CHAPTER 5

BLOOD BIOCHEMISTRY OF NILE CROCODILES IN THE OLIFANTS RIVER

INTRODUCTION

Blood biochemistry and haematology is used by veterinary surgeons to detect conditions affecting the health of many animals including reptiles (Campbell, 2006). Conditions detected through evaluation of the blood biochemistry and haematology of reptiles includes anaemia, inflammatory diseases, parasistemias, hematopoietic disorders and haemostatic alterations (Campbell, 2006). According to Campbell (2006), normal hematologic values for reptiles (including crocodilians), as determined by different laboratories, vary significantly due to differences in blood sampling, handling, analytic techniques, differences in the environmental conditions of the reptiles' habitat, physiologic status of the reptile, its age, gender and nutrition and the use of anaesthetics. Although blood biochemistry profiles are often used to assess the physiological status of reptilians, the clinical chemistry has not achieved the same degree of critical evaluation as seen in mammals (Campbell, 2006). Reference ranges for biochemical and also haematological values in Nile crocodiles are currently limited (Lovely, Pittman and Leslie, 2007) whereas similar reference ranges for biochemical and haematological values for saltwater crocodiles (*Crocodylus porosus*) was established for 1 to 2 year old animals only (Millan *et al.*, 1997) and some parameters have been established for female American alligators (*Alligator mississippiensis*) only (Guilette *et al*., 1997). Because of the difficulty in obtaining meaningful reference intervals for each species of reptile (Campbell, 2006), decision levels are often used when assessing reptilians. The Concise Dictionary of Modern Medicine (Segen, 2002) defines decision levels as an alternative to a reference value for reporting laboratory results; when decision levels are exceeded, a response by a clinician is required. Baseline health assessments of wild reptiles are, however, vital for detection of underlying health problems in reptilian populations. Studies that provide insight into the blood biochemistry and haematological values of reptilian populations provide important reference and comparative values for future health assessments (Gillespie *et al.,* 2000). Apart from being relevant in veterinary medicine, blood biochemistry is also an important tool in providing physiological indicators in wildlife and conservation programmes (Elamin, 2004). The usefulness of this tool in ecological studies was demonstrated by Le Resche *et al.* (1974) as well as by Seal *et al.* (1981) in Franzmann (1985). It is expected that evaluation of blood biochemistry results will indicate the basic general health of Nile crocodiles in the Loskop Dam, the Flag Boshielo Dam, the Blyderivierspoort Dam and the Olifants Gorge populations and thus allow speculation regarding the overall health of crocodile populations in the Olifants River. These populations have been chosen as research sites because they represent populations in the upper, middle and lower Olifants River with the Blyderivierspoort Dam, which is considered to be an unpolluted tributary of the Olifants River, being the control population.

METHODS

A total of 30 Nile crocodiles were captured during the period October 2007 to September 2008 at selected sites (Loskop Dam, Flag Boshielo Dam, Blyderivierspoort Dam and Olifants Gorge) in the Olifants River. No large and very large sized crocodiles occur in the Loskop Dam, therefore only small and medium sized crocodiles were caught in this area. The small number of animals in the Loskop Dam population severely limited our ability to capture a large sample of crocodiles from this lake and consequently we settled for a sample size of four animals out of an estimated population of 18 animals (Chapter 3) meaning that 22.2% of the population was sampled.

The change in habitat due to the increase in height of the Flag Boshielo Dam-wall also made it difficult to capture crocodiles in that lake. Flooding of large stands of *Acacia burkei* due to the increased height of the wall effectively prevented the capture crew from approaching crocodiles during capture operations and increased the possibility that crocodiles would be lost as a result of entanglement of the capture rope in the dense submerged *Acacias*. As a direct result of these problems, only 6 Nile crocodiles were successfully captured in the Flag Boshielo Dam for this study.

The Nile crocodile population in the Blyderivierspoort Dam was chosen as a control population since the Blyde River catchment is situated in a protected area and the river is therefore minimally affected by pollutants. The Blyde River is currently regarded as a class B river by the river component of the South African National Spatial Biodiversity Assessment (Nel *et al.* 2004). Class B rivers are categorised as largely natural while the Blyde River is also regarded as endangered in terms of its conservation status (Nel *et al.*, 2004). The Blyderivierspoort Dam population is however quite small due to the habitat not being able to sustain large numbers of Nile crocodiles. The survey sample of 3 animals from a total population of about 15 (20%) was deemed sufficient.

All Nile crocodiles studied were captured using a modified version of the methods described by Chabreck (1965), Kofron (1989) and the Florida Fish and Wildlife Conservation Commission (2003), adapted to suit local conditions and tried and tested in a previous study (Botha, 2005). Nile crocodiles were located at night by their reflective eye-shine that is a characteristic red glow using an 800 000 candlepower halogen spotlight. Animals identified for capture were approached with a boat (eight metre fibreglass hull with 80 hp Yamaha outboard motor). Capture equipment consisted of a standard self-locking 3S-72" Thompson steel snare (Thompson Snares, Lynnwood, Washington, USA) attached to a 15 mm heavy duty braided rope by a steel coupling. The snare is kept open by stretching it over a Y-shaped frame attached to a five metre aluminium catchpole. During capture, the snare is positioned just behind the head of the crocodile and pulled tight. The self-locking mechanism on the snare prevents the crocodile from opening the snare and escaping. Crocodiles less than 2.1 m in TL were pulled onto the boat while bigger animals were pulled onto shore for measuring and sampling. All crocodiles caught were physically restrained without the use of narcotics and were released within 15 minutes of being caught.

Once captured and restrained, blood samples ranging between 7 and 10 ml each were collected from the post-occipital venous sinus, dorsal midline and just caudal to the base of the head using a 20 gauge needle and a 10 ml syringe as described by Guillette *et al*. (1996; 1997) and Millan *et al*. (1997). The volume of blood collected depended on the size of the animal. All blood collected was immediately transferred to heparinized blood tubes and kept cool with ice packs. Samples were centrifuged at the end of each evening and plasma samples placed in Cryovails and frozen in liquid nitrogen until analysed in the Clinical Pathology Laboratory at the Department of Companion Animal Clinical Studies at the Veterinary Faculty of the University of Pretoria at Onderstepoort. Blood samples were only collected from living animals and all dead animals found in the study area were intentionally disregarded and did not form any part of the study.

Blood samples were analysed for Total Serum Protein (TSP), Albumin, Globulin, Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Glucose, Sodium (Na), Potassium (K), Calcium (Ca^{2+}), Total Calcium (Ca^{Total}), Magnesium (Mg), Serum Inorganic Phosphate (SIP), Cholesterol, Creatinine, Chloride (Cl), Uric Acid, Triglycyrides, Vitamin A and Vitamin E since these are the blood biochemical tests that appear to be the most useful in reptilian diagnostics (Campbell, 2006).

Biochemical analyses were done using a Next/Vetex Alfa Wassermann Analyser (Alfa Wassermann B.V., Woerden, The Netherlands). Total protein was determined using a modified Weichselbaum biuret method (Weichselbaum, 1946). Albumin was measured using the bromocresol green method (Cheesbrough, 2005) while globulin and the albumin:globulin ratio were calculated (Johnson *et al.,* 2002). Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were determined by using standard IFCC methods (Bergmeyer *et al.*, 1977; Tietz *et al.,* 1983; Bergmeyer *et al.,* 1986). The glucose oxidase method (Marks, 1996) was used to determine glucose in the samples. Sodium, potassium, ionised calcium and chloride were measured using an 865 pH/Blood Gas Analyser (Chiron Diagnostics Limited, Halstead), by means of ion selective electrodes. Total calcium was measured by the Arsenazo method (Weissman *et al.,* 1980), magnesium was measured by the zylidyl blue method (American Chemical Society, 1987), cholesterol was determined by enzymatic methods (Abell *et al.,* 1952; Bergmeyer and Grassl, 1983), creatinine was determined by the picrate method (Cheesbrough, 2005) and uric acid by the uricase method (Bauer, 1982).

Analysis of variance (ANOVA) was used to test for statistically significant differences ($P < 0.05$) between location (site) and biochemical values of the blood samples collected, size class and biochemical values of the blood samples collected and also gender and biochemical values of the blood samples collected. Each parameter was tested for normality by applying the Ryan-Joiner test (similar to the Shapiro-Wilk test) using MINITAB

15 STATISTICAL SOFTWARE (Minitab Ltd, Coventry, United Kingdom). Some of the Blyderivierspoort Dam, Loskop Dam and Flag Boshielo Dam results were run twice by the Clinical Pathology Laboratory at the Department of Companion Animal Clinical Studies at the Veterinary Faculty of the University of Pretoria at Onderstepoort to ensure replication of results, in these cases the mean values of the two tests from one sample were used for statistical analyses. Data collected for this study is compared to the work done by Lovely *et al*. (2007) in Botswana because according to their published article, they used the same capture methods, sampling techniques and used the same laboratory as this study did.

RESULTS

The results of the biochemical analysis of blood samples collected from 30 Nile crocodiles in the Loskop Dam, Flag Boshielo Dam, Blyderivierspoort Dam and the Olifants River Gorge are given in table 13.

Collection of blood samples from the Olifants River Gorge took place during August 2008 and September 2008 when an outbreak of pansteatitis caused an estimated 60% of the Nile crocodile in that area to die suddenly. Where data is compared to other author's work this refers only to Nile crocodile blood biochemistry since it would be impossible to compare reptile blood values to those of mammals for instance.

Table 13: Blood biochemical parameters of Nile crocodiles at selected localities in the Olifants River (*n* = 30) compared to other recent studies of Nile crocodile blood biochemistry in southern Africa.

