Capture of farmed Nile crocodiles (*Crocodylus niloticus*): comparison of physiological parameters after manual capture and after capture with electrical stunning

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Key words: ALP, ALT, AST, CK, crocodilians, corticosterone, electrical stunning, glucose, lactate, noosing

Abstract

The electric stunner (e-stunner) is commonly used to handle Nile crocodiles (*Crocodylus niloticus*) on commercial farms in South Africa, but while it seems to improve handling and safety for the keepers, no information regarding physiological reactions to e-stunning is currently available. The aim of this study was therefore to compare various physiological parameters in farmed Nile crocodiles captured either manually (noosing) or by using an e-stunner. A total of 45 crocodiles were captured at a South African farm by either e-stunning or noosing, and blood

samples were taken immediately as well as four hours after capture. Parameters monitored were serum corticosterone, lactate, glucose, as well as alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and creatinine kinase. Lactate concentrations were significantly higher in noosed compared to e-stunned animals ($P < 0.001$). No other blood parameter differed significantly between the two capture methods. In addition, recorded capture time confirmed that noosing takes significantly longer compared to e-stunning ($P < 0.001$), overall indicating that e-stunning seems the better option for restraint of especially large numbers of crocodiles in a commercial setup because it is quicker, safer, and did not cause a significant increase in any of the parameters measured.

Introduction

The belly skins of farmed crocodiles are the main product and are used to produce luxury goods including handbags and leather garments (Fuchs and others 1989, Caldwell 2012). The value of raw skins is adversely affected by the presence of defects including scratches and bite marks (Manolis and Webb 2011). Crocodiles approaching slaughtering size are handled more intensively, namely for regular evaluation of belly skin quality in order to decide when to slaughter specific individuals.

Effective and safe handling of crocodiles can be performed through the correct use of an electrical stunner (e-stunner) (Franklin and others 2003). The e-stunner for crocodiles is based on the same principle as the electric stunner for pigs and sheep (Gregory and Wotton 1985; Anil 1991). It is presumed that apart from immobilisation, electric stunning also causes a short period of temporary insensibility – at least in domestic mammals (Grandin 2013). When a crocodile is completely stunned it shows reactions similar to those seen in mammals (Davis and others 2000). It has a relaxed body, with legs splayed backwards parallel to the body, eyes closed and shows no reaction to prodding (Davis and others 2000). This suggests that – just like domestic mammals – completely stunned crocodiles are unconscious (Davis and others 2000). This period of temporary “electrical anaesthesia” of crocodiles for five to ten minutes is usually enough to complete most management tasks on commercial crocodile farms. The use of the e-stunner has thus contributed to an improvement in the safety of crocodile workers when handling large numbers of crocodiles (Davis 2001). The e-stunner was first introduced to the crocodile industry in Australia during 1999 (Davis and others 2000). The stunning equipment uses a combination of limited amperes (A) and low voltage to reduce the risk of skin damage and electrical shock to the animal (Davis and others 2000). Battery operated e-stunners are

often used on commercial crocodile farms in southern Africa (R. Reader personal communication). The advantage of a battery operated stunner is that no electricity or extension cables are needed. This eliminates construction costs, especially on large farms with outdoor enclosures and it also creates a safer environment for workers because there are no electrical cables near the wet crocodile environment (Davis and others 2000).

Franklin and others (2003) investigated the level and duration of the acute stress response of Australian saltwater crocodiles (*C. porosus*) when traditionally captured by noosing (manual capture) compared to using an e-stunner. They showed that physiological parameters such as plasma corticosterone and glucose were significantly increased only in manually restrained animals, whereas lactate levels were significantly increased regardless of capture method. While serum corticosterone and elevated glucose levels give an indication of the stress levels experienced by crocodiles due to capture (Lance and others 2001; Jessop and others 2003), lactate levels indicate anaerobic metabolism which would result from physical struggle during capture and in extreme cases can lead to metabolic acidosis and death (Bennett and others 1985).

