of 437 U/L in a chronically stressed captive Nile crocodile population while a captive population that was not stressed had a mean ALP value of 64.2 U/L. ALP in our study ranged from 16.0 to 263 U/L with a mean of 57.1 U/L, which coincides with values cited in literature (Table 10).

No significant differences could be found between samples taken at T0 and samples taken at T1 (Table 5) with the exception of stunned crocodiles on D2 where ALP concentrations were significantly lower after four hours as this is the only occasion where ALP changed significantly within four hours; it has to be concluded that in general ALP was either not affected by the capture or not within the four hour time period. ALP seemed higher more often in noosed animals if compared to electrically immobilised animals (Table 6).

While there seemed to be a strong positive correlation at first between ALP concentrations and the duration from capture of the crocodile to the first blood collection (T0); this correlation seemed to be driven by outliners and therefore is questionable (Figure 5).

In conclusion, the enzyme ALP possibly did not change significantly due to capture but it might also have to be examined within a shorter time period – maybe the peak was reached

within the four hour period and it was already decreasing by the time the second blood sample was collected. In domestic cats, e.g.; ALP has a half-life of six hours (Last *et al.* 2010). However, this would also mean that any potential organ damage was not significant enough to be long lasting.

Elevated AST can be found in various myopathies. Amongst others, AST is elevated in capture myopathy. However, AST is not as organ specific as CK. High AST activity can also be found in liver and other organs as well as in red blood cells (Kaneko *et al.* 1997). If there is liver damage however, ALT usually rises before AST (Last *et al.* 2010). Haemolysis can cause false increases of AST (Last *et al.* 2010). AST together with ALT was found to be elevated in runting crocodiles on farms in Zimbabwe (Foggin 1987) where healthy crocodile hatchlings had a mean AST of 16.6 U/L while runts had a mean AST value of 42.7 U/L. In wild Nile crocodiles AST measured 14.0 to 211 U/L in the Okavango Delta (Lovely *et al.* 2007) and 24.0 to 47.0 U/L in various wild Nile crocodile populations in South Africa (Botha 2010). AST values in our study were measured at 14.0 to 75.0 U/L with a mean of 39.4 U/L.

CK is highly active in skeletal and myocardial muscle and was used to monitor muscle damage. It has a short half-life of 60 to 90 minutes and after a single injuring event, concentrations return back to normal within 24 to 48 hours (Kaneko *et al.* 1997; Last *et al.* 2010) making it ideal for our project as changes can be expected within our four hour time limit, should muscles be affected by the capture method. Watson (1990) measured CK in chronically stressed captive bred Nile crocodiles and compared the values with healthy Nile crocodiles. CK concentrations of the healthy control group were 211 U/L, while in chronically stressed animals the CK values increased to levels as high as 9 187 U/L. This is well above levels observed in mammals and it coincides with levels in birds. Rested mallards in a study by Dabbert & Powell (1993) had AST values of 19 U/L and CK values of 225 U/L. Wild captured mallards in the same study developed AST values of 330 U/L and CK values of 12 035 U/L. CK values in this study averaged at 1 014 U/L with a range of 93.0 to 7 075 U/L (Table 10).

The combination of CK and AST is a good indicator for muscle necrosis and is used in horses to monitor paralytic myoglobinuria (Kaneko *et al.* 1987). These two enzymes were also used in numerous wildlife studies in fish, birds and mammals as indicators of muscle exertion and capture myopathy (Wells *et al.* 1986; Bollinger *et al.* 1989; Dabbert & Powell 1993;

Nicholson *et al*. 2000; Cattet *et al.* 2003). In our study AST as well as CK values increased in most groups within the four hour period (Table 5). This indicates that both these enzymes were influenced by capturing the animals and our chosen time interval of four hours was suitable to indicate this change. These results coincide with other studies were AST and CK also took time to rise after capture. Bollinger *et al.* (1989) found a significant rise of CK and AST in mallards (*Anas platyrhynchos*) between samples taken immediately after capture and samples taken one hour later. Dabbert & Powell (1993) also had a time interval of 45 minutes between capture of mallards and blood sampling.

