

**Morphometrics, ecotoxicology and stable isotope  
ecology of Nile crocodiles (*Crocodylus niloticus*) in  
KwaZulu-Natal, South Africa**

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**Submitted in fulfilment of the academic requirements for the degree of**

**Doctorate of Philosophy**

**in the Discipline of Ecological Sciences**

**School of Life Sciences**

**College of Agriculture, Science and Engineering**

**University of KwaZulu-Natal**

**Pietermaritzburg Campus**

**2015**



## ABSTRACT

Although Nile crocodiles (*Crocodylus niloticus*) are apex predators that occur throughout Africa in many aquatic ecosystems, their feeding ecology is poorly understood, along with the species' risk of exposure to environmental contaminants such as heavy metals. Recent population declines of Nile crocodiles in South Africa have underscored the ecological value of this important predator to aquatic ecosystem health and function. Consequently aspects of Nile crocodile ecotoxicology and diet based on the analyses of various body tissues sampled from free-ranging individuals at the remaining viable crocodile populations in the province of KwaZulu-Natal, South Africa, were examined. Detailed analysis of free-ranging Nile crocodile morphometrics and demography are also presented.

Knowledge of individual body size and sex are necessary data for analyses of animal population demographics and size structure, but little information exists regarding free-ranging Nile crocodile morphometrics, sex ratios of wild populations, sexual size dimorphism, and standing crop biomass. We captured 322 Nile crocodiles at Lake St Lucia and Ndumo Game Reserve, the two largest crocodile populations in KwaZulu-Natal, and measured a suite of physical characteristics to create predictive models of body length from other morphological attributes and body mass. Our sample included 118 hatchlings, 91 subadults and 113 adults. Strong positive allometric relationships were found between body length metrics (total length and snout-vent length) and other morphometrics ( $r^2 \geq 0.91$ ). All morphometric regressions were linear, with the exception of the relationship body length to body mass, which was logarithmic. Among relationships of cranial morphology and body length we found considerable individual variation among all size classes. The mean head width-to-head length ratio was  $1.9 \pm 1.6$ , and mean head length-to-total length ratio was  $0.14 \pm 0.005$ . The sex ratios for non-hatchling

individuals at both populations were essentially 1:1, but the adult sex ratios were both male-biased. We calculated a total standing crop biomass of 96867.18 kgs (161.45 kg/km) and 52640.40 kgs (1504.01 kg/km) for Nile crocodile at Lake St Lucia and Ndumo Game Reserve, respectively, and an estimated 3400 non-hatchling individuals for the province of KwaZulu-Natal based on airplane and spotlight surveys with built-in correction factors.

As organisms that utilize both aquatic and terrestrial habitats and predators that bioaccumulate the toxins found in their prey, crocodilians are thereby exposed to a potentially wide range of environmental contaminants. During July – October 2010 we collected whole blood from 34 sub-adult and adult free-ranging Nile crocodiles from three separate populations in northeastern South Africa in order to analyze their blood lead concentrations (BPb). Concentrations ranged from below detectability ( $< 3 \mu\text{g/dL}$ ,  $n = 8$ ) to  $960 \mu\text{g/dL}$  for an adult male at the Lake St Lucia Estuary. Blood lead concentrations averaged  $8.15 \mu\text{g/dL}$  ( $\text{SD} = 7.47$ ) for females and  $98.10 \mu\text{g/dL}$  ( $\text{SD} = 217.42$ ) for males. Eighteen individuals (53%) had elevated BPbs ( $\geq 10 \mu\text{g/dL}$ ). We assessed 12 general linear models using Akaike's Information Criterion (AIC) and found no significant effects among the parameters of sex, crocodile size and population sampled. On average, crocodiles had higher BPbs at Lake St Lucia than at Ndumo Game Reserve or Kosi Bay, which we attribute to lead sinker ingestion during gastrolith acquisition and to a lesser extent, fishing-bait theft. No clinical effects of lead toxicosis were observed in these crocodiles, even though the highest BPb ( $960 \mu\text{g/dL}$ ) we report represents the most elevated BPb recorded to date for any tissue in any free-ranging vertebrate. Our data and field observations suggest adult Nile crocodiles are likely tolerant of elevated Pb body burdens, but experimental studies on other crocodilian species suggest the BPb levels reported here may have harmful or fatal effects to egg development and hatchling health. In light of recent Nile

crocodile nesting declines in South Africa we urge further BPb monitoring and ecotoxicology research on reproductive females and embryos. Lead monitoring of crocodile populations will likely also benefit other wildlife taxa.

To examine aspects of Nile crocodile diet and feeding ecology, we conducted stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis (SIA) on crocodile tissues. Because different tissues within the same consumer can incorporate dietary isotopes over different time periods and at different isotopic enrichment levels, we first conducted SIA on blood plasma and scute collagen from the same individual for 38 crocodiles resident to differing macrohabitats (freshwater vs. estuarine). Adult dietary  $\delta^{15}\text{N}$  values were higher than those for sub-adults for both tissues, but  $\delta^{15}\text{N}$  values between populations and sexes were not significantly different. The high variance among  $\delta^{15}\text{N}$  values in plasma and scutes suggests Nile crocodiles at the sites we sampled are generalist feeders. Isotopic  $\delta^{13}\text{C}$  signatures were generally more depleted in sub-adults and individuals from freshwater habitats. Crocodile  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were both isotopically enriched in scutes compared to plasma. Although it is possible this pattern was due to an unobserved dietary shift for most individuals across both habitats during our sampling timeframe, it is more likely that the isotopic variance we observed between tissues represents a marked difference in isotope enrichment, with diet-tissue discrimination values for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  higher in crocodile scute tissue. This illustrates the importance of not relying on a single tissue type when interpreting food web and trophic processes, but suggests the isotope signature in crocodile blood plasma is probably closer to actual prey isotope signature. Before discrete trophic positions can be assigned in Nile crocodile field studies, isotope discrimination values and tissue turnover times need to be established by way of controlled experiment.

Before implementing effective conservation strategies an understanding of a species' feeding ecology and trophic niche is needed, but this knowledge can be hard to come by for elusive, large-bodied predators whose life history is difficult to observe over space and time. To further examine the isotopic structure and dietary niche width for Nile crocodiles we obtained blood plasma from 106 individuals at varying microhabitats from four populations in KwaZulu-Natal and assessed the structure and variance of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios. We found a very large isotopic range (niche width) of 9.99‰ among all individuals, indicative of a generalist predator that feeds throughout the entirety of the food web. Although evidence of dietary specialism among individuals or populations was not found, principal components analysis and linear models revealed that both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  increased with crocodile size (snout-to-vent length). We interpret this trend to known dietary and habitat use differences between subadults and adults. This pattern should not be considered axiomatic, however, because the smallest and largest individuals in this study were isotopically similar with regard to dietary nitrogen in their tissues, indicative of a diet composed of primary consumers (likely small rodents for juveniles, large ungulates for adults). The significant variance of  $\delta^{13}\text{C}$  values among capture sites illustrates the heterogeneity of aquatic and terrestrial carbon inputs Nile crocodile utilizes and has practical applications for crocodile conservation management in South Africa, for example as a forensic tool to determine the geographic origin of poached individuals on the black market.

The study helps to further our understanding of Nile crocodiles in sub-Saharan Africa, and provides conceptual frameworks and quantitative baselines for future ecotoxicological and stable isotope ecology studies for the species.

## PREFACE

The data described in this thesis were collected in Pietermaritzburg, Republic of South Africa from February 2009 to May 2013. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Colleen T. Downs, and co-supervision of Dr Jan Myburgh.

This thesis, submitted for the degree of Doctorate of Philosophy in Science in the College of Agriculture, Science and Engineering, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.



.....  
Jonathan K. Warner

December 2015

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.



.....  
Professor Colleen T. Downs

Supervisor

December 2015

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## DECLARATION 1 - PLAGIARISM

I, Jonathan K. Warner, declare that

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## DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

**Jonathan K. Warner, X. Combrink, P. Calverley, G. Champion, C.T. Downs**

**Morphometrics, sex ratio, sexual size dimorphism, biomass, and population size of the Nile Crocodile (*Crocodylus niloticus*) at its southern range limit in KwaZulu-Natal, South Africa**

*Author contributions:*

JKW conceived paper with CTD. JKW, XC, PC, and GC collected data. JKW analyzed data and wrote the paper. CTD, XC, PC, and GC contributed valuable comments to the manuscript.

Publication 2 (Provisionally accepted)

**Jonathan K. Warner, X. Combrink, J.G. Myburgh, C.T. Downs**

**Blood lead concentrations in free-ranging Nile crocodiles (*Crocodylus niloticus*) from South Africa**

*Author contributions:*

JKW conceived paper with CTD. JKW and XC collected data. JKW analyzed data and wrote the paper. JKW, XC, PC, JGM and CTD contributed valuable comments to the manuscript.

Publication 3

**Jonathan K. Warner, X. Combrink, S. Woodborne, G. Hall, J.G. Myburgh, C.T. Downs**

**Stable isotope values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in blood plasma and scute tissue from Nile crocodiles (*Crocodylus niloticus*) at freshwater and estuarine habitats in South Africa**

*Author contributions:*

JKW conceived paper with CTD. JKW and XC collected data. GH, SW and JKW were responsible for lab analyses. JKW analyzed data and wrote the paper. SW, GH, XC, JGM and CTD contributed valuable comments to the manuscript.

Publication 4

**Jonathan K. Warner, X Combrink, S. Woodborne, G. Hall, J.G. Myburgh, C.T. Downs**

**Trophic niche width and dietary stable isotope structure of Nile crocodile (*Crocodylus niloticus*) populations in KwaZulu-Natal, South Africa**

*Author contributions:*

JKW conceived paper with CTD. JKW and XC collected data. GH, SW and JKW were responsible for lab analyses. JKW analyzed data and wrote the paper. SW, GH, XC, JGM and CTD contributed valuable comments to the manuscript.



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December 2015

## ACKNOWLEDGEMENTS

I thank my primary supervisor, Prof. Colleen Downs, for making this research possible. Colleen invested an inordinate amount of financial resources, academic risk and supernatural patience into the study, and I thank her for the fortitude and vision to see this project through to the end. Colleen's competence as world-class biologist is only matched by her uncommon kindness as a human being.

Nobody is perhaps more deserving of thanks than Xander Combrink (long-time technician/ecologist at St Lucia, now senior lecturer at Tshwane University of Technology), a great friend and research colleague at St Lucia for almost a decade. I have been lucky to spend countless days and nights with Xander exploring the field, herping, and collecting crocodile data by foot, boat, and airplane. Xander's enthusiasm for crocodiles is infectious, and his rare combination of humility, intelligence, humor and absolute fearlessness in the field will long be remembered in the history of Zululand naturalists. It is also impossible for me to properly express my gratitude to Xander's wife, Susan Combrink, and their boys Stanis, Anno and Bernard for their friendship, camaraderie and hospitality over the years. I would not have been able to do this work without the Combrink family, and am grateful for all the exciting times we shared.

I thank Ricky Taylor, career Ezemvelo KZN Wildlife ecologist at St Lucia until his retirement in 2012, for bringing awareness to the need for this research in KwaZulu-Natal and using his official capacity as ecologist to initiate the study. Thanks also to Ricky for sharing his ecological knowledge of St Lucia on many different occasions. Special thanks also go to Wendy Taylor for her friendship and support in St Lucia.

I thank Jan Myburgh (Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort) for acting as my co-supervisor. Jan's veterinary experience, toxicological background, and in-depth knowledge of crocodiles contributed greatly to this study. Jan and his wife, Susan, welcomed me into their home in Pretoria on numerous occasions and they are sincerely thanked for their hospitality.

Peter Calverley and Garreth Champion are thanked for their hard work at Ndumo Game Reserve and Jozini Dam, and for helping me collect morphometric data and obtain tissue

samples from those localities. The many late nights catching crocodiles, spinning down blood, and braaing until dawn are fond memories. I'm glad we all made it out alive.

Sincere thanks to Scotty, Diane, Ewan and Kirsty Kyle for assistance with capture and sampling at Kosi Bay, and for sharing their home in paradise with me innumerable times over the years. The many conversations and adventures with the Kyles enriched my life significantly during the course of this study, and I thank them for their friendship.

I thank Nick and Freya van der Wiel (Tailor Made Safaris) for their friendship and camaraderie in St Lucia, and for always being there to lend a helping hand or listening ear.

Stephan Woodborne and Grant Hall are thanked for their laboratory work for the stable isotope analysis.

I thank Danie Pienaar, Markus Hofmeyr, Danny Govender and Sam Ferreira for allowing us to participate in the crocodile fieldwork at the Kruger National Park. Sorry again for getting lost in the Gorge.

Special thanks to the late Lou Guillete and Russ Lowers for their interest in this study and for sharing their capture methods on alligators in the field at NASA Kennedy Space Center at the outset of our research.

The late Tony Pooley and the late Fritz Huchzermeyer are acknowledged for their important pioneering contributions to Nile crocodile ecology and veterinary science in South Africa.

In addition to those named above, throughout the course of this research there were many individuals that assisted logistically, intellectually, administratively, financially, and emotionally. In no particular order these people are gratefully acknowledged as follows, with my sincere apologies for any unintentional omissions: Ferdi Myburgh, Mark Robertson, Hannes Botha, Dirk Rossouw, Daphne Avenstrup, Chris Van de Berg, Frans Radloff, Chantal Dickson, Johan Steyl, Caroline Fox, Lawrence and Imelda Du Plessis, Greg Nanni, Dave Cooper, Alison Leslie, Jenny Calverley, Rob and Amy Kyle, Samantha Lavin, the late Greg Fleming, Jelger Herder and Maaïke Pouwels, Roland Zoer, Ashley Percy, the late Oupa Blokkies and Ouma Cora de Beer, Johan Gerber, Rob Taylor, Janet Taylor, James Wood, Grant and Beverley Burden, Carrie Kaarre, Xolani Mpanza, Pete Laver, Steve Slater, Mike Dreslik, Sarah Baker, Dan Wylie, the Chris Phillips herp lab, the Graham Alexander herp lab, Sven Bourquin, Vince Shacks, Gavin Masterson, Jonathan Retzlaff, Brady Barr, Kester Vickery, Joe Townsend, Matt

Shirley, Adam Rosenblatt, Kevin and San-Marie Jollife, Sean Tilden, members of the IUCN Crocodile Specialist Group, and African Impact staff and volunteers.

Tony Conway and staff (Ezemvelo KZN Wildlife) and Andrew Zaloumis and staff (iSimangaliso Wetland Park Authority) are thanked for hosting this project, which in large part occurred within the iSimangaliso Wetland Park. Thanks also to the operational and management staff for Pongola Game Reserve, Pongola Nature Reserve and Ndumo Game Reserve.

The Ford Wildlife Foundation is gratefully acknowledged for providing 4x4 vehicles, along with the South African Water Research Commission and the University of KwaZulu-Natal for financial scholarship and assistance.

I thank my parents, Dr. Richard and Zoe Warner, whose unwavering support in every facet of my life allowed me to pursue this project. I also thank Susanna Warner, Chris, Becka, Aidan, Teddy and Weston Warner, and Brock, Elizabeth, Edie, Emma and Violet Angelo. I love you guys.

**This work is dedicated to my parents, Richard and Zoe**

**If I fought wild beasts in Ephesus with no more than human hopes, what have  
I gained?**

**1 Corinthians 15:32**

**A fact is not a truth until you love it**

**John Keats**

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# CHAPTER 1

## Introduction

### **The Nile crocodile**

Although understudied compared with birds and mammals, ecological research on the world's ~25 species of crocodylians (alligators, caimans, crocodiles, gharials) is steadily increasing. Recent studies have illustrated the importance of crocodylians as top predators (Hanson et al. 2015), ecological “umbrella” species (Martin 2008) and sentinels of ecosystem health (Mazzotti et al. 2009, Milnes and Guillete 2008). Crocodylians have also emerged as excellent models for studying trophic linkages between ecosystems (Rosenblatt et al. 2015), animal movement within complex and foreign habitats (Combrink 2014) and ecosystem change (Woodborne et al. 2012). Because of their strong ecological influence and ubiquity in tropical and subtropical ecosystems worldwide, crocodylians are exceptional study subjects with regard to information accrued versus research investment. As charismatic megafauna, crocodylians are also banner organisms for aquatic ecosystem conservation programs (Shirley et al. 2009).

Economically, crocodylians are also valuable. Crocodile farming and sustainable use programs for meat (Da Silveira and Thorbjarnarson 1999) and the exotic leather trade (Thorbjarnarson 1999) are important regional drivers of economic stability. Novel innovations such as “source to skin” traceability systems pioneered by developing countries like South Africa will hopefully increase the positive impacts of industry both for local communities and crocodile conservation in the near future. Additionally, crocodylians still hold untapped commercial potential for local and international tourism (Ryan and Harvey 2000).

Of the seven proposed crocodylian species in Africa (Shirley et al. 2014) the Nile crocodile *Crocodylus niloticus* (Laurenti, 1768) is the most widespread and recognizable. From the Nile River basin of its nomenclatural origin, throughout sub-Saharan Africa to subtropical South Africa, the Nile crocodile inhabits almost every type of aquatic ecosystem. However, the distribution and abundance of free-ranging Nile crocodiles have decreased overall in the past century (Fergusson 2010), and recent genetic studies indicate individuals in western Africa constitute a different species (*C. suchus*; Hekkala et al. 2011). Like many crocodylians, the Nile crocodile is an apex predator that has far-reaching ecological impacts on both aquatic and terrestrial food webs (Combrink 2014). Nile crocodiles also provide economic value to communities due primarily to the commercial leather trade (Cott 1961). Importantly, despite the species' fearsome reputation, the Nile crocodile is still an understated human-health concern in rural Africa where the death toll is usually only guessed at, but probably approaches multiple hundreds of individuals annually.

At the species' southern limit in South Africa, the Nile crocodile has benefited from an inordinate amount of research focus. While the research of Hugh Cott (1961) and Jon Hutton (1984) helped pioneer the study of crocodile ecology in Africa, the legacy of Nile crocodile research in South Africa can definitively be traced back to Tony Pooley (1938–2004), a well-known naturalist, conservationist and crocodile expert. Since Tony's initial work (Pooley 1982), most published ecological information on the Nile crocodile in South Africa has stemmed from a number of important postgraduate studies (Jacobsen 1984, Leslie 1997, Botha 2010, Champion 2011, Calverley 2013, Combrink 2014).

Despite valuable research and conservation efforts, the Nile crocodile is currently under threat in South Africa and IUCN red-listed as Vulnerable (Marais 2014). Multiple small

fragmented populations currently exist in the northeastern part of the country, but the long-term viability of the species is likely restricted to two large populations located at Lake St Lucia within the iSimangaliso Wetland Park (~ 1000 non-hatchling individuals) and at the Kruger National Park (KNP) (population unknown, but likely > 1000 individuals). Historically, large numbers of Nile crocodiles in South Africa were lost to widespread habitat destruction, indiscriminate killing and exploitation for leather (Cott, 1961, Pooley 1982). Current threats to the species are more nuanced but just as serious. Indirect threats include the sedimentation of crocodile habitats, uncontrolled water abstraction, drought, invasive vegetation, climate change and loss of suitable nesting habitat (Leslie and Spotila 2001, Calverley and Downs 2014, Combrink 2014). Direct threats to Nile crocodiles mostly involve human conflict in the form of snaring, gillnetting and other forms of poaching. Crocodiles are targeted because they are viewed as threats to livestock or competitors for aquatic resources, as incidental offtake, or for traditional medicine (Blake and Jacobsen 1992, Kyle 1999, Combrink et al. 2011). In recent years, environmental pollution has emerged as an insidious new threat to Nile crocodiles, highlighted by the pansteatitis outbreak and resultant deaths of hundreds of crocodiles from Loskop Dam and the Olifants, Letaba and Sabie Rivers in KNP (Ashton 2010, Botha et al. 2011, Ferreira and Pienaar 2011, Woodborne et al. 2012, Lane et al. 2013). The die-offs in KNP served in large part as catalyst for the research presented in this dissertation, which was conducted as part of the Zululand Nile Crocodile Research Programme.

### **Thesis structure and conceptual framework**

The following chapters examine aspects of Nile crocodile morphometrics, sex ratios, sexual size dimorphism, biomass, ecotoxicology and feeding ecology based on the analyses of morphological and demographic data, and various body tissues sampled from free-ranging

individuals at the remaining viable crocodile populations in the province of KwaZulu-Natal, South Africa. The thesis is composed of a general introduction, four main data chapters, and a concluding chapter. Each data chapter is formatted as a stand-alone manuscript for a specific scientific journal. As such, there is some unavoidable repetition of information among chapters, particularly in the Introduction and Methods sections.

### **Nile crocodile morphometrics**

Chapter 2 assesses the morphometrics, sex ratio, sexual size dimorphism, biomass and population size of Nile crocodiles in KwaZulu-Natal. Knowledge of individual body size and sex are important data for analyses of animal population ecology and structure. Basic morphometric parameters (e.g. head shape, total length, mass) can help researchers answer complex questions about sexual size dimorphism (Shine 1986, Zamudio 1998), energetic constraints (Wikelski et al. 1997), hybridization (Brede et al. 2000), adaptation to different ecological niches (McMaster and Downs 2006, Ousterhout 2015), and the identification and conservation of cryptic species (Ennen et al. 2010, Sanders et al. 2015). Conservation management of crocodylians in particular relies heavily on knowledge of individual body size and population size-class structure because size, rather than age, is the primary driver of demographic and reproductive processes (Webb and Smith 1987). For South Africa, this information is lacking. In this chapter, we present a rigorous analysis of morphometric and demographic data collected in the field in Zululand. Additionally, we estimate the current metapopulation size and standing crop biomass of the Nile crocodile at its African range limit in KwaZulu-Natal based on recent ecological and population studies. Our findings are presented in light of their significance to crocodile research and management in South Africa.

### **Nile crocodile blood lead concentrations (BPbs)**

Chapter 3 deals exclusively with whole blood lead concentrations (BPb) in free-ranging Nile crocodiles. Lead (Pb) is an inert heavy metal that is ubiquitous in the environment but has no known physiological or metabolic benefit to animals (Buekers et al. 2009). Acute and chronic cases of Pb exposure can be fatal or lead to disorders of the nervous, gastrointestinal, reproductive and circulatory systems. The epidemiology of Pb poisoning and toxicokinetics of Pb circulation and sequestration in analogous tissues and organs among different species, however, are poorly understood (Fisher et al. 2006, Sparling et al. 2010). Recent research has established clinical concentration levels in bird populations due to the direct and indirect ingestion of Pb shot and fishing tackle, which has prompted several countries to ban recreationally-used Pb-based bullets and sinkers (Thomas 1997).

Several studies (Twining et al. 1999, Jeffree et al. 2001, Rainwater et al. 2007) report background or elevated Pb concentrations in the flesh and osteoderms of free-ranging crocodilian species, but information about the clinical symptoms and reproductive effects of Pb toxicosis is poorly understood and limited to studies on captive crocodilians fed meat contaminated with Pb shot (Hammerton et al. 2003, Lance et al. 2005). For Nile crocodiles background, subclinical and clinical Pb concentrations in blood have not been recorded and Pb blood kinetics are not understood, despite several crocodile populations in South Africa affected by mining operations, metals pollution and heavy recreational fishing pressure (Ashton 2010, Botha et al. 2011, this study). Biomagnification of anthropogenically introduced Pb through the food chain is therefore a concern for Nile crocodiles in South Africa.

At the outset of this study in 2009 a cohort of severely emaciated crocodiles was observed at Lake St Lucia in the iSimangaliso (Warner et al. unpublished data). Two individuals in very poor condition were subsequently euthanized and elevated Pb concentrations were found in liver tissue and blood plasma, in addition to fishing sinkers found in the stomachs. Consequently, a program of Pb surveillance in the blood of crocodiles from KwaZulu-Natal was initiated. Chapter 3 provides the findings and analysis of this research, and the conservation management implications for Nile crocodiles.

### **Nile crocodile stable isotope analysis**

Very little information exists regarding the feeding ecology of Nile crocodiles. Although they are presumed apex predators, most feeding records are collated by way of incidental and direct observation (Whitfield and Blaber 1979). Stomach content studies can provide important insight into prey taxonomy (Leslie 1997, Wallace and Leslie 2008) but stomach lavaging is labor-intensive and extremely difficult in the field for individuals > 2.5 m (Radloff et al. 2012). Stomach content analysis also provides only a snapshot in time of an individual's aggregate diet; a snapshot that carries a number of intrinsic biases mainly due to differential digestion and assimilation rates within and among prey items (Bearhop et al. 2002, Radloff et al. 2012).

Over the past three decades, stable isotope analysis of diet (SIA) has become a powerful ecological tool for helping to clarify food web structure (Hanson et al. 2015), trophic interactions (Kurle 2002), habitat connectivity (Rosenblatt et al. 2015), species' niche width and overlap (Willson et al. 2010) and individual niche specialization (Rosenblatt et al. 2015), among other applications. The underlying theory of SIA is that the isotopic composition of consumer tissues reflects assimilated diet in predictable ways (Post 2002). Isotopic variation within a consumer population can be therefore be used to examine dietary patterns and niche characteristics through

space and time (Bearhop et al. 2004). Carbon (C) and nitrogen (N) are the two most commonly used elements in animal SIA studies with  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios standardly reported as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively (DeNiro and Epstein 1978, 1981).

Generally  $\delta^{15}\text{N}$  composition is an indicator of trophic position, with higher values reflecting higher trophic status (usually between +2‰ and +6‰ enrichment per trophic level) (Minagawa and Wada 1984, Peterson and Fry 1987, Caut 2009). Because carbon remains relatively unchanged (between -1‰ and +1‰) within food chains, consumer  $\delta^{13}\text{C}$  values are useful for determining primary nutrient source(s) at the base of food webs (Hobson and Clark 1992, Post 2002, Rosenblatt et al. 2015). The variation in  $\delta^{13}\text{C}$  values is directly linked primarily to differing photosynthetic pathways found among  $\text{C}_3$  and  $\text{C}_4$  plants (Farquhar et al. 1989).

In Chapters 4 and 5, the SIA findings for Nile crocodiles at four different populations in KwaZulu-Natal are presented. Chapter 4 provides a comprehensive review of ecological isotope research for crocodylians, and addresses  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic differences for Nile crocodiles in differing macrohabitats (freshwater vs. estuarine). The variance and isotopic enrichment differences between tissue types (blood plasma vs. scute collagen) are also discussed within the context of Nile crocodile trophic position, and what  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values can and cannot tell us about the species' feeding ecology. Chapter 5 addresses the trophic niche width and isotopic structure of Nile crocodile populations in the province, specifically looking at differences among crocodile size, sex and population. The quantitative niche width derived for the species is analyzed for its ecological importance and practical application to Nile crocodile conservation and management.