Enzymes are located in cells and some enzymes are relatively organ specific while others can be present in multiple organs (Halsted 2004). Monitoring enzyme activity is a diagnostic tool used to recognize alteration of cellular integrity which would accelerate the release of enzymes into the circulation (Halsted 2004). Elevated enzyme levels can potentially give an indication of any acute organ damage such as might result from capture. Elevated creatinine kinase (CK) and aspartate aminotransferase (AST) levels for example could be an indication for muscle activity or damage to muscle cells (Last and others 2010) while high alanine aminotransferase (ALT) values could be an indicator for liver disease (Last and others 2010) and alkaline phosphatase (ALP) can be found in many different tissues. Watson (1990) reported elevated ALP values in chronically stressed Nile crocodiles.

In 2009, e-stunning was approved by the South African Bureau of Standards (National Standard on crocodiles in captivity; SANS 631: 2009) as a tool for “electrical immobilisation” of the Nile crocodile (*Crocodylus niloticus*), based on the work that was done by Franklin and others (2003). However, while this technique seems sufficiently investigated for *C. porosus* (Franklin and others 2003), no information regarding potential physiological stress reactions to e-stunning is available for the Nile crocodile.

As an initial step, the aim of this project was therefore to compare physiological parameters in farmed Nile crocodiles captured either manually (noosing) or by using an e-stunner. More specifically, the study aimed to compare serum corticosterone, blood lactate, blood glucose, and the serum enzyme concentrations of ALT, ALP, AST and CK of noosed and e-stunned Nile crocodiles at the time of capture and again four hours later while still restrained.

Materials and Methods

Animals and experimental design

The study was carried out in January / February 2012 on a crocodile farm near Pongola in northern Kwazulu Natal, South Africa. At this time, the farm accommodated around 365 young captive bred Nile crocodiles of both sexes in an open air enclosure consisting of two connected freshwater ponds. The crocodiles had been in these ponds for the past two years and were accustomed to people due to frequent cleaning of the ponds and other maintenance activities. Forty-five of these crocodiles (approximately four years of age with a total length of 160 to 210 cm) were used for the study. Sampling occurred on two days that were two weeks apart. During the first sampling day (D1), 19 January 2012, 12 animals were e-stunned and thereafter 11 animals were manually captured with a noose. On the second sampling day (D2), 2 February 2012, 11 animals were manually captured and thereafter 11 animals were e-stunned. The e-stunner used for this study consisted of a pair of electrodes at the end of a forced wand, with the electrodes connected to a modified inverter which allows a choice of voltages. The electronic design was similar to the stunner described by Davis and others 2000. The battery-operated e-stunner was set on 135 V at 50 Hz (capable of producing 120 Watts) and the electrodes were applied for 5 to 11 seconds behind the head of each individual. Individual tagging eliminated the possibility of re-capturing the same animals on D2. Food was withdrawn for four days prior to the two respective sampling days. The study was approved by the Animal Use and Care Committee of the University of Pretoria (project number: V029/11).

Sample and data collection

Individual blood sampling took place immediately after capture as well as four hours post-capture. In between, the crocodiles were kept tied-up and blindfolded, and were moved to a quiet climate controlled place (±30^{o}C), to minimise further exposure to stressors. Blood samples were collected from the post-occipital spinal venous sinus according to Myburgh and others (in press). Blood samples were placed in the shade for approximately 60 minutes until clotted, and

then were centrifuged for ten minutes. After transferring the serum into cryotubes, the samples were stored in liquid nitrogen until analysis.

Sample analysis

Immediately after collection, a drop of blood was used to determine lactate and glucose concentrations using a hand held Cobas® glucose and lactate meter (Accutrend® Plus, Roche Diagnostics). The measurements base on reflectance photometry and sensitivity for the monitored parameters range from 1.1 to 33.3 mmol/L for glucose and 0.8 mmol/L to 22 mmol/L for lactate, respectively.

Serum corticosterone levels were determined by using a Coat-A-Count© Corticosterone Radio-Immunoassay (Diagnostic Products Coat-a-Count Rat-Corticosterone). In brief, 50 µl standards, controls, and samples were transferred in duplicates into coated tubes. 1 ml ^{125}L corticosterone solution was added, and the tubes were incubated for two hours at room temperature. Subsequently, the liquid was removed; the tubes patted dry, and counted for one minute in a gamma counter (Wallac Wizzard, 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%.