AST and CK values rose to higher levels after four hours in noosed animals compared to stunned animals (Table 6). This is possibly due to higher muscle activity and higher lactate levels in noosed animals. It could indicate that there was some muscle damage due to exertion. When animals were noosed, they struggled on average for one minute longer than crocodiles that were electrically immobilised. The length of physical handling time seems to be closely correlated in birds with the elevation of CK and AST (Bollinger *et al*. 1989; Dabbert & Powell 1993). Physical handling of animals leads to muscle activity which is sustained by anaerobic metabolism and therefore results in production of lactate which leads to local and systemic acidosis. The resulting lower PH level causes increased cell permeability and cell lysis, which leads to release of enzymes such as CK and AST into the blood stream (Bollinger *et al*. 1989). An exercise-induced CK increase is normal but the increase varies greatly between individuals (Morandi *et al*. 2006). If damage was caused due to eccentric muscle activity and lactacidosis, it can lead to capture myopathy and even death of the animal. Unfortunately, it is not clear at which enzyme concentration it can be expected that severe damage was caused. None of the crocodiles in our study showed any signs of lameness or muscle damage within the next two to three weeks. The highest CK level in our study was 7 075 U/L (Table 10) which was in the very first crocodile captured (stunning method) and this value decreased to 1 806 U/L within four hours. The AST level of this animal was also comparatively high at 55 U/L but decreased to 41 U/L. Because these high enzyme values dropped very quickly, we can conclude that no permanent muscle damage was caused.

There were just weak and inconsistent correlations of AST concentrations with the length time it took from capture to first time blood collection. CK concentrations show one strong positive correlation to this time interval on D2 in electrically immobilised animals. Overall, it

can be concluded that the length of time from capture to first blood collection did not influence AST or CK blood concentrations in a consistent manner (Table 7). The relationship of AST blood concentrations with the overall disturbance during the duration of the trial were also not clear. In some instances AST concentrations increased while in others, it decreased. In contrast, CK showed two strong negative correlations. CK values of electrically immobilised animals on D1 and of noosed animals on D2 decreased with the on-going duration of the trial (Table 8).

5.2. Strong and weak points of this study

Some of the measured blood parameters seemed to be affected by our research model and therefore differences were detected between D1 and D2.

Technical factors that influenced the study was the fact that on D2 there were a lot of lipaemic serum samples and the blood did not clot as quickly. Animals were not fed for four days before the trial to avoid lipaemic samples. However, cannibalism is a common occurrence in communal crocodile pens (Rootes & Chabreck 1993; Huchzermeyer 2003) and the remains (pieces of scutes) of a dead crocodile were found later when the pond was cleaned. The fact that blood samples of crocodilians can be lipaemic was also reported by other researchers (Watson 1990; Millan *et al.* 1997; Swanepoel *et al.*2000). Unfortunately cannibalism can't be controlled in communal pens and therefore this factor could only be excluded by using individual pens. This was not a practical option for us at the time. Fortunately, all the parameters that were measured were not influenced by lipaemia.

On D2 blood tubes had to stand for about 30 minutes longer until blood clotted and serum could be spun down and frozen in liquid nitrogen. The reason for this could not be determined. The longer clotting time in return could have led to increased enzyme activity due to haemolysis. However ALT activity instead of being elevated was reduced on D2 (Table 4).

Corticosterone and glucose were most probably also influenced by the research model due to crocodile behaviour. We suspect that on D1 animals saw people moving around their pen but did not know yet what was coming and therefore only started to show a stress reaction later during the trial of D1. In contrast, on D2 they possibly remembered D1 and started to feel

stressed from the beginning when the researchers approached the enclosure. Therefore, despite the fact that we had a flip-over trial design, we could not avoid this kind if interference. The only way to exclude such behavioural stress reaction would be to carry out a study like this in two completely separated populations. However, if this was done, there might be more environmental factors that would differ between the populations and interfere with results. Again, individual pens, might also help to remedy this problem.