## **Study sites**

Nile crocodiles were captured at four populations in northeastern KwaZulu-Natal, South Africa. These sampling sites represent the largest remaining protected aquatic ecosystems that harbor the species in the province. Research was conducted during 2009 – 13 in concordance with other regional crocodile studies under the umbrella of the Zululand Nile Crocodile Research Programme. Full descriptions of crocodile ecology and habitat at the following study sites can be found in ancillary studies (Champion 2011, Calverley 2013, Combrink 2014).

### *Lake St Lucia*

The St Lucia estuarine system enclosed within the iSimangaliso Wetland Park World Heritage Site contains the largest estuarine crocodile population (~1000 individuals) in Africa (Leslie and Spotila 2001, Combrink 2014), and is also the most southern breeding population for the species. At 67 km in total length the system contains a main lake basin (6 km wide at capacity) connected to the Indian Ocean via a 27 km long “Narrows” channel. Lake St Lucia is a highly dynamic environment over space and time with cyclical, but often unpredictable annual fluctuations between drought and high rainfall (Stretch et al. 2013). Due to a decade-long drought, at the time of our sampling the estuary mouth was closed, there were limited freshwater inputs, and significant sections of the lake were hypersaline or exposed due to evaporation. Nile crocodiles were captured from eight distinct habitats at Lake St Lucia.

Catalina Bay and Dead Tree Bay are shallow basins connected to the eastern shoreline. Freshwater seeps and small wetlands at the periphery of these two areas are important crocodile habitat components and refugia from saline lake conditions during drought periods (Combrink et al. 2013). The perennially-flowing Nkazana Stream drains seepage from the Mfabeni wetland into Catalina Bay, and the 2 km stretch that flows into the Catalina mouth is a critical nesting

area for Nile crocodiles at Lake St Lucia (Combrink et al. 2013). Just north but isolated from Nkazana is eSengeni; also an important nesting area. At the northern reach of the lake Tewati Bay harbours large congregations of common hippopotamuses *Hippopotamus amphibius* (hereafter hippos) and Nile crocodiles and is a regional freshwater oasis during hypersaline conditions. Lake Bhangazi South is a small (262 ha), landlocked freshwater lake nested between Lake St Lucia and the Indian Ocean. The Mphate River feeds into Lake St Lucia on the ecologically-distinct western shoreline and is connected to the Narrows, a winding channel that links the lake to the ocean. The Narrows section is a critical feeding area for Nile crocodiles but fish densities fluctuate depending on whether the estuary mouth is opened or closed (Whitfield and Blaber 1979, Govender et al. 2011).

#### *Kosi Bay*

Also within the iSimangliso Wetland Park, Kosi Bay sits on the southern Mozambique border. The ecosystem consists of an estuary mouth that flows into a linear series of four lakes on a salinity gradient interconnected by narrow channels. Kosi Bay harbors < 20 Nile crocodiles (Warner, unpubl. data) which in this study were captured at the Sihadla Channel, a small waterway at the southern terminus of the fourth lake. Due to its distance from the Indian Ocean, Sihadla is unaffected by tidal activity or salinity and is functionally a freshwater-mangrove ecosystem (Kyle 1999).

#### *Ndumo Game Reserve*

The 10,000 ha Ndumo Game Reserve is found inland at the western edge of the Mozambique Coastal Plain. The Usuthu and Phongola Rivers form the northern and eastern boundaries of the

reserve, respectively. During the rainy season (Nov – Mar) up to 40% of the reserve may be inundated, including 12 permanent and semi-permanent floodplain lakes that are critical habitats year-round for the approximately 900 crocodiles in the area. Lake Inyamithi harbors the highest density of crocodiles, where all Nile crocodiles for this study at Ndumo were captured (Calverley 2013).

### *Jozini Dam*

The ~13,500 ha freshwater Jozini (or Pongolapoort) Dam was constructed in 1973 along the Pongolo River near the southern Swaziland border. Conservative estimates place the Jozini Dam crocodile population at 275 individuals and it is likely this waterbody supports the only stable or increasing population in South Africa (Champion 2011).

### **Nile crocodile capture and sampling**

Capturing Nile crocodiles can be difficult under the most favorable conditions, given the inherent potential danger of the animals and the heterogeneity of terrestrial and aquatic habitats that must be traversed during field research. The major safety issue working with free-ranging Nile crocodiles in Zululand is not usually the animals themselves, nor the difficulty of the terrain or field conditions, but is the abundance of hippos resident to the same waterbodies as crocodiles. Many routine fieldwork activities utilized in other crocodylian studies had to be altered or amended because of the risks associated with hippos, especially capture activities at night. This problem is mentioned in passing here so future researchers that study Nile crocodiles in the field in South Africa will remember to take into account the challenges hippos can present to study design, researcher safety, field work, and capture success.

Permission to catch Nile crocodiles and take blood samples at all sites were obtained under permit from Ezemvelo KZN Wildlife and ethical clearance from the University of KwaZulu-Natal. A variety of techniques were employed to capture and restrain Nile crocodiles. Most commonly a noosing rig composed of a self-locking cable attached to a bamboo or aluminum pole was used. Noosing was effective at night from a boat with spotlight, and during the day for crocodiles encountered in small pools or shallow waterbodies. Once the noose was securely over a crocodile's head it was tightened around the neck, leaving the individual attached to the capture rope. In deeper waterbodies, a small detachable harpoon head (Webb and Messel 1977) or 8/0 barbless weighted treble hook (R. Lowers, pers. comm.) attached to a 50 – 80 m Kevlar rope were used to spear or snag crocodiles. Small weighted barbless treble hooks (3/0 or 4/0) with fishing rod and reel were also occasionally used. These techniques were non-invasive to crocodiles, with penetration occurring just beneath the scaly epidermis, usually < 2 cm.

Once an individual was blindfolded and restrained, blood samples for ecotoxicology and stable isotope analyses were collected before body measurements and tagging activities were conducted (usually < 10 min. after capture). Blood was drawn from the post-occipital spinal venous sinus using a 20 ml syringe with 18G needle. Either a 1.5” hypodermic or 3.5” spinal needle was used depending on the body size and/or neck fat of individual crocodiles (Myburgh et al. 2014). Between 2 and 20 ml of blood was collected dependent on crocodile size and the needs of ancillary bloodwork studies. The capture and sampling methods used in this study were recently published in the South African National Parks' standard operating procedure for the monitoring, capture and sampling of Nile crocodiles (Combrink et al. 2012).

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## CHAPTER 2

### **Morphometrics, sex ratio, sexual size dimorphism, biomass, and population size of the Nile crocodile (*Crocodylus niloticus*) at its southern range limit in KwaZulu-Natal, South Africa**

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#### **ABSTRACT**

Knowledge of individual body size and sex are necessary data for analyses of animal population demographics and size structure, but little information exists regarding free-ranging Nile crocodile *Crocodylus niloticus* morphometrics, sex ratios of wild populations, sexual size dimorphism, and standing crop biomass. We captured 322 *C. niloticus* at Lake St Lucia and Ndumo Game Reserve, the two largest crocodile populations in KwaZulu-Natal, and measured a suite of physical characteristics to create predictive models of body length from other morphological attributes and body mass. Our sample included 118 hatchlings, 91 subadults and 113 adults. Strong positive allometric relationships were found between body length metrics (total length and snout-vent length) and other morphometrics ( $r^2 \geq 0.91$ ). All morphometric regressions were linear, with the exception of the relationship of body length to body mass,

which was logarithmic. Among relationships of cranial morphology and body length we found considerable individual variation among all size classes. The mean head width-to-head length ratio was  $1.9 \pm 1.6$ , and mean head length-to-total length ratio was  $0.14 \pm 0.005$ . The sex ratios for non-hatchling individuals at both populations were essentially 1:1, but the adult sex ratios were both male-biased. We calculated a total standing crop biomass of 96867.18 kgs (161.45 kg/km) and 52640.40 kgs (1504.01 kg/km) for *C. niloticus* at Lake St Lucia and Ndumo Game Reserve, respectively, and an estimated 3400 non-hatchling individuals for the province of KwaZulu-Natal.

## INTRODUCTION

Of the seven proposed crocodylian species in Africa (Shirley et al. 2014) the Nile crocodile *Crocodylus niloticus* is the most iconic and widespread, ranging throughout sub-Saharan Africa to its southern geographic range limit in subtropical KwaZulu-Natal, South Africa. As an apex predator *C. niloticus* has extensive ecological influences on both aquatic and terrestrial food webs (Chapters 4, 5). The species is also economically valuable in the commercial leather trade (Cott 1961, Blake and Jacobsen 1992). Furthermore, despite the species' fearsome reputation, *C. niloticus* is still an understated human-health concern in rural Africa where hundreds of communities must partition water resources with crocodiles and their prey.

*Crocodylus niloticus* is currently under threat in South Africa and IUCN red-listed as Vulnerable (Marais 2014). In addition to traditional threats of habitat alteration and destruction (Leslie and Spotila 2001, Combrink et al. 2011) and poaching (Calverley and Downs 2014a), environmental pollution has emerged as an insidious new threat to *C. niloticus*, highlighted by

the pancreatitis outbreak and resultant deaths of hundreds of crocodiles from the greater Olifants River watershed (Ferreira and Pienaar 2011, Lane et al. 2013).

In South Africa outside of the Kruger National Park, remaining viable crocodile populations are limited to northern KwaZulu-Natal, where the highest densities of *C. niloticus* occur in protected areas at Lake St Lucia within the UNESCO World Heritage iSimangaliso Wetland Park (Combrink 2014), Ndumo Game Reserve (Calverley 2013), and at the Pongola River inlet at Jozini Dam (Champion 2013). Numerous smaller populations persist at the periphery of these areas, including uMkhuze Game Reserve, Tembe Elephant Reserve, Hluhluwe-iMfolozi Park, various waterbodies in greater iSimangaliso (Kosi Bay, Lake Sibaya, Lake Bhangazi North and South), and in the Enseleni, Zinkwazi and Tugela Rivers.

Knowledge of individual body size and sex are requisite data for analyses of animal population ecology and structure. Basic morphometric parameters (e.g. head shape, total length, mass) can help researchers answer complex questions about sexual size dimorphism (Shine 1986, Zamudio 1998), energetic constraints (Wikelski et al. 1997), hybridization (Brede et al. 2000), adaptation to different ecological niches (McMaster and Downs 2006, Ousterhout 2015), and the identification and conservation of cryptic species (Ennen et al. 2010, Sanders et al. 2015). Conservation management of crocodylians in particular relies heavily on knowledge of individual body size and population size-class structure because size, rather than age, is the primary driver of demographic and reproductive processes (Webb and Smith 1987).

Despite a legacy of conservation-based research on the species (Cott 1961, Pooley 1982, Leslie 1997, Champion 2011, Calverley 2013, Combrink 2014), with the exception of Hutton (1987 a,b,c) scant published information exists regarding morphometrics of free-ranging *C. niloticus*, sex ratios of wild populations, and sexual size dimorphism. In this study, we present a

rigorous analysis of these variables from data collected in field studies. Additionally, we estimate the current metapopulation size and standing crop biomass of *C. niloticus* at its African range limit in KwaZulu-Natal based on recent ecological and population studies. Our findings are presented in light of their significance to crocodile research and management in South Africa.

## **MATERIALS AND METHODS**

We captured *C. niloticus* during 2009 – 13 at Lake St Lucia (STL) and Ndumo Game Reserve (NGR) in conjunction with crocodile population surveys (Champion 2011), and studies of habitat use (Calverley 2013), spatial ecology (Combrink 2014), ecotoxicology (Chapter 3), and stable isotope ecology (Chapters 4, 5). The STL estuarine system is 67 km in total length and contains a main lake basin (6 km wide at capacity) connected to the Indian Ocean via a 27 km long “Narrows” channel. Lake St Lucia is a highly dynamic environment over space and time with cyclical, but often unpredictable annual fluctuations between drought and high rainfall (Stretch et al. 2013). Due to a decade-long drought, at the time of our sampling the estuary mouth was closed and significant sections of the lake were hypersaline or exposed due to evaporation, thereby rendering the surrounding freshwater pools and feeder streams important habitat for crocodiles (Combrink et al. 2013). The 10,000 ha Ndumo Game Reserve (NGR) is located on the southern Mozambique border, with the Usuthu and Phongola Rivers forming the northern and eastern boundaries of the reserve, respectively. During the rainy season (Nov – Mar) up to 40% of the reserve may be inundated, including 12 permanent and semi-permanent floodplain lakes that host large congregations of crocodiles (Calverley and Downs 2014b). Lake St Lucia and NGR represent the two largest *C. niloticus* populations in KwaZulu-Natal.

We captured crocodiles at night and during the day, usually by direct noosing or snagging with a barbless weighted treble hook attached to Kevlar rope or fishing line (Combrink et al. 2012). Hatchlings were captured by hand in nursery areas in late February, March, and early April within 30 days of nest emergence. After individuals were safely restrained and tissue samples were taken, we recorded the following measurements:

1. Total length (TL): distance from the tip of the snout to the tip of the tail, measured dorsally.
2. Snout-vent length (SVL): distance from the tip of the snout to the posterior margin of the cloacal vent, measured dorsally.
3. Head width (HW): maximum distance between the surangular bones at the level of jaw articulation (Fig. 1).
4. Head length (HL): maximum distance between the tip of the snout to the posterior edge of the supraoccipital bone.
5. Neck girth (NG): maximum circumference of the neck, measured between the base of the skull and front legs.
6. Front leg body girth (FBG): circumference of the pectoral region, measured immediately posterior to the front legs.
7. Middle body girth (MBG): maximum circumference of the middle torso.
8. Hind leg body girth (HBG): circumference of the pelvic region, measured immediately anterior to the hind legs.
9. Tail girth (TG): maximum circumference of the tail posterior to the hind legs.
10. Horizontal-ventral scale girth (HVG): tail circumference where the two rows of horizontal tail scutes transition into a single vertical scute row.

## 11. Body mass (BM).

Head length, TL and SVL are used as body length indicators in crocodylian studies (Fukuda et al. 2013), whereas NG, FBG, MBG, HBG, TG and HVG are volumetric indicators of body condition (Zweig et al. 2004). The body measurements we collected during this study are consistent with other crocodylian morphometric studies (Webb and Messel 1978, Montague 1984, Hutton 1987a, Hall and Portier 1994, Platt et al. 2009, Platt et al. 2011), and for comparative purposes with other species we generally followed the statistical approach of Hutton (1987a) and Platt et al (2009, 2011). Cranial morphometrics (HW, HL) were measured with tree calipers for adults ( $\pm 1$  mm) and digital calipers for hatchlings ( $\pm 0.1$ mm). All other body measurements were collected with a fiberglass measuring tape or plastic sewing tape ( $\pm 1$  mm). Non-hatchling crocodiles were weighed in the field with a digital crane scale ( $\pm 0.1$  kg) attached to an adjustable aluminum tripod. Individuals were first stabilized with towing bands and then hoisted with a block and tackle until they were suspended from the ground, allowing accurate mass to be recorded (Fig. 1). The mass of the bands and d-ring attached to the scale were subtracted from the total mass. Hatchling crocodiles were weighed with an Ohaus digital scale ( $\pm 0.1$  g). Non-hatchling *C. niloticus* were sexed by manual probing of the cloacal cavity (Brazaitis 1968).

We used regression analysis to determine predictive relationships between morphometric data (HW, HL, NG, FBG, MBG, HBG, TG, HVG, BM) and body size (TL and SVL), with the former treated as independent variables. For comparison with other crocodylian species, we also regressed the ratio of HL:HW against SVL, and the ratio of HL:TL against TL to examine potential ontogenetic changes in cranial morphology and the consistency of the HL:TL ratio across size classes (Tucker et al. 1996, Platt et al. 2009, 2011).

Similar to other crocodylians, male *C. niloticus* attain greater body sizes than females (Cott 1961, Pooley 1982a, Hutton 1987b). To confirm this quantitatively, we used a t-test to test the hypothesis that adult male SVL is significantly greater than adult female SVL (for free-ranging crocodiles in South Africa both sexes reach maturity at approximately 1400 mm SVL; Combrink 2014). We used a compressed sexual size dimorphism index (SDI) to quantify the degree of sexual size dimorphism between adult males and females (Lovich and Gibbons 1992, Platt et al. 2009, 2011). SDI is a dimensionless number calculated by dividing the mean size of the larger sex by the mean size of the smaller sex and then adding one to this value if males are the larger sex, or subtracting one if females are larger (Lovich and Gibbons 1992). Although SDI can theoretically be based on any measurement or mass, we used SVL given its ubiquity in herpetological studies and in keeping with previous crocodylian studies (Platt et al. 2009, 2011).

We estimated standing crop biomass (total mass of all individuals) for *C. niloticus* at NGR and STL by calculating the mean body mass for subadults and adults using the TL-BM regression equation. This value was then multiplied by the estimated number of individuals for each cohort for each population, and then summed to determine standing crop biomass (Thorbjarnarson 1988, Platt et al. 2009, 2011). The estimated number of individuals for each population was derived from spotlight and aerial census data with correction factors (Calverley 2013, Combrink 2014). Biomass estimates for crocodylians are usually reported as kilogram per kilometer of shoreline (kg/km; Hutton 1987b, Thorbjarnarson 1988, Platt et al. 2009, 2011, Fukuda et al. 2011); we report biomass in both kg/km and kg/ha. Lake St Lucia is approximately 35000 ha at mean lake level (Taylor 2006) with 600 km of shoreline surveyed during crocodile censuses (Combrink 2014). Shoreline length varies over time at the 10117 ha NGR, as it is bordered by two meandering rivers and contains numerous ephemeral floodplain habitats. Taking

into account the boundaries of the reserve and seasonal movement ecology of crocodiles at NGR (Calverley 2013) we established a shoreline length of 35 km for biomass estimates, although this number is likely conservative. Population size for the province of KwaZulu-Natal was aggregated from census data in Champion (2011), Calverley (2013), Combrink et al. (2011), Combrink (2014), and unpublished reports of standardized aerial and spotlight counts.

## RESULTS

We captured 322 *C. niloticus* from STL and NGR for morphometric analyses (Fig. 2). Our sample included 118 hatchlings with a mean TL, SVL and BM ( $\pm$  SD) of  $316 \pm 25$  mm (range = 272 to 414 mm),  $146 \pm 12$  mm (range = 131 to 193 mm), and  $73 \pm 19$  g (range = 17 to 182 g), respectively. For 91 subadults captured, mean TL and SVL were  $1958 \pm 486$  mm (range = 753 to 2702 mm) and  $1001 \pm 258$  (range = 358 to 1392), respectively, and for 113 adults mean TL and SVL were  $3172 \pm 389$  mm (range = 2590 to 4136 mm) and  $1710 \pm 225$  mm (range = 1408 to 2460 mm), respectively. Mean BM for non-hatchlings was  $90.3 \pm 70.6$  kgs (range = 7.0 to 341.0 kgs), hatchlings were excluded from body mass regressions and biomass calculations due to extensive variance. Strong positive allometric relationships were found between body length metrics (TL and SVL) and other morphometrics ( $r^2 \geq 0.91$ ); not all variables were recorded for each individual (Table 1, Figs. 3 & 4). Morphometric regressions were linear, with the exception of the relationship of TL and SVL to BM, which was logarithmic (Table 1, Figs. 3 & 4). For the relationships of HW:HL to SVL and HL:TL to TL we found considerable individual variation among all size classes, although there were slight negative linear trends for both relationships (Figs. 5 & 6). The mean HW:HL ratio was  $1.9 \pm 1.6$  (range = 1.39 to 2.50), and mean HL:TL was  $0.14 \pm 0.005$  (range = 0.12 to 0.16).

We determined the sex of 191 non-hatchling *C. niloticus* (96 female, 95 male). At NGR we captured 35 subadult and 9 adult females, and 23 subadult and 20 adult males. The female-to-male observed sex ratio for all individuals at NGR was almost even (1:0.97) but the observed adult sex ratio was male biased (0.45:1). At STL we captured 15 subadult and 37 adult females, and 5 subadult and 47 adult males, with an even sex ratio (1:1) for all individuals. The observed adult sex ratio was male biased (0.79:1). For both the NGR and STL populations (which represent the majority of *C. niloticus* in KwaZulu-Natal), the observed sex ratio for all individuals was essentially even (1:0.99). Males were larger than females; the largest female SVL was 1782 mm, while 49% of adult males captured were greater than 1800 mm. The mean SVL of adult males was significantly greater than that of adult females ( $t = -6.52$ ,  $df = 111$ ,  $p < 0.001$ ; Fig. 7) and an SDI of 2.19 was calculated for *C. niloticus*.

Equation 18 from Table 1 was used to calculate standing crop biomass of *C. niloticus* at NGR (52640.40 kgs; Table 2) and STL (96867.18 kgs; Table 3). These values were then divided by the respective ha of habitat and km of shoreline for both populations, and reported in relation to other studies (Table 4). We estimate the total *C. niloticus* non-hatchling metapopulation size in KwaZulu-Natal to be approximately 3400 individuals, with STL ( $1005 \pm 137$  individuals; Combrink 2014), NGR ( $846 \pm 59$  individuals; Calverley 2013) and Jozini Dam (273 individuals) accounting for most crocodiles in the province.

## **DISCUSSION**

We did not include an assessment of morphometric variance between STL and NGR in our study because although these two populations are allopatric, there is a documented history in Zululand of *C. niloticus* being transported from source populations and released into foreign ones,

particularly between uMkhuze Game Reserve, NGR, and STL (Pooley 1982a,b). Furthermore, “problem” crocodiles captured by local conservation authorities in peripheral waterbodies outside of protected areas are frequently released at STL, as well as presumed farmed and pet escapees captured as far away as the uMsunduzi River near Durban (unpubl. data). Discussion of the conservation merit of these practices is beyond the scope of this manuscript, but any minor morphological or especially genetic differences found between current Zululand *C. niloticus* populations are almost certainly not a reflection of ecological or evolutionary processes.

Similar to other morphometric studies of *C. porosus* (Webb and Messel 1978), *C. siamensis* (Chentanez et al. 1983), *C. novaeguineae* (Montague 1984), *C. niloticus* (Hutton 1987a), *C. moreletii* (Platt et al. 2009) and *C. acutus* (Platt et al. 2011) we found strong allometric relationships between measures of body length (TL and SVL) and other physical attributes, with greater variance in body form found among larger individuals (Figs. 3 and 4). The considerable individual variation found among all size classes for the relationships of HW:HL, HW:HL to SVL, and HL:TL to TL revealed a significant degree of morphological plasticity for *C. niloticus* in KwaZulu-Natal. While such variability is not unusual for crocodilian hatchlings (Milnes et al. 2001, Murray et al. 2013; Figs. 5 & 6), our study adds to a growing body of literature that suggests there is an inherent high degree of variation between and among *C. niloticus* populations that probably exceeds that of all other crocodilian species (Salem 2011, Percy and Wijtten 2011, Hekkala et al. 2011, Nestler 2012). For example, Nestler (2012) found that based on statistical analyses of just skull characteristics, *C. niloticus* in the Congo River Basin alone showed as much morphological variation as *C. porosus* did across the entirety of its geographic range (the largest among crocodilians). It is therefore likely that *C. niloticus* is actually a cryptic species complex consisting of multiple taxa (Hekkala et al. 2010, 2011), and

the recent elevation of *C. suchus* in West Africa to specific status supports this (Hekkala et al. 2011). Additional morphometric and molecular data for *C. niloticus* across the species' range is urgently needed to delineate potential taxa synonymized under *C. niloticus* and to properly address the region-specific conservation challenges these crocodiles face (Shirley et al. 2014). For southern Africa, the predictive models we present here (Table 1) will be helpful to future studies in assessing *C. niloticus* body length from skulls found in the field, detecting ontogenetic shifts in morphology, and estimating crocodile biomass at population and regional scales.

The neutral sex ratio found among non-hatchlings at both NGR and STL was unexpected. We hypothesized some degree of skewness among males and females simply because we could not capture and account for the sex of every individual in the population. Additionally, sex among *C. niloticus* embryos is determined by temperature (similar to other crocodylians), and the nesting substrate and aspects of the species' nesting ecology differs between the two populations (Calverley 2013, Combrink 2014). The observed even ratio we found for both populations is indicative that our sampling was probably truly random and reflective of the actual sex ratio, as observed deviations from a 1:1 pairing in reptile populations with temperature-sex determination is often due to sampling bias (Mrosovsky and Pieau 1991). However, intraspecific population biases do occur in crocodylians (Thorbjarnarson 1997) and for *C. niloticus* at a higher (colder) elevation at Lake Ngezi in Zimbabwe, Hutton (1987b) found a strongly female-biased sex ratio among all size classes and embryos.

Despite congruity in the overall sex ratio at NGR, the strong male bias we observed among adults is concerning with regard to long-term recruitment in the face of the ongoing *C. niloticus* population decline there (Calverley and Downs 2014a). For STL, Leslie and Spotila (2001) documented the shading effect of invasive trifid weed or paraffin bush (*Chromolaena*

*odorata*) at *C. niloticus* nesting sites and warned that “unless immediate action is taken, a female-biased sex ratio in all nesting areas will result in eventual extirpation of the Nile crocodile from the Lake St. Lucia ecosystem.” Fifteen years on, the sex ratios found in our study do not support this claim, and in fact we found a male-biased adult population. However, this should not diminish the ongoing serious threat of *C. odorata* invasion to suitable nesting habitat at STL, which requires active management in the form of plant removal and other proactive strategies.