Parameters	Current study										Lovely *et al.* (2007)			Swanepoel *et al.* (2000)	
	Loskop Dam (n = 4)		Flag Boshielo Dam (n = 6)		Blyde River Dam (n = 3)		Olifants River Gorge (n = 17)		Olifants River Combined (n = 30)		Okavango Delta (n = 35)		Okavango Delta Range (n = 35)	Olifants River (n = 6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Min-Max	Mean	SD
TSP (g/L)	55.64	18.44	44.94	21.82	51.02	23.77	54.90	13.79	52.62	16.67	41.2	-	28.9 - 57.1	-	-
Albumin (g/L)	21.70	2.55	16.23	7.46	17.20	3.39	15.55	2.62	16.28	3.67	14.7	1.8	11.1 - 19.4	11.72	2.69
Globulin (g/L)	51.30	0.28	34.87	22.69	35.80	26.16	39.35	12.05	39.49	13.85	26.5	6.8	16.5 - 42.6	39.20	8.75
A/G	0.43	0.05	0.59	0.28	0.61	0.35	0.42	0.10	0.46	0.16	0.58	0.12	0.34 - 0.79	0.30	0.02
ALT (IU/L)	19.50	0.71	13.00	12.29	30.00	42.43	22.94	15.94	22.00	16.90	43.9	13.1	15 - 63	-	-
ALP (IU/L	28.00	7.07	18.67	12.66	13.00	1.41	9.18	6.94	12.25	9.15	21.1	13.7	3 - 72	-	-
AST (IU/L)	40.00	0.00	24.00	7.00	38.00	45.25	47.00	24.93	42.79	24.24	66.5	56.4	14 - 211	-	-
Glucose (mmol/L)	5.68	2.76	4.01	1.17	3.87	0.65	5.45	2.54	5.04	2.27	3.8	0.5	1.8 - 4.8	5.68	4.01
Na (mmol/L)	144.25	6.03	154.25	16.53	148.67	6.90	145.97	8.75	147.67	10.43	147.9	8.3	122 - 164	141.50	17.17
K (mmol/L)	5.52	0.37	5.42	1.91	5.02	0.39	13.37	7.07	9.90	6.67	4.88	1.03	3.30 - 7.65	4.59	0.70
Ca^{2+} (mmol/L)	0.57	0.44	0.86	0.56	0.56	0.52	0.53	0.66	0.61	0.59	1.35	0.12	1.08 - 1.61	-	-

Table 13 (continued): Blood biochemical parameters of Nile crocodiles at selected localities in the Olifants River (*n* = 30) compared to other recent studies of Nile crocodile blood biochemistry in southern Africa.

Parameters	Current study										Lovely *et al.* (2007)			Swanepoel *et al.* (2000)	
	Loskop Dam (n = 4)		Flag Boshielo Dam (n = 6)		Blyde River Dam (n = 3)		Olifants River Gorge (n = 17)		Olifants River Combined (n = 30)		Okavango Delta (n = 35)		Okavango Delta Range (n = 35)	Olifants River (n = 6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Min-Max	Mean	SD
Ca^{Total} (mmol/L)	3.10	0.18	2.77	0.60	2.85	0.08	1.38	1.32	2.04	1.27	2.73	0.19	2.34 - 3.15	3.74	2.28
Mg (mmol/L)	1.10	0.11	1.01	0.27	1.06	0.23	0.57	0.59	0.78	0.52	1.15	0.26	0.65 - 1.72	2.24	2.58
SIP (mmol/L)	2.09	0.99	1.64	0.43	2.07	0.46	1.94	0.84	1.91	0.75	-	-	-	1.17	0.50
Cholesterol (mmol/L)	3.23	2.02	4.83	2.56	7.65	0.37	3.77	2.56	4.14	2.56	5.49	2.08	0.0 - 9.86	-	-
Creatinine (µmol/L)	29.38	20.73	26.67	11.52	15.33	12.83	30.18	20.12	27.88	17.94	34.0	10.2	17 - 56	77.67	39.64
Chloride (mmol/L)	108.63	14.63	116.28	3.98	75.85	66.42	115.92	7.86	110.84	22.96	120.3	9.6	97 - 135	113.40	11.95
Uric Acid (mmol/L)	0.17	0.10	0.15	0.07	0.27	0.06	0.20	0.10	0.19	0.09	0.12	0.05	0.04 - 0.30	-	-
Triglycerides (mmol/L)	1.13	-	0.41	0.21	4.38	5.24	1.28	2.10	1.43	2.33	-	-	-	-	-
Vitamin A (µg/L)	330.61	8.41	326.37	18.40	335.81	7.41	200.79	213.62	255.66	172.87	-	-	-	-	-
Vitamin E (mg/L)	7.10	1.48	9.65	3.28	11.00	0.22	6.21	2.97	7.38	3.12	-	-	-	-	-

Total Serum Protein (TSP) (Figure 31):

The mean TSP concentration of all the sample sites combined (52.62 g/L) are much higher than the mean range (41.2 g/L) for Nile crocodiles reported by Lovely, *et al.* (2007) in the Okavango Delta of Botswana (Table 13).

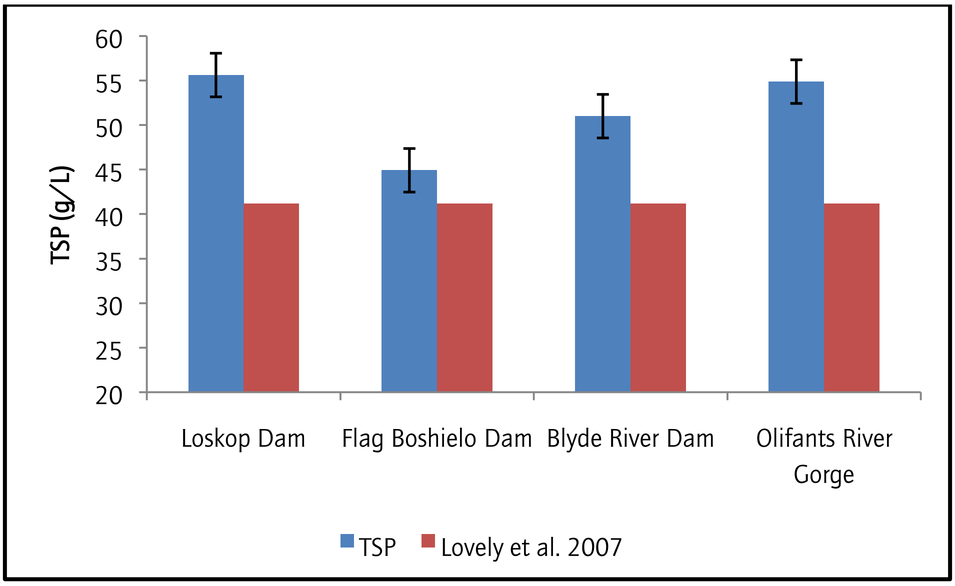

Figure 31: Mean TSP concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

Albumin (Figure 32):

Mean albumin concentration at Loskop Dam (21.7 g/L) are higher than the maximum concentration for Nile crocodiles (Lovely, *et al.*, 2007) in the Okavango Delta but the mean concentration for the Olifants River are only slightly higher than those reported for the Okavango Delta (Table 15). However, the mean albumin concentration (16.28 g/L) are near the maximum concentration found in Botswana while Swanepoel *et al.* (2000) reported concentrations (11.72 g/L) during 2000 (Table 13) that correspond much closer to the latest minimum concentrations reported from Botswana. Since all other variables such as the size of animals, capture technique and season of capture where the same when Swanepoel *et al.* (2000) did their study, this could indicate that conditions have changed in the river over the last nine years.

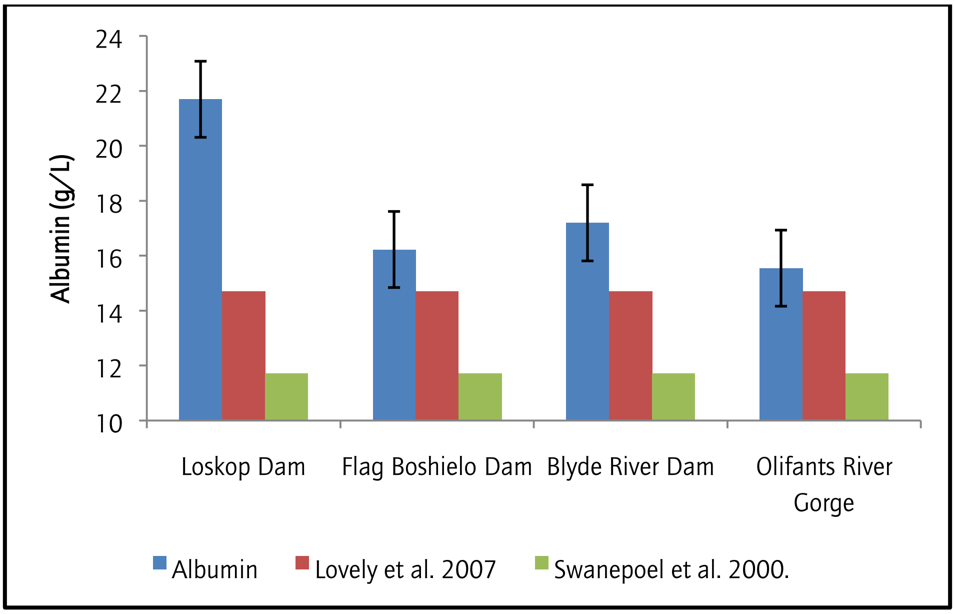

Figure 32: Mean albumin concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

<u>**Globulin (Figure 33):**</u>

Globulin concentrations at Loskop Dam (51.3 g/L) are substantially higher than the maximum concentration for Nile crocodiles in the Okavango Delta as published by Lovely, *et* *al.* (2007). All concentrations except for the Flag Boshielo Dam also exceed those reported by Swanepoel *et al*. (2000) for the Olifants River in the Kruger National Park.

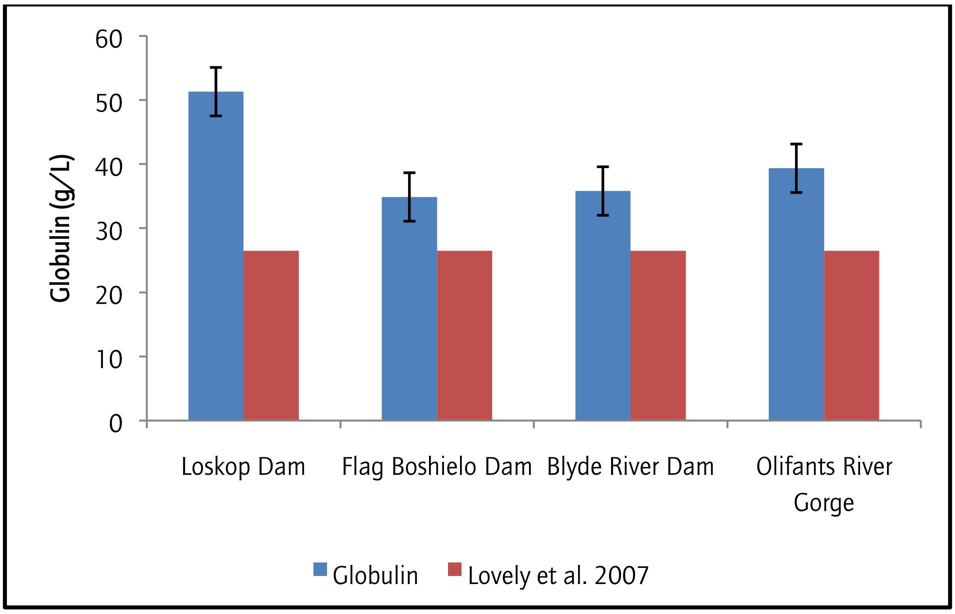

Figure 33: Mean globulin concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

Albumin/Globulin ratio (A/G) (Figure 34):

The mean albumin/globulin ratio of plasma samples taken from crocodiles in the Flag Boshielo Dam (0.59) and the Blyderivierspoort Dam (0.61) corresponds closely to the mean concentration reported (0.58) for the Okavango Delta (Table 13). Swanepoel *et al.* (2000) reported a mean albumin/globulin ratio (0.30) from the Olifants River that was lower than the mean minimum concentration found in the Okavango Delta (0.34 - 0.79) by Lovely *et al*. (2007). Results from the Loskop Dam (0.43) and the Olifants River Gorge (0.42) were fairly low although still well within the range (0.34 - 0.79) reported from the Okavango Delta (Table 13).