Lactate, ALT and ALP did not show any clear difference between the samples taken at T0 and again four hours later (Table 5). This might be due to the fact that the time period or the method was not suitable to show the difference in Nile crocodiles. Animals had to be restrained to take the second blood sample and this might have caused a second rise of these parameters which might have masked a decrease compared to the sample taken at T0. This is unlikely for enzymes however, as animals were tied up and blindfolded and the period between the second restrained and blood collection was very short and would possibly not leave enough time for enzymes to be released and circulate in the blood stream. For lactate however, this might be a possibility as the animals thrashed their bodies as soon as they were held for the second blood collection. Therefore any lactate values that might have started to decrease might have gone up again.

The only way around this problem would be to take blood only once and possibly use individual pens so that animals don't have to be restrained at all. A research model similar to that of Franklin *et al* (2003) might be more appropriate. Franklin and co-workers used individual pens and therefore did not have to restrain the crocodiles after the capture event took place. He then took blood samples at different time intervals but each crocodile was only bled once.

Our study was hardly affected by environmental factors. We were very lucky as the weather during the research period did not change significantly and our research team stayed exactly the same. Neither did we have to deal with any disturbances that might have influenced our research – such as loud noises from aeroplanes, wild animals breaking into enclosures, etc.

Physiological responses in crocodiles are only apparent at their preferred temperature range which is 25 to 35 $^{\circ}$C (Lang 1987; Lance *et al.* 2001). In crocodiles, the stress response might differ with different environmental temperatures (Lance 1994; Lance & Elsey 1999b).

Temperatures in this study were nearly the same at D1 (25 to 30 °C) and D2 (24 to 31 °C) and within the comfort range of crocodiles. Therefore temperature would not have influenced our research results. The only way to reliably exclude environmental factors is to carry out the research in climate controlled houses.

In summary, while we did not have too many external disturbances that could have influenced our research model, this could have been different and beyond our control. Therefor it is recommended that stress research on crocodiles should be carried out in a temperature controlled closed environment and preferably in individual pens. The construction of a suitable climate controlled research facility with individual pens in South Africa is recommended.

5.3. Advantages and disadvantages of the electrical immobilisation technique

One advantage of electrical immobilisation is the fact that a large number of crocodiles can be processed in a short time. For an experienced team, it is on average one minute quicker to electrically immobilise a crocodile than to capture it by hand. If work is carried out in a pond with 200 crocodiles – this saves 200 minutes – nearly 3.5 hours.

As a result, operations are faster and crocodiles will be less stressed as corticosterone levels rise with the time period that operations are carried out in the enclosures. Further, because the capture procedure is quicker and crocodiles struggle less when they are immobilised with a stunner – compared to manual capture, less lactic acid is released. If manual capture takes too long, crocodiles can potentially suffer from lactacidosis and muscle damage and will take a long time to recover (Bennet *et al.* 1985). This is supported by anecdotal reports from South African crocodile farmers who stated that since they started using the stunner, crocodiles that have been handled, start eating the next day while previously, crocodiles took at least a week until they had sufficiently recovered from handling stress and started eating again (personal communication Coen Labuschagne 2011). Davis *et al*. (2000) also reported this phenomenon in their report on electric immobilisation of *C. porosus* in Australia.

By electrical immobilisation, more crocodiles can be handled within a short period of time. The management can therefore be intensified and skin quality can be improved. Further, animals can be examined before slaughter and thus animals with insufficient skin quality and

scars can be left to heal. Previously crocodiles had to be shot in the ponds, because it was impossible to hand capture and examine all of them. Often skins turned out to be not of sufficient good quality for the international market.