The significant sexual size dimorphism we found for *C. niloticus* (Fig. 7), with males obtaining larger body sizes, conforms to the findings of previous research on the species (Hutton 1987a,b) and the normal trend among crocodylians (Webb and Messel 1978, Platt et al. 2009, 2011). Numerous hypotheses have been posited for the crocodylian size differential among sexes but the most likely explanation is that female growth slows at sexual maturity so that resources can be diverted into reproduction, whereas large body size in males confers a fitness advantage in mating competition and territory defense (Platt et al. 2009, 2011). Across species males are approximately 20% larger than females (Platt et al. 2009), and the SDI of 2.19 we calculated for *C. niloticus* is comparable to *C. moreletii* (2.12; Platt et al. 2009) and *C. acutus* (2.10; Platt et al. 2011). From stable isotope analyses, we found no dietary differences between sexes that could be responsible for differences in growth (Chapters 4, 5). For Zululand, the largest female captured in this study was 3.22 m TL, which probably represents the maximum female size for the region. Although male *C. niloticus* reach lengths of 5 m in the Kruger National Park in South Africa (D. Pienaar, pers. comm.), a 4 m male is a very large crocodile for KwaZulu-Natal. For our study at NGR and STL there were only five individuals captured > 4 m, none of which were > 4.2 m. However, at the outset of our research in 2009 two males > 4.2 m (not included in morphometric

analyses) were captured; a 4.30 m male from Jozini Dam and a 4.72 m male from NGR. The latter, “Beauty,” was a relatively famous crocodile in the region known for his enormous body size, severe facial scarring and reputation as a man-eater. Beauty was in noticeably poor body condition at the time of capture and died several months later at Lake Inyamithi in NGR, presumably of old age. From years of *C. niloticus* surveillance and monitoring data in Zululand we find it unlikely that there are currently more than five crocodiles > 4.3 m, and extremely unlikely that there are any individuals > 5.0 m.

Our estimate of biomass for STL (161.45 kg/km) is comparable with other *C. niloticus* populations (Table 4), although it is important to note that given the current severe drought in the region most individuals are now restricted to southern portions of the lake exposed to freshwater inputs (e.g. the Nkazana stream mouth at Catalina Bay) and the 27 km long Narrows channel. In light of the current adult bias at STL (Table 3), the survival of hatchlings to the subadult stage is a critical factor to the stability of the *C. niloticus* population over the next twenty years. For NGR, the biomass of 1504.01 kg/km we found is over four times the amount recorded for any other crocodilian population in the world (Table 4), but this is potentially due to a restocking program for the species in the late 1960s and early 1970s. Unless mitigative management actions are taken in the area, we predict the biomass at NGR to decline in the future due primarily to poaching and the destruction of nesting habitat (Calverley and Downs 2014a). The future of the estimated 3400 *C. niloticus* in KwaZulu-Natal will largely depend on proper crocodile management practice and protection underpinned by continuing demographic studies at STL, NGR and Jozini Dam.

## Acknowledgements

We thank Ricky Taylor and Scotty Kyle for advisory support. The success of this project was reliant on a great number of volunteers that assisted with fieldwork, but we would like to thank Ferdi Myburgh and Mark Robertson in particular for their logistical support and provision of boats. The Ford Wildlife Foundation and the South African Water Research Commission provided vehicle and financial support. We thank for Ezemvelo KwaZulu-Natal Wildlife and the iSimangaliso Wetland Park Authority for hosting this research.

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## Figure legends

**Figure 1.** Researchers taking morphometric measurements of *Crocodylus niloticus* in KwaZulu-Natal, South Africa.

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**Figure 3.** Relationship between total length (TL) and all other morphometric parameters for *Crocodylus niloticus* captured 2009 – 2013 at Lake St Lucia and Ndumo Game Reserve in KwaZulu-Natal, South Africa.

**Figure 4.** Relationship between snout-vent length (SVL) and all other morphometric parameters for *Crocodylus niloticus* captured 2009 – 2013 at Lake St Lucia and Ndumo Game Reserve in KwaZulu-Natal, South Africa.

**Figure 5.** Relationship of the ratio of head length:head width (HL:HW) to snout-vent length (SVL) for *Crocodylus niloticus* (n = 322) at Ndumo Game Reserve and Lake St Lucia, South Africa.

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**Figure 7.** Sexual size dimorphism for adult male (n = 67) and female (n = 46) *Crocodylus niloticus* in KwaZulu-Natal, South Africa quantified as the variance between snout-vent length means ( $t = -6.52$ ,  $df = 111$ ,  $p < 0.001$ ).

## Tables

**Table 1.** Regression equations for predicting total length (TL) and snout-vent length (SVL) in millimeters for *Crocodylus niloticus* from other morphometric features (in mm except for body mass). (HW = head width, HL = head length, NG = neck girth, FBG = front-leg body girth, MBG = mid-body girth, HBG = hind-leg body girth, TG = tail girth, HVG = horizontal-ventral scale girth, BM = body mass (kg)).

Equation no. and predictor X	Estimated value Y	Equation	R <sup>2</sup>	n
1. TL	SVL	$SVL = 0.55(TL) - 35.05$	0.99	322
2. HW	TL	$TL = 12.79(HW) + 90.30$	0.99	322
3. HW	SVL	$SVL = 6.99(HW) + 11.42$	0.99	322
4. HL	TL	$TL = 7.25(HL) - 7.234$	0.99	322
5. HL	SVL	$SVL = 3.96(HL) - 40.07$	0.99	322
6. NG	TL	$TL = 3.01(NG) + 589.92$	0.96	204
7. NG	SVL	$SVL = 1.72(NG) + 227.43$	0.96	204
8. FBG	TL	$TL = 2.71(FBG) + 466.43$	0.98	80
9. FBG	SVL	$SVL = 1.57(FBG) + 145.21$	0.98	80
10. MBG	TL	$TL = 2.24(MBG) + 407.31$	0.98	80
11. MBG	SVL	$SVL = 1.29(MBG) + 117.40$	0.97	80
12. HBG	TL	$TL = 2.89(HBG) + 362.26$	0.97	80
13. HBG	SVL	$SVL = 1.68(HBG) + 84.81$	0.96	80
14. TG	TL	$TL = 3.64(TG) + 354.14$	0.95	204
15. TG	SVL	$SVL = 2.07(TG) + 100.48$	0.94	204
16. HVG	TL	$TL = 8.47(HVG) + 107.01$	0.94	80
17. HVG	SVL	$SVL = 4.84(HVG) - 41.24$	0.92	80
18. BM	TL	$TL = 1722.24(\log BM) - 393.97$	0.91	47
19. BM	SVL	$SVL = 990.81(\log BM) - 344.71$	0.91	47

**Table 2.** Estimated standing crop biomass of non-hatchling *Crocodylus niloticus* for Ndumo Game Reserve (NGR), South Africa. Population size data extrapolated from Calverley (2013). Individual body mass estimated from equation 18 in Table 1. Biomass estimates based on 10117 ha of habitat and 35 km of shoreline available to *C. niloticus* at NGR (see text for rationale).

Size class	Total length (mm)	Mean length (mm)	Predicted body mass (kgs)	Population size	Estimated biomass (kgs)
Subadults	750 – 2700	1955	23.12	440	10172.80
Adults	2701 – 5000	3084	104.60	406	42467.60
Total standing crop biomass (kgs)					52640.40
Biomass/ha (kg/ha)					5.20
Biomass/km shoreline (kg/km)					1504.01

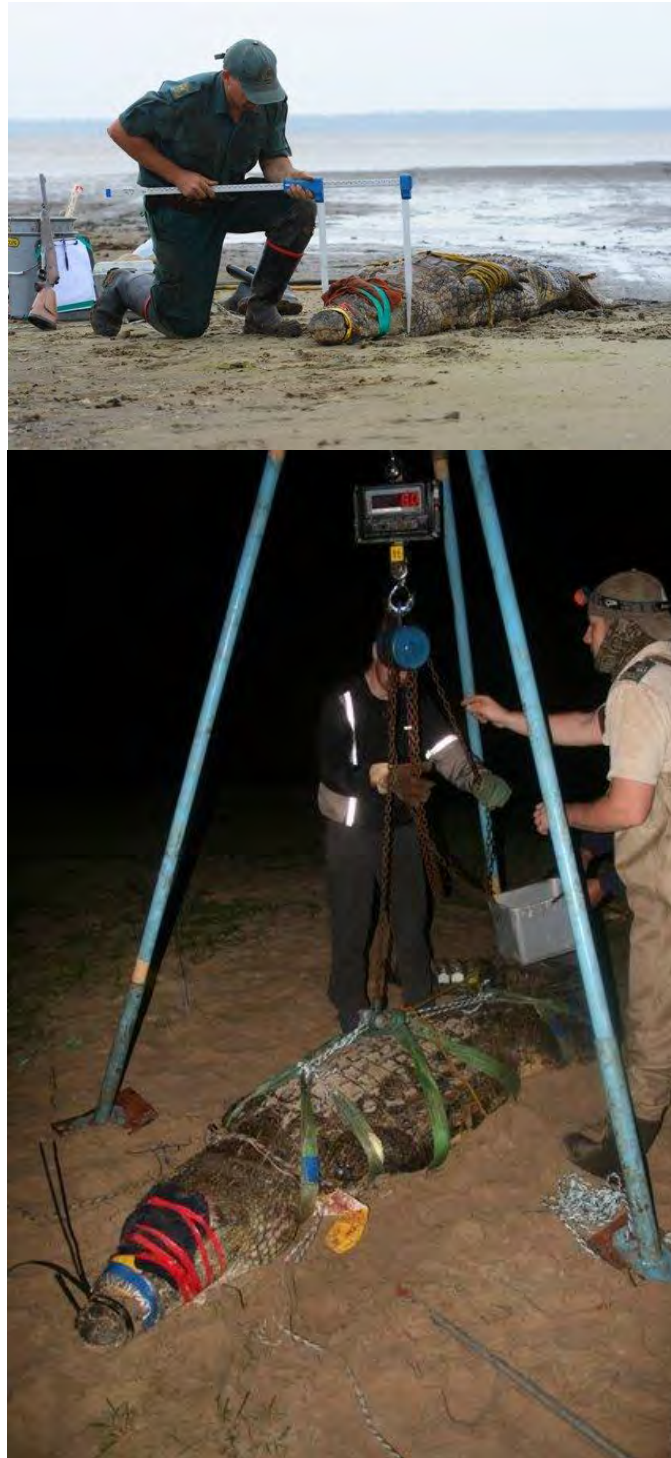
**Table 3.** Estimated standing crop biomass of non-hatchling *Crocodylus niloticus* at Lake St Lucia (STL), South Africa. Population size data extrapolated from Combrink (2014). Individual body mass estimated from equation 18 in Table 1. Biomass estimates based on 35000 ha of habitat at mean lake level (Taylor 2006) and 600 km of shoreline (Combrink 2014) available to *C. niloticus* at STL.

Size class	Total length (mm)	Mean length (mm)	Predicted body mass (kgs)	Population size	Estimated biomass (kgs)
Subadults	750 – 2700	1967	23.50	266	6251.00
Adults	2701 – 5000	3203	122.62	739	90616.18
Total standing crop biomass (kgs)					96867.18
Biomass/ha (kg/ha)					2.77
Biomass/km shoreline (kg/km)					161.45

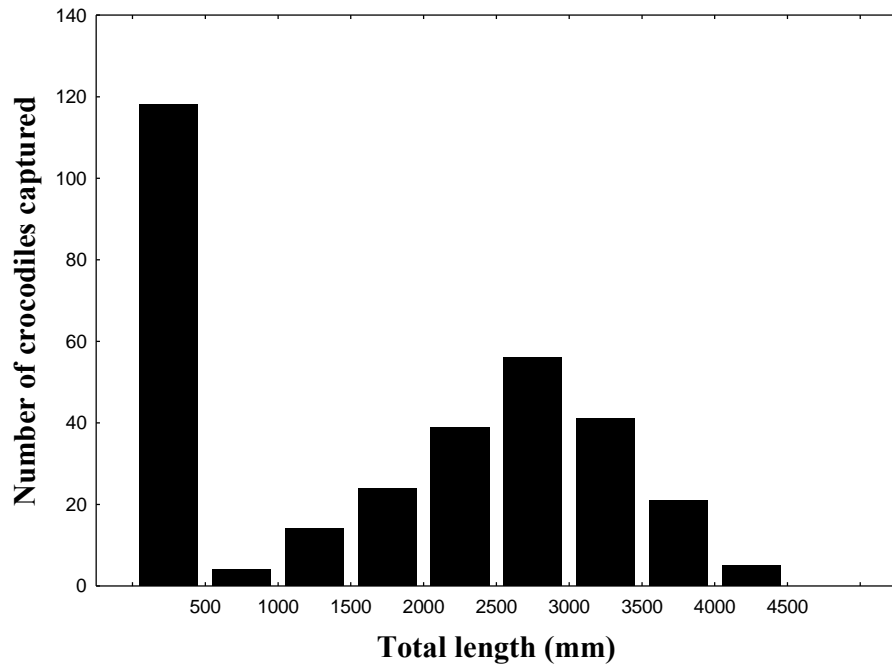
**Table 4.** Standing crop biomass estimates (kg/km of shoreline) for crocodylian populations.

Species and locality	Estimated biomass (kg/km)	Source
<i>Crocodylus acutus</i>		
Lake Etang Saumatre (Haiti)	66.6	Thorbjarnarson (1988)
Turneffe Atoll (Belize)	35.2	Platt et. al (2011)
<i>Crocodylus moreletii</i>		
Gold Button Lagoon (Belize)	187.2	Platt et al. (2009)
<i>Crocodylus niloticus</i>		
Lake Turkana (Kenya)	350.20	Graham (1968)
Upper Murchison Falls (Uganda)	75.3	Parker & Watson (1970)
Lower Murchison Falls (Uganda)	76.03	Parker & Watson (1970)
Lake Ngezi (Zimbabwe)	171.88	Hutton (1987b)
White and Black Umfolozi Rivers (South Africa)	124.97	Blake & Jacobsen (1992)
Olifants River excl. Flag Boshielo (South Africa)	14.74	Kleynhans & Engelbrecht (1993)
Flag Boshielo Dam (South Africa)	142.83	Botha (2006)
Lake St Lucia (South Africa)	161.45	This study
Ndumo Game Reserve (South Africa)	1504.01	This study
<i>Crocodylus porosus</i>		
Blyth River (Australia)	203.6	Fukuda et al. (2011)
Cadell River (Australia)	109.1	Fukuda et al. (2011)
Liverpool River (Australia)	104.5	Fukuda et al. (2011)
South Alligator River (Australia)	304.4	Fukuda et al. (2011)
West Alligator River (Australia)	146.1	Fukuda et al. (2011)
Wildman River (Australia)	370.3	Fukuda et al. (2011)

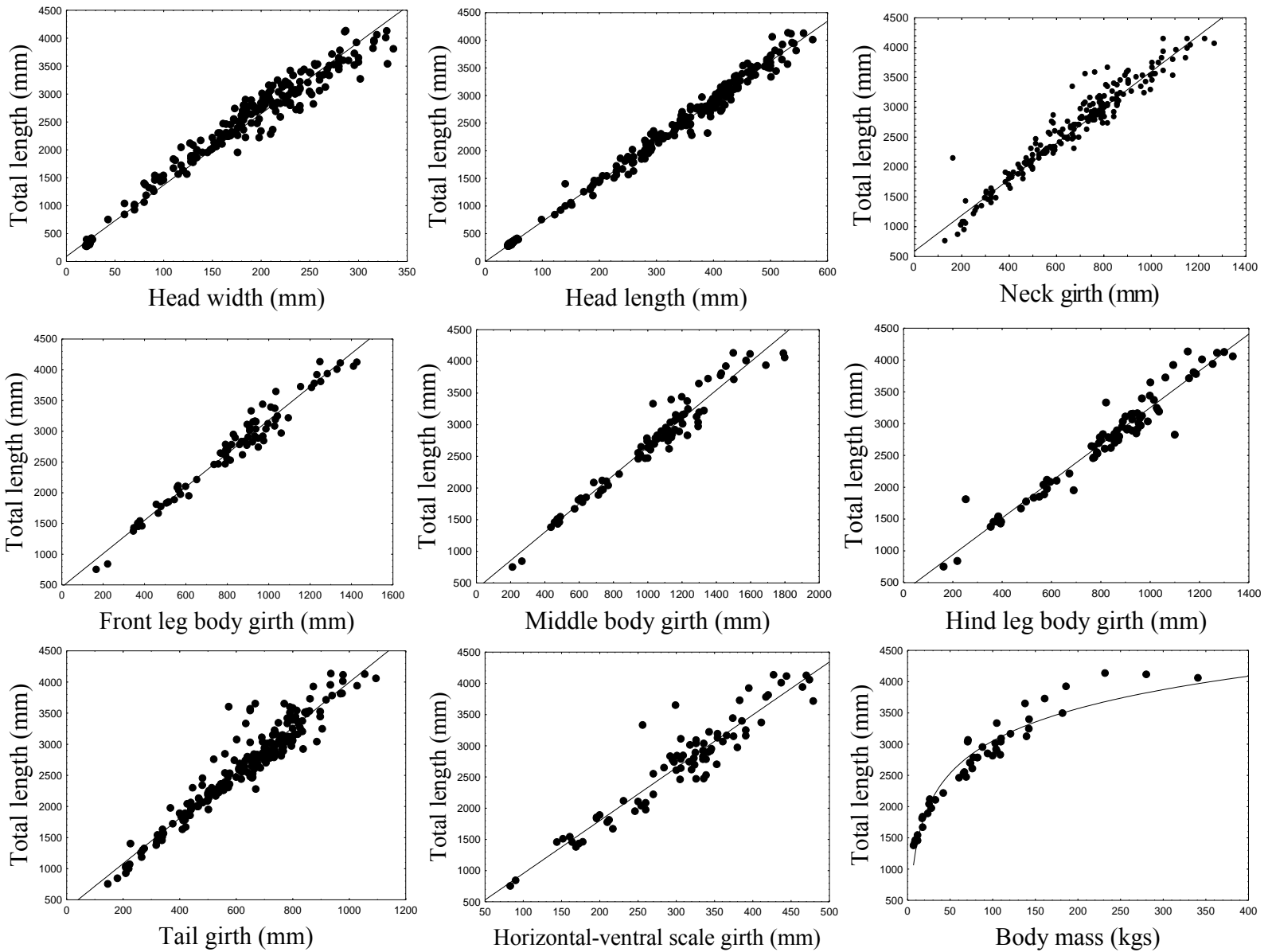
## Figures



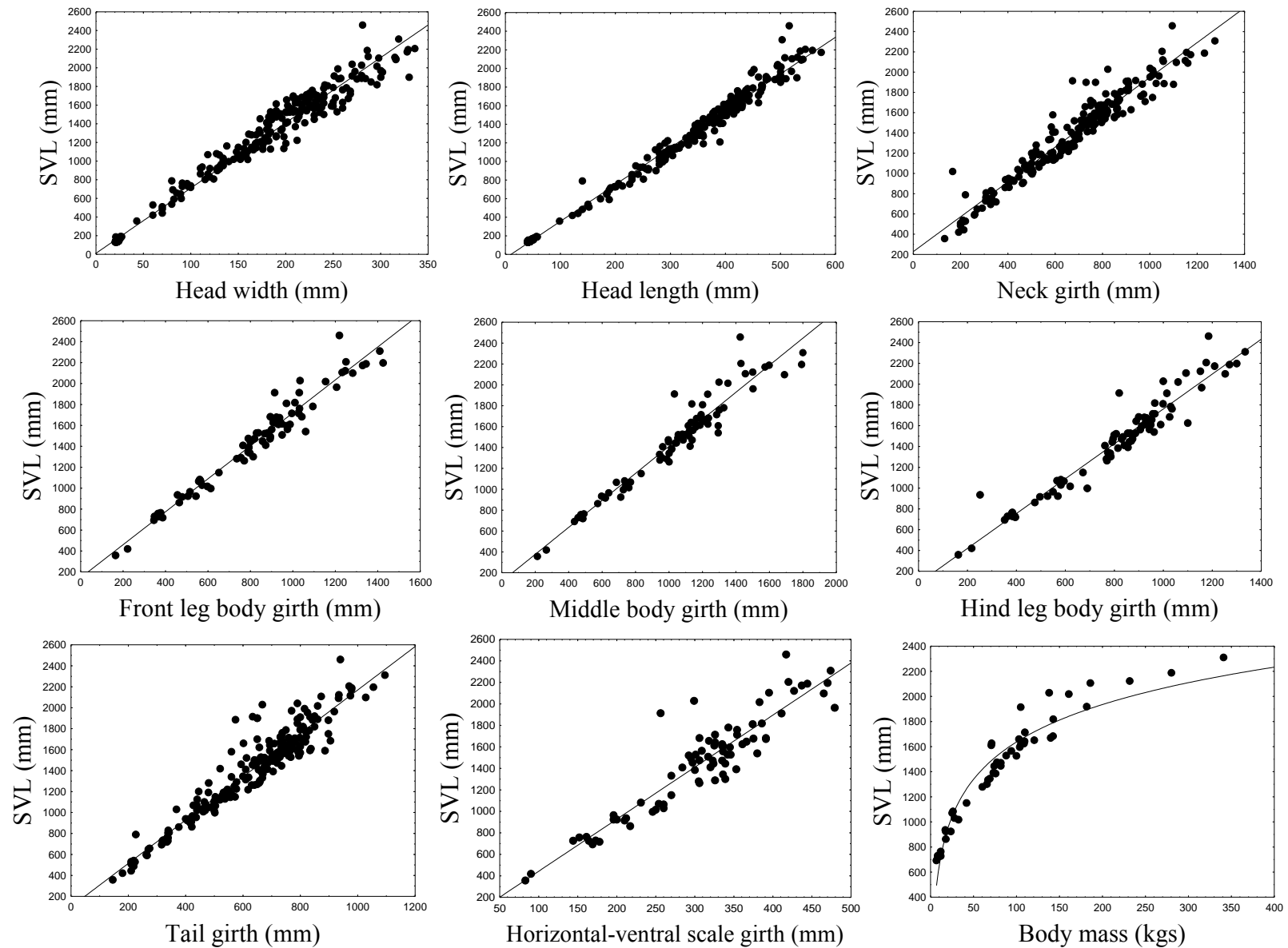
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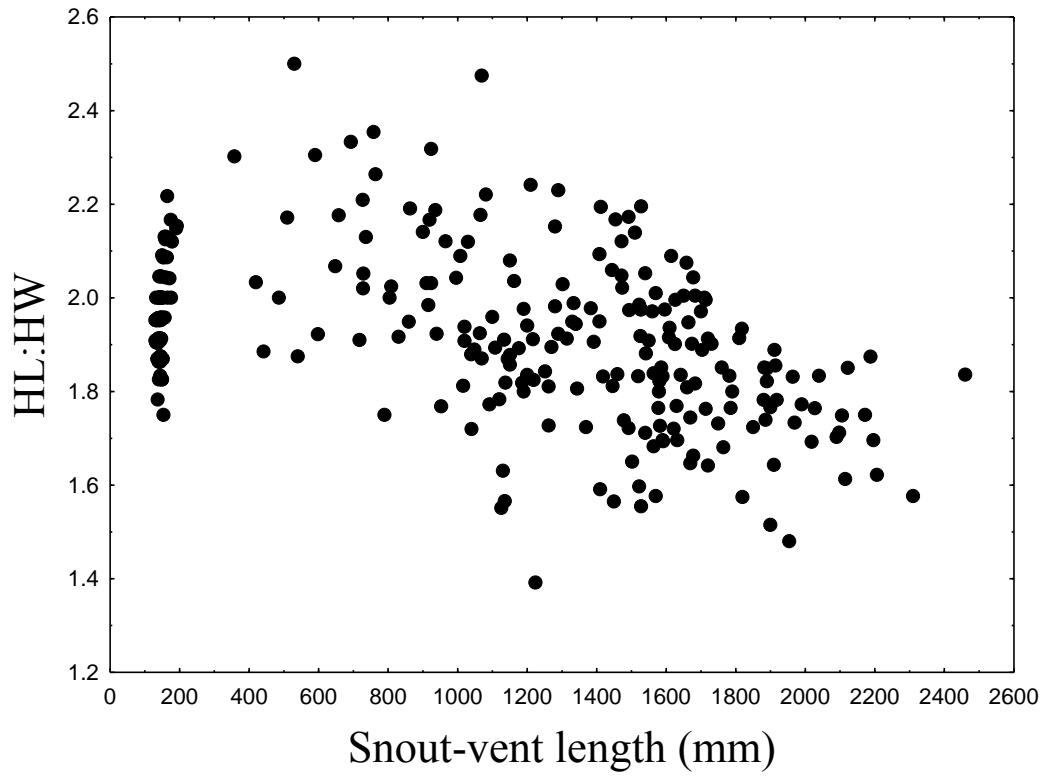
**Figure 2.** Size class distribution of 322 *Crocodylus niloticus* captured 2009 – 2013 at Lake St Lucia and Ndumo Game Reserve in KwaZulu-Natal, South Africa.



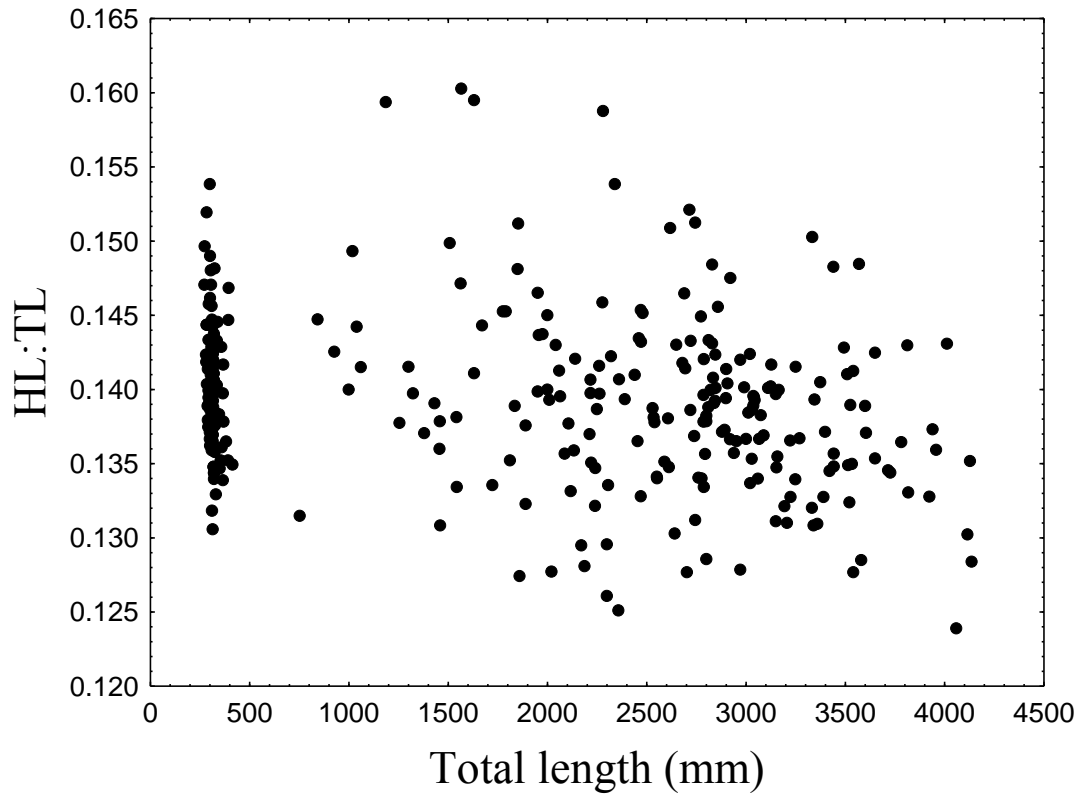
**Figure 3.** Relationship between total length (TL) and all other morphometric parameters for *Crocodylus niloticus* captured 2009 – 2013 at Lake St Lucia and Ndumo Game Reserve in KwaZulu-Natal, South Africa.