Blood enzyme concentrations (ALT, ALP, AST and CK) were determined via absorbance photometry using the Cobas Integra 400 plus (Roche Diagnostics 2008). ALT catalytic activity was measured at 340 nm during reduction of L-alanine to 2-oxoglutarate. The measuring range for ALT was 2 to 2700 Units / Liter (U/L). ALP activity was monitored at 409 nm during the conversion of p-nitrophenyl phosphate into phosphate and p-nitrophenol. The measuring range for ALP was 3.0 to 1200 U/L. AST activity was determined at 340 nm during the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The measuring range for AST was 2 to 700 U/L. CK concentration was measured during the formation of adenosine-tri-phosphate from creatinine phosphate and adenosine-di-phosphate at 340 nm (Roche Diagnostics 2008). The measuring range of CK was 7 to 2000 U/L (Roche Diagnostics 2008).

Data analysis

Data were assessed for normality by assessing histograms, calculating descriptive statistics and using the Anderson-Darling test (MINITAB Statistical Software, Release 13.32, Minitab Inc., State College, Pennsylvania, USA). Data violating the normality assumption were modified using the natural logarithm or square root transformation prior to statistical analysis. The effect of capture method was evaluated using a repeated measures ANOVA with sample time (first capture versus subsequent capture four hours later) as a within subject effect and capture method as a between subjects effect. Sampling day, study duration, capture time and the interaction between capture method and sample time were included in all statistical models to adjust for potential confounding. Study duration was defined as the time from when the research team first entered the ponds until the time blood was successfully collected from each individual animal. Capture time was defined as the amount of time from when an individual animal was targeted for capture until successful collection of the blood sample. Capture time was further compared using a two-way ANOVA including sampling day and method of restraint as fixed factors. Post-hoc power calculations were performed for corticosterone levels when comparisons between capture methods were not statistically significant. Statistical modelling was performed using IBM SPSS Statistics Version 21 (International Business Machines Corp., Armonk, NY, USA) and results interpreted at $P < 0.05$.

Results

After adjusting for sampling day, study duration and capture time, a comparison of respective corticosterone, glucose, ALT, ALP, AST and CK levels revealed no significant difference ($P > 0.05$) between the two capture methods (Table 1). While the difference in corticosterone levels was not different between capture methods, the power of the statistical test was low (35%). Lactate concentrations were significantly higher in noosed animals compared to e-stunned animals ($P < 0.001$). On D1, the overall median blood lactate concentration directly after capture (T0) was 4.0 mmol/L (interquartile range (IQR): 3.6, 5.4) in e-stunned animals and 10.2 mmol/L (IQR: 9.0, 11.3) in noosed crocodiles. On D2, e-stunned crocodiles had an overall median lactate level of 3.8 mmol/L (IQR: 2.1, 4.8) at T0 while noosed animals had an overall median lactate level of 9.8 mmol/L (IQR: 8.2, 12.3). Four hours after capture, stunned crocodiles had an overall median blood lactate concentration of 5.7 mmol/L (IQR: 4.4, 6.4) on D1 and 3.4 mmol/L (IQR: 2.9, 5.0) on D2, whereas noosed animals had an overall median blood lactate levels of 5.7 mmol/L (IQR: 5.7, 9.3) and 8.2 mmol/L (IQR: 5.8, 13.1).

TABLE 1: Comparison of blood parameters (corticosterone, lactate, glucose, ALT, ALP, AST and CK) of in total 45 crocodiles either captured by stunning or noosing on two different days (D1 and D2).