With the electric stunner, it is not necessary any more to use a firearm to cull crocodiles in their ponds. The use of a firearm on a crocodile farming environment with densely populated crocodile ponds and many workers around is always a safety issue.

Electrical immobilisation saves labour time and therefore costs. It also facilitates that crocodiles are motionless when handled and therefore less injuries of crocodile handlers occur. In the past crocodile handlers were often injured during capture procedures and it has to be born in mind that even a small laceration caused by a crocodiles tooth can be a huge health risk due to the oral flora of crocodiles (Wamisho *et al.* 2009).

A device similar to the electric stunner used in animals was also in use on humans for the treatment of patients with certain psychiatric disorders (Clare 1978; Lebensohn 1999). Although slightly modified and refined; up to today, this instrument is still in use to conduct electro convulsive therapy (ECT) on patients, mostly for treatment of depression. A negative side effect reported in some patients is amnesia (Lebensohn 1999; Royal College of Psychiatrists 2012 webpage). While this is not desirable in humans, it would be very desirable if crocodiles could not remember the capture procedure. Unfortunately this would be difficult to prove scientifically. However, the fact that crocodiles are relaxed and start eating quickly after capture by stunning, in direct contrast to crocodiles that were captured manually (Davis *et al.* 2000) might indicate that they do not remember the capture procedure. However, this is just speculation.

On the other hand, the electric crocodile stunner is an economic investment of about R 2000.00 per stunner plus the battery and charger (R 500.00). Like any equipment it has to be cleaned and maintained.

The stunner can easily be abused or misused by untrained personnel. It is therefore imperative that only well trained workers should operate the stunning device. If a crocodile is stunned at the wrong voltage, for too long or in the wrong position, this could lead to serious injury or death of the crocodile. Electrically immobilised crocodiles must be removed

immediately from the water and observed so they don't go back into the water too soon and drown. All handlers should be well trained in these animal welfare issues.

It seems to be safe to operate the described immobilisation device in a wet environment as both electrodes have to touch the skin in order to pass the electric current through an animal or a person. Crocodiles in the immediate vicinity of an individual that is being stunned don't seem to be disturbed by this procedure and therefore it can be concluded that the electric current is not conducted through water sufficiently that it can be felt by other individuals unless there is proper skin contact with both electrodes. (Davis *et al.* 2000). We are not aware of any human accidents due to the use of this equipment and it has been witnessed that the operator was standing in the crocodile pond while stunning crocodiles. However, as a safety precaution it is recommended that the operator wears rubber boots (Davis *et al.* 2000).

5.4. Recommendations for the use of the stunner in crocodiles

In this study, we used one of the most common stunners used on farms in South Africa which works on 120 Watts and 50 Hz (D7 electronics, Pongola). We used a battery stunner as there was no electricity supply near the ponds we worked in. Numerous farms, especially with large outdoor enclosures use the battery operated stunner. The advantage is that no electricity or extension cables are needed close to the ponds – this saves construction costs but also creates a safer work environment. The disadvantage of battery operated stunners is that the battery can go flat. A flat battery can lead to malfunctioning of the stunner and as a result to injury of the crocodile. Therefore it is also imperative, if battery operated stunners are used to have several fully charged batteries on standby and to test the battery charge regularly. In this study the stunner was set on 135 Volts which were applied for five to 11 seconds behind the head. It has to be born in mind that voltage has to be lower in smaller crocodiles and higher in larger crocodiles to achieve good results. Stunning with grossly different machines might lead to different results. A higher voltage could lead to fractures and high frequencies (over 500 Hz) seem to cause pain because the current stays on the surface of the animal (Grandin 1997). It also has to be kept in mind that the handler who carries out the stunning has to be well trained not to stun the animals on areas of the body where stunning could lead to cardiac arrest because the electric current will affect the heart (Grandin 1997). The crocodile also has to be sufficiently wet to insure good contact with the stunner. The electrodes should be kept

clean and not touch the ground otherwise stunning will be ineffective and could lead to burn marks.