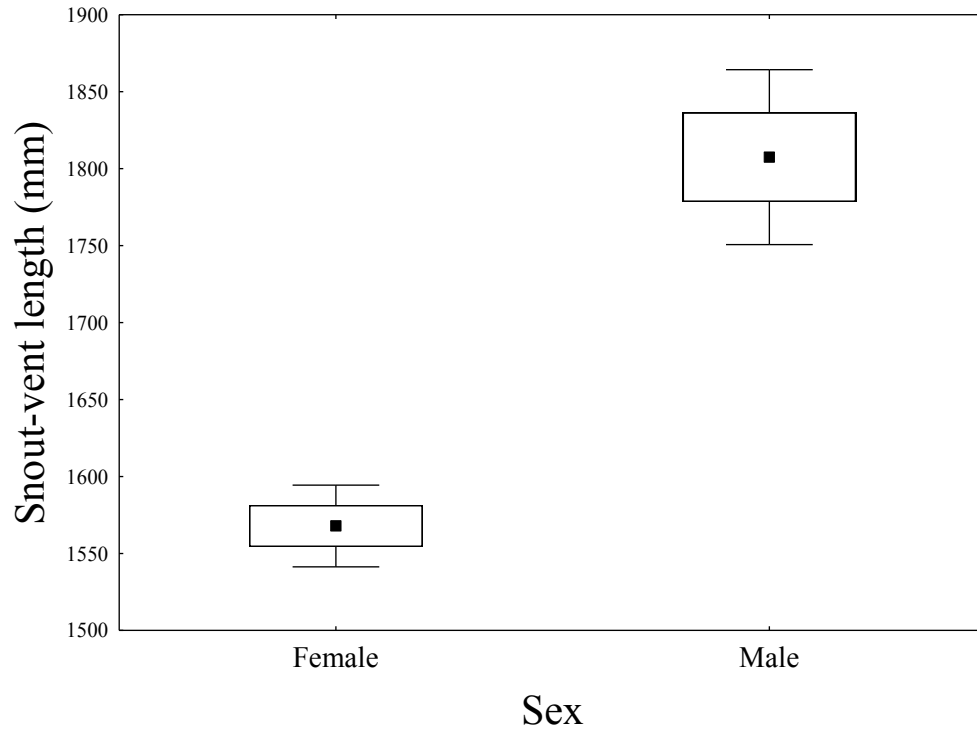
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**Figure 5.** Relationship of the ratio of head length-to-head width (HL:HW) to snout-vent length (SVL) for *Crocodylus niloticus* (n = 322) at Ndumo Game Reserve and Lake St Lucia, South Africa.



**Figure 6.** Relationship of the ratio of head length-to-total length (HL:TL) to total length (TL) for *Crocodylus niloticus* (n = 322) at Ndumo Game Reserve and Lake St Lucia, South Africa.



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## CHAPTER 3

### **Blood lead concentrations in free-ranging Nile crocodiles (*Crocodylus niloticus*) from South Africa**

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Provisionally accepted Ecotoxicology

**Abstract** Generally crocodylians have received little attention with regard to the effects of lead toxicity despite their trophic status as apex, generalist predators that utilize both aquatic and terrestrial habitats, thereby exposing them to a potentially wide range of environmental contaminants. During July – October 2010 we collected whole blood from 34 sub-adult and adult free-ranging Nile crocodiles (*Crocodylus niloticus*) from three separate populations in northeastern South Africa in order to analyze their blood lead concentrations (BPb). Concentrations ranged from below detectability (< 3 µg/dL, n = 8) to 960 µg/dL for an adult male at the Lake St Lucia Estuary. Blood lead concentrations averaged 8.15 µg/dL (SD = 7.47) for females and 98.10 µg/dL (SD = 217.42) for males. Eighteen individuals (53%) had elevated

BPbs ( $\geq 10 \mu\text{g/dL}$ ). We assessed 12 general linear models using Akaike's Information Criterion (AIC) and found no significant statistical effects among the parameters of sex, crocodile size and population sampled. On average, crocodiles had higher BPbs at Lake St Lucia than at Ndumo Game Reserve or Kosi Bay, which we attribute to lead sinker ingestion during normal gastrolith acquisition. No clinical effects of lead toxicosis were observed in these crocodiles, even though the highest concentration ( $960 \mu\text{g/dL}$ ) we report represents the most elevated BPb recorded to date for a free-ranging vertebrate. Although we suggest adult Nile crocodiles are likely tolerant of elevated Pb body burdens, experimental studies on other crocodylian species suggest the BPb levels reported here may have harmful or fatal effects to egg development and hatchling health. In light of recent Nile crocodile nesting declines in South Africa we urge further BPb monitoring and ecotoxicology research on reproductive females and embryos.

**Keywords** Crocodile · Crocodylus · Heavy metal · Lead · Ecotoxicology · Environment · South Africa

## **Introduction**

Lead (Pb) is an inert heavy metal that is ubiquitous in the environment but has no known physiological or metabolic benefit to animals (Buekers et al. 2009). Acute and chronic cases of Pb exposure can be fatal or lead to disorders of the nervous, gastrointestinal, reproductive, and circulatory systems. The epidemiology of Pb poisoning and toxicokinetics of Pb circulation and sequestration in analogous tissues and organs among different species, however, are poorly understood (Fisher et al. 2006; Sparling et al. 2010). Although many environmental contaminants are accumulated within organisms through inhalation or permeation of the

integument, Pb intoxication in wildlife occurs primarily through oral ingestion (Nadjafzadeh et al. 2013). After humans, most of the literature that addresses Pb as a metabolic poison focuses on Aves. The link between birds spanning a wide-variety of taxa and the ingestion of Pb is well documented (Fisher et al. 2006; Lambertucci et al. 2011; Naidoo et al. 2012; and references therein). Recent research has established clinical concentration levels in bird populations due to the direct and indirect ingestion of Pb shot and fishing tackle, which has prompted several countries to ban recreationally-used Pb-based bullets and sinkers (Thomas 1997).

Compared with birds and mammals, ectothermic organisms like reptiles have received relatively little research attention with regard to the potential and actual effects of Pb toxicity caused by polluted environments. Such attention is warranted given that many squamate, testudine and crocodylian species partition their lifespans between aquatic and terrestrial habitats, occupy different trophic positions dependent on size or age, and employ multiple foraging strategies. Their behavior potentially exposes them to a wide range of environmental contaminants due to their complex life histories (Sparling et al. 2010).

As long-lived vertebrates and apex predators, crocodylians (gharials, caimans, alligators and crocodiles) in particular are excellent study subjects for understanding environmental and ecosystem health (Campbell 2003; Milnes and Guillette 2008). Multiple studies (Twining et al. 1999; Jeffree et al. 2001; Rainwater et al. 2007) report background or elevated Pb concentrations in the flesh and osteoderms of several species in the wild, but information about the clinical symptoms and reproductive effects of Pb toxicosis is mainly limited to studies on captive crocodylians fed meat contaminated with Pb shot (Camus et al. 1998; Hammerton et al. 2003; Lance et al. 2006). One experimental study of four juvenile saltwater crocodiles (*Crocodylus porosus*) dosed with Pb shot suggested a half-life for Pb in blood of about 3.4 days (Hammerton

et al. 2003). Quantifying Pb body burdens and making relevant comparisons between tissues and species has been hindered by various non-standardized documentations of Pb concentrations using a variety of ratios (e.g.  $\mu\text{g/g}$ ,  $\text{ng/g}$ , ppm) reported in wet, dry or unspecified masses. Clinical studies of Pb intoxication in living humans, domestic animals and wildlife are standardly reported in  $\mu\text{g/dL}$  from whole blood samples because Pb is mainly present in erythrocyte (red blood) cells.

In Africa, Almi et al. (2005) found hepatic Pb concentrations ranging from 0.71 – 17  $\mu\text{g/g}$  and renal concentrations ranging from 0.07 -2.2  $\mu\text{g/g}$  from tissue samples harvested from nine Nile crocodiles (*Crocodylus niloticus*) in the Kafue and Luangwa Rivers in Zambia. Swanepoel et al. (2000) noted comparable values in Pb concentrations from kidney and liver tissues collected from 15 Nile crocodiles in the Kruger National Park, South Africa, with the highest concentration (19.85  $\mu\text{g/g}$ ) occurring in the liver of an individual from the Olifants River. The respective river systems in the two studies are associated with mining operations and metal pollution along sections of their reaches. Potential biomagnification of anthropogenically introduced Pb through the food chain was suggested by the authors as the cause of elevated Pb levels in the crocodile tissues. For Nile crocodiles the background, subclinical and clinical Pb concentrations in blood have not been recorded and Pb blood kinetics are not understood.

In 2009-10 approximately a dozen severely emaciated Nile crocodiles were observed during field capture excursions and aerial and boat surveys at Lake St Lucia in the iSimangaliso Wetland Park (a UNESCO World Heritage Site), South Africa. Emaciated Nile crocodiles had protruding scapulae and spinal columns, and open abscesses where the anterior gastralia and sternum contact the substrate (indicating long periods of immobility) (Warner et al. unpublished data). Two individuals in very poor condition were euthanized and elevated Pb concentrations

were found in liver tissue and blood plasma in addition to fishing sinkers found in the stomachs (Warner et al. unpublished data). Consequently a program of Pb surveillance in the whole blood of living Nile crocodiles at three populations in northeastern South Africa was initiated. We predicted elevated Pb concentrations in Nile crocodiles at Lake St Lucia compared with the other two populations. We examine through statistical modelling if BPb concentration is related to sex or body size, and discuss the ramifications of Pb ingestion and tolerance as it potentially relates to reproduction and nesting.

## **Methods**

### **Study animals**

The Nile crocodile is an apex predator widespread throughout many aquatic ecosystems in sub-Saharan Africa. In South Africa the species is IUCN red-listed as Vulnerable (Marais 2014), with remaining viable populations restricted to protected areas and waterbodies under some degree of conservation management. There are recent declines in major populations (Kruger National Park, Flag Boshielo Dam, Loskop Dam, Ndumo Game Reserve, iSimangaliso Wetland Park) due to poaching and indiscriminate killing (Calverley and Downs 2014a), loss of nesting habitat (Combrink et al. 2011) and environmental degradation (Botha et al. 2011; Ferreira and Pienaar 2011). These trends are concerning not only with regard to the long-term persistence of the species but also for the ecological integrity and environmental health of aquatic ecosystems in northeastern South Africa (Combrink et al. 2013).

## **Sampling sites**

We collected blood samples from individuals at three allopatric Nile crocodile populations in the province of KwaZulu-Natal, South Africa. The 10,000 ha Ndumo Game Reserve (MGRS 36JVR3036825394) occurs inland at the western edge of the Mozambique Coastal Plain. The Usuthu and Phongola Rivers form the northern and eastern boundaries of the reserve, respectively. During the rainy season (Nov – Mar) up to 40% of the reserve may be inundated, including 12 permanent and semi-permanent pans that are critical habitats year-round for the approximately 900 crocodiles in the area (Calverley and Downs 2014b). Anthropogenic pressures and habitat loss have largely extirpated the species from the greater floodplain outside the reserve (Calverley and Downs 2014a).

East of Ndumo Game Reserve (50 km) is Kosi Bay (MGRS 36JVR8210609928) which sits adjacent to the Indian Ocean as the northernmost component of the 328,000 ha iSimangaliso Wetland Park, a UNESCO World Heritage Site. The ecosystem consists of an estuary mouth that flows into a linear series of four lakes on a salinity gradient interconnected by narrow channels. Legal mass-scale fishtrapping by local Tsonga people and illegal gillnets are significant threats to Nile crocodiles (Kyle 1999) and aerial and boat spotlight surveys show Kosi Bay currently harbors < 20 crocodiles (Warner et al. unpublished data).

South of Kosi Bay (down the coast 120 km), within the iSimangaliso Wetland Park, is the St Lucia estuarine system (MGRS 36JVP4779196119) known as Lake St Lucia. At 67 km in total length, the main lake basin (6 km wide at capacity) is connected to the ocean via a 27 km long “Narrows” channel. Lake St Lucia is a highly dynamic environment over space and time with cyclical, but often unpredictable annual fluctuations between drought and high rainfall (Stretch et al. 2013). Inputs of freshwater from five feeder rivers and seeps on the eastern lake

edge are critical components in the feeding, nesting and movement ecology of Nile crocodiles (~1,000 individuals) at Lake St Lucia (Combrink 2014). Due to a decade-long drought, at the time of our sampling in 2010 the estuary mouth was closed, there were limited freshwater inputs, and significant sections of the lake were exposed due to evaporation.

Recreational (line and tackle) fishing is permitted at Kosi Bay but prohibited at Ndumo Game Reserve. Lake St Lucia historically and up to present is a major regional fishing destination with most angling activities confined to the estuary mouth area and southern portion of the Narrows.

### **Crocodile capture and blood collection**

We captured Nile crocodiles from boats and/or on foot during both nocturnal and diurnal hours dependent on environmental conditions and researcher accessibility to crocodile habitat under permit from Ezemvelo KZN Wildlife and ethics permission from the University of KwaZulu-Natal. Crocodiles were secured using a variety of noosing snares, ropes, hooks and traps according to standard operating procedure (Combrink et al. 2012). Once an individual was safely restrained, blood was collected immediately before body measurements and tagging activities were undertaken. Blood for Pb analysis was drawn from the post-occipital spinal venous sinus using a 20 ml syringe with a 18G needle (Myburgh et al. 2014). Either a 1.5” hypodermic needle or 3.5” spinal needle (Terumo Corporation, Tokyo, Japan) was used depending on the body size and/or neck fat of individual crocodiles. The blood samples were deposited into spray-coated K<sup>2</sup>EDTA 6 ml plastic tubes (BD Vacutainer®), slowly inverted to mix contents, and refrigerated whole (not centrifuged) at 1.6°C until delivered for analysis 1 – 7 days post-collection. We determined sex for each crocodile by inserting a finger into the cloaca and palpating for the

presence or absence of the penis (Brazaitis 1968). Snout-to-vent length (SVL) was measured dorsally as the distance from the tip of the snout to the posterior end of the cloacal opening for each individual. For crocodylians, SVL is the standard measurement of body length because their tails can be partially missing, damaged or deformed. As part of an ancillary study of *C. niloticus* demographics and morphology a suite of ten additional morphometric and body condition measurements were also collected (Warner 2016).

### **Clinical analyses**

Nile crocodile blood samples were analyzed for their Pb concentration (BPb) by Ampath Laboratories, The Bay Hospital, Richards Bay, South Africa. The determination of BPb was performed using a Graphite Furnace Atomic Absorption Spectrometer (GFAAS, Varian AAZ 220). Standard blood processing and AAS operational parameters were observed according to Zinterhofer et al. (1971) and Perkin-Elmer (1996). Blood aliquot analysis was performed twice for each sample to ensure BPb validity. Detection limit of BPb for GFAAS was 3 µg/dL. As such, some samples that registered as 3 µg/dL (Table 1) may have in fact represented BPbs slightly less than this value, but were demarcated as 3 µg/dL for statistical modelling.

### **Statistical methods**

All analyses were performed using R version 2.12.1 (R Development Core Team, 2011). Blood Pb concentration and SVL values were first z-transformed as to be on equivalent scales. We then assessed 12 General Linear Models using Akaike's Information Criterion (AIC), an information theoretic approach (Burnham and Anderson 2002) which is described as

$$AIC = -2(\log - likelihood) + 2K$$

where  $K$  is the number of estimated parameters included in the model (i.e., SVL + sex + the intercept). The log-likelihood was generated from the statistical output and reflected the overall fit of the model (smaller values indicate worse fit). Because of our relative small sample size for each population, the second-order Akaike Information Criterion (AICc) was used where  $n$  is the sample size

$$AICc = -2(\log - likelihood) + 2K + \frac{2K(K + 1)}{(n - K - 1)}$$

The 12 candidate set included a null model (intercept only), all main effects models (sex, SVL, population), all 2-way models with and without interactions, and a global model which included all main effects, all 2-way interactions and all 3-way interactions. If model support was low, we used model averaging on the 95% confidence set of models (Akaike Weights summing to 0.95).

## Results

During July – October 2010 we collected whole blood for Pb analysis from 34 subadult and adult Nile crocodiles (Kosi Bay:  $n = 5$ , Ndumo Game Reserve:  $n = 11$ , Lake St Lucia:  $n = 18$ ; Table 1). Concentrations ranged from below detectability ( $< 3 \mu\text{g/dL}$ ,  $n = 8$ ) to  $960 \mu\text{g/dL}$  (croc no. 111, Lake St Lucia). For Lake St Lucia, 11 individuals had Pb blood concentrations  $> 20 \mu\text{g/dL}$  while Ndumo Game Reserve and Kosi Bay collectively had no sample  $> 18 \mu\text{g/dL}$  (Table 1). Blood Pb concentrations averaged  $8.15 \mu\text{g/dL}$  ( $SD = 7.47$ ) for females and  $98.10 \mu\text{g/dL}$  ( $SD = 217.42$ ) for males (also see boxplots with SEM in Fig. 1).

Multiple competing models showed that no single model explained a significant amount of the statistical variance (Table 2), and model averaging revealed that data slopes were not

significantly different from zero for the 95% confidence set of models (Table 3). Statistical variance found for Nile crocodile BPb values among the three populations is presented in Fig. 2. The relationship between male and female crocodile size (SVL) and blood lead concentration is plotted in Fig. 3.

## **Discussion**

Our study is the first to report whole blood Pb concentrations (BPb) for live free-ranging Nile crocodiles. We assessed 12 general linear models using Akaike's Information Criterion (AIC) and found no significant effects among the parameters of sex, crocodile size and population sampled. Given the robustness of the AIC analysis and our relative small sample size, the lack of significant statistical effects was not surprising despite 53% (18 individuals) of samples registering elevated BPb ( $\geq 10 \mu\text{g/dL}$ ). The extensive range of BPbs without quantitative clustering among groups hindered the selection of a successful model using AIC. The male crocodile from Lake St Lucia with a BPb of 960  $\mu\text{g/dL}$  is to our knowledge the highest recorded BPb for a free-ranging vertebrate species. For clinical reference, in falconiformes and waterfowl BPb levels  $> 100 \mu\text{g/dL}$  are considered toxic (Franson 1996, Pain 1996), while concentrations greater than 60 to 80  $\mu\text{g/dL}$  represent toxicosis in mammals (Ma 1996). The BPb threshold of concern for human children as set by the US Centers for Disease Control and Prevention is 5  $\mu\text{g/dL}$ .

We attribute the elevated levels observed at Lake St Lucia to the widespread and longstanding recreational use of Pb fishing sinkers (pers. obs.), albeit largely by process of elimination of other potential sources. The geology of the area consists of quaternary dune sands and sandy coastal sediment deposits and lacks mineral deposits containing Pb ore (Botha et al.

2013). As a World Heritage Site, hunting and recreational shooting are prohibited and there are no industrial or mining waste inputs into the estuary ecosystem. The major cause of sedimentation deposited by freshwater rivers is agricultural runoff from the numerous sugarcane fields in the greater coastal plain (Stretch et al. 2013). Furthermore, during the course of this study from 2009 – 14 all crocodiles (n = 8) examined that died of natural causes, incidental or intentional killing were found to have multiple fishing sinkers in their stomach cavities (Fig. 3). Unfortunately, the logistics of working in a remote location prohibited us from radiographing captured crocodiles for the presence of stomach Pb.

While secondary ingestion of Pb fishing tackle through consumption of fish that contain sinkers cannot be ruled out, crocodiles are likely swallowing Pb sinkers through normal gastrolith acquisition and perhaps occasional theft of fishing bait. The function of crocodile gastroliths (stomach stones) is not fully known but they likely help serve as ballast and/or grind down food items (Wallace and Leslie 2008). The small crocodilian pylorus prevents gastroliths from passing into the duodenum and being excreted as waste (Chiasson 1962), but small metal fragments that are ingested but not absorbed may be eliminated through the feces (Xu et al. 2006). The rate of retention and erosion of Pb solids in the crocodilian gastrointestinal tract is not well understood. However it is clear that low stomach pH aids the dissolution of Pb (Hammerton et al. 2003), which after entering systemic circulation is carried by red blood cells and sequestered into organs (especially the kidney and liver), soft tissues, and ultimately bone, which bears 90% of the body burden (Hammerton et al. 2003, Grillitsch and Schiesari 2010).

In a controlled experiment where three captive juvenile saltwater crocodiles (*Crocodylus porosus*) were fed five Pb shot each (mean mass  $377 \pm 17$  mg) BPbs increased 10 – 20 fold during the first seven days after exposure and attained steady-equilibrium after 20 days at 278 –

363  $\mu\text{g}/\text{dL}$ . A fourth juvenile fed ten Pb shot had an upper plateau BPb of  $514 \pm 33 \mu\text{g}/\text{dL}$  at 85 to 140 days after exposure (Hammerton et al. 2003). If similar Pb blood kinetics hold for Nile crocodiles, the highly elevated BPbs (e.g. 126, 280, 344, 960  $\mu\text{g}/\text{dL}$ ) we found in individuals at Lake St Lucia could reasonably be explained by either single or multiple events of Pb sinker ingestion in the weeks or months prior to capture. While our results show possible Pb intoxication we cannot adduce clinical significance. At time of capture none of the Nile crocodiles we sampled had any observable or quantifiable signs of physical attrition, deformation, or abnormal behavior compared to other individuals captured and not sampled for BPb throughout the course of the study (Warner 2016). As part of concurrent ecological research, the individual with the highest BPb (no. 111; Table 1) was fitted with a GPS-GSM transmitter and exhibited normal movements within his home range for the duration of the study (Combrink 2014). Our findings therefore support the previous hypothesis of Cook et al. (1988) and Hammerton et al. (2003) that crocodilians likely possess a high degree of resistance to Pb toxicity. That notwithstanding, some of the BPbs reported in this study are concerning for a largely pristine, protected environment.

In light of recent nesting declines at STL (Combrink 2014), breeding females and embryos merit a more intensive research focus with regard to environmental contaminants, especially Pb. Although adult *C. niloticus* may be able to tolerate elevated Pb body burdens, there is evidence that female crocodilians shunt Pb into developing embryos, leading to eggshell thinning, deformities or death even at low concentrations (Heinz et al. 1991; Manolis et al. 2002; Lance et al. 2006). There is one recorded instance of elevated Pb concentrations in Nile crocodile eggs but the cause and health of the hatchlings is unknown (Phelps et al. 1986). The sequestration of contaminants into gonadal tissues and the significance of intergenerational

transfer in reducing maternal body burden require further study in crocodylians. The deleterious effects of Pb on reproductive success, egg production, eggshell thinning, and embryo health has been more extensively documented in Aves (Fisher et al. 2006 and refs. therein, Watson et al. 2009), and the toxicokinetic processes between mother and oocyte are likely similar among crocodiles (Grillitsch and Schiesari 2010).

Sex related differences in BPb were 10-fold in our study, with males registering higher concentrations (Fig. 1). This discrepancy may not be upheld with a larger dataset, but we also cannot rule out the possibility that low BPbs observed among breeding-aged females could be attributed to mothers passing Pb body burdens off to their embryos. The presence of BPb and its ramifications for other species at Lake St Lucia have not been investigated. Such work is urgently needed especially given the longstanding severe drought in the region. Symptoms of physiological or nutritional stress may be exacerbated by ingested Pb in animals with lesser degrees of tolerance, and drought conditions have been shown to enhance the negative effects of heavy metals on wildlife (Rumbold et al. 2002). Future Nile crocodile research efforts in Africa would benefit from including BPb monitoring as part of standard protocol, especially in habitats where recreational fishing occurs.

**Acknowledgments** We thank R. Taylor, P. Calverley, G. Champion, F. Myburgh, S. Kyle and family, the many volunteers who assisted with fieldwork, M. Dreslik for statistical guidance, Ezemvelo KwaZulu-Natal Wildlife and the iSimangaliso Wetland Park Authority. The Ford Wildlife Foundation and the South African Water Research Commission are thanked for their vehicle/financial support.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Statement of Human and Animal Rights** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

**Informed Consent** There were no human subjects, thus informed consent is not applicable for this study.

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## Figure legends

**Fig. 1** For 34 Nile crocodiles sampled in South Africa mean lead concentration ( $\mu\text{g/dL}$ ) in whole blood was higher for males (98.10) than females (8.15), but not statistically significant ( $t = -1.48$ ,  $df = 32$ ,  $p = 0.15$ ). Boxes are mean  $\pm$  SE, error bars are mean  $\pm$  SE(1.96).

**Fig. 2** Non-significant statistical effects were found for Nile crocodile blood lead concentration (BPb) values among three allopatric populations in KwaZulu-Natal, South Africa (Abbreviations are KB = Kosi Bay, NGR = Ndumo Game Reserve, and STL = Lake St Lucia). Error bars are mean  $\pm$  SD.

**Fig. 3** Male and female wild Nile crocodile snout-to-vent length (SVL) (bold lines) plotted against their blood lead concentrations showed overlapping confidence intervals (dashed lines).

**Fig. 4** Stomach contents from a large (4.1m total length) adult male Nile crocodile from the Lake St Lucia Estuary, iSimangaliso Wetland Park, South Africa: four lead fishing sinkers, broken glass, a spark plug, assorted stone gastroliths (Combrink 2014).

## Tables

**Table 1** Whole blood lead concentrations from wild Nile crocodiles in KwaZulu-Natal, South Africa. (Abbreviations are as follows: KB = Kosi Bay, NGR = Ndumo Game Reserve, and the following from Lake St Lucia where STL = St Lucia Estuary, SC = Sihadla Channel, 4L = Fourth Lake, IP = Inyamithi Pan, NC = Narrows Channel, PC = Potter's Channel, NS = Nkazana Stream, MR = Mphate River. SVL is snout-to-vent length).