		E-Stunned (n = 12 D1; n = 11 D2)		Noosed (n = 11 D1; n = 11 D2)		
Variable	**Day**	**T0 Median (IQR)**	**T4 Median (IQR)**	**T0 Median (IQR)**	**T4 Median (IQR)**	**P value***
Corticosterone (ng/ml)	1	42 (19, 48)	67 (48, 95)	32 (21, 46)	68 (62, 79)	0.117
	2	40 (32, 83)	123 (85, 126)	33 (25, 58)	96 (53, 128)	
Lactate (mmol/L)	1	4 (3.6, 5.4)	5.7 (4.4, 6.4)	10.2 (9, 11.3	5.7 (5.7, 9.3)	<0.001
	2	3.8 (2.1, 4.8)	3.4 (2.9, 5)	9.8 (8.2, 12.3)	8.2 (5.8, 13.1)	
Glucose (mmol/L)	1	2.7 (2, 3.2)	6.1 (5.1, 6.8)	3.8 (3.5, 4)	6.1 (5.7, 6.5)	0.696
	2	3.8 (3.6, 4)	6.3 (6.1, 6.9)	4.4 (3.7, 4.9)	5.5 (4.9, 5.8)	
ALT (U/L)	1	45 (43, 47)	45 (33, 50)	45 (41, 54)	46 (40, 60)	0.830
	2	36 (26, 42)	42 (29, 46)	36 (30, 40)	27 (25, 40)	
ALP (U/L)	1	48 (29, 77)	48 (37, 67)	41 (38, 50)	77 (61, 87)	0.142
	2	36 (32, 45)	35 (30, 45)	55 (45, 66)	49 (35, 111)	
AST (U/L)	1	35 (28, 38)	44 (37, 48)	34 (32, 39)	51 (46, 63)	0.097
	2	26 (18, 31)	37 (31, 40)	33 (26, 35)	43 (39, 62)	
CK (U/L)	1	460 (286, 3033)	1051 (575, 2125)	479 (436, 985)	1116 (665, 1903)	0.967
	2	190 (149, 384)	422 (258, 609)	327 (230, 528)	1012 (834, 1471)	

IQR = interquartile range

* P value is the comparison between capture techniques while adjusting for sampling day, time elapsed from study start, and time necessary to perform capture.

Overall median individual capture time was 101 seconds (s) (range: 67 to 359 s) for stunned animals and 177 s (range: 123 to 380 s) for noosed crocodiles and the difference was statistically significant ($P < 0.001$).

All crocodiles were stunned completely as described by Davis and others (2000) and recovered uneventfully from both, e-stunning and noosing.

Discussion

In general, T0 blood biochemistry results compared favourably with the normal reference ranges for the Nile crocodile published by other authors (Foggin 1987, Watson 1990, Swanepoel and others 2000, Franklin and others 2003, Lovely and others 2007, Botha 2010). Median T0 corticosterone values in this study ranged from 32 to 42 ng/ml. This seems much higher than values given for crocodilians in literature which range from 5 ng/ml in Nile crocodiles (Balment and Loveridge 1989) to 2 ng/ml in American alligators (*Alligator mississippiensis*) (Guillette and others 1997) and 1 ng/ml in saltwater crocodiles (Franklin and others 2003). Corticosterone levels of Caiman (*Caiman crocodylus*) also measured comparatively high at 20 ng/ml (Gist and Kaplan 1976). However, this discrepancy most possibly has to do with different research models and test methods. Species of crocodilians investigated and other extrinsic factors, like housing conditions, laboratory procedures, or environmental conditions could also have an influence on absolute hormone values (Romero 2004). In this study, preparations like draining the pond took place about thirty minutes before capture. Despite greatest caution not to stress the crocodiles beforehand, we cannot exclude the possibility that the experimental animals had perceived a stressor prior to the actual procedure. Blood corticosterone levels presumably take only three minutes to rise in these animals as shown for other reptiles and birds (Romero and Reed 2005). The main interest in this study laid in examining the capture–related differences in corticosterone concentrations as well as in the change of corticosterone values over time. When T0 and T4 results were compared, increases within the four hour time frame in the concentrations of most of the enzyme parameters as well as of blood glucose and serum corticosterone were found. This increase over four hours appeared in both study groups irrespective of the capture method and could be due to the fact that animals had to be kept restrained and blind folded over this period in order to facilitate the second sample collection after four hours. Our study only had 35% power to detect a difference in corticosterone levels between the capture methods. Corticosterone levels only differed by 2.34 ng/ml after adjustment within the statistical model. A difference of 4.59 ng/ml between capture methods would have been necessary to give a statistical test with sufficient power (80%) to detect a significant difference. The observed difference between groups (2.34 ng/ml) does not appear to be clinically relevant based on the absolute corticosterone levels and the differences observed over

time and sampling day (Table 1). The study therefore had low power for the detection of the observed difference between capture methods but had adequate power to detect clinically more important differences (> 4.59 ng/ml).