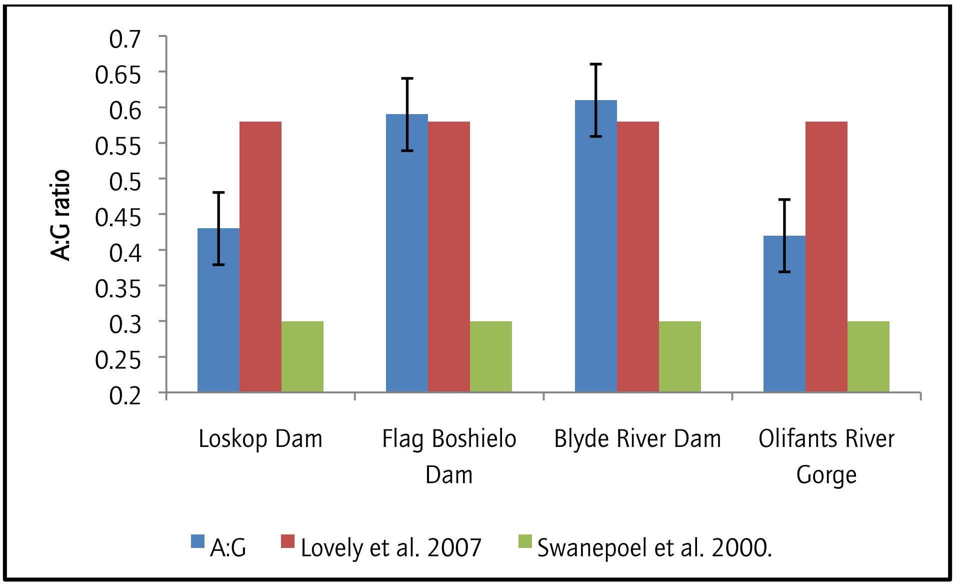

Figure 34: Mean albumin/globulin ratio measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

Alanine transaminase (ALT) (Figure 35):

The mean concentration for alanine transaminase (ALT) at Flag Boshielo Dam (13.0 IU/L) are lower than the minimum concentration for Nile crocodiles in the Okavango reported by Lovely, *et al.* (2007). ALT concentrations at all other sampling sites in the Olifants River registered at concentrations below the mean concentrations measured in the Okavango (Table 13).

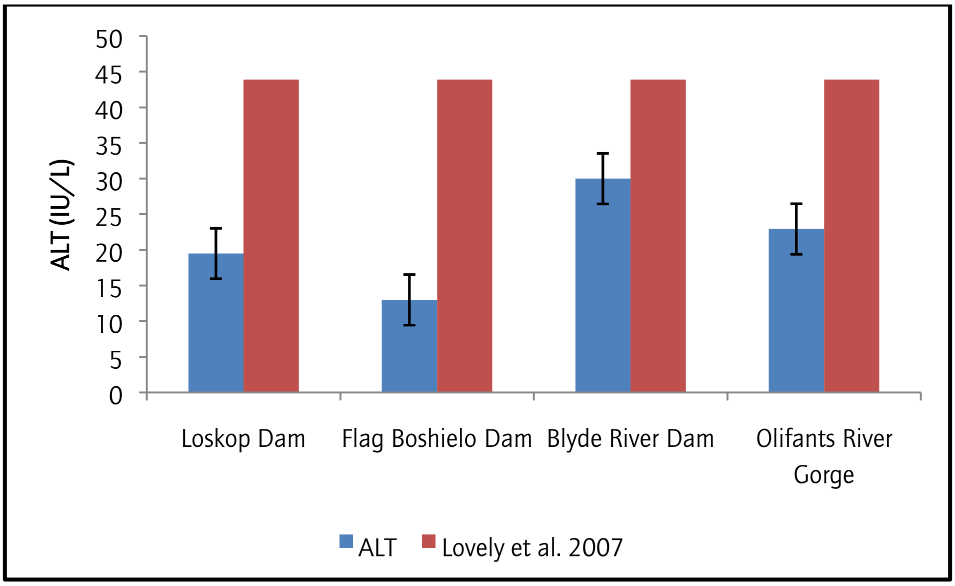

Figure 35: Mean alanine transaminase (ALT) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

<u>**Alkaline phosphatase (ALP) (Figure 36):**</u>

Alkaline phosphatase (ALP) concentrations in the plasma of Nile crocodiles exhibit a significant difference ($P = 0.014$) over the length of the Olifants River, in the downstream direction, with the Olifants River Gorge concentrations being much lower than those of the Loskop Dam. The ALP values at Flag Boshielo Dam (18.67 IU/L), Blyderivierspoort Dam (13.00 IU/L) and Olifants River Gorge (9.18 IU/L) are lower than the mean concentrations measured in the Okavango Delta (21.1 IU/L). Analysis of the August and September plasma samples from crocodiles in the Olifants River Gorge showed that ALP concentrations was substantially lower during August 2008 ($\bar{x} = 4.500$) compared to September 2008 ($\bar{x} = 17.000$).

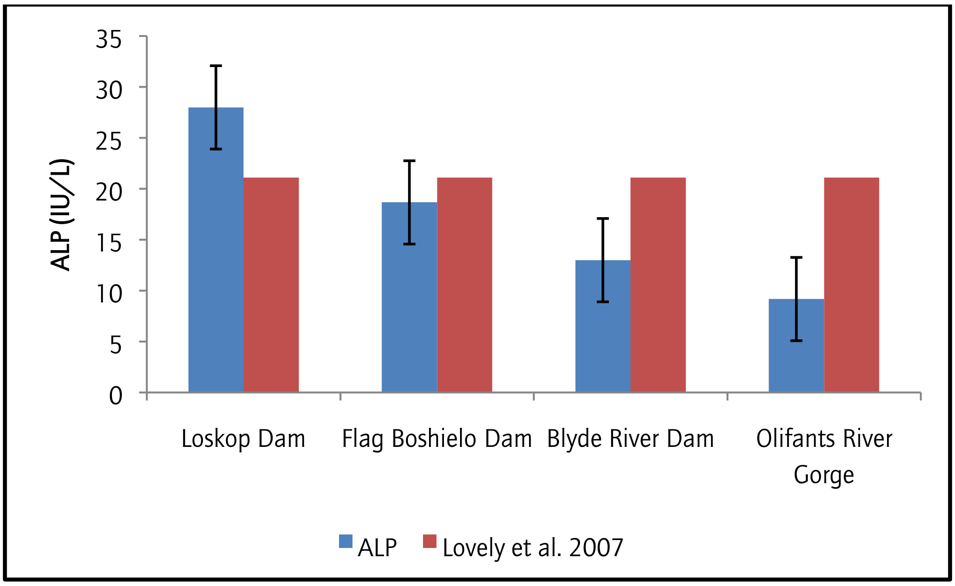

Figure 36: Mean alkaline phosphatase (ALP) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

<u>**Aspartate aminotransferase (AST) (Figure 37):**</u>

All plasma aspartate aminotransferase (AST) concentrations measured in the plasma of Nile crocodiles in the Olifants River where much lower than the mean AST concentrations found in the Okavango Delta Nile crocodile population (Table 13). In the case of the Flag Boshielo Dam population, the concentration of AST where found to be 24.00 IU/L which is substantially lower than the mean concentration for the entire Olifants River (42.79 IU/L). The Olifants Gorge registered the highest concentration at 47.00 IU/L but this was still substantially lower than the Okavango Delta mean concentration of 66.5 IU/L (Table 13).

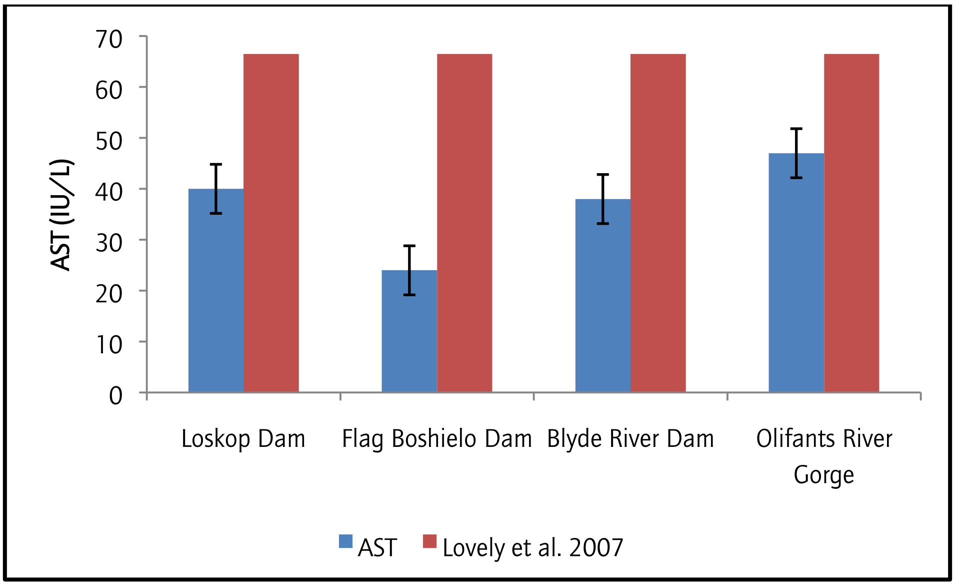

Figure 37: Mean aspartate aminotransferase (AST) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

__Glucose (Figure 38):__

Mean plasma glucose concentrations in the Olifants River Gorge (5.45 mmol/L) and Loskop Dam (5.68 mmol/L) are outside the maximum concentrations (1.8 - 4.8 mmol/L) for Nile crocodiles found in the Okavango Delta as reported by Lovely, *et al.* (2007). However, the mean glucose concentrations found in the plasma of the Blyderivierspoort Dam population (3.87 mmol/L) matched the mean plasma glucose concentrations reported from the Okavango Delta study (3.8 mmol/L) by Lovely, *et al.* (2007). Glucose was recorded at higher concentrations in the plasma of crocodiles in the Olifants River Gorge during August 2008 compared to September 2008 (August $\bar{x} = 6.420$ and September $\bar{x} = 3.100$).