<b>Croc ID</b>	<b>Blood Pb conc. (µg/dL)</b>	<b>Population</b>	<b>Locality</b>	<b>Date (2010)</b>	<b>SVL (mm)</b>	<b>Sex</b>
3	3	KB	SC	07-02	495	F
4	10	KB	SC	07-02	1060	M
6	8	KB	SC	07-02	898	M
7	3	KB	4L	07-03	693	F
8	8	KB	4L	07-03	681	M
86	8	NGR	IP	06-21	1760	F
87	16	NGR	IP	06-21	996	M
88	3	NGR	IP	06-21	758	F
90	3	NGR	IP	06-23	924	M
91	10	NGR	IP	06-23	1344	M
92	6	NGR	IP	06-21	916	F
94	3	NGR	IP	06-23	1384	F
95	3	NGR	IP	06-23	1150	F
96	6	NGR	IP	06-24	1964	M
98	3	NGR	IP	06-24	1030	M
99	18	NGR	IP	06-24	1334	M
111	960	STL	NC	10-22	1527	M
504	20	STL	LBS	06-09	2310	M
505	4	STL	LBS	06-19	727	M
506	3	STL	LBS	06-19	764	F
508	26	STL	NS	07-21	1810	M
509	344	STL	NC	08-21	1678	M
510	18	STL	NC	10-22	1642	F
511	8	STL	PC	10-07	863	F
512	32	STL	NS	10-08	1818	M
513	16	STL	NS	10-08	1684	M
514	8	STL	MR	10-23	1392	F
515	12	STL	MR	10-23	1610	F
516	280	STL	MR	10-25	1626	M
517	28	STL	MR	10-25	1658	F
518	64	STL	MR	10-26	1444	M
519	42	STL	MR	10-26	1474	M
520	126	STL	MR	10-27	2122	M
521	64	STL	MR	10-29	2188	M

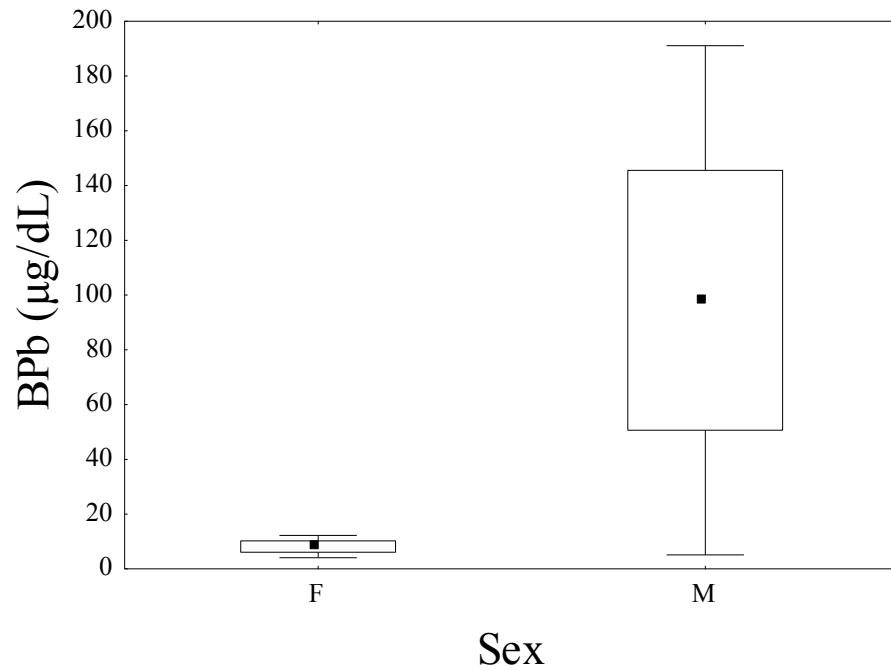
**Table 2** Twelve models as determined by AIC for General Linear Model analysis of Nile crocodile whole blood lead concentration ( $n = 34$ ) sampled at 3 northeastern South African populations in 2010. (Abbrevitions are as follows:  $K$  is the number of parameters, LL is log-likelihood, AICc is second-order Akaike Information Criterion,  $w_i$  is the Akaike weight, and  $\sum w_i$  is the cumulative sum of the Akaike weights. For the models, zSVL is z-transformed snout-to-vent length and P is population).

Rank	Model	$K$	-2LL	AICc	$\Delta$ AICc	$w_i$	$\sum w_i$
1	Null	2	-47.736	99.860	0.000	0.250	0.250
2	Sex	3	-46.608	100.015	0.155	0.232	0.482
3	zSVL	3	-47.003	100.806	0.946	0.156	0.638
4	P	4	-46.014	101.408	1.548	0.116	0.754
5	zSVL + Sex	4	-46.276	101.932	2.072	0.089	0.843
6	Sex + P	5	-45.063	102.270	2.410	0.075	0.918
7	zSVL + P	5	-45.953	104.048	4.188	0.031	0.949
8	zSVL + Sex	5	-46.177	104.497	4.637	0.025	0.973
	Int						
9	zSVL + P + Sex	6	-45.058	105.227	5.367	0.017	0.990
10	Sex + P Int	7	-44.199	106.707	6.847	0.008	0.999
11	zSVL + P Int	7	-45.930	110.168	10.308	0.001	1.000
12	Global	13	-44.031	132.263	32.403	0.000	1.000

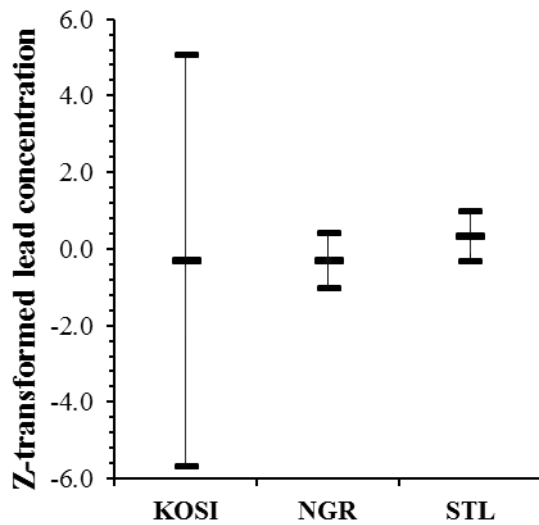
**Table 3** Average parameters for the 95% confidence set of models determined by AIC for wild Nile crocodile whole blood lead analysis with all non-significant effects.

<b>Parameter</b>	<b>Beta</b>	<b>SE</b>	<b>Lower C.I.</b>	<b>Upper C.I.</b>	<b>Significance</b>
(Intercept)	-0.200	0.351	-0.889	0.489	N.S.
zSVL	0.158	0.204	-0.242	0.559	N.S.
LeadSexMale	0.481	0.354	-0.214	1.176	N.S.
LeadPopNGR	0.003	0.535	-1.045	1.051	N.S.
LeadPopSTL	0.589	0.515	-0.421	1.598	N.S.

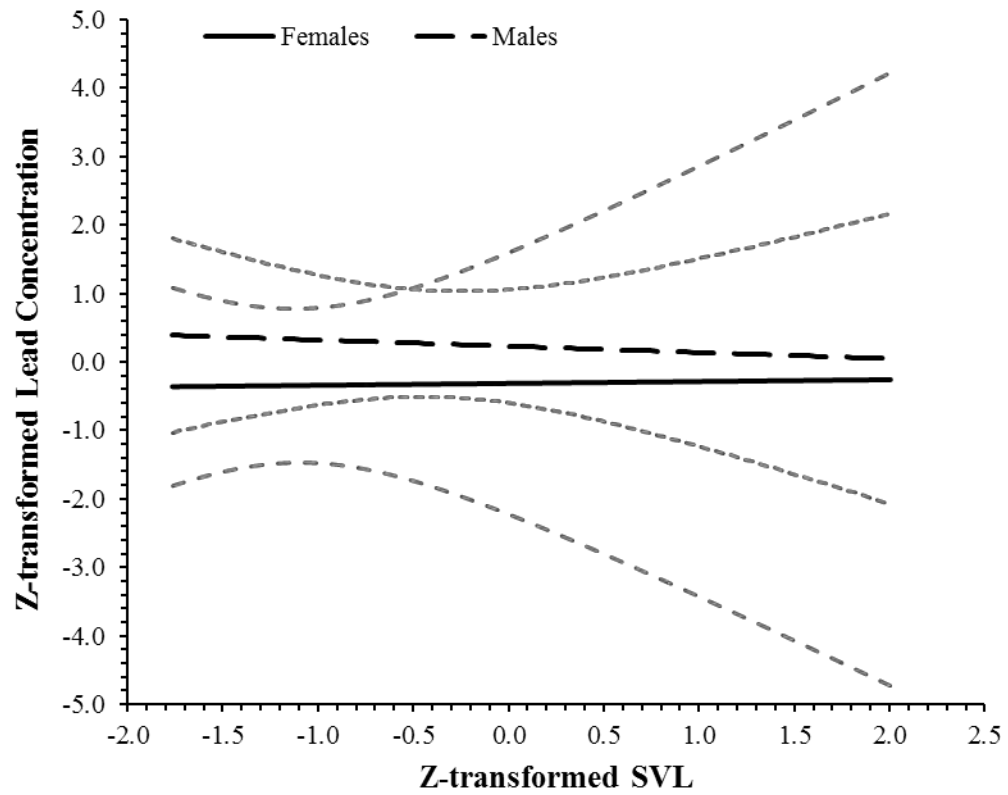
## Figures



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**Fig. 4** Stomach contents from a large (4.10 m total length) adult male Nile crocodile from the Lake St Lucia Estuary, iSimangaliso Wetland Park, South Africa: four lead fishing sinkers, broken glass, a spark plug, assorted stone gastroliths (Combrink 2014).

## CHAPTER 4

### **Stable isotope values ( $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ ) in blood plasma and scute tissue from Nile crocodiles (*Crocodylus niloticus*) at freshwater and estuarine habitats in South Africa**

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#### **Abstract**

Nile crocodiles (*Crocodylus niloticus*) are apex predators that occur in many African aquatic ecosystems but their feeding ecology is poorly understood. We conducted stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis of blood plasma and scute collagen from 38 crocodiles

resident to freshwater and estuarine habitats in KwaZulu-Natal, South Africa. Adult dietary  $\delta^{15}\text{N}$  values were higher than those for sub-adults, but  $\delta^{15}\text{N}$  values between populations and sexes were not significantly different. The high variance among  $\delta^{15}\text{N}$  values in plasma and scutes suggests Nile crocodiles at the sites we sampled are generalist feeders. Isotopic  $\delta^{13}\text{C}$  signatures were generally more depleted in sub-adults and individuals from freshwater habitats. Crocodile  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were both isotopically enriched in scutes compared with plasma, indicating the importance of not relying on a single tissue type when interpreting food web and trophic processes. Isotope discrimination values and tissue turnover times are needed for Nile crocodiles before accurately interpreting trophic position in free-ranging individuals. In this study we also reviewed the experimental and field stable isotope studies to date for crocodylians and make recommendations for future research.

**Keywords:** crocodile, *Crocodylus niloticus*, stable isotopes, diet, food webs, trophic structure

## **Introduction**

Over the past three decades stable isotope analysis (SIA) has become an effective and increasingly widespread method used in dietary tracing in animal ecology studies. The underlying principle of SIA is that consumers incorporate dietary isotopic values into their own tissues and can therefore provide quantitative insight into food web dynamics and trophic interactions (Post 2002). Carbon (C) and nitrogen (N) are the two most commonly used elements in animal SIA studies with  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios standardly reported as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively (DeNiro and Epstein 1978, 1981).

Generally  $\delta^{15}\text{N}$  composition is an indicator of trophic position, with higher values reflecting higher trophic status (usually between +2‰ and +6‰ enrichment per trophic level) (Minagawa and Wada 1984, Peterson and Fry 1987, Caut 2009). Because carbon remains relatively unchanged (between -1‰ and +1‰) within food chains, consumer  $\delta^{13}\text{C}$  values are useful for determining primary nutrient source(s) at the base of food webs (Hobson and Clark 1992, Post 2002, Rosenblatt et al. 2015). The variation in  $\delta^{13}\text{C}$  values is directly linked primarily to differing photosynthetic pathways found among  $\text{C}_3$  and  $\text{C}_4$  plants (Farquhar et al. 1989).

Although there is a wealth of ecological SIA studies, few exist for large-bodied apex predators such as crocodylians, primarily because of the inherent difficulties in capturing adequate tissue sample numbers from free-ranging animals that are elusive and/or potentially dangerous. Isotopic studies of crocodylian diets are also particularly challenged because many species (e.g., *Crocodylus niloticus*, *C. porosus*, *Alligator mississippiensis*) are generalist predators that occupy complex food webs and feed on freshwater, marine, brackish, terrestrial and migratory organisms (Grigg and Kirshner 2015, refs. therein). Constructing isotopic profiles for all potential prey (prey that also has among-individual variation in tissue isotopic composition) or even a reliable cohort available to crocodylians in the ecosystems they occupy is therefore extremely difficult. While stomach content studies can provide important insight into prey taxonomy, lavaging the gut contents of multiple individuals (especially those > 2.5m total length) is logistically difficult in the field (Leslie 1997, Wallace and Leslie 2008). More critically, stomach content analysis provides only a snapshot in time of an individual's aggregate diet; a snapshot that carries a number of intrinsic biases mainly due to differential digestion and assimilation rates within and among prey items (Bearhop et al. 2002, Radloff et al. 2012).

Despite the clear challenges and built-in ambiguity attached to crocodylian dietary studies SIA remains a powerful tool for examining energy flow in aquatic ecosystems, and crocodylians are ideal candidates for examining general trophic patterns and linkages because of their robust influence on food webs (Hanson et al. 2014), established role as indicators of environmental health (Milnes and Guillette 2008), and relative longevity. Although there are only seven published ecological SIA dietary studies on free-ranging crocodylians to date (Table 1), these investigations have made important strides with regard to understanding species' ontogenetic niche shifts (Radloff et al. 2012, Marques et al. 2013), trophic connections to primary producers in variable habitats (Rosenblatt and Heithaus 2011, Wheatley et al. 2012, Hanson et al. 2015), individual foraging specialization and temporal diet stability (Rosenblatt et al. 2015), and invasive alterations to food web hierarchy and structure (Woodborne et al. 2012).

Four SIA experimental “diet-switch” studies on captive crocodylians (Table 2) have investigated discrimination values (changes in  $\delta$  ratios between trophic transfers) and turnover rates (the time it takes for tissues to incorporate dietary  $\delta$  values). These experiments all found variable but surprisingly low  $\delta^{15}\text{N}$  discrimination values ( $\Delta^{15}\text{N}$  range among tissues for all species: - 2.24 to 3.3‰; Table 2) compared to other top predators and the standard trophic enrichment metric of +2‰ to +6‰ reported in the literature (e.g. Lesage et al. 2002, Kurle 2002). Conversely, compared even with other ectotherms, isotopic turnover rates were generally slower by a time period of days or weeks for all tissues (Rosenblatt and Heithaus 2013, refs. therein), with blood plasma < scutes < RBCs (Table 2).

With this research backdrop in mind, we collected blood plasma and scute tissue samples to investigate isotopic dynamics in free-ranging Nile crocodiles (*C. niloticus*), an ecologically important but understudied top predator with a wide distribution in Africa (Cott and Pooley

1972). Specifically, we sought to examine  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and variances among *C. niloticus*, sex, size, age class, and habitat (freshwater vs. estuarine/brackish). More importantly, we wanted to analyze isotopic differences between blood plasma and scute tissue types, as this has not been examined yet for Nile crocodiles and few SIA studies of free-ranging animals incorporate multiple tissues. As Nile crocodile observational and stomach content studies (Pooley and Gans 1976, Leslie 1997, Wallace and Leslie 2008) suggest the species to be a largely opportunistic feeder, we hypothesized they show a broad range of  $\delta^{15}\text{N}$  values. As larger individuals generally have more prey options available to them and may occupy higher trophic levels, we expected  $\delta^{15}\text{N}$  to be higher in adults and increase with body size similar to the study of Radloff et al. (2012). Due to extensive movements and home ranges among individuals (Combrink et al. 2014) and therefore potential exposure to different microhabitats and prey sources from different carbon pools, we had no a priori hypotheses regarding  $\delta^{13}\text{C}$  values.

## **Methods**

### **Study animal**

Nile crocodiles are widespread throughout many aquatic ecosystems in sub-Saharan Africa. In South Africa, crocodile distribution and abundance are in states of reduction and the species is IUCN red-listed as Vulnerable (Marais 2014), with remaining viable populations restricted to protected areas and waterbodies under some degree of conservation management. Challenges to the persistence of the species include poaching and indiscriminate killing (Calverley and Downs 2014), loss of nesting habitat (Combrink et al. 2011) and environmental degradation (Botha et al. 2011; Ferreira and Pienaar 2011).

Nile Crocodiles are apex predators that undergo ontological shifts in diet based on body size, and to a lesser extent, skull morphology (Radloff et al. 2012). The species is an opportunistic, generalist predator that feeds on a range of prey taxa and sizes from insects to large ungulates and human beings (Wallace and Leslie 2008). Depending on the ecology and availability of their prey, Nile crocodiles employ both ambush and active foraging strategies. In subtropical South Africa, Nile crocodile feeding activity decreases or stops entirely with concomitant decreases in movement and body temperature during cooler weather (Downs et al. 2008).

### **Study sites**

We collected blood plasma and scute tissue for stable isotope analysis from Nile crocodiles at estuarine and freshwater populations in KwaZulu-Natal, South Africa. Freshwater habitats included Jozini Dam (Pongolapoort Dam); a large man-made reservoir that is fed by the Pongola River, Inyamithi Pan; a protected Pongola River floodplain lake inside Ndumo Game Reserve, and the Sihadla Channel; a small waterway at the southern terminus of the Kosi Bay Lakes. Kosi Bay encloses an estuary connected to the Indian Ocean but our sampling site at Sihadla was unaffected by tidal activity or salinity (Kyle 1998). These localities represent or are components of the largest freshwater habitats to still support crocodiles in KwaZulu-Natal.

All estuarine crocodile samples were collected from Lake St Lucia in the iSimangaliso Wetland Park, the largest population in the province (~1000 individuals). At 67 km in total length the system contains a main lake basin (6 km wide at capacity) connected to the ocean via a 27 km long “Narrows” channel. The largest estuarine system in Africa, Lake St Lucia is a highly dynamic environment over space and time with cyclical, but often unpredictable annual

fluctuations between drought and high rainfall (Stretch et al. 2013). Due to a decade-long drought, at the time of our sampling in 2009-10 the estuary mouth was closed, there were limited freshwater inputs, and significant sections of the lake were hypersaline or exposed due to evaporation. Freshwater inputs from five feeder rivers and seeps on the eastern lake edge are important components to the nesting ecology of crocodiles at Lake St Lucia, but a comprehensive spatial ecology study that ran concurrently with this one showed that crocodiles move extensively throughout the entire system (Combrink et al. 2014).

### **Sample collection**

Nile crocodiles were captured under permit from Ezemvelo KZN Wildlife and ethics permission from the University of KwaZulu-Natal and secured using a variety of noosing snares, ropes and traps according to standard operating procedure (Combrink et al. 2012). Once an individual was safely restrained, blood was collected immediately before body measurements and tagging activities were conducted. Blood for stable isotope analysis was drawn from the post-occipital spinal venous sinus using a 20 ml syringe with an 18G needle (Myburgh et al. 2014). Either a 1.5” hypodermic needle or 3.5” spinal needle (Terumo Corporation, Tokyo, Japan) was used depending on the body size and/or neck fat of individual crocodiles (Myburgh et al. 2014). The blood samples were deposited into spray-coated lithium heparin plastic tubes (BD Vacutainer®), and centrifuged at 3000 rpm to separate red blood cell and plasma components. Dorsal tail scutes for stable isotope analysis were removed using a sharp knife or scalpel, double-sealed in Ziploc® freezer bags and then frozen at -80°C until lab analysis. Necrospray (Bayer AG) was applied to the area of scute removal before crocodile release.

Sex was determined for each Nile crocodile by inserting a finger into the cloaca and palpating for the presence or absence of the penis (Ziegler and Olbort 2007). Snout-to-vent length (SVL) was measured dorsally as the distance from the tip of the snout to the posterior end of the cloacal opening for each individual. Similar to squamates, SVL is the standard measurement of body length for crocodylians because tails can be partially missing, damaged or deformed.

### **Stable isotope analysis**

Isotopic analyses were performed at the Mammal Research Institute, University of Pretoria, using a Flash Elemental Analyser (1112 series) integrated via a ConFlo IV system with a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany). Because plasma and scute C:N ratios were not within acceptable limits for aquatic samples ( $> 3.5$ ) we conducted lipid extraction on the samples before analysis (Post et al. 2007).

The pyramid-shaped scute samples consisted of an inert, keratinized epidermal outer layer and an inner core of dense collagen. We chose to perform SIA on scute collagen because the tissue is metabolically active and continuously remodeled, and therefore indicative of long-term dietary integration (Radloff et al. 2012).

Scute samples were weighed into tin cups that had been pre-cleaned in Toluene, and plasma samples were pipetted into smooth walled tin capsules for liquid analyses. The samples were combusted at  $1020^{\circ}\text{C}$ . Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel  $\delta^{13}\text{C} = -20.57\text{‰}$ ;  $\delta^{15}\text{N} = +6.80\text{‰}$ ), run after every 12 unknowns in a sequence. During scute analyses the standard was introduced into the elemental analyzer as a solid, while the standards for the plasma analysis were diluted with deionized water in the

capsules in order to compensate for any matrix effects. The precision ( $\pm$  SD) of the standards was 0.18‰ for  $\delta^{13}\text{C}$  and 0.37‰ for  $\delta^{15}\text{N}$  ( $n = 14$ ) for the plasma analyses, and 0.11‰ for  $\delta^{13}\text{C}$  and 0.08‰ for  $\delta^{15}\text{N}$  ( $n = 16$ ) for the scute analyses. A blank was also run after every 12 unknowns to confirm that there was no sample-to-sample memory effect from incomplete combustion.

Isotopic differences between samples typically occur at the third significant figure of the isotope ratio, therefore stable isotope ratios are reported using the conventional delta ( $\delta$ ) notation as parts per thousand deviation (‰)

$$\delta X_{\text{STD}} = ((R_{\text{Sample}} / R_{\text{STD}}) - 1) \times 1000$$

where R is either  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ , and  $\delta$  expresses the isotopic abundance of X (either  $^{15}\text{N}$  or  $^{13}\text{C}$ ) in a sample relative to the abundance of the international isotopic standard (PeeDee Belemnite for C, atmospheric nitrogen for N).

### **Statistical analyses**

Statistical analyses were conducted using Statistica 12.0 for Windows. Conditions for distribution normality were not met, so the nonparametric Kruskal-Wallis H Test was used to determine if there were significant differences between independent variable groups (sex, age class, population) and  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C:N ratio values for plasma and scute tissue. For the population variable, crocodiles were grouped as either “estuarine” or “freshwater” dependent on capture locality. For age class, individuals were grouped as “adult” ( $\geq 1400$  mm) or “sub-adult” ( $< 1400$  mm). Snout-to-vent length of 1400 mm was chosen as the cohort delineation metric

because it represents the smallest known nesting female for free-ranging *C. niloticus* in the province of KwaZulu-Natal (males and females become reproductively active at approximately the same size).

A Wilcoxon Matched Pairs Test was used to analyze signature values of the same isotope among scutes and plasma, and Spearman's Rank-Order Correlation was used to assess the strength of the relationship between crocodile SVLs and isotopic values. The significance threshold was set at  $\alpha = 0.05$  for all analyses.

## Results

Stable isotope analysis was performed on plasma and scute tissues from 38 adult ( $n = 29$ ) and sub-adult ( $n = 9$ ) Nile crocodiles captured from estuarine ( $n = 26$ ) and freshwater ( $n = 12$ ) populations in northern KwaZulu-Natal, South Africa (Table 1). The mean SVL (mm) for all individuals was 1562 (min = 681, max = 2310, SD = 412). Average isotopic signature was 8.35‰ (min = 4.37‰, max = 13.02‰, SD = 1.98‰) for  $\delta^{15}\text{N}$  plasma and -18.25‰ (min = -27.30‰, max = -15.20‰, SD = 2.97‰) for  $\delta^{13}\text{C}$  plasma, with a mean C:N ratio of 3.75 (min = 2.35, max = 5.31, SD = 0.61). With the exception of three individuals (nos. 101, 127, PO2)  $\delta^{15}\text{N}$  was enriched in scute samples compared to plasma (mean = 10.83‰, min = 5.29‰, max = 14.13‰, SD = 2.26‰). Mean scute  $\delta^{13}\text{C}$  was -16.84‰ (min = -23.74‰, max = -12.54‰, SD = 2.90‰) and the mean C:N ratio for scutes was 3.74 (min = 3.31, max = 7.50, SD = 0.88). Among individuals, the Wilcoxon Matched Pairs Test determined a significant difference between  $\delta^{15}\text{N}$  plasma and scute values ( $T = 27$ ,  $z = 4.98$ ,  $p = 0.00$ ) and between  $\delta^{13}\text{C}$  plasma and scute values ( $T = 131$ ,  $z = 3.47$ ,  $p = 0.00$ ) (Fig. 1).

For age class and  $\delta^{15}\text{N}$  there were significant differences for both plasma (Kruskal-Wallis  $H = 5.53$ ,  $p = 0.0187$ ) and scutes ( $H = 5.37$ ,  $p = 0.0205$ ) with adults having higher  $\delta^{15}\text{N}$  signatures for both tissues (Fig. 2). Crocodile  $\delta^{13}\text{C}$  values were significantly different between estuarine and freshwater populations for plasma ( $H = 13.5$ ,  $p = 0.0002$ ) and scutes ( $H = 8.90$ ,  $p = 0.0028$ ). There was a weak monotonic but significant effect between crocodile SVL for both plasma  $\delta^{13}\text{C}$  (Spearman  $R = 0.37$ ,  $p = 0.02$ ) and scute  $\delta^{13}\text{C}$  ( $R = 0.38$ ,  $p = 0.02$ ), with smaller crocodiles having more negative (isotopically lighter) carbon signatures. No significant effects were found among sexes, for C:N ratios and any grouping variable, between age classes and  $\delta^{13}\text{C}$  values in tissue, or for  $\delta^{15}\text{N}$  tissue values among populations.