Although we cannot discount the possibility that our experimental procedures might have influenced our corticosterone levels at T0, our results are in accordance with previously published data. Franklin and others (2003) also did not find a significant difference in corticosterone levels between the two capture methods immediately after capture. However, corticosterone levels of manually restrained animals rose for about 0.5 to 1 hour after capture while corticosterone levels of stunned animals stayed low. These researchers also found a significant increase in blood glucose concentrations of manually restrained saltwater crocodiles which stayed elevated and only returned to baseline levels after eight hours while the blood glucose concentrations of e-stunned saltwater crocodiles did not increase significantly. Franklin and others (2003) used individual pens in their study set up and did not have to keep crocodiles restrained over a period of time in order to repeat the periodic sample collection.

Franklin and others (2003) reported that lactate concentrations of e-stunned saltwater crocodiles returned to baseline levels within four hours, lactate levels of manually captured saltwater crocodiles, however, experienced much higher elevations of up to 21.0 mmol/L and only returned to baseline levels after eight hours (Franklin and others 2003). In contrast, lactate concentrations in this study generally decreased between T0 and T4. In accordance with Franklin and others (2003) this investigation also revealed significantly higher lactate concentration in manually captured crocodiles compared to e-stunned Nile crocodiles after results were adjusted for sampling day, study duration and capture time. Median lactate concentrations at T0 of noosed crocodiles were 9.8 and 10.2 mmol/L respectively, while median lactate concentrations of e-stunned crocodiles at T0 were 4.0 and 3.8 mmol/L respectively. The most likely explanation for this difference was that crocodiles struggled less when they were immobilised with the e-stunner. The median individual capture time with the e-stunner was 101 s. In contrast, the median capture time by noosing was 177 s – this is 76 s longer during which time crocodiles thrashed around vigorously until they could be overpowered and restrained well enough manually to take the first blood sample. Lance and others (2001) reported that the rise of lactate in blood is a reaction to physical restraint. If manual capture takes too long, crocodiles can potentially suffer from lacto-acidosis and muscle damage and will take a long time to recover (Bennet and others 1985). This is supported by results of this study as well as 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 (Coen Labuschagne personal communication). Crocodile farmers in Australia also did not report any decreased appetite or increased nervousness after stunning had been performed and crocodiles returned to feed and water sooner compared to the noosing method (Davis and others 2000, Peucker and others 2005).

The median individual capture time by electrical stunning was distinctively shorter (76 s per individual) compared to noosing. When working 100 crocodiles using an e-stunner would not only reduce crocodiles from exposure to stress due to capture activity inside the pond, but the e-stunner would also save 126 minutes of labour. While previously crocodiles had to be randomly shot in the ponds, because it was impossible to hand capture so many animals for examination; crocodiles now can be regularly examined and those with good enough skins can be slaughtered immediately while animals with insufficient skin quality can be returned to the pond for the skin quality to improve. E-stunning also insures that crocodiles are motionless when handled and therefore the risk is lower for crocodile handlers to be bitten.

Misuse or use of a malfunctioning stunner can lead to heart failure, fracture and trauma of the animals (Grandin 1997). It is therefore imperative that only well-trained handlers operate the stunning device and that it is well maintained and locked away when not in use. If there is not enough moisture to facilitate good contact or if the contact plates are dirty, stunning can be ineffective, painful and lead to burns. E-stunned crocodiles must be removed immediately from the water and observed so they don't go back into the water too soon and drown (Davis and others 2000). Another concern is whether e-stunning simply immobilises crocodiles or if it also causes unconsciousness and thus produces a short term "electrical anaesthesia". It is accepted that, based on experience in man, a grand mal type epileptiform activity in the brain is indicative of unconsciousness (Gregory 1994). To the best of our knowledge this epileptiform activity in crocodiles has not yet been confirmed by electroencephalogram or electrocorticogram 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. For example in domestic animals, these kind of studies lead to recommendations on the maximum allowable time interval in from stunning to sticking (bleeding) in abattoirs (Anil 1991, Anil and others 2000).