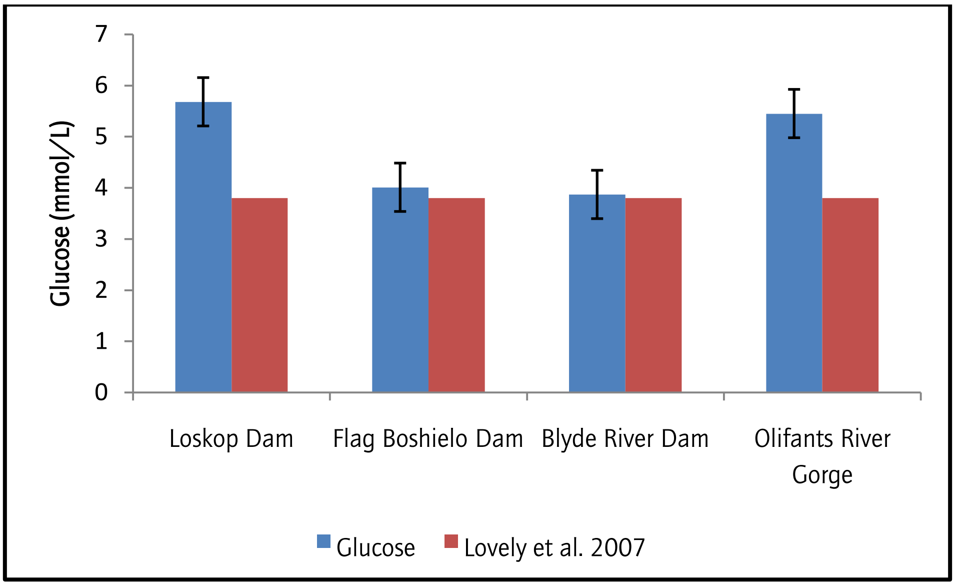

Figure 38: Mean glucose concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

<u>Sodium (Na) (Figure 39):</u>

The mean sodium (Na) concentration in the Olifants River (147.67 mmol/L) correspond well with values reported by Lovely *et al.* (2007) in the Okavango Delta (147.9 mmol/L). Mean concentrations at all the sampling sites in the Olifants River also fall well within the range reported for the Okavango Delta (Table 13). Sodium plasma concentrations in crocodiles from the Olifants River Gorge were substantially lower during August 2008 ($\bar{x} = 141.25$) compared to September 2008 ($\bar{x} = 149.80$).

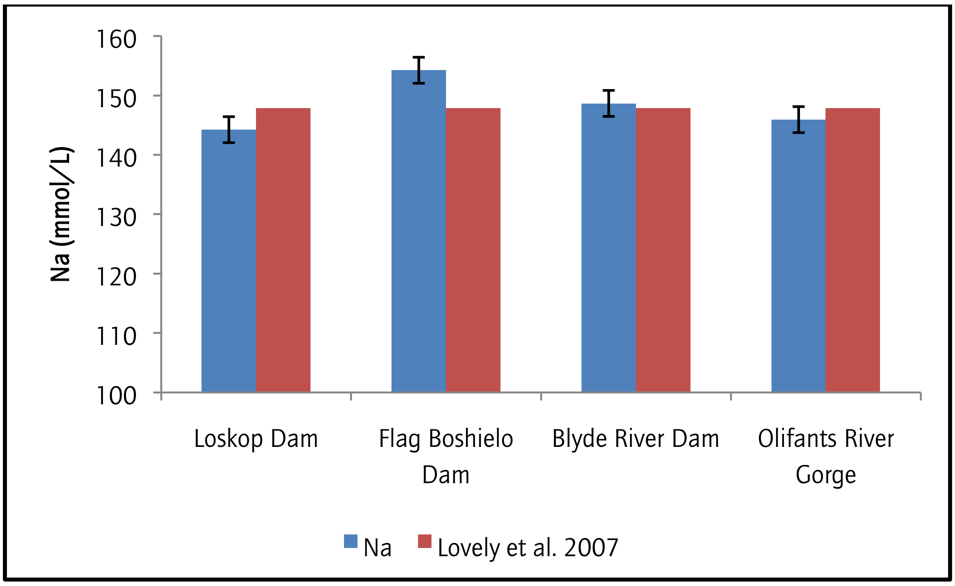

Figure 39: Mean sodium (Na) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

<u>Potassium (K) (Figure 40):</u>

Plasma potassium (K) concentration from Nile crocodiles in the Olifants River Gorge were between 58.71% and 62.45% higher than the concentrations measured at other sites in the Olifants River. A test of significance showed this difference to be significant ($P = 0.007$). Mean plasma potassium (K) concentrations in the Olifants River Gorge (13.37 mmol/L) are much higher than the maximum concentration for Nile crocodiles in the Okavango Delta (7.65 mmol/L): the concentrations reported here represent a marked increase of 63.50% over the mean Okavango concentrations published by Lovely *et al*. (2007) and a 65.66% increase over the mean concentration reported for the Olifants River by Swanepoel *et al.* (2000).

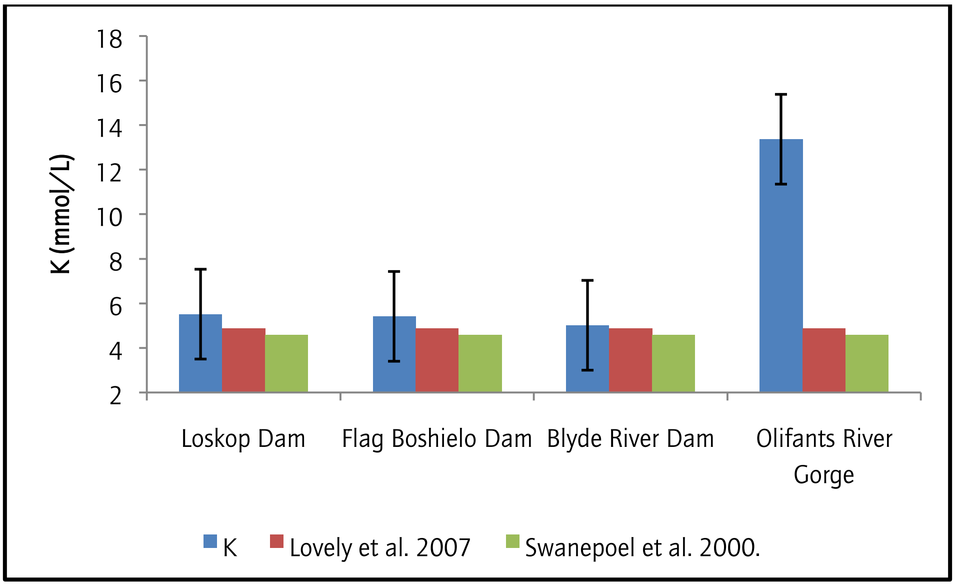

Figure 40: Mean potassium (K) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

Plasma potassium were recorded at higher concentrations in the plasma of crocodiles from the Olifants River Gorge during August 2008 compared to September 2008 (August $\bar{x} =$ 18.674 and September $\bar{x} = 5.388$).

Ionised calcium (Ca^{2+}) (Figure 41):

The mean concentration for ionised calcium (Ca^{2+}) are much lower at all sampling sites in the Olifants River including the control population in the Blyderivierspoort Dam (Olifants River Gorge = 0.53 mmol/L; Blyderivierspoort Dam = 0.56 mmol/L; Loskop Dam = 0.57 mmol/L; Flag Boshielo Dam = 0.86 mmol/L) when compared to the minimum concentration for Nile crocodiles in the Okavango Delta (Lovely *et al.*, 2007).

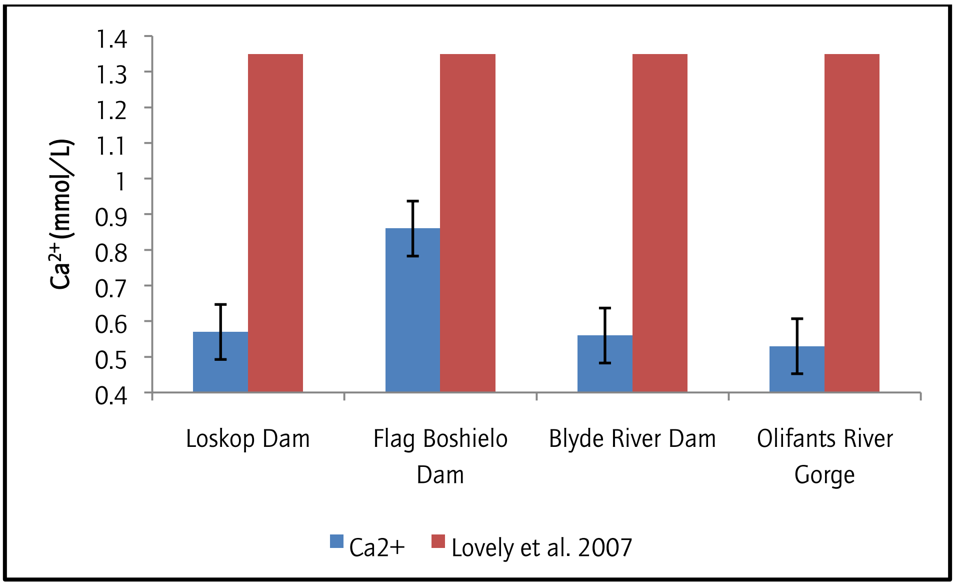

Figure 41: Mean calcium (Ca^{2+}) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

Total calcium (Ca^{Total}) (Figure 42):

Concentrations of total calcium (Ca^{Total}) were significantly lower (P = 0.007) in the plasma of Nile crocodiles in the Olifants River Gorge when compared to all other sites in the river. The mean concentration for Ca^{Total} for Loskop Dam (3.10 mmol/L) is close to the maximum concentration while the mean concentration recorded in the Olifants River Gorge (1.38 mmol/L) is substantially lower than the minimum concentration for Nile crocodiles (2.34 - 3.15 mmol/L) established in the Okavango delta (Lovely *et al.*, 2007). Total calcium concentrations in the Olifants River therefore, decreases from very high to very low in the downstream direction (Table 13). Concentrations in the Olifants River Gorge have declined by 63.10% over the intervening years since Swanepoel *et al.* (2000) completed their studies into certain chemical parameters in the blood of Nile crocodiles in the Kruger National Park. Total calcium concentrations was found to be substantially lower in the plasma of crocodiles from the Olifants River Gorge during August 2008 ($\bar{x} = 0.3230$) compared to September 2008 ($\bar{x} = 2.8800$).