## **Discussion**

### **$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values**

This study is the first to report  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures for different tissues (blood plasma and scutes) in free-ranging Nile crocodiles. As hypothesized we observed higher  $\delta^{15}\text{N}$  values in adults than for sub-adults, which we attribute to previously documented ontogenetic dietary shifts found in Nile crocodiles, with adults occupying higher trophic positions (Radloff et al. 2012). While this appears to be a general trend for Nile crocodiles, it is important to note that elevated trophic position does not always correspond with increasing  $\delta^{15}\text{N}$  (Chapter 5). Surprisingly, we did not find significant effects for  $\delta^{15}\text{N}$  among freshwater and estuarine crocodile samples despite these ecosystems having markedly different aquatic food web structures (Merron et al. 1993, Carrasco et al. 2012). This could be attributed to differing estuarine and freshwater fish assemblages having similar or overlapping  $\delta^{15}\text{N}$  averages, or perhaps individuals from the allopatric Nile crocodile populations we studied incorporated

identical terrestrial prey taxa in their diets at frequencies high enough to mask dietary isotopic distinction among groups. Ungulate species that Nile crocodiles are known to prey upon common to all sampled sites include buffalo (*Syncerus caffer*), kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), nyala (*Tragelaphus angasii*), duiker (*Sylvicapra grimmia* and *Cephalophus natalensis*) and juvenile common hippopotamus (*Hippopotamus amphibious*).

Given the highly conserved morphology of the species between sexes (Hutton 1987, Warner unpubl. data), the lack of dietary variance among females and males was not surprising. Because quantifying the isotopic structures of the estuarine and freshwater food webs where we sampled Nile crocodiles was well beyond the capacity of this study, we did not include mixing models or attempt to assign discrete trophic positions to the crocodile cohorts we analyzed. However, our data clearly showed that given the species' broad trophic width (scute  $\delta^{15}\text{N} = 5.29 - 14.13\text{‰}$ ) Nile crocodiles in KwaZulu-Natal are highly generalist predators when assessed as an ecological cohort. Along with the largest  $\delta^{15}\text{N}$  values recorded among wild crocodylians, the  $\delta^{15}\text{N}$  spectrum of 8.84‰ we quantified in this study is also the widest recorded to date.

In contrast to our  $\delta^{15}\text{N}$  findings, crocodile  $\delta^{13}\text{C}$  plasma and scute values were significantly different between freshwater Nile crocodile populations and those from estuarine Lake St Lucia (Fig. 3) (Aside from the statistical analysis presented here, this finding was also separately corroborated in a more robust analysis for just blood plasma in 134 different crocodiles from the same freshwater and estuarine populations; (Chapter 5). Generally crocodiles from freshwater habitats had more isotopically depleted  $\delta^{13}\text{C}$  values, implying that the basal food web structure and primary nutrient sources were different between sites. The isotopic contribution of  $\text{C}_3$  and  $\text{C}_4$  plants to the base of a foodweb are unambiguously different ( $\text{C}_3$ : -35 to -22‰, average 27‰;  $\text{C}_4$ : -16 to - 9‰, average -12.5 ‰; Farquhar et al. 1989, Jardine et al.

2012), so these inputs are relatively easy to distinguish given that  $\delta^{13}\text{C}$  is highly conserved as it travels up food webs (Post et al. 2002). We attribute the more negative  $\delta^{13}\text{C}$  values found in crocodile tissues from freshwater areas in this study in part to the nutrients propagated through the food web from  $\text{C}_3$  *Vachellia xanthophloea*, *Barringtonia sp.*, and *Acacia sp.* trees that border these habitats. Although  $\text{C}_3$  trees are present along parts of the Lake St Lucia shoreline, *Phragmites* reeds and other wetland  $\text{C}_4$  sedges and grasses are dominant, in addition to marine carbon inputs (Adams et al. 2013). Our assessment of the contributing carbon sources to these ecosystems is undoubtedly a simplified generalization (e.g. previous SIA studies at Lake St Lucia have shown marked seasonal variation at the isotopic base of the food web; Dyer et al. 2015). However, we conclude that further  $\delta^{13}\text{C}$  SIA for Nile crocodiles over a longer period of time would be a powerful method for examining crocodile habitat partitioning and movement among carbon pools (e.g. Rosenblatt et al. 2015).

### **Differences between blood plasma and scute collagen**

Dietary  $\delta^{15}\text{N}$  signatures were enriched in Nile crocodile scute tissue (mean  $\delta^{15}\text{N} = 10.83\text{‰}$ ) compared with plasma (mean  $\delta^{15}\text{N} = 8.35\text{‰}$ ) by approximately + 2.50‰. If similar to other crocodylians (Table 2) Nile crocodile discrimination values ( $\Delta^{15}\text{N}$ ) are higher in scutes compared with plasma at dietary equilibrium (which is likely, A. Rosenblatt pers. comm.), the discrepancy we observed is probably a true reflection of isotopic enrichment between the tissues. An alternative explanation is that almost every individual in the study undertook a recent dietary shift at both the freshwater and estuarine populations (within the previous year if turnover rates for Nile crocodiles are roughly equivalent to other crocodylians), which is highly unlikely. We therefore present good evidence here that scute tissue in Nile crocodiles is naturally enriched

compared with plasma, likely as a result of the metabolic and biochemical processes associated with isotopic routing in the species (Gannes et al. 1997).

The general isotopic enrichment differences we observed between Nile crocodile plasma and scute tissues highlights the need for future SIA studies on crocodylians to strongly consider sampling a suite of tissues, especially when attempting to answer questions relating to dietary  $\delta^{15}\text{N}$  and crocodile trophic position. Going forward, collecting blood plasma should be the first prerequisite for addressing dietary and trophic dynamics, as experimental studies to date (Table 2) suggest plasma discrimination values ( $\Delta^{15}\text{N}$ ) are the smallest among other commonly sampled tissues (e.g. claw, scute) and therefore likely closer to actual prey  $\delta^{15}\text{N}$ . As a practical example, depending on seasonal and other environmental factors, the most common fish species at Lake St Lucia (*Oreochromis mossambicus*, *Liza* spp., *Ambassis ambassis*) have  $\delta^{15}\text{N}$  values ranging from  $8.7 \pm 0.2\text{‰}$  to  $14.17 \pm 0.95\text{‰}$  (Govender et al. 2011, Dyer et al. 2015). Therefore, in theory, if scute tissue from a fish-eating crocodile was collected with a  $\delta^{15}\text{N}$  value of  $10\text{‰}$ , based off the findings of this study the blood plasma  $\delta^{15}\text{N}$  value might be closer to  $7.50\text{‰}$ , and the dietary interpretation derived from scute tissue alone would be wrong, possibly by several trophic levels.

To conclude, the most important research need to advance SIA applications in ecological studies for Nile crocodiles is a robust diet-switch study for sub-adult (sexually immature) and especially adult individuals because of large ontogenetic changes in body mass ( $> 1000$ -fold). Another species may possibly serve as a suitable proxy, but diet-switch studies to date have only been conducted on crocodylians  $< 1$  m (e.g. Thiago et al. 2014, Rosenblatt & Heithaus 2015). Until reliable discrimination values and turnover rates for all tissues currently sampled as protocol for field studies in South Africa are determined, assigning absolute trophic levels to crocodiles among different populations will remain speculative.

## Acknowledgements

We sincerely thank R. Taylor, P. Calverley, G. Champion, F. Myburgh, S. Kyle and family, the many volunteers who assisted with fieldwork, Ezemvelo KwaZulu-Natal Wildlife and the iSimangaliso Wetland Park Authority. The Ford Wildlife Foundation and the South African Water Research Commission are thanked for their vehicle and financial support.

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## Figure legends

**Figure 1.** Boxplots for isotopic a.)  $\delta^{13}\text{C}$  blood plasma and scute values (Wilcoxon Matched Pairs  $T = 131$ ,  $z = 3.47$ ,  $p = 0.00$ ) and b.)  $\delta^{15}\text{N}$  plasma and scutes ( $T = 27$ ,  $z = 4.98$ ,  $p = 0.00$ ) for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

**Figure 2.** Variance between  $\delta^{15}\text{N}$  isotopic signatures among age classes for a.) scute (Kruskal-Wallis  $H = 5.37$ ,  $p = 0.02$ ) and b.) plasma ( $H = 5.53$ ,  $p = 0.02$ ) tissues for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

**Figure 3.** Crocodile  $\delta^{13}\text{C}$  values were significantly different between estuarine and freshwater populations for a.) scutes ( $H = 8.90$ ,  $p = 0.00$ ) and b.) plasma ( $H = 13.5$ ,  $p = 0.00$ ) for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

## Tables

**Table 1.** Ecological stable isotope analysis studies of crocodylian diet (free-ranging individuals).

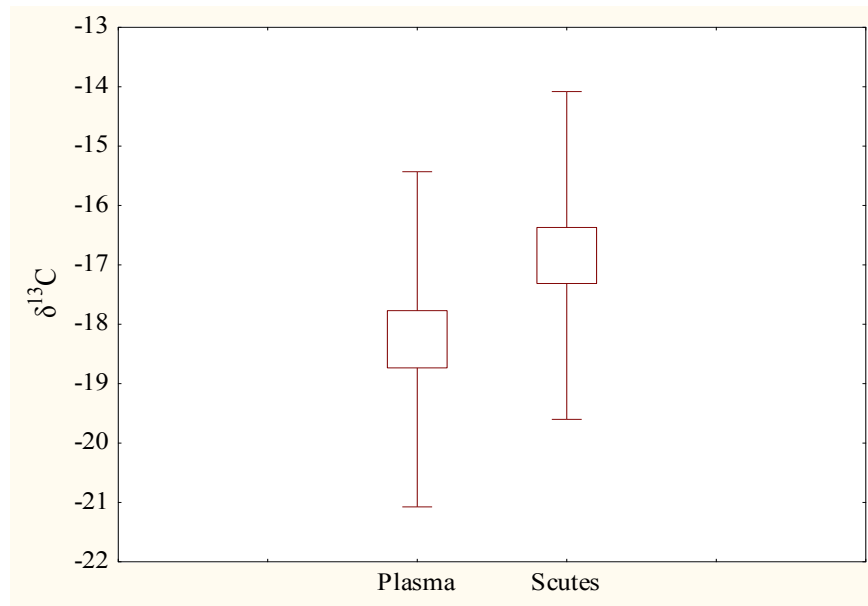
<b>Study</b>	<b>Species</b>	<b>Isotope <math>\delta</math> analyzed</b>	<b>Tissue analyzed</b>
Hanson et al. 2015	<i>Crocodylus porosus</i>	$\delta^{15}\text{N}$ , $\delta^{13}\text{C}$	Plasma, RBC, scutes
Marques et al. 2013	<i>Caiman latirostris</i>	$\delta^{15}\text{N}$ , $\delta^{13}\text{C}$	Claw
Radloff et al. 2012	<i>Crocodylus niloticus</i>	$\delta^{15}\text{N}$ , $\delta^{13}\text{C}$	Scute
Rosenblatt & Heithaus 2011	<i>Alligator mississippiensis</i>	$\delta^{15}\text{N}$ , $\delta^{13}\text{C}$	Scute
Rosenblatt et al. 2015	<i>A. mississippiensis</i>	$\delta^{13}\text{C}$	Plasma, RBC
Wheatley et al. 2012	<i>Crocodylus acutus</i> , <i>A. mississippiensis</i>	$\delta^{13}\text{C}$	Tooth (from museum samples)
Woodborne et al. 2012	<i>C. niloticus</i>	$\delta^{15}\text{N}$ , $\delta^{13}\text{C}$	Claw

**Table 2.** Experimental stable isotope analysis studies of crocodylian diet (captive individuals): discrimination ( $\Delta$ ) values and turnover rates.

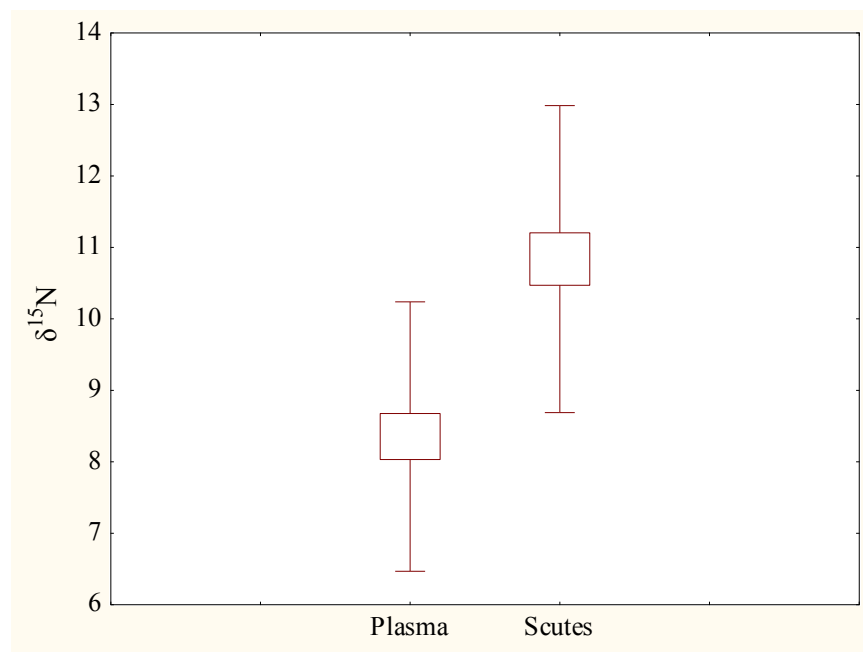
Study	Species	No. individuals	$\Delta^{13}\text{C}_{\text{tissue-diet}}$ ‰ (range or $\pm$ SE)	$\Delta^{15}\text{N}_{\text{tissue-diet}}$ ‰ (range or $\pm$ SE)	Complete turnover rate (days)	Tissue
Caut 2013	<i>Caiman latirostris</i>	23	-0.11 – 0.08	-2.24 – 0.08	83	Plasma
Caut 2013	<i>C. latirostris</i>	23	-0.52 – 0.66	-0.93 – 0.39	256 – 306	RBC
Caut 2013	<i>C. latirostris</i>	23	-0.04 – 1.06	-2.50 – -1.59	134	Muscle
Hanson et al. 2015	<i>Crocodylus porosus</i>	8	$0.8 \pm 1.0$	$2.7 \pm 0.1$	n/a	Plasma
Hanson et al. 2015	<i>C. porosus</i>	8	$0.6 \pm 1.0$	$1.9 \pm 0.1$	n/a	RBC
Hanson et al. 2015	<i>C. porosus</i>	8	$2.0 \pm 1.0$	$3.3 \pm 0.3$	n/a	Scute (collagen)
Hanson et al. 2015	<i>C. porosus</i>	8	$1.4 \pm 1.0$	$3.0 \pm 0.5$	n/a	Scute (keratin)
Marques et al. 2014	<i>C. latirostris</i>	18	$1.2 \pm 0.1$	$1.1 \pm 0.1$	n/a	Claw
Marques et al. 2014	<i>C. latirostris</i>	18	$0.9 \pm 0.2$	$0.8 \pm 0.2$	n/a	Scute
Rosenblatt & Heithaus 2015	<i>Alligator mississippiensis</i>	14	-0.04	0.35	249.6	Plasma
Rosenblatt & Heithaus 2015	<i>A. mississippiensis</i>	14	0.03	0.95	1,109.2	RBC
Rosenblatt & Heithaus 2015	<i>A. mississippiensis</i>	14	0.61	1.22	414.0	Scute

## Figures

a.

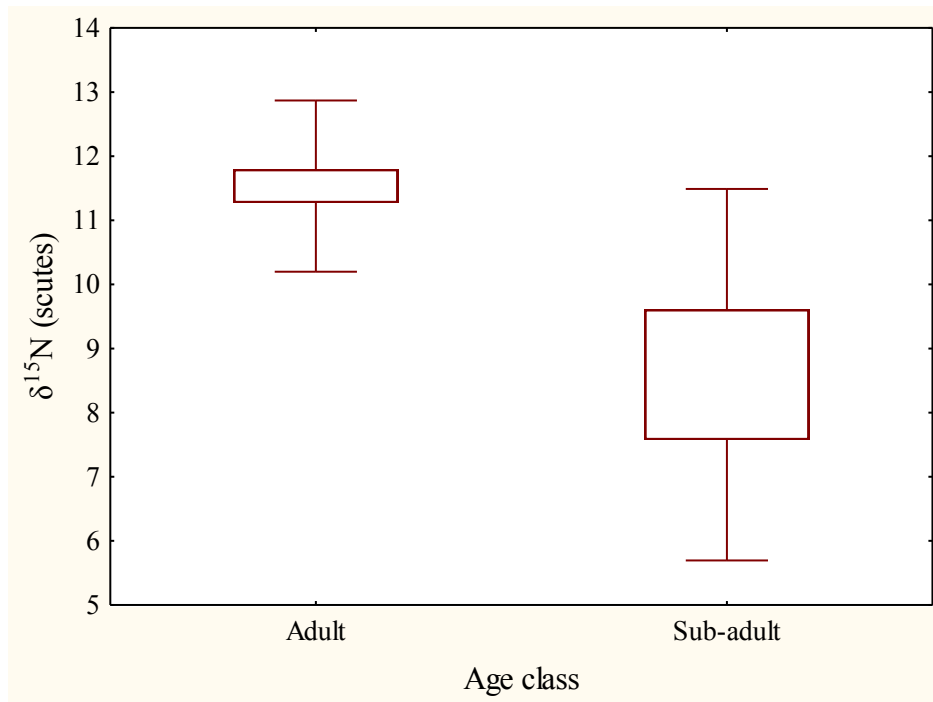


b.

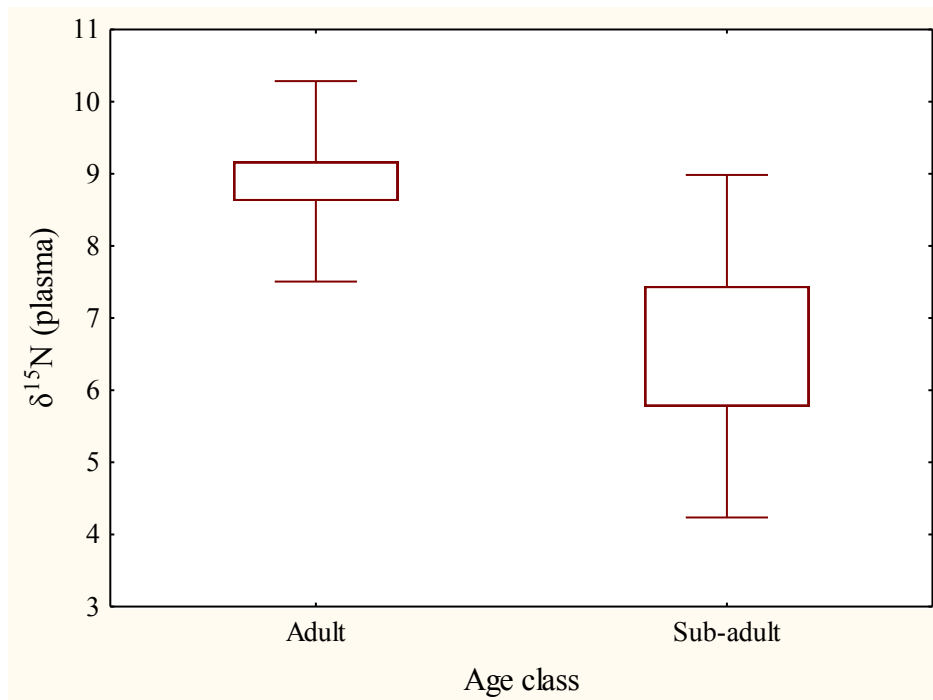


**Figure 1.** Boxplots for isotopic a.)  $\delta^{13}\text{C}$  blood plasma and scute values (Wilcoxon Matched Pairs  $T = 131$ ,  $z = 3.47$ ,  $p = 0.00$ ) and b.)  $\delta^{15}\text{N}$  plasma and scutes ( $T = 27$ ,  $z = 4.98$ ,  $p = 0.00$ ) for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

a.

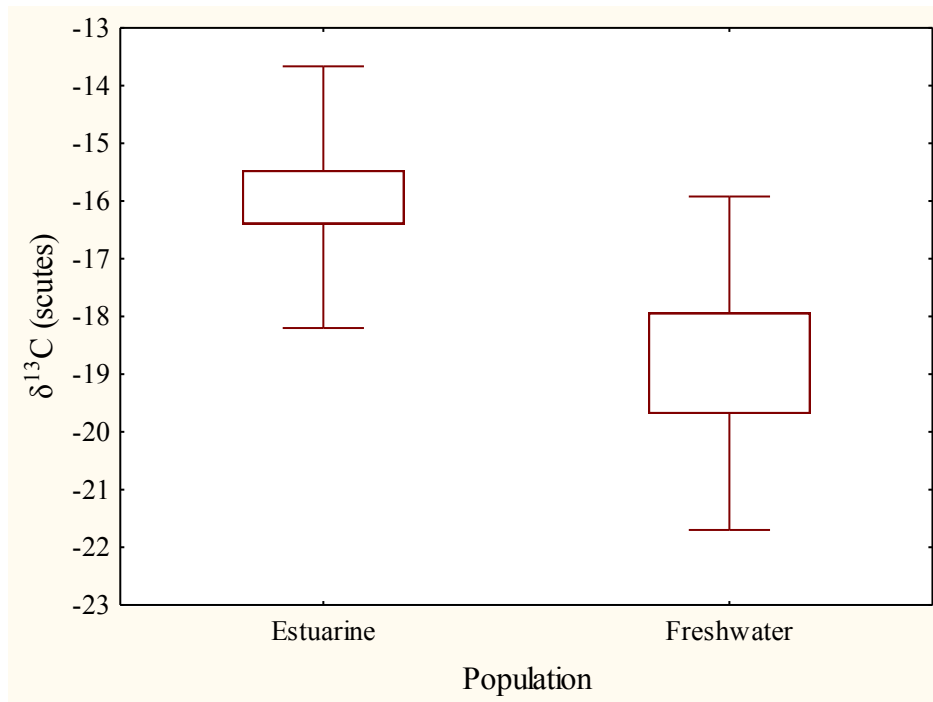


b.

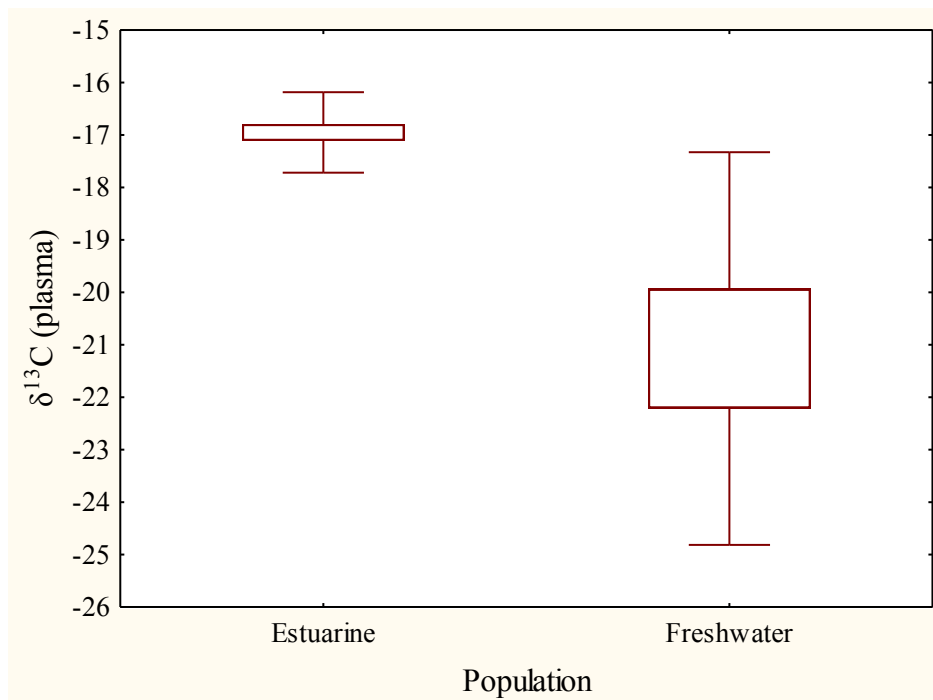


**Figure 2.** Variance between  $\delta^{15}\text{N}$  isotopic signatures among age classes for a.) scute (Kruskal-Wallis  $H = 5.37$ ,  $p = 0.02$ ) and b.) plasma ( $H = 5.53$ ,  $p = 0.02$ ) tissues for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

a.



b.



**Figure 3.** Crocodile  $\delta^{13}\text{C}$  values were significantly different between estuarine and freshwater populations for a.) scutes ( $H = 8.90$ ,  $p = 0.00$ ) and b.) plasma ( $H = 13.5$ ,  $p = 0.00$ ) for 38 *Crocodylus niloticus* from KwaZulu-Natal, South Africa. (Boxes = mean  $\pm$  SE, error bars = mean  $\pm$  SD).