In this context, it would also be of interest to investigate if there is any difference in epileptiform activity when crocodiles are stunned with a battery operated stunner which produces a modified sine wave compared to a stunner operated on alternating current through a transformer, which produces a proper sine wave. Further, while it seems that crocodiles as well as pigs (Franklin and others, 2003; Mc Kinstry and Anil, 2004) recover from electric head stunning without any identifiable animal welfare issues, the question still remains whether repeated head stunning over a period of several months – as it is carried during the finishing period of crocodiles for slaughter – does not cause brain lesions.

In conclusion, the most significant physiological difference between the two capture methods was the higher blood lactate concentrations of noosed crocodiles. For this reason, we propose that capture by means of e-stunning compares favourably with the traditional manual capture method by noosing and that the additional advantages of e-stunning make it the method of choice for Nile crocodiles on commercial farms. At the same time we propose more research into various issues with regards to the functionality and repeated use of crocodile e-stunners and to standardise these tools and facilitate handler training to insure animal welfare.

Acknowledgements

The authors would like to acknowledge Prof. Amelia Goddard from the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria for her contributions and advice with regards to chemical pathology. The authors would also like to acknowledge Cliff Dearden for his input on the electrical stunner and Coen Labuschagne from Metcroc and his team for their help with the immobilisation of crocodiles. The authors would further like to thank Ian Colenbrander and his team.

References

ANIL, M. H. (1991) Studies on the return of physical reflexes in pigs following electrical stunning. Meat Science 30, 13-21.

ANIL, M. H., RAJ, A. B. M & MCKINSTRY, J. L. (2000) Evaluation of electrical stunning in commercial rabbits: effect on brain function. Meat Science 54, 217-220.

BALMENT, R. J. & LOVERIDGE, J. P. (1989) Endocrines and osmoregulatory mechanisms in the Nile crocodile, *Crocodylus niloticus*. General and Comparative Endocrinology 73, 361-367.

BENNETT, A. F, SEYMOUR, R. S., BRADFORD, D. F. & WEBB, G. J. W. (1985) Mass-dependence of anaerobic metabolism and acid-based disturbance during activity in the salt-water crocodile, *C. porosus*. Journal of Experimental Biology 117, 161-171.

BOTHA, P. J. (2010) The Distribution, Conservation Status and Blood Biochemistry of Nile Crocodiles in the Olifants River System, Mpumalanga, South Africa. PhD thesis, University of Pretoria.

CALDWELL, J. (2012) World Trade in Crocodilian Skins 2008 – 2010. UNEP World Conservation Monitoring Centre. www.unep-wcmc.org/medialibrary/2012/10/02/82849f21/World%20Trade%20in%20Crocodilian%20Skins%202008-2010.pdf. Accessed January 26, 2014.

DAVIS, B. M., PEUCKER, S. K. J. & MAYER, R. J. (2000) Crocodiles Restraining & Meat quality. RIRDC Publication No. 00/105.

DAVIS, B. M. (2001) From hatching to harvest. RIRDC Publication No 01/123, 83-84.

FOGGIN, C. M. (1987) Disease and disease control on crocodile farms in Zimbabwe. In: Wildlife Management: Crocodiles and Alligators. Ed. G.J.W. Webb; S.C. Manolis; P.J. Whitehead. Surrey Beatty& Sons Pty Limited, Australia. pp 351-362.

FRANKLIN, C. E., DAVIS, B. M., PEUCKER, S. K. J.; STEPHENSON, H., MAYER, R., WHITTIER, J., LEVER, J. & GRIGG, G. C. (2003) Comparison of stress induced by manual restraint and immobilisation in the estuarine crocodile, *Crocodylus porosus.* Journal of Experimental Zoology 298 A, 86-92.

FUCHS, H. H. P, ROSS, A. C., POOLEY T. & Whitaker, R. (1989) Crocodile-skin Products in: Crocodiles & Alligators Ed. Ross A.C. 1st Ed. Facts on File Inc. Kyodo Printing Singapore, 188-195.

GIST, D. H. & KAPLAN, M. L. (1976) Effects of stress and ACTH on plasma corticosterone levels in the caiman *Caiman crocodilus*. General and Comparative Endocrinology 28, 413-419.