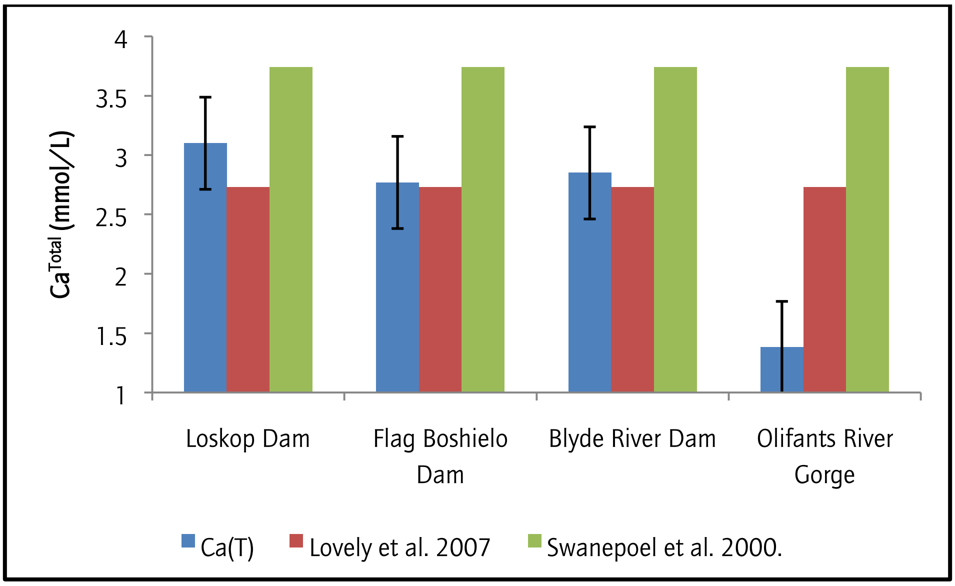

Figure 42: Mean calcium (Ca^{Total}) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

Magnesium (Mg) (Figure 43):

The mean concentrations for magnesium (Mg) in the Olifants River Gorge (0.57 mmol/L) are only slightly lower than the minimum concentration for Nile crocodiles in the Okavango Delta. However the Olifants River Gorge concentrations are now substantially lower than the 2.24 mmol/L recorded during the study of Swanepoel *et al.* (2000). Further, a general trend was observed of decreasing plasma Mg concentrations in the downstream direction of the river (Table 13). Magnesium concentrations in the blood of crocodiles from the Olifants River Gorge was substantially lower during August 2008 ($\bar{x}$ = 0.1020) compared to September 2008 ($\bar{x}$ = 1.2840).

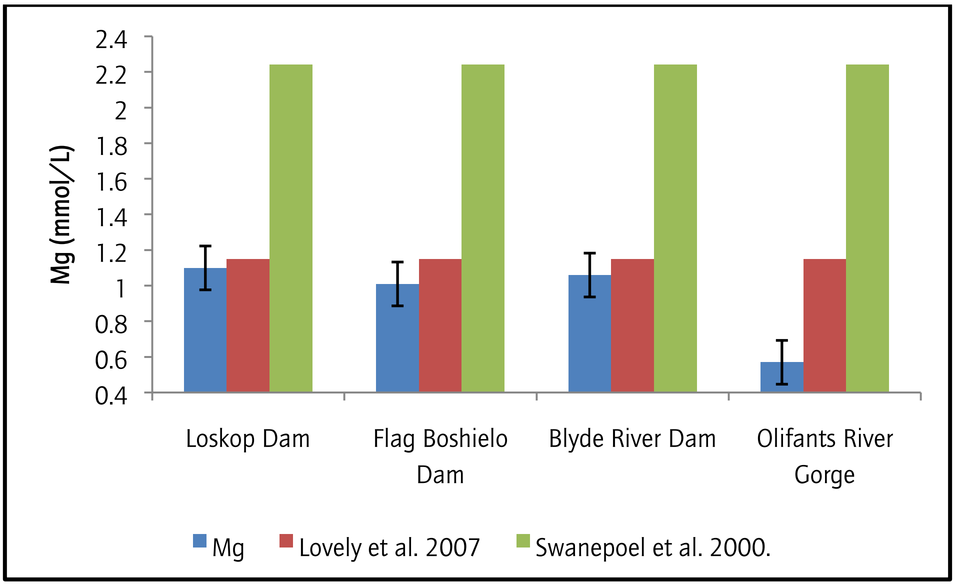

Figure 43: Mean magnesium (Mg) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

<u>Serum Inorganic Phosphate (SIP) (Figure 44):</u>

Serum Inorganic Phosphate (SIP) concentrations of the Loskop Dam (2.09 mmol/L), Blyderivierspoort Dam (2.07 mmol/L0 and Olifants River Gorge (1.94 mmol/L) all correspond closely to each other (Table 13). The concentrations recorded in the Flag Boshielo Dam are however lower at 1.64 mmol/L than all other sampling sites. Overall, the

SIP concentrations in the Olifants River are higher than those measured by Swanepoel *et al*. (2000) during their study.

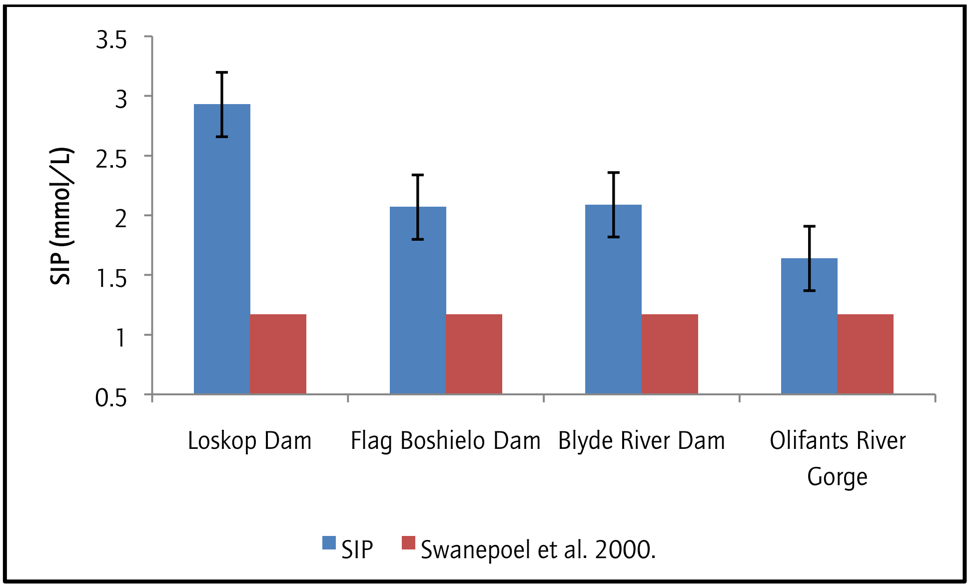

Figure 44: Mean serum inorganic phosphate (SIP) concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Swanepoel *et al.* (2000) in the Olifants River.

Cholesterol (Figure 45):

Lovely *et al*. (2007) reported that they had measured a mean cholesterol concentration of 5.49 mmol/L in the Nile crocodile population of the Okavango Delta. In the Olifants River, cholesterol in the plasma of crocodiles in the Loskop Dam (3.23 mmol/L), Flag Boshielo Dam (4.83 mmol/L) and Olifants River Gorge (3.77 mmol/L) were all measured at lower concentrations (Table 13). However, cholesterol concentrations in plasma samples collected from the unpolluted Blyderivierspoort Dam were elevated at a mean of 7.65 mmol/L (Table

13). All cholesterol concentrations in the Olifants River were however still well within the range (0.0 - 9.86 mmol/L) for Nile crocodiles in the Okavango Delta (Lovely *et al*. 2007). Cholesterol concentrations in the plasma of crocodiles from the Olifants River Gorge was substantially lower during August 2008 ($\bar{x} = 2.939$) compared to September 2008 ($\bar{x} = 5.788$).

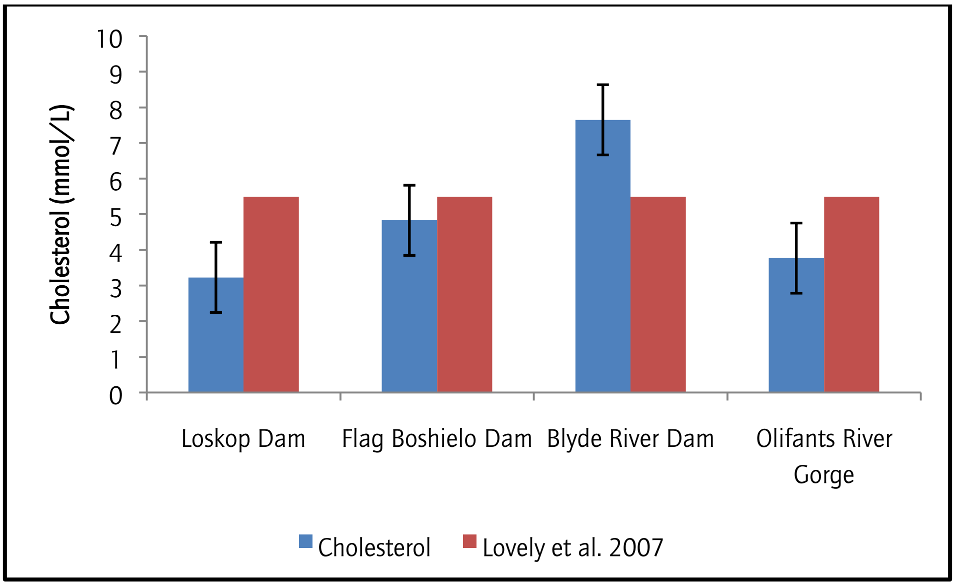

Figure 45: Mean cholesterol concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

<u>**Creatinine (Figure 46):**</u>

Mean concentrations for creatinine at all sampling sites in the Olifants River are well below the mean concentrations measured in the Okavango Delta (Table 13). At 15.33 µmol/L, the mean creatinine concentration in the plasma of Nile crocodiles in the Blyderivierspoort

Dam are 54.91% lower than the mean concentration measured in Nile crocodiles in the Okavango Delta (Table 13). Creatinine concentrations in the Olifants River have also declined by 64.10% since 2000 (Table 13).