It is common practice to tie the legs together on the back of crocodiles for transport. It must be kept in mind that crocodiles that are tied up should not be stunned as the resulting muscle contraction could result in fracture of the spine and of limbs. Further, immobilised crocodiles should be kept out of direct sunlight to prevent overheating during the recovery period.

With these basic rules in mind, electric stunning should be the method of choice when a large amount of crocodiles have to be captured and handled.

5.5. Potential future research projects

5.5.1. To further insure that repeated electrical immobilisation during the lifetime of a crocodile on a commercial farm does not lead to permanent tissue damage, the authors would suggest that the organs, especially the brain and muscles of some crocodiles from a farm that uses electrical immobilisation are examined histologically and compared to crocodiles of the same age that have never been stunned.

5.5.2. Faecal corticosterone values of electrically immobilised and manually captured crocodiles could be compared for several days after capture without disturbing the crocodiles.

5.5.3. The respiratory and the heart rate could be compared between resting animals and stunned animals to gain some more insight into the physiology of the stunning procedure. Heart rate monitors that are commonly used for fitness training could be used for this purpose.

5.5.4. It is accepted that, based on experience in man, a grand mal type epileptiform activity in the brain is indicative of unconsciousness (Gregory 1994). Up to date to the best of our knowledge this epileptiforme activity in crocodiles has not yet been confirmed by EEG or ECOG during and after stunning. The confirmation and duration of epileptiform activity and unconsciousness is an important factor and would indicate if painful procedures could be carried out while crocodiles are under the influence of the electric stunner and for how long.

In domestic animals, these kind of studies lead to recommendations on e.g. the maximum stunning to sticking interval (Anil 1991; Anil *et al*. 2000).

5.5.5. Potentially there is a wide field of practical application of the electric stunner - not only in the crocodile industry but also in other livestock industries where a short complete immobilisation with insensibility is required to carry out a relative painless procedure. While there are anecdotal reports of the successful use of the crocodile stunner on e.g. African buffalo (*Syncerus caffer*) for quick painless procedures such as blood collection (personal communication: Andrew Cader 2013), no scientific work has been carried out to evaluate these applications yet.

6. CONCLUSIONS

No significant differences could be detected between Nile crocodiles that were electrically immobilised and those that were captured by noosing. All animals recovered from the capture and therefore we can conclude that no animal welfare issues could be identified when using the stunner to electrically immobilise crocodiles. This coincides with finding by Franklin *et al.* (2003) who compared the electric stunner with manual capture of *C. porosus* in Australia. This is also in agreement with findings of McKinstry & Anil (2004) who studied the recovery of pigs after electrical stunning with 50 Hz and 200 Volts as part of the slaughter process. These researchers could not identify any obvious animal welfare issues should pigs recover from a head stunning procedure.

Higher lactate and enzyme values identified in our study in manually captured crocodiles would indicate that manually captured crocodiles might take longer to recover. This is supported by Franklin *et al*. (2003) who also came to the conclusion that Australian saltwater crocodiles recovered faster from capture by electric immobilisation compared to manually captured animals.

Capture by electrical immobilisation was on average 68 seconds faster compared to noosing. If 100 crocodiles were worked on in a pond, this would save 113 minutes of exposure to stress as well as of labour expenses. We have to keep in mind that some communal ponds on commercial farms accommodate 500 crocodiles and more; this would mean a saving of over nine hours. In this study we found that disturbance of longer duration leads to higher corticosterone levels in *C. niloticus* (Figure 3). Therefore we have to conclude that the time saving when an electric stunner is used to work on a large amount of crocodiles will also lead to drastically reduced corticosterone levels, indicating that animals were less stressed. For this reason alone electrical immobilisation would be the method of choice in a commercial set up where large amounts of crocodiles have to be handled on a regular basis.

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