## Appendix 1.

Fractionation-corrected blood plasma and tail scute isotope  $\delta$  ratios with metadata for thirty-eight *Crocodylus niloticus* from estuarine and freshwater habitats in northern KwaZulu-Natal, South Africa

Croc ID	Plasma			Scutes			Population	Locality	Habitat	SVL	Sex	Age Class
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	C:N	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	C:N						
74	8.74976 389	- 16.7607 143	3.76843 763	10.7191 407	- 13.8258	3.39939 601	St Lucia	Nkaza na	Estuari ne	17 00	F	Ad ult
75	4.91635 86	- 17.2047 143	3.73699 942	5.29441 068	- 16.2638	3.51855 46	St Lucia	Nkaza na	Estuari ne	95 3	F	Sub - adu lt
78	7.95684 418	- 16.9087 143	3.27658 053	12.4137 36	- 15.4278	3.36847 164	St Lucia	Nkaza na	Estuari ne	14 94	F	Ad ult
80	6.61409 729	- 17.0767 143	2.82119 322	11.3037 109	- 14.2568	3.37775 908	St Lucia	Nkaza na	Estuari ne	13 70	F	Sub - adu lt
83	8.95024 493	- 17.1957 143	4.03527 992	12.7192 08	- 15.5928	3.40811 792	St Lucia	Catali na Bay	Estuari ne	17 86	F	Ad ult
94	10.1564 356	- 16.8927 143	4.57553 176	12.1673 984	- 19.1798	3.50527 45	St Lucia	Dead Tree Bay	Estuari ne	16 70	F	Ad ult
95	10.0371 306	- 17.2077 143	4.30462 609	11.5148 321	- 15.0718	3.54308 943	St Lucia	Dead Tree Bay	Estuari ne	16 32	F	Ad ult
98	10.3691 05	- 17.0637 143	5.30845 887	11.9383 502	- 18.6738	3.44674 356	St Lucia	Dead Tree Bay	Estuari ne	14 92	F	Ad ult
99	9.33201 945	- 16.9747 143	4.15102 63	11.8137 177	- 15.8408	3.40343 651	St Lucia	Dead Tree Bay	Estuari ne	15 02	F	Ad ult
103	8.65454 864	- 17.3247 143	3.85279 522	11.2141 421	- 13.8408	3.44068 529	St Lucia	Nkaza na	Estuari ne	15 90	F	Ad ult
108	8.57499 612	- 17.1797 143	3.47751 305	12.7695 032	- 20.1098	3.40517 615	St Lucia	Dead Tree Bay	Estuari ne	94 0	F	Sub - adu lt
502	8.45542 202	- 17.4217 143	3.38361 662	11.7502 616	- 14.6858	3.44521 065	St Lucia	Narro ws	Estuari ne	16 84	F	Ad ult
510	8.39744 458	- 18.1527 143	3.42973 758	11.1688 385	- 14.4038	3.31407 846	St Lucia	Narro ws	Estuari ne	16 42	F	Ad ult

<b>76</b>	8.76030 275	- 17.4057 143	4.43954 992	8.90779 711	- 16.5228	3.73714 371	St Lucia	Nkaza na	Estuari ne	16 22	M	Ad ult
<b>81</b>	9.31298 11	- 16.5607 143	3.93495 258	13.0337 733	- 14.8928	3.35999 261	St Lucia	Nkaza na	Estuari ne	19 90	M	Ad ult
<b>82</b>	7.04878 05	- 17.3317 143	2.90160 559	12.2721 459	- 14.3118	3.37870 518	St Lucia	Catali na Bay	Estuari ne	19 70	M	Ad ult
<b>84</b>	7.04544 617	- 15.2237 143	4.20181 521	11.4201 467	- 14.7108	3.38313 345	St Lucia	Catali na Bay	Estuari ne	18 90	M	Ad ult
<b>86</b>	10.1627 404	- 17.0087 143	4.27513 016	11.3813 459	- 15.0798	3.48682 913	St Lucia	Nkaza na	Estuari ne	18 80	M	Ad ult
<b>92</b>	9.79777 198	- 17.2947 143	3.37229 946	11.4830 385	- 23.7428	7.50722 657	St Lucia	Dead Tree Bay	Estuari ne	18 50	M	Ad ult
<b>10 1</b>	9.21079 301	- 17.9077 143	3.05887 493	7.74006 128	- 18.2478	4.49166 157	St Lucia	Dead Tree Bay	Estuari ne	19 54	M	Ad ult
<b>10 9</b>	9.65169 73	- 16.8747 143	4.00702 737	11.4150 281	- 15.0798	3.43847 042	St Lucia	Nkaza na	Estuari ne	15 82	M	Ad ult
<b>11 0</b>	6.96113 565	- 16.8037 143	3.73843 378	11.6687 316	- 16.8967 143	4.64757 652	St Lucia	Nkaza na	Estuari ne	19 18	M	Ad ult
<b>12 7</b>	10.6644 929	- 15.3627 143	3.59558 156	9.29649 312	- 12.5358	3.41612 089	St Lucia	Narro ws	Estuari ne	93 2	M	Sub - adu lt
<b>12 8</b>	7.92315 884	- 15.8067 143	4.03380 278	11.7084 522	- 14.3248	3.40669 991	St Lucia	Narro ws	Estuari ne	22 06	M	Ad ult
<b>50 3</b>	9.99731 61	- 18.6007 143	3.46074 649	12.7327 424	- 16.1068	3.46441 747	St Lucia	Narro ws	Estuari ne	10 26	M	Sub - adu lt
<b>50 4</b>	6.37575 922	- 15.1967 143	4.14335 65	9.24054 267	- 14.6188	3.42610 508	St Lucia	Lake Bhang azi	Estuari ne	23 10	M	Ad ult
<b>K4</b>	4.72293 149	- 25.5727 143	3.79170 462	6.87479 924	- 21.5255	3.51741 083	Kosi Bay	Sihadl a	Freshw ater	10 60	F	Sub - adu lt
<b>K6</b>	4.36689 183	- 27.0887 143	2.50199 634	5.95165 578	- 22.6098	3.54968 282	Kosi Bay	Sihadl a	Freshw ater	89 8	M	Sub - adu lt
<b>K7</b>	4.43373 036	- 24.0587 143	4.03543 769	5.36105 139	- 23.4198	3.45186 798	Kosi Bay	4th Lake	Freshw ater	69 3	M	Sub - adu lt
<b>K8</b>	5.19673 623	- 27.2987 143	5.09679 56	7.74652 375	- 21.5528	3.40839 416	Kosi Bay	4th Lake	Freshw ater	68 1	F	Sub - adu

<b>J1</b>	5.58904 584	- 17.3947 143	2.97205 155	12.1933 671	- 15.5938	3.43763 18	Jozini	Pongo la Inlet	Freshw ater	18 36	M	Ad ult
<b>J2</b>	8.71312 729	- 16.9387 143	3.78389 188	11.3776 085	- 19.0688	6.89839 771	Jozini	Pongo la Inlet	Freshw ater	22 36	M	Ad ult
<b>J3</b>	9.83521 382	- 18.3677 143	3.51467 917	13.0975 973	- 16.0468	3.70653 188	Jozini	Pongo la Inlet	Freshw ater	15 76	F	Ad ult
<b>J4</b>	10.0791 856	- 19.5417 143	3.80171 72	14.0504 898	- 17.4428	4.21388 138	Jozini	Pongo la Inlet	Freshw ater	15 18	F	Ad ult
<b>J5</b>	13.0172 828	- 18.0167 143	3.75700 053	11.0268 368	- 15.7788	3.38115 396	Jozini	Pongo la Inlet	Freshw ater	15 76	F	Ad ult
<b>J6</b>	8.91799 801	- 21.1947 143	2.34660 378	9.12108 058	- 15.5418	3.44766 737	Jozini	Pongo la Inlet	Freshw ater	15 55	M	Ad ult
<b>J7</b>	10.1409 439	- 17.0017 143	3.68637 675	14.1250 344	- 16.2048	3.42486 764	Jozini	Pongo la Inlet	Freshw ater	14 61	F	Ad ult
<b>N6 2</b>	8.36736 812	- 20.3957 143	3.89096 77	11.7867 745	- 20.9418	4.13630 731	Ndumo	Inyam ithi Pan	Freshw ater	16 64	M	Ad ult

## CHAPTER 5

### **Trophic niche width and dietary stable isotope structure of Nile crocodile populations in KwaZulu-Natal, South Africa**

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#### **Abstract**

Recent population declines of Nile crocodiles (*Crocodylus niloticus*) in South Africa are environmental red flags regarding the future of sustainable aquatic ecosystem health and function in the region. Before implementing effective conservation strategies an understanding of a species' feeding ecology and trophic niche is needed, but this knowledge can be hard to come by

for elusive, large-bodied predators whose life history is difficult to observe over space and time. To quantify the trophic niche width of Nile crocodiles we obtained blood plasma from 106 individuals in four populations in the province of KwaZulu-Natal, South Africa and assessed the structure and variance of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios. We found a relatively large isotopic range (niche width) of 9.99‰ among individuals, indicative of a generalist predator that feeds throughout the entirety of the food web. Although evidence of dietary specialism among individuals or populations was not found, principal components analysis and linear models revealed that both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  increased with crocodile size (snout-to-vent length). We interpret this trend to known dietary and habitat use differences between subadults and adults. This pattern should not be considered axiomatic, however, because the smallest and largest individuals in this study were isotopically similar with regard to dietary nitrogen in their tissues, indicative of a diet composed of primary consumers (likely small rodents for juveniles, large ungulates for adults). The significant variance of  $\delta^{13}\text{C}$  values among capture sites illustrated the heterogeneity of aquatic and terrestrial carbon inputs Nile crocodiles utilize and has practical applications for crocodile conservation management in South Africa.

**Keywords:** crocodilian; trophic niche; diet; isotope analysis

## **Introduction**

The global battle against biodiversity loss includes the widespread endangerment and extinction of many reptile and amphibian taxa (Gibbons et al. 2000, Beebee and Griffiths 2005). Among these are numerous crocodilian species and populations in significant decline largely due to unsustainable commercial use, historic and present overhunting, and landscape-scale habitat destruction (Ross 1998, Martin 2008). The loss of crocodilians (alligators, caimans, crocodiles

and gharials) and their habitat is a genuine environmental crisis, namely because many of the tropical and subtropical wetland ecosystems these iconic animals occupy are among the most imperiled on earth. Despite increasing conservation challenges, crocodylians are ecologically hearty and generally respond well to management and protection efforts (Da Silveira and Thorbjarnarson 1999, Mazzotti et al. 2009, Fukuda et al. 2011), and as both indicators of environmental health (Milnes and Guillete 2008) and ecosystem “umbrella” species (Martin 2008), they are therefore exceptional study subjects with regard to information accrued versus research investment.

Among the proposed seven African crocodile species (Shirley et al. 2014) the Nile crocodile (*Crocodylus niloticus*) is the most widespread, occurring in up to 42 countries (Fergusson 2010). Despite its large distribution, ecological importance, and potential threat to humans, the Nile crocodile remains almost entirely unstudied throughout most waterbodies in Africa. A notable exception to this is the Republic of South Africa, where despite crocodiles being hunted as vermin within nature reserves until the 1970s, recent decades have seen important research detailing the ecology and environmental importance of Nile crocodiles (Pooley 1982, Jacobsen 1984 Leslie 1997, Champion 2011, Calverley 2013, Combrink 2014).

As crocodile research has gained traction unfortunately so have the threats to and degradation of viable Nile crocodile populations. Although multiple fragmented populations currently exist in the northeastern part of the country, the long-term regional viability of the species is likely restricted to two large populations located at the Kruger National Park (KNP) and at Lake St Lucia within the iSimangaliso Wetland Park. Threats to crocodiles in South Africa are multitudinous, and include ecosystem alteration (sedimentation of waterbodies, uncontrolled water abstraction, invasive vegetation), loss of suitable nesting habitat, direct

human conflict (poaching, snaring, gillnetting) and climate change (Blake and Jacobsen 1992, Kyle 1999, Leslie and Spotila 2001, Combrink et al. 2011, Calverley and Downs 2014). More recently, environmental pollution has emerged as an insidious threat to Nile crocodiles, highlighted by the pansteatitis outbreak and resultant deaths of hundreds of crocodiles from Loskop Dam and the Olifants, Letaba and Sabie Rivers in KNP (Ashton 2010, Botha et al. 2011, Ferreira and Pienaar 2011, Woodborne et al. 2012, Lane et al. 2013, Chapter 3). The species is IUCN red-listed as Vulnerable in South Africa (Marais 2014) but should be reevaluated within the next ten years.

Critical to conservation management of threatened biota is knowledge of fundamental ecological processes. While recent headway has been made toward understanding the movement patterns and home ranges of Nile crocodiles (Champion 2011, Calverley 2013, Combrink 2014) very little information exists regarding feeding ecology; the biological cornerstone that supports all other crocodile activity. Although Nile crocodiles are presumed apex predators, most feeding records are collated by way of direct observation (Whitfield and Blaber 1979). Stomach content studies can provide important insight into prey taxonomy (Leslie 1997, Wallace and Leslie 2008) but lavaging the gut contents of individuals (especially adult crocodiles) of sufficient sample size over multiple populations is labour-intensive and impossible without adequate field staff support. Stomach content analysis also provides only a snapshot in time of an individual's aggregate diet; a snapshot that carries a number of intrinsic biases mainly due to differential digestion and assimilation rates within and among prey items (Bearhop et al. 2002, Radloff et al. 2012). Additionally, the inordinately high biodiversity of tropical and subtropical African ecosystems presents an absolute number of potential prey taxa available to Nile crocodiles that is likely higher than that for other crocodylian species. Among the populations in KwaZulu-Natal under

examination in this study, Nile crocodiles are known to eat a wide variety of prey including insects and other invertebrates, reptiles and amphibians, small mammals, birds, freshwater, estuarine and marine fish (including rays, sharks and eels), ungulates ranging from small antelope to common hippopotamus *Hippopotamus amphibius* and giraffe *Giraffa camelopardalis*, other crocodiles (Fig. 1), and human beings (Pooley 1982, Leslie 1997, Warner and Combrink pers. obs.). Radloff (et al. 2012) found in Botswana that although *C. niloticus* is a generalist feeder as a species, different size cohorts may specialize on different prey groups, with prey size usually increasing with crocodile size.

Facing these challenges, we used an alternative approach to assess the dietary trophic niche width and feeding patterns of Nile crocodiles in South Africa: determining the variance of carbon and nitrogen stable isotope ratios in crocodile blood plasma (Bearhop et al. 2004, Willson et al. 2010). The underlying principle of stable isotope analysis (SIA) is that consumers incorporate the stable isotope ratios from prey into their own tissues through stepwise enrichment (Hobson 1999, Post 2002). Carbon and nitrogen are the two most commonly used elements in dietary SIA studies, with  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios conventionally reported as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Generally  $\delta^{15}\text{N}$  composition is an indicator of trophic position, with higher values reflecting higher trophic status (usually between +2‰ and +6‰ enrichment per trophic level) (Minagawa and Wada 1984, Caut and Courchamp 2009). Because carbon remains relatively unchanged within food chains (between -1‰ and +1‰), consumer  $\delta^{13}\text{C}$  values are useful for determining primary nutrient source(s) at the base of food webs (Hobson and Clark 1992, Post 2002, Rosenblatt et al. 2015). Crocodilian blood plasma has a much slower isotopic turnover time than that for endothermic animals (89 – 250 days; Caut 2013, Rosenblatt and Heithaus 2013) rendering it a good representation of an individual's average isotopic dietary signature for

the months preceding capture and sampling (detailed information regarding isotopic discrimination values, turnover rates and the use of SIA in crocodylian studies is provided in Chapter 4).

Although obtaining  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for Nile crocodiles potential prey was well beyond the reach of this study, and therefore isotopic construction of a complete food web with discrete trophic positions not feasible, we specifically wanted to employ SIA to analyze the entire  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic spectrum across a variety of habitats that support Nile crocodile populations in KwaZulu-Natal. We predicted that if individuals generally shared the same diet across sites, we would find a narrow range of  $\delta^{15}\text{N}$  values. Similarly, if crocodiles occupy a specific trophic position we expected to find mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values roughly equivalent over space and time. We examine how our findings about the isotopic structure of Nile crocodile populations in South Africa can benefit practical conservation and management efforts for the species.

## **Materials and methods**

### **Study sites**

We collected blood plasma for SIA from four allopatric Nile crocodiles populations in KwaZulu-Natal, South Africa (Fig. 2). Our sampling sites represent the largest remaining protected aquatic ecosystems that harbor Nile crocodiles in the province of KwaZulu-Natal (for South Africa, the only other viable population is found at the ~20,000 km<sup>2</sup> Kruger National Park).

### *Lake St Lucia*

The St Lucia estuarine system (Fig. 3a) enclosed within the iSimangaliso Wetland Park World Heritage Site contains the largest estuarine Nile crocodile population (~1000 individuals) in Africa (Leslie and Spotila 2001, Combrink 2014), and is also the most southern breeding population for the species. At 67 km in total length the system contains a main lake basin (6 km wide at capacity) connected to the Indian Ocean via a 27 km long “Narrows” channel. Lake St Lucia is a highly dynamic environment over space and time with cyclical, but often unpredictable annual fluctuations between drought and high rainfall (Stretch et al. 2013). Due to a decade-long drought, at the time of our sampling the estuary mouth was closed, there were limited freshwater inputs, and significant sections of the lake were hypersaline or exposed due to evaporation. We captured crocodiles from eight distinct habitats at Lake St Lucia (Fig. 3a).

Catalina Bay and Dead Tree Bay are shallow basins connected to the eastern shoreline. Freshwater seeps and small wetlands at the periphery of these two areas are important crocodile habitat components and refugia from saline lake conditions during drought periods (Combrink et al. 2013). The perennially-flowing Nkazana Stream drains seepage from the Mfabeni wetland into Catalina Bay, and the 2 km stretch that flows into the Catalina mouth is a critical nesting area for crocodiles at Lake St Lucia (Combrink et al. 2013). Just north but isolated from Nkazana is eSengeni; also an important nesting area. At the northern reach of the lake Tewati Bay harbours large congregations of hippopotami and crocodiles and is a regional freshwater oasis during hypersaline conditions. Lake Bhangazi South is a small (262 ha), landlocked freshwater lake nested between Lake St Lucia and the Indian Ocean. The Mphate River feeds into Lake St Lucia on the ecologically-distinct western shoreline and is connected to the Narrows, a winding channel that links the lake to the ocean. The Narrows section is a critical feeding area for Nile

crocodiles but fish densities fluctuate depending on whether the estuary mouth is opened or closed (Whitfield and Blaber 1979, Govender et al. 2011). Extensive information on crocodile habitat and ecology at Lake St Lucia can be found in Combrink (2014).

Although these eight areas represent distinct habitats used by Nile crocodiles at Lake St Lucia, GPS/GSM and satellite transmitters fitted to individuals during this study revealed large movements throughout the lake for some individuals but regional site fidelity for others (Combrink 2014). Lake St Lucia is one of the most complex estuarine ecosystems in the world (Perissinotto et al. 2013), and the ecology of Nile crocodiles over space and time is correspondingly complex (Leslie 1997, Combrink 2014).

#### *Kosi Bay*

Also within the iSimangliso Wetland Park, Kosi Bay sits on the southern Mozambique border (Fig. 3b). The ecosystem consists of an estuary mouth that flows into a linear series of four lakes on a salinity gradient interconnected by narrow channels. Kosi Bay harbours < 20 Nile crocodiles (Warner, unpubl. data) which in this study were captured at the Sihadla Channel, a small waterway at the southern terminus of the fourth lake (Fig. 4). Due to its distance from the Indian Ocean, Sihadla is unaffected by tidal activity or salinity and is functionally a freshwater-mangrove ecosystem (Kyle 1999).

#### *Ndumo Game Reserve*

The 10,000 ha Ndumo Game Reserve (NGR; Fig. 4a) is found inland at the western edge of the Mozambique Coastal Plain. The Usuthu and Phongola Rivers form the northern and eastern boundaries of the reserve, respectively. During the rainy season (Nov – Mar) up to 40% of the

reserve may be inundated, including 12 permanent and semi-permanent floodplain lakes that are critical habitats year-round for the approximately 900 crocodiles in the area (Calverley and Downs 2014b). Lake Inyamithi harbours the highest density of crocodiles, and all individuals for SIA analysis were captured at that locality. Extensive information on crocodile habitat and ecology at NGR can be found in Calverley (2013).

### *Jozini Dam*

The ~13,500 ha Jozini (or Pongolapoort) Dam was constructed in 1973 along the freshwater Phongolo River near the southern Swaziland border (Fig. 4b). After the Domoina floods in 1984, the water level rose by over 70% and crocodiles moved into the Phongolo inlet section on the western side where the current population is still concentrated (Champion 2011). Conservative estimates place the Jozini Dam crocodile population at 275 individuals and it is likely this waterbody supports the only stable or increasing population in South Africa (Champion 2011). Information on crocodile habitat and ecology at Jozini Dam can be found in Champion (2011).

### **Sample collection**

Permission to catch Nile crocodiles and take blood samples at all sites were obtained under permit from Ezemvelo KZN Wildlife and ethical clearance from the University of KwaZulu-Natal. Individuals were captured and secured using a variety of noosing snares, ropes and traps according to standard operating procedure (Combrink et al. 2012). Because of logistical issues and field safety constraints due to potentially dangerous large animals, Nile crocodiles at Jozini Dam, Ndumo Game Reserve and Kosi Bay were all caught at night from a boat, whereas most individuals at St Lucia were captured during the day by researchers on foot. Once an individual

was restrained, blood was collected before body measurements and tagging activities were conducted (usually < 10 min. after capture). Blood for stable isotope analysis was drawn from the post-occipital spinal venous sinus using a 20 ml syringe with 18G needle. Either a 1.5” hypodermic or 3.5” spinal needle (Terumo Corporation, Tokyo, Japan) was used depending on the body size and/or neck fat of individual crocodiles (Myburgh et al. 2014). Between 2 and 20 ml of blood was collected dependent on crocodile size and the needs of ancillary bloodwork studies (< 1 ml is necessary for SIA). Blood samples were immediately deposited into heparinized plastic tubes (4 ml lithium green caps; BD Vacutainer®) and placed in a portable cooler until they could be centrifuged at 3000 rpm. Depending on the length of the field excursion, the time between blood collection and centrifuge ranged from 1 to 6 h. Once red blood cell and plasma components were separated, plasma was manually pipetted off and frozen in cryovials at -80°C until lab analysis. Necrospray (Bayer AG) was applied to the area of scute removal before crocodile release.

Sex was determined for each Nile crocodile by palpating for the presence of the penis in the cloacal cavity (Ziegler and Olbort 2007). Snout-to-vent length (SVL) was measured dorsally as the distance from the tip of the snout to the posterior end of the cloacal opening for each individual. Similar to squamates, SVL is the standard measurement of body length for crocodilians because tails can be partially missing, damaged or deformed. For age class, individuals were grouped as “adult” ( $\geq 140$  cm) or “subadult” (< 140 cm). Although Radloff et al. (2012) reported size at sexual maturity to be ~120 cm for Nile crocodiles in Botswana, we selected 140 cm as the cohort delineation metric because it represented the smallest known nesting female for free-ranging individuals in the province of KwaZulu-Natal at the species’

southern range limit (males and females become reproductively active at approximately the same size).

### **Stable isotope analysis**

A potential confounding factor in the laboratory analysis of isotopes is the lipid content of samples. Lipids can misleadingly produce more negative  $\delta^{13}\text{C}$  values because fats are generally more depleted in  $^{13}\text{C}$  compared with proteins and carbohydrates (DeNiro and Epstein 1978, Gannes et al. 1997, Post et al. 2007). Therefore lipid extraction is usually performed on samples from aquatic organisms that have a C:N ratio of  $> 3.5$  (Post et al. 2007). The mean C:N for our dataset was  $3.6 (\pm 0.9 \text{ SD})$  so lipid extraction was done before analysis.

Isotopic analyses were performed at the Mammal Research Institute, University of Pretoria, using a Flash Elemental Analyser (1112 series) integrated via a ConFlo IV system with a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany). Blood plasma samples were pipetted into smooth walled tin capsules for liquid analyses. The samples were combusted at  $1020^\circ\text{C}$ . Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel  $\delta^{13}\text{C} = -20.57\text{‰}$ ;  $\delta^{15}\text{N} = + 6.80\text{‰}$ ), run after every 12 unknowns in a sequence. The standards for the plasma analysis were diluted with deionized water in the capsules in order to compensate for any matrix effects. The precision ( $\pm \text{SD}$ ) of the standards was  $0.18\text{‰}$  for  $\delta^{13}\text{C}$  and  $0.37\text{‰}$  for  $\delta^{15}\text{N}$  ( $n = 14$ ) for the plasma analyses. A blank was also run after every 12 unknowns to confirm that there was no sample-to-sample memory effect from incomplete combustion.

Isotopic differences between samples typically occur at the third significant figure of the isotope ratio, therefore stable isotope ratios are reported using the conventional delta ( $\delta$ ) notation as parts per thousand deviation ( $\text{‰}$ )

$$\delta X_{\text{STD}} = ((R_{\text{Sample}} / R_{\text{STD}}) - 1) \times 1000$$

where R is either  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ , and  $\delta$  expresses the isotopic abundance of X (either  $^{15}\text{N}$  or  $^{13}\text{C}$ ) in a sample relative to the abundance of the international isotopic standard (PeeDee Belemnite for C, atmospheric nitrogen for N).

### **Data analysis**

To avoid temporal pseudoreplication, Nile crocodile that were captured more than once over the course of the study only contributed the first blood sample taken for the analysis. Plasma from hatchling crocodiles were deliberately excluded from SIA as their tissues may reflect the dietary isotopic signature of mother (Pilgrim 2007). All data were first summarized in bi-plots for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  by sex, stage, year, site, and SVL. We then conducted a principal component analysis (PCA) to create one independent variable from  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for further analysis with linear models. Next, we constructed a candidate set of linear models with PCA score as the dependent variable and sex, stage, year, and site as main effects. Snout-vent length was analyzed as a continuous covariate. Our candidate set included a null model (intercept only), global model (all main effects and two-way interactions), all main effects models, and all two-way effects models with and without interactions. Statistical analyses were performed in R (R Core Team 2015). We assessed model fit using Akaike's Information Criterion (AIC) (an information theoretic approach; Burnham and Anderson 1998) with the AICcmodavg package (Mazzerolle 2015). The top models were delineated as those within 2  $\Delta\text{AIC}_c$  units. We used the effects package to

generate confidence limits for each variable (Fox 2003) and constructed all plots using ggplot2 (Wickham, 2009).

## Results

From 2009 – 12 we obtained blood plasma samples for SIA from 106 Nile crocodile individuals constituting 54 females (13 subadults, 42 adults) and 52 males (14 subadults, 37 adults) in KwaZulu-Natal (Lake St Lucia n = 80, Kosi Bay n = 4, Jozini Dam n = 9, Ndumo Game Reserve n = 13). Snout-vent length for all individuals averaged 154.5 cm (range: 68.1 – 231.0 cm). Mean  $\delta^{15}\text{N}$  was 8.58‰ ( $\pm 2.15$  SD, range: 3.54 to 13.53‰) and mean  $\delta^{13}\text{C}$  was -18.12‰ ( $\pm 2.29$  SD, range: -27.30 to -14.55‰) (Appendix 1).

Bi-plots revealed there was overlap among sexes, years, and most of the sites (Fig. 5). The three groups that parsed out from the main clade constituting the Pongola River inlet at Jozini Dam and most of the Lake St Lucia sites were two freshwater areas at St Lucia (Lake Bengazi south and Tewati Bay), Inyamithi Pan at Ndumo, and the Sihadla Channel at Kosi Bay (Fig. 2). We found marked isotopic separation between subadult and adult individuals (Fig. 5) but this pattern was better illustrated in the SVL bi-plots, with both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  levels increasing concomitantly with SVL (Fig. 6).

The Kaiser-Meyer-Olkin test of sampling adequacy (score = 0.50) and Bartlett's test of sphericity ( $\chi^2 = 2.60$ ,  $df = 1$ ,  $p = 0.107$ ) deemed that variable reduction for the PCA was warranted. Principal component analysis revealed that the first component (PC1) had an eigenvalue of 1.157 (explaining 57.9% of the variance) and PC2 had an eigenvalue of 0.842 (42.1% of the variance). Thus we retained PC1 and used those scores as new orthogonal and

centered dependent variables. We found  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  loaded positively (0.707) with PC1 whereby as scores increased, so did  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Fig. 7).