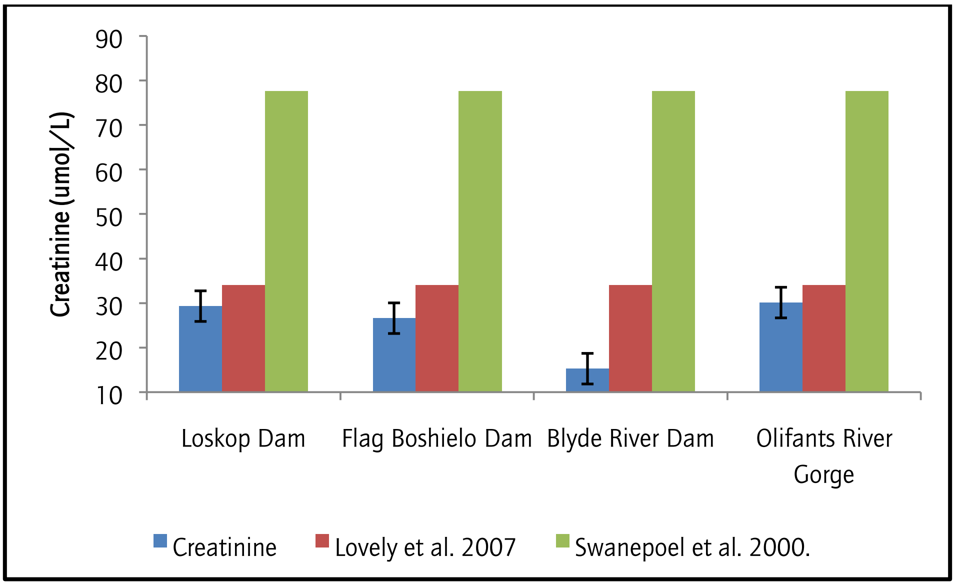

Figure 46: Mean creatinine concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

<u>Chloride (Cl) (Figure 47):</u>

The mean chloride (Cl) concentrations in the blood of Nile crocodiles in the Blyderivierspoort Dam population (75.85 mmol/L) were substantially lower (test of significance: $P = 0.034$) than in any of the other populations sampled during this study and were also substantially below the minimum concentration for Nile crocodiles in the Okavango Delta reported by Lovely *et al.* (2007). All other sites compared well to each other (Loskop Dam = 108.63

mmol/L; Flag Boshielo Dam = 116.28 mmol/L; Olifants River Gorge = 115.92 mmol/L) (Table 13).

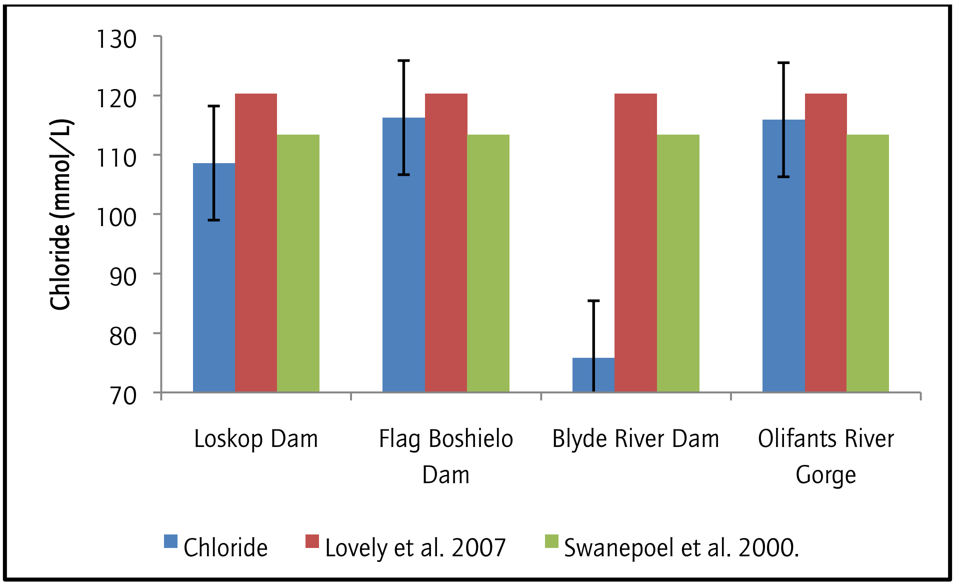

Figure 47: Mean chloride concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) and Swanepoel *et al.* (2000) for the Okavango Delta and Olifants River.

However, the mean chloride concentration in the plasma of Nile crocodiles from the Olifants River (110.84 mmol/L) compare well to that reported by Swanepoel *et al.* (2000) for the Olifants River in the Kruger National Park (113.40 mmol/L). Chloride concentrations in the plasma collected from Nile crocodiles in the Olifants River Gorge were substantially lower during August 2008 ($\bar{x} = 110.84$) compared to September 2008 ($\bar{x} = 124.08$).

Uric acid (Figure 48):

The mean concentration for uric acid recorded in the plasma of Nile crocodiles in the Blyderivierspoort Dam (0.27 mmol/L) is almost at the maximum concentration for Nile crocodiles in the Okavango Delta (0.04 - 0.30 mmol/L) as reported by Lovely *et al.* (2007).

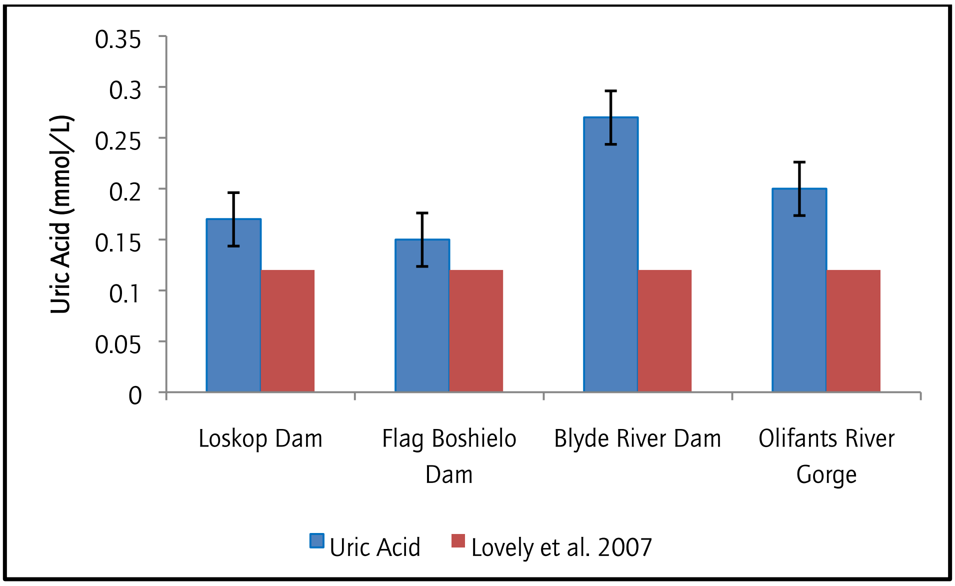

Figure 48: Mean uric acid concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River compared to concentrations reported by Lovely, *et al.* (2007) for the Okavango Delta.

Uric acid mean concentrations in the Olifants River (Loskop Dam = 0.17; Flag Boshielo = 0.15; Blyderivierspoort Dam = 0.27 mmol/L; Olifants River Gorge = 0.20 mmol/L) were higher at all sampling sites than the mean concentration recorded in the Okavango Delta (Table 13). Concentrations in the Lower Olifants River (Blyderivierspoort Dam and Olifants

River Gorge) were substantially higher than those in the Upper Olifants River (Loskop Dam and Flag Boshielo Dam) (Table 13).

Triglycerides (Figure 49):

The mean concentrations for triglycerides at the Blyderivierspoort Dam (4.38 mmol/L) are 67.35% higher than the concentrations measured at the other three sample sites (Table 13).

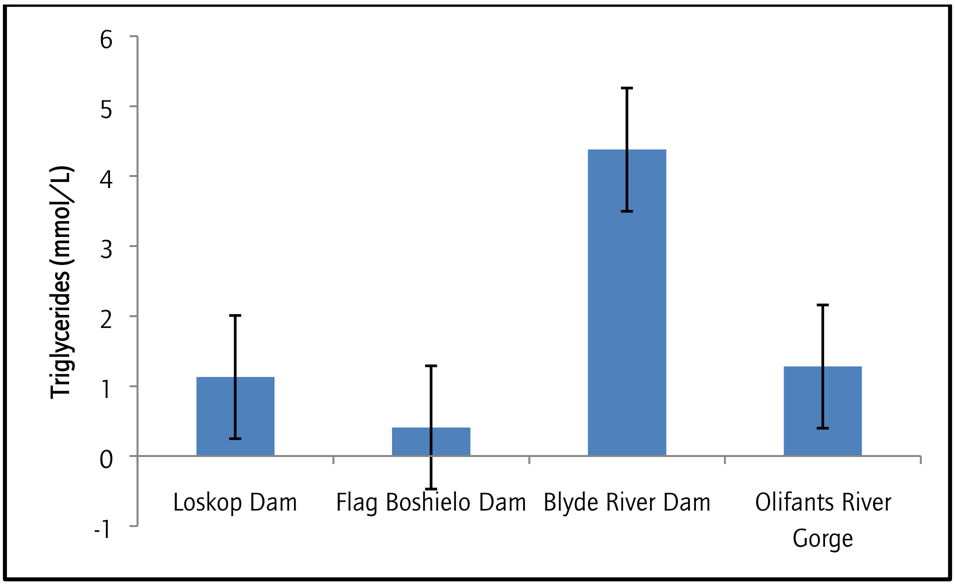

Figure 49: Mean triglyceride concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River.

Vitamin A (Figure 50):

The mean concentrations for vitamin A in the Olifants River Gorge (200.79 µg/L) are much lower than the concentrations measured at the other three sample sites (Loskop Dam =

330.61 µg/L; Flag Boshielo Dam = 326.37 µg/L; Blyderivierspoort Dam = 335.81 µg/L) (Table 13). Concentrations for the Blyderivierspoort Dam are 40.21% higher than in the Olifants River Gorge whereas the mean concentration for the entire Olifants River is 21.46% higher than values measured in the Gorge.