GRANDIN, T. (1997) Cardiac arrest stunning of livestock and poultry with 1997 updates. In: Advances in Animal Welfare Science. Ed.: M.W. Fox; L.D. Mickley. Published by Martinus Nijhoff Publishers. www.grandin.com/humane/cardiac.arest.html. Accessed January 26, 2014.

GRANDIN, T. (2013) Electric stunning of pigs and sheep, updated March 2013. www.grandin.com/humane/elec.stun.html. Accessed January 26, 2014.

GREGORY, N.G. (1994) Preslaughter handling, stunning and slaughter. Meat Science 36, 45-56.

GREGORY, N.G. & WOTTON, S.B. (1985) Sheep slaughtering procedures IV. Responsiveness of the brain following electrical stunning. British Veterinary Journal 141, 74-81.

GUILLETTE, L. J., CRAIN, D. A., ROONEY, A. A. & WOODWARD, A.R. (1997) Effect of acute stress on plasma concentrations of sex and stress hormones in juvenile alligators living in control and contaminated lakes. Journal of Herpetology 31, 347-353.

HALSTED, J.A. (2004) Clinical chemistry. In: Veterinary Laboratory Medicine Interpretation and Diagnosis. Ed.: D.J. Meyer, J.W. Harvey. Saunders, USA. pp 145-155.

JESSOP, T. S., TUCKER, A. D., LIMPUS, C. L. & WHITTIER, J. M. (2003) Interactions between ecology, demography, capture stress and profiles of corticosterone and glucose in a free-living population of Australian freshwater crocodiles. General and Comparative Endocrinology 132, 161-170.

LANCE, V. A., MORICI, L. A. & ELSEY, R. M. (2001) Physiology and endocrinology of stress in crocodilians. In: Crocodilian Biology and Evolution. Ed.: G.C. Grigg, F. Seebacher, C.E. Franklin. Surrey Beatty & Sons Pty Limited, Australia. pp 327-340.

LAST, R. D., HILL, J. M., VORSTER, J. H., BOSCH, S. J. & GRIFFITHS, C. (2010) Vetdiagnostix Laboratory Manual. Vetlink Media Solution, South Africa.

LOVELY, C. J., PITTMAN, J. M. & LESLIE, A. J. (2007) Normal haematology and blood biochemistry of wild Nile crocodiles (*C. niloticus*) in the Okavango Delta, Botswana. Journal of the South African Veterinary Association 78, 137-144.

MANOLIS, S. C. & WEBB, G. J. W. (2011) Tracking crocodile skin defects – from farm to product. RIRDC Publication No. 11/012.

MCKINSTRY, J. L. & ANIL, M. H. (2004) The effect of repeat application of electrical stunning on the welfare of pigs. Meat Science 67, 121-128.

MYBURGH, J. G., KIRBERGER, R. M., STEYL, J. C. A., SOLEY, J. T., BOOYSE, D. G., HUCHZERMEYER, F. W., LOWERS, R. H. & GUILLETTE, L. J. (2014) The post-occipital spinal venous sinus of the Nile crocodile (*Crocodylus niloticus*): anatomy and its use for blood sample collection and intravenous infusions. Journal of the South African Veterinary Association. In press.

PEUCKER, S. K. J., DAVIS, B. M. & VAN BARNEVELD, R. J. (2005) From hatching to harvest. RIRDC Publication No 05/152, 81-82.

ROCHE DIAGNOSTICS. 2008. Method manual for Cobus Integra 400.

ROMERO, L. M. (2004) Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19, 249-255.

ROMERO, L. M. & REED, J. M. (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comparative Biochemistry and Physiology Part A 140, 73-79.

SWANEPOEL, D., BOOMKER, J. & Kriek, N. P. K. (2000) Selected chemical parameters in the blood and metals in the organs of the Nile crocodile*, C. niloticus*, in the Kruger Park. Onderstepoort Journal of Veterinary Research 67, 141-149.

WATSON, P. A. I. (1990) Effects of blasting on Nile crocodiles. Proceedings of the 10th working meeting of the CSG, IUCN the world conservation union, Gland, Switzerland Vol. 2, 240-252.