Of the 27 linear models we examined the top model carried a high Akaike Weight (0.56), had strong predictive power ( $r^2_{\text{adj}} = 0.603$ ; Table 1), and included the additive effects of site and SVL (Table 1). The null model performed poorly (ranked 22) as did the global model (ranked 27; Table 1). The effects of most sites were weak, as their confidence intervals overlapped zero, except for Lake Inyamithi and the Sihadla Channel which had negative effects (Table 2). Most of the sites overlapped, with the exception of Kosi Bay, which had highly depleted  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Fig. 3). We found that SVL had a weaker positive effect on PC1 (Table 2), and that as crocodile SVL increased so did  $\delta\text{N}^{15}$  and  $\delta\text{C}^{13}$  signatures in the PCA singular component (Fig. 8).

## **Discussion**

### **Trophic niche width: $\delta^{15}\text{N}$ variance**

Niche width is broadly defined as the variety of resources a species or population exploits. Hutchinson's (1957) conceptualization of ecological niche as an  $n$ -dimensional hypervolume is a foundational concept still used in the study of ecological community structure (Tilman 2004), ecosystem functioning and change (Pajeulo et al. 2010), and evolutionary processes (Wiens and Graham 2005). While the concept of niche width is conceptually robust, finding practical measures to estimate niche parameters can be difficult (Bearhop 2004). Over the past 25 years, carbon and nitrogen stable isotopes have been increasingly used in animal ecology studies to analyze food web patterns and ecological niche. The power of SIA resides in using isotope data (usually  $\delta^{15}\text{N}$ ) from animal tissues to construct quantitative parameters for dietary niche width

(Bearhop et al. 1999, Layman et al. 2007). This is most easily done for a species or population by analyzing the variation among individual samples or sample means (Bearhop et al. 2004, Willson et al. 2010). Newsome et al. (2007) suggested that an organism's "isotopic niche," its range of stable isotope values expressed in  $\delta$ -space, is comparable to Hutchinson's (1957) n-dimensional hypervolume representation of the niche. Using the same theoretical approach, the term "trophic niche width" used here equates to the difference between the highest (= isotopically enriched) and lowest Nile crocodile dietary  $\delta^{15}\text{N}$  values collected from all individuals (for comparisons of means see Fig. 5).

For Nile crocodiles in KwaZulu-Natal, we found a surprisingly wide trophic niche among dietary  $\delta^{15}\text{N}$  values, with a total spectrum across populations of 9.99‰ (range = 3.54 to 13.53‰). For comparison, in the only other study to assess the species diet using SIA, Radloff et al. (2012) found that  $\delta^{15}\text{N}$  niche width was over twice as narrow in scute tissue from Botswana (total width = 4.90‰, range = 4.2 to 9.0‰). The isotopic range for  $\delta^{15}\text{N}$  presented here for Nile crocodile is also the largest recorded to date among crocodylians, and for large-bodied African predators (Codron et al. 2007).

Without isotope data from crocodile prey (or knowing Nile crocodile tissue-prey discrimination values; Chapter 4) it is not possible to reliably assign discrete trophic positions to Nile crocodile, and any attempt to link the  $\delta^{15}\text{N}$  values we found to specific prey would be meretricious. For example, primary consumers at the base of food webs in KwaZulu-Natal generally have relatively low  $\delta^{15}\text{N}$  values (e.g. between 1 and 2‰ for gastropods; Miranda and Perissinotto 2012) but in some cases may be as high as 14.5‰ (mysid shrimp; Carrasco and Perissinotto 2011). Dietary  $\delta^{15}\text{N}$  values for a species are relatively meaningless without ecological context (e.g. an estuarine shrimp with  $\delta^{15}\text{N} = 10$ ‰ does not indicate that it consumed

a crocodile with  $\delta^{15}\text{N} = 9\text{‰}$ ). In general, though, the crocodile  $\delta^{15}\text{N}$  values presented here overlap with previously recorded isotopic values in South Africa for suspected frequent prey items such as ungulate browsers and grazers ( $\sim 4 - 8\text{‰}$ ; Codron et al. 2007) and omnivorous fish ( $\sim 8 - 14\text{‰}$ ; Govender et al. 2011, Dyer et al. 2015).

### **General isotopic dietary structure among Nile crocodiles in Zululand**

Although the large isotopic variance we observed implicates the Nile crocodile as a generalist predator that appears to feed across a broad swath of the food web over space and time, statistical analyses revealed some general trends. Linear modeling and PCA analysis showed that as crocodile SVL increased so did  $\delta\text{N}^{15}$  values. This conforms to the general pattern in the literature that trophic dietary nitrogen ratios increase with trophic position. Larger crocodiles have more prey options available to them, and can be expected to consume prey with a wider variety of  $\delta\text{N}^{15}$  values, therefore increasing  $\delta\text{N}^{15}$  values in their own tissues (which represents a dietary average over time; Chapter 4). For example, a subadult Nile crocodile that is restricted to eating mostly primary consumers (e.g. small mammals; Radloff et al. 2012) will likely have a lower  $\delta\text{N}^{15}$  signature than a larger individual that can consume omnivorous (protein-eating) fish or birds. Even without incorporating knowledge of specific Nile crocodile prey taxa, our data clearly showed that as crocodile body size increased, the statistical trend was for  $\delta\text{N}^{15}$  to also increase.

That considered, an interesting caveat to this trend emerged among isotope data for relatively small ( $< 1$  m total length) and very large ( $> 4$  m TL) Nile crocodile individuals. Among all crocodiles, the smallest subadults (Kosi Bay) and the largest adults sampled (Tewati Bay, Lake Bhangazi) shared the lowest  $\delta\text{N}^{15}$  values when grouped as a single cohort (mean =  $4.8\text{‰}$ , range =  $3.8$  to  $5.5\text{‰}$ ). Despite a four-fold length disparity and mass difference of over 350

kgs, these particular animals were essentially isotopically identical with regard to dietary nitrogen in blood plasma. The most parsimonious explanation for this based on known Nile crocodile feeding ecology (Leslie 1997, Wallace and Leslie 2008, Radloff et al. 2012) is that young juveniles and dominant male adults captured during this study were both feeding on plant-eating primary consumers; likely small rodents for young crocodiles at Kosi Bay, and antelope or hippopotamus for large adults at Tewate Bay and Lake Bhangazi (Warner and Combrink, unpubl. data). This observation is important because it shows that low  $\delta\text{N}^{15}$  values for crocodilians are not always indicative of small individual size or low food web position (by any ecological metric, a crocodile eating a hippopotamus is an apex predator). For protected African ecosystems that harbour intact food webs (e.g. Lake St Lucia, Kruger National Park, Serengeti National Park) the complete  $\delta\text{N}^{15}$  food web structure for Nile crocodiles may be parabolic in nature, with juveniles and large adults registering roughly equivalent  $\delta\text{N}^{15}$  signatures (indicative of preying on  $\text{C}_3$  and  $\text{C}_4$  plant and grass-eating mammals, but of strongly disparate sizes) and crocodile size classes in between responsible for higher  $\delta\text{N}^{15}$  values linked to protein eating prey such as tigerfish (*Hydrocynus spp.*) and catfish (Clariidae).

The variation in  $\delta^{13}\text{C}$  values is directly linked to differing photosynthetic pathways found among  $\text{C}_3$  and  $\text{C}_4$  plants (Farquhar et al. 1989), and because carbon does not significantly change as it travels in food webs,  $\delta^{13}\text{C}$  values are useful for determining primary nutrient source(s) at the base of those food webs. For Nile crocodiles we found that  $\delta^{13}\text{C}$ , similar to  $\delta\text{N}^{15}$ , increased concomitantly with crocodile size but that there were discernable differences among populations and site means. The isotopic differences for  $\delta^{13}\text{C}$  among ecosystems were not surprising given differing freshwater and estuarine carbon inputs (Chapter 4). While Ndumo Game Reserve, Kosi Bay, and freshwater habitats at Lake St Lucia clearly parsed out into separate clades, Jozini Dam

surprisingly clustered with the majority of the Lake St Lucia sites for both  $\delta^{13}\text{C}$  and  $\delta\text{N}^{15}$ . We attribute the significant effect of increasing  $\delta^{13}\text{C}$  with larger SVL to differing habitat use patterns between subadults and adults, with smaller crocodiles preferring secluded nursery areas and heavily sheltered reeds for predator avoidance (Combrink et al. 2013). The wide variance among  $\delta^{13}\text{C}$  tissue values illustrated the broad predatory impact Nile crocodiles have on aquatic and terrestrial ecosystems, and is an indicator of how the species is ultimately linked to a range of habitats, including those outside of what the species' physically occupies. Similar to  $\delta\text{N}^{15}$ ,  $\delta^{13}\text{C}$  values in Nile crocodile blood plasma should be interpreted as a dietary average, but the variances we observed among these averages bolsters our interpretation based on  $\delta\text{N}^{15}$  data that the Nile crocodile as a species is a true generalist predator.

### **Conservation and management implications**

Stable isotope analysis of Nile crocodile tissue has several practical applications for further research and management of threatened crocodile populations in Africa. Among scientists, conservation managers, and the general public, Nile crocodiles are usually viewed as top predators that dominate the aquatic ecosystems in which they reside; an intellectual framework that is not entirely without merit. While large Nile crocodile indeed occupy the upper echelons of food webs and exert strong top-down ecological influences in many African ecosystems, we suggest the isotope data presented in this study calls for a more nuanced interpretation of Nile crocodile feeding ecology. As a species the Nile crocodile feeds throughout the entirety of the food web, and  $\delta^{13}\text{C}$  and  $\delta\text{N}^{15}$  data show the species' biological reach as a generalist predator is probably even larger than previously assumed, and should be taken into account in management of terrestrial and aquatic crocodile habitats. Crocodile importance as prey within African ecosystems is also vastly underrated, with perhaps as few as 2% of hatchlings attaining sexual

maturity due to predation (Pooley 1982). As a natural resource, we are just beginning to understand the ecological importance of crocodiles in Africa. Future SIA research on Nile crocodile ontogenetic dietary shifts and individual dietary specialization within and among populations (e.g. Radloff et al. 2012, Rosenblatt et al. 2015) will help further the understanding of ecosystem dynamics where crocodiles are currently under threat in South Africa.

As the use of SIA becomes more accessible and widespread in South Africa, there are intriguing forensic applications for the technology going forward with regard to crocodiles. Nile crocodile poaching and indiscriminate killing is an eminent threat to the viability of some populations (Calverley and Downs 2014a), primarily in areas where humans and livestock must partition water resources (Combrink et al. 2011). The species is also targeted for use in traditional medicine practices (Combrink et al. 2013). Often live individuals and body parts are recovered by authorities far from capture sites, e.g. at markets and during raids on poachers. The remaining crocodile populations in KwaZulu-Natal have markedly different carbon inputs, as indicated by the  $\delta^{13}\text{C}$  signatures obtained from Nile crocodile tissues in this study. It would therefore be theoretically possible, if tissue could be obtained and sent for lab analysis within a reasonable time frame, to identify the localities where poaching pressure is greatest, and even return recovered live animals back to their source populations.

### **Acknowledgments**

We thank R. Taylor, P. Calverley, G. Champion, F. Myburgh, S. Kyle and family, the many volunteers who assisted with fieldwork, M. Dreslik for statistical guidance, Ezemvelo KwaZulu-Natal Wildlife and the iSimangaliso Wetland Park Authority. The Ford Wildlife Foundation and the South African Water Research Commission are thanked for their vehicle/ financial support.

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### Figure legends

**Figure 1.** Nile crocodile (*Crocodylus niloticus*) cannibalism at Lake St Lucia, South Africa  
(Photo: Frans Lemmer).

**Figure 2.** Nile crocodile (*C. niloticus*) populations in KwaZulu-Natal, South Africa: Jozini Dam  
(1), Ndumo Game Reserve (2), Kosi Bay (3), and Lake St Lucia (4).

**Figure 3.** Nile crocodile sampling sites at a.) Lake St Lucia, and b.) Kosi Bay.

**Figure 4.** Nile crocodile sampling sites at a.) Ndumo Game Reserve, and b.) Jozini Dam  
(Pongolapoort Dam).

**Figure 5.** Bi-plots of mean and standard error for  $\delta N^{15}$  and  $\delta C^{13}$  values by a.) sex, b.) capture  
site, c.) stage, and d.) year (2009-2012).

**Figure 6.** Scatter plots of the relationship of a.)  $\delta N^{15}$  and b.)  $\delta C^{13}$  to SVL in Nile crocodiles.

**Figure 7.** Scatter plots of the relationship of a.)  $\delta N^{15}$  and b.)  $\delta C^{13}$  to the isotope component  
(PC1) in Nile crocodiles.

**Figure 8.** The effects of a.) site (mean, bar = standard error, whiskers = 95% C.I.) and b.) SVL  
(mean line and 95% confidence limits) on the isotope component in Nile crocodiles.

## Tables

**Table 1:** Model selection table for 27 candidate models of the effects of sex, stage, site, year, and SVL on the composite isotope component (principal component score of  $\delta N^{15}$  and  $\delta C^{13}$ ). Table includes the model rank and name, number of parameters (K), -2 log-likelihood, AICc and  $\Delta AICc$ , likelihood of model, Akaike weights ( $w_i$ ) and cumulative weights ( $\Sigma w_i$ ).

Rank	Model	K	-2LL	AICc	$\Delta AICc$	Likelihood	$w_i$	$\Sigma w_i$
1	Site+SVL	14	-102.26	237.14	0.00	1.00	0.56	0.56
2	Site	13	-104.69	239.35	2.20	0.33	0.19	0.75
3	Site+Sex	14	-103.69	239.99	2.84	0.24	0.14	0.89
4	Site+Stage	14	-104.01	240.63	3.49	0.18	0.10	0.99
5	Year+Site	15	-104.67	244.68	7.54	0.02	0.01	1.00
22	Null	2	-157.65	319.42	82.28	0.00	0.00	1.00
27	Global	50	-83.93	360.58	123.44	0.00	0.00	1.00

**Table 2:** Parameter estimates, standard errors, and confidence intervals of the top linear model of the effects of site and SVL on the isotope component (PC1).

Parameter	Est.	S <sub>err</sub>	95% C. I.	
			Lower	Upper
Intercept	-0.5810	0.4827	-1.5395	0.3776
$\beta_{\text{DeadTreeBay}}$	0.4568	0.2895	-0.1181	1.0316
$\beta_{\text{Sengeni}}$	0.3147	0.3267	-0.3341	0.9635
$\beta_{\text{Inlet}}$	-0.0254	0.3243	-0.6694	0.6185
$\beta_{\text{Inyamithi}}$	-0.7463	0.3212	-1.3840	-0.1085
$\beta_{\text{LakeBhangazi}}$	-0.5190	0.4720	-1.4563	0.4183
$\beta_{\text{Mphate}}$	-0.5026	0.3608	-1.2190	0.2139
$\beta_{\text{Narrows}}$	0.1967	0.3126	-0.4241	0.8174
$\beta_{\text{Nkazana}}$	-0.2007	0.3096	-0.8156	0.4141
$\beta_{\text{NkazanaStream}}$	0.1951	0.3047	-0.4099	0.8002
$\beta_{\text{Sihadla}}$	-3.5348	0.4706	-4.4693	-2.6003
$\beta_{\text{TewatiBay}}$	-0.3928	0.7187	-1.8199	1.0344
$\beta_{\text{SVL}}$	0.0005	0.0002	0.0000	0.0009

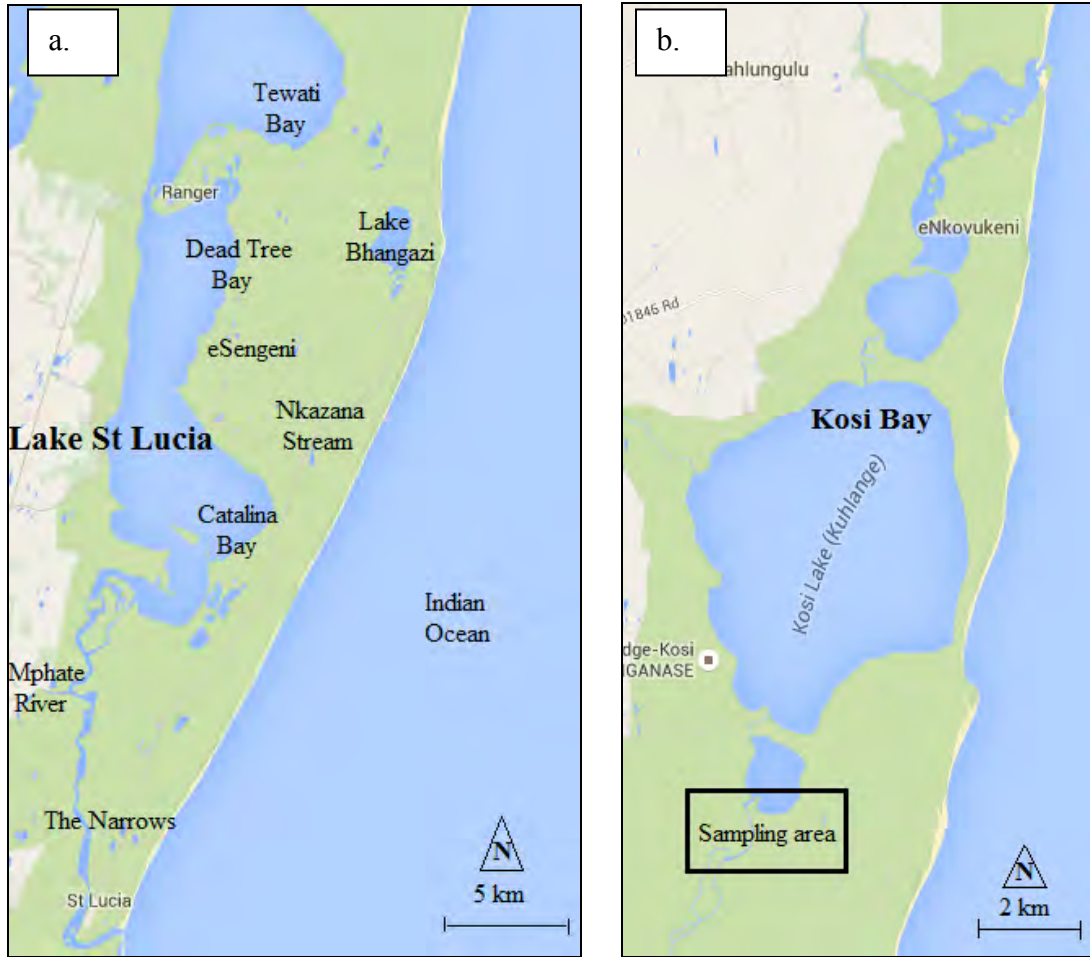
## Figures



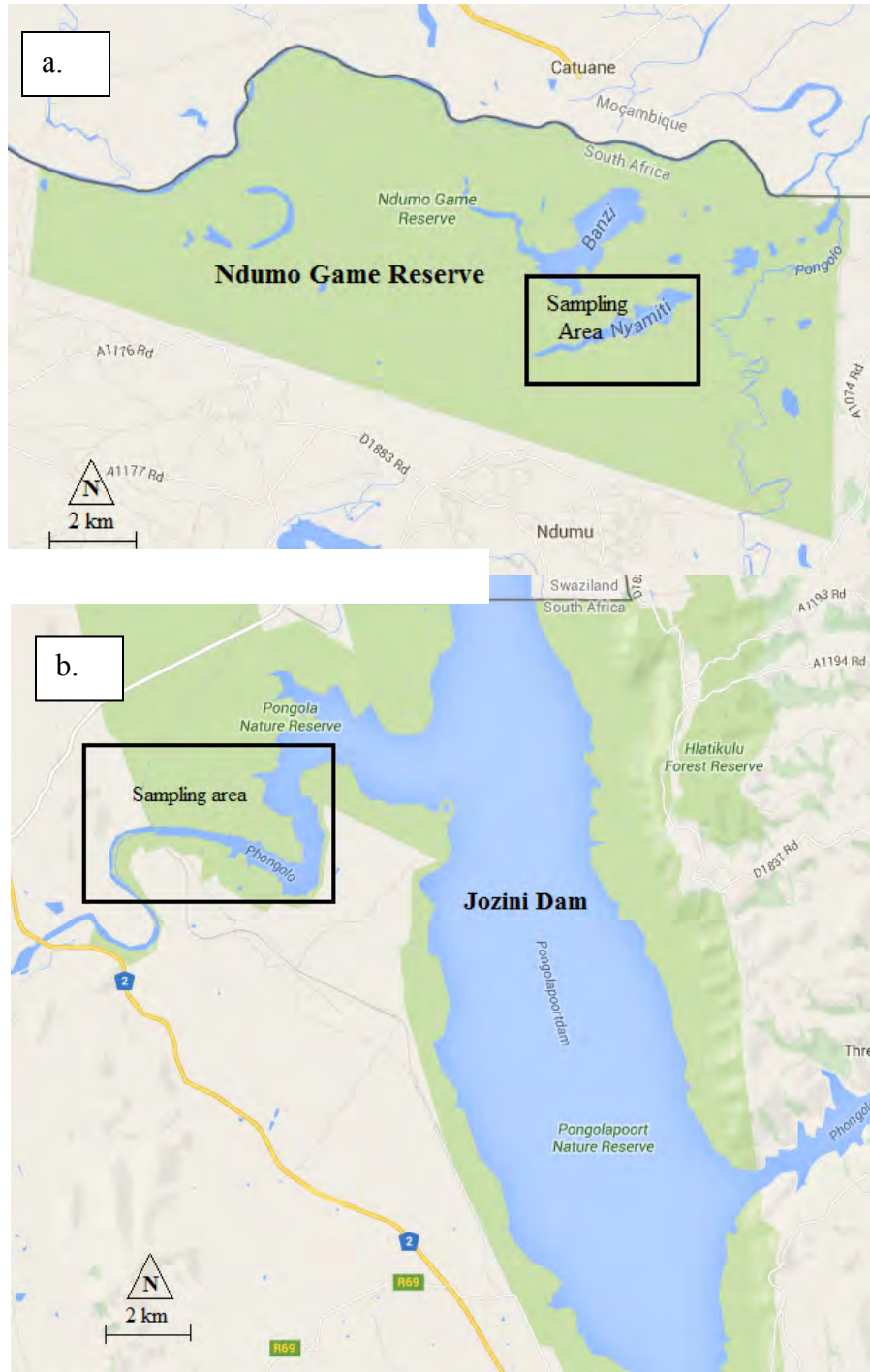
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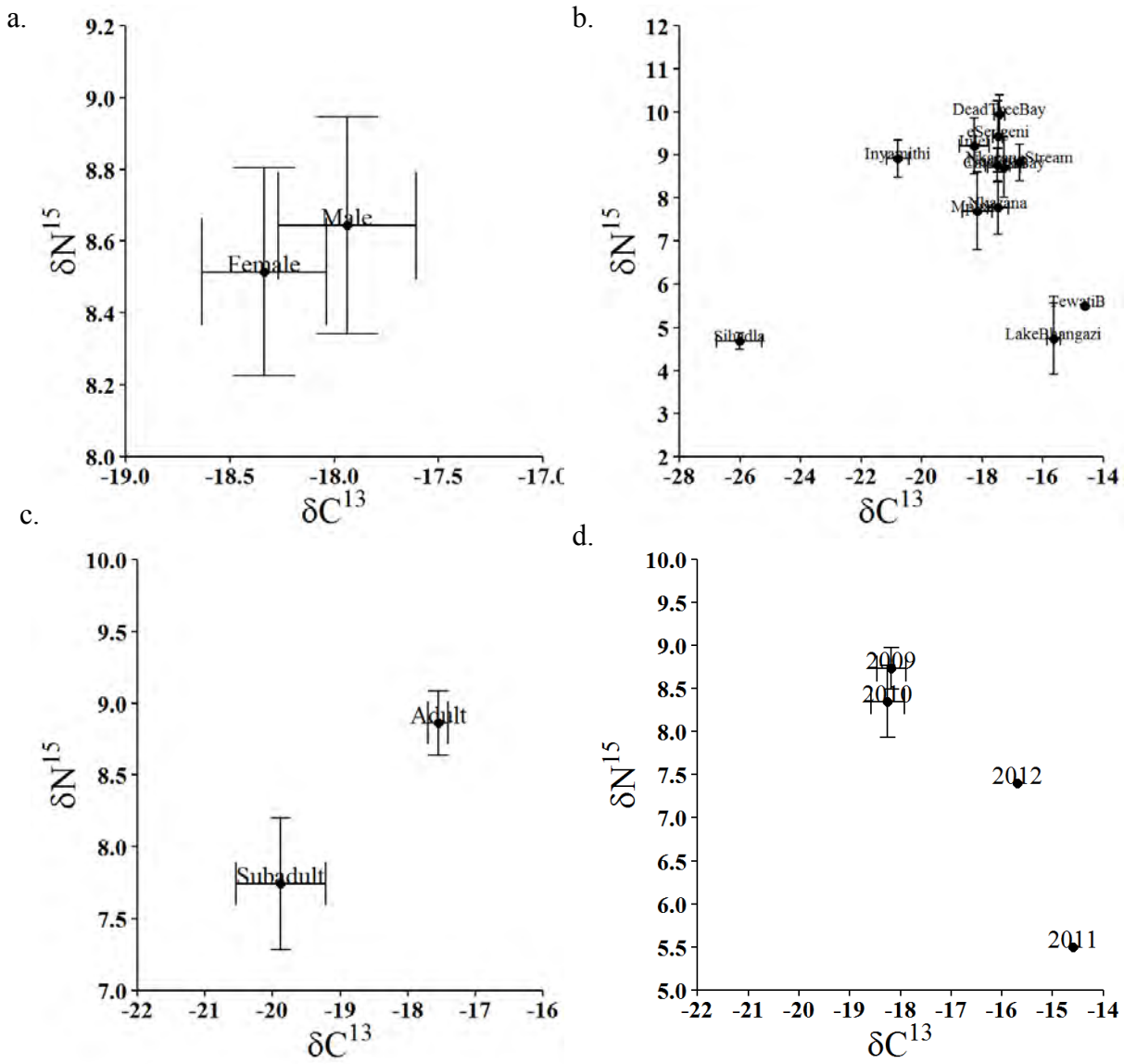
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