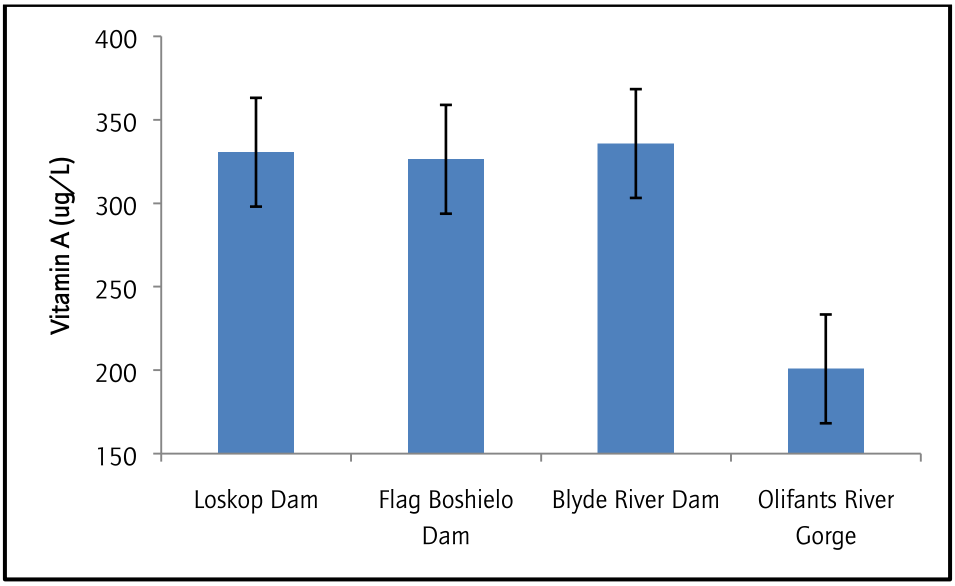

Figure 50: Mean vitamin A concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River.

Vitamin E (Figure 51):

A definite pattern in vitamin E values emerged with Loskop Dam ($\bar{x} = 7.102$) and the Olifants River Gorge ($\bar{x} = 6.210$) having the lowest concentrations whereas the Flag Boshielo Dam ($\bar{x} = 9.648$) had slightly higher concentrations and the Blyderivierspoort Dam ($\bar{x} = 11.000$) showed the highest concentrations of the four sample sites (Table 13). Vitamin E concentrations measured in the plasma of Nile crocodiles occurring in the Olifants

River Gorge were significantly lower ($P = 0.027$) than at any other site sampled in the river. No significant difference was found to exist in vitamin E concentrations of plasma collected from crocodiles in the Olifants River Gorge during August 2008 and September 2008.

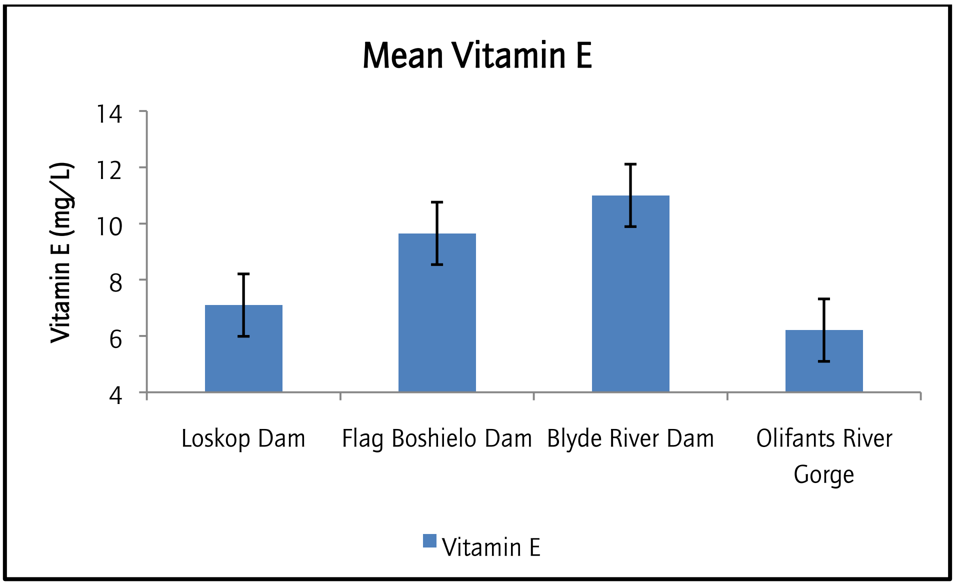

Figure 51: Mean vitamin E concentration measured in the plasma of Nile crocodiles at various sampling sites in the Olifants River.

DISCUSSION

Species, age, gender, nutritional status, season and physiological status influence the blood biochemistry of reptiles (Dessauer, 1970; Lawrence, 1987; Samour, Hawkey, Pugsley and Ball, 1986 and Thrall, Baker, Campbell, Lassen, Alan, DeNicola, Fettman and Weiser, 2004). This makes interpretation of blood biochemistry results in reptiles very challenging especially in the case of Nile crocodiles because very little previous work has been done in terms of establishing normal reference ranges for wild Nile crocodiles. Much of the work that has been published refers to captive populations or to other crocodilian species such as *Crocodylus porosus, Crocodylus palustris* and *Alligator mississippiensis* (Foggin, 1987; Millan *et al.*, 1997; Guillette *et al.* 1997; Stacy and Whitaker, 2000).

Considerable variation has been reported in biochemical and haematological values among the different crocodilian species (Millan *et al.*, 1997; Stacy and Whitaker, 2000; Lovely *et al.*, 2007) and in their review Lovely *et al.* (2007) suggest that a species-specific reference range must be developed if clinical pathology is to be used as a successfully diagnostic tool.

Mean total serum protein TSP plasma concentrations were found to be in the upper concentrations of the range as reported by Lovely *et al.* (2007). According to Campbell (2006), elevated plasma TSP concentrations could indicate possible chronic inflammation or other infection as it does in mammals and birds (Cray *et al.*, 2005).

Mean albumin concentrations (Figure 31) for the Olifants River Nile crocodile population registered in the above average, upper concentration of the range recently established in Botswana (Lovely *et al.*, 2007). The mean albumin concentrations established by Swanepoel *et al.*, (2000) correspond closely with the lower concentrations reported from Botswana (Lovely *et al.*, 2007). This indicates that conditions have changed since Swanepoel *et al.* (2000) did their study to the effect that albumin concentrations in the crocodiles of the Olifants River are now much higher with the Loskop Dam population showing concentrations above the maximum concentration recorded in Botswana. Albumin is a common plasma protein that serves as a carrier protein for other in the blood, protecting them from hepatic degradation (Tully *et al.*, 2003). Serum protein electrophoresis is used as a non-specific diagnostic tool to determine immune function and drastic changes in plasma albumin concentrations have been reported in other reptiles indicating serious cases of disease (Kaneko, 1980; Lutz *et al.*, 2001).

Mean globulin concentrations (Table 13) measured in the Olifants River Nile crocodile population were established near the upper limits of the range recently reported for Nile crocodiles in Botswana (Lovely *et al.*, 2007). These values correspond very well with the mean plasma concentration measured by Swanepoel *et al.*, (2000) which also reported concentrations just below the upper limit reported for Nile crocodiles in Botswana (Lovely *et al.*, 2007). As with the plasma albumin concentrations reported here, the globulin concentrations in the Loskop Dam Nile crocodile population were much higher than any of the other populations in the Olifants River and also well above the maximum concentrations measured in Botswana. Elevated globulin concentrations are indicative in reptiles of altered immune activity and the presence of an infectious disease in the population (Thrall *et al.*, 2004; Campbell, 2006).

Alkaline phosphatase (ALP) mean plasma concentrations in Nile crocodiles from the Olifants River show a general decline along the gradient of the river with the highest concentrations recorded at Loskop Dam and the lowest concentrations found in the Olifants River Gorge. Concentrations for Loskop Dam and Flag Boshielo Dam were just above and just below the mean concentration from Botswana whereas the mean plasma concentration from Nile crocodiles captured at the Olifants River Gorge was very close to the minimum concentration reported from Botswana. Although decreased plasma ALP concentrations is not necessarily associated with veterinary disease, it has been shown that low ALP activity in plasma can be attributed to environmental contamination in birds (Kertész and Hlubik, 2002). Therefore, the low ALP concentrations from the plasma of crocodiles in the Olifants River support the hypothesis that the river is suffering from environmental contamination and that the pollution is worse in the downstream direction of the river. This is possibly due to very few crocodiles being able to survive in the upper Olifants River thereby restricting our ability to test a large sample as was possible in the lower Olifants River.

Aspartate aminotransferase (AST) mean concentrations recorded in the plasma of Nile crocodiles from the Olifants River do not show a big difference over the gradient of the river. The concentrations are however fairly low and registered below the mean concentrations reported from Botswana but still above the minimum concentration established by Lovely *et al.* (2007). Plasma AST concentrations were higher in medium sized (TL = 1.5 – 2.0m) crocodiles compared to small sized (TL < 1.5m) crocodiles indicating that size class could play an important role in the interpretation of these values.

The mean glucose concentrations found in the plasma of Nile crocodiles from the Olifants River show elevated concentrations at Loskop Dam and in the Olifants River Gorge. These are the populations where recent catastrophic population crashes have been documented. Mean concentrations at the Flag Boshielo Dam and the Blyderivierspoort Dam correspond with mean concentrations reported from the Botswana Nile crocodile population. Concentrations described by Swanepoel *et al*. in 2000 in the Olifants River (Kruger National Park) were the same as those measured in the Loskop Dam and in the Olifants River Gorge by this study. Elevated concentrations of blood glucose in reptiles are often related to metabolic conditions, systemic diseases and stress associated hyperclycemia resulting from glucocortocoid and epinephrine release (Thrall *et al.,* 2004; Campbell, 2006) During the August 2008 pansteatitis related die-off of Nile crocodiles in the Olifants River Gorge, the animals were known to be under considerable stress due to the debilitating and painful effects of the disease. Elevated blood glucose concentrations of the Loskop Dam and Olifants River Gorge Nile crocodiles suggests that conditions in the Olifants River, especially in terms of diet which plays a central role in glucose concentrations, have changed over a long period of time leading up to the catastrophic population crash in the Olifants River Gorge during 2008.

Mean potassium (K) concentrations from the plasma of Nile crocodiles in the Olifants River were registered at concentrations close to the mean concentrations reported from the Botswana population. However, the potassium concentrations measured in the Olifants River Gorge were higher than those measured at all the other sample sites, higher than the maximum concentration from Botswana and also higher than the Olifants River mean concentrations recorded by Swanepoel *et al.* (2000). The sudden dramatic rise in potassium concentrations in the Olifants River Gorge is possibly indicative of an inadequate diet especially during August/September 2008 when most of the samples were taken and when the animals were suffering from the effects of disease. Clinical symptoms associated with elevated potassium concentrations could include tissue necrosis, hypo-adrenocorticism, acidosis and could affect the heart muscle of affected animals (Thrall *et al.*, 2004; Campbell, 2006).

The mean ionised calcium (Ca^{2+}) concentrations recorded from Nile crocodiles at all sample sites in the Olifants River, registered at concentrations even lower than the minimum concentration from the Botswana population. Concentrations of Ca^{2+} in the plasma from Nile crocodiles in the Flag Boshielo Dam were substantially below the Botswana minimum concentration. Hypocalcemia indicate dietary problems and specifically a deficiency in calcium (Thrall *et al.*, 2004; Campbell, 2006). Extremely low concentrations of calcium can result in calcium mobilisation from the skeletal bones, increased calcium absorption from the intestines and increased re-absorption of calcium from the kidneys (Thrall *et al.,* 2004;

Campbell, 2006). The results also appear to indicate that the decreased Ca^{2+} concentrations correspond with increasing size class.

Mean concentrations for total calcium (Ca^{Total}) in the plasma of Nile crocodiles occurring in the Olifants River show very low concentrations in the Olifants River Gorge. These concentrations are well below the minimum concentration described by Lovely *et al.* (2007). The very low Ca^{Total} concentrations support the low Ca^{2+} values and the conclusion that the Olifants River Gorge population suffer from hypocalcemia. All other sample sites produced concentrations that are near the mean concentration for the Okavango Delta population, except for the Loskop dam population which are at the maximum concentration measured in the Okavango Delta population. Total calcium concentrations reported by Swanepoel *et al.* (2002) were at concentrations much higher than the reported maximum concentration in the Okavango Delta population. This indicates a massive drop in Ca^{Total} concentrations in the Olifants River Gorge and Kruger National Park with extreme implications for nesting females who increase their plasma calcium concentrations for the production of eggshells (Elsey and Wink, 1986; Huchzermeyer, 2003). Other consequences of low calcium include ovarian haemorrhage, shell defects, diaphanous teeth, weak growing of bones and difficulty moving on land, spine fractures due to violent spasms and osteomalacia (Huchzermeyer, 2003).

The mean magnesium (Mg) concentrations of plasma collected from Nile crocodiles occurring in the Olifants River and especially the Olifants River Gorge, are substantially lower than those reported for the Olifants River by Swanepoel *et al.* (2000). Decreased concentrations of Mg are considered by veterinarians to contribute greatly to affected animals not eating and associated weakness (Thrall *et al.*, 2004;Campbell, 2006).

Serum inorganic phosphate (SIP) showed a drop in mean concentrations over the gradient of the Olifants River decreasing in the downstream direction. However, SIP concentrations in the river were still at higher concentrations than during 2000 when Swanepoel *et al.* studied the chemical parameters in the plasma of Nile crocodiles in the Kruger National Park. The high SIP concentrations in the Loskop Dam indicates that the current poor conditions observed in that area has probably not migrated downstream but it is my opinion that it is only a question of time before these spread beyond the wall of the Loskop Dam downstream into the Olifants River.

Mean cholesterol concentrations for Nile crocodiles in the Olifants River registered at concentrations near the mean concentrations reported by Lovely *et al.* (2007) in the Okavango Delta. However concentrations for Loskop Dam and the Olifants River Gorge were below the mean concentration of the Okavango Delta Nile crocodile population whereas at the Blyderivierspoort Dam, they were above the mean concentration of the Okavango Delta Nile crocodile population. However, since plasma cholesterol concentrations are easily influenced by the elapsed time between the crocodile's previous meal and collection of the blood sample, the implications of plasma cholesterol concentration results are often difficult to interpret.

Mean creatinine concentrations for Nile crocodiles in the Olifants River registered at concentrations near the mean reported by Lovely *et al.* (2007) in Nile crocodiles obtained in the Okavango Delta. Mean concentrations from the Blyderivierspoort Dam were the lowest of all sample sites at just below the minimum concentration for the Okavango Delta Nile crocodile population. Creatinine is a breakdown product of creatine which is an important part of muscle tissue. Low creatinine is indicative of low muscle mass (Thrall *et al.*, 2004; Campbell, 2006;). Due to the population characteristics of the specific population, most of the crocodiles captured in the Blyderivierspoort Dam were small sized animals of less than 1.0m TL with consequently less muscle mass than larger animals. This could explain the drop in creatinine concentrations registered at the Blyderivierspoort Dam.

The mean plasma chloride values for Nile crocodiles in the Olifants River registered at concentrations near to the mean reference range values reported by Lovely *et al.* (2007). The Blyderivierspoort Dam had the lowest mean values of all sample sites at concentrations much lower than the minimum reference range. Chloride values from the current study did not differ markedly from those reported by Swanepoel *et al.* (2000) from the Olifants River in the Kruger National Park. Chloride is a principle anion in blood but hypochloremia is considered to be rare in reptiles (Thrall *et al.*, 2004; Campbell, 2006) and would suggest the loss of chloride ions through overhydration with fluids low in chloride ions. It therefore remains difficult to explain the low chloride values in the Blyderivierspoort Dam samples unless low chloride is a condition that occurs in the whole Blyde River system.

The mean uric acid concentrations for Nile crocodiles in the Olifants River registered at concentrations slightly higher than the mean reported by Lovely *et al.* (2007) for the Okavango Delta Nile crocodile population. The Blyderivierspoort Dam showed a substantially higher concentration which was just below the maximum concentration recorded in the Okavango Delta. Uric acid is the primary catabolic end product of protein, non-protein nitrogen and purines and represents 80 - 90% of the total nitrogen excreted by the kidneys (Frye, 1991). Carnivorous reptiles tend to have higher blood uric acid concentrations which generally peak one day after a meal resulting in a 1.5 - 2.0 fold increase in uric acid (Frye, 1991). This likely explains the high blood uric acid concentrations measured in samples from the Blyderivierspoort Dam especially if viewed with the elevated blood cholesterol concentrations from the same sample site keeping the known effects of recent feeding on increasing blood cholesterol concentrations in mind.

Mean triglyceride concentrations measured in plasma samples collected from Nile crocodiles occurring in the Olifants River was recorded at very low concentrations at all sample sites except in the Blyderivierspoort Dam where they were substantially higher. Low triglyceride concentrations are generally indicative of malnutrition in reptiles (Thrall *et al.*, 2004; Campbell, 2006). Unfortunately, the Okavango Delta study in Botswana did not measure the triglyceride concentrations present in the plasma of that population of Nile crocodiles. It is generally accepted that it is not good practise to compare plasma values of different species because blood biochemistries can vary substantially between species and this is especially so in reptiles (Samour *et al.*, 1986; Lawrence, 1987; Dessauer, 1970 and Thrall *et al.*, 2004). However, due to the lack of relevant Nile crocodile data, comparison of triglyceride data to a similar species namely the saltwater crocodile (*Crocodylus porosus*) allows at least some interpretation of the collected information. With this in mind, the Nile crocodile data for Loskop Dam, Flag Boshielo Dam and the Olifants River Gorge seem to be near the minimum concentration. The concentrations for the Blyderivierspoort Dam are closer to the probable average of the range when compared to data from studies on *C. porosus* (Millan *et al.,* 1997) which was taken from similar sized animals. Based on these data, it is probable to hypothesize that most of the Nile crocodile population in the Olifants River is suffering from some degree of malnutrition.

The mean vitamin A concentrations measured in plasma samples collected from Nile crocodiles occurring in the Olifants River were recorded at much lower concentrations in the Olifants River Gorge than at any of the other sample sites (Loskop Dam, Flag Boshielo Dam, Blyderivierspoort Dam). Low vitamin A concentrations could indicate a vitamin A deficiency which in turn could be the result of an infectious disease.

Mean vitamin E concentrations measured in blood samples collected from Nile crocodiles occurring in the Olifants River were recorded at much lower concentrations in the Loskop Dam and Olifants River Gorge than at the other two sample sites (Flag Boshielo Dam, Blyderivierspoort Dam). Statistical analysis showed a significant difference in the low vitamin E concentrations and the different sampling sites (P = 0.027). Vitamin E is an important antioxidant which protects body tissue from damage by free radicals which in turn can harm all tissues and organs. Therefore, the low vitamin E concentrations encountered at sites where catastrophic events are known to have occurred within the Nile crocodile populations almost certainly indicate serious disease in the sampled animals.

CONCLUSION

Based on this study, the conclusions that can be drawn suggest, that, on average, the blood biochemistry of most of the Nile crocodiles in the Olifants River fall within the range for healthy Nile crocodiles. However, there are pertinent exceptions to this statement which when viewed in more detail certainly suggest that changes to the dynamics of the river over the longer term did have a negative impact on the Nile crocodile population. These impacts can be described as follows:

- Elevated TSP values suggest possible chronic inflammation (especially in the Loskop Dam and Olifants River Gorge populations).

- Albumin values increased substantially over an eight year period indicating changing conditions in the habitat.
- Elevated globulin values indicate the possible presence of an infectious disease (particularly in the Loskop Dam population but all other sites also showed elevated values);
- Elevated blood glucose values indicate that conditions particularly in the Loskop Dam and Olifants River Gorge populations where changing drastically over a long term and could be related to metabolic conditions, systemic diseases and stress associated hyperclycemia resulting from glucocortocoid and epinephrine release.
- The sudden drastic rise in potassium (K) values in the Olifants River Gorge indicates possible inadequate diet (especially during the pansteatitis outbreak of August/September 2008).
- Decreased concentrations of ionised calcium (Ca^{2+}) and total calcium (Ca^{Total}) support the theory of dietary problems.
- Magnesium (Mg) concentrations showed a substantial drop in concentrations in the Olifants River Gorge over a period of eight years and are considered to contribute greatly to affected animals not eating and associated weakness.
- Low triglyceride concentrations support the theory that dietary problems and malnutrition could be a factor in the health of Nile crocodiles in the Loskop Dam, Flag Boshielo Dam and Olifants River Gorge populations.

- Low vitamin A concentrations could indicate a vitamin A deficiency which in turn could be the result of an infectious disease.
- Low vitamin E concentrations could indicate serious immune problems in the Olifants River Gorge population.

Therefore the blood biochemistry of the Nile crocodile population in the Olifants River indicate that these animals have suffered and continue to suffer from chronic inflammation and infection possibly due to inadequate diet and malnutrition with associated weakness and serious immune problems over the last eight years and possibly longer.

Results from this study show that while establishing reference ranges for blood biochemistry in wild crocodiles is needed, the existing very limited information has ranges that are most often too wide and that further study in this direction is needed. It is important for future studies to note that the data must come from free ranging animals if we are to know what "normal" is. Other variables that must be investigated in future if we are to use this type of data as predictive markers of population health include the annual cycle in each of these parameters already examined, the role of temperature, dietary changes with season, reproductive activity and growth cycles. The data from this study is a start that can be added to the previous studies but much further work is needed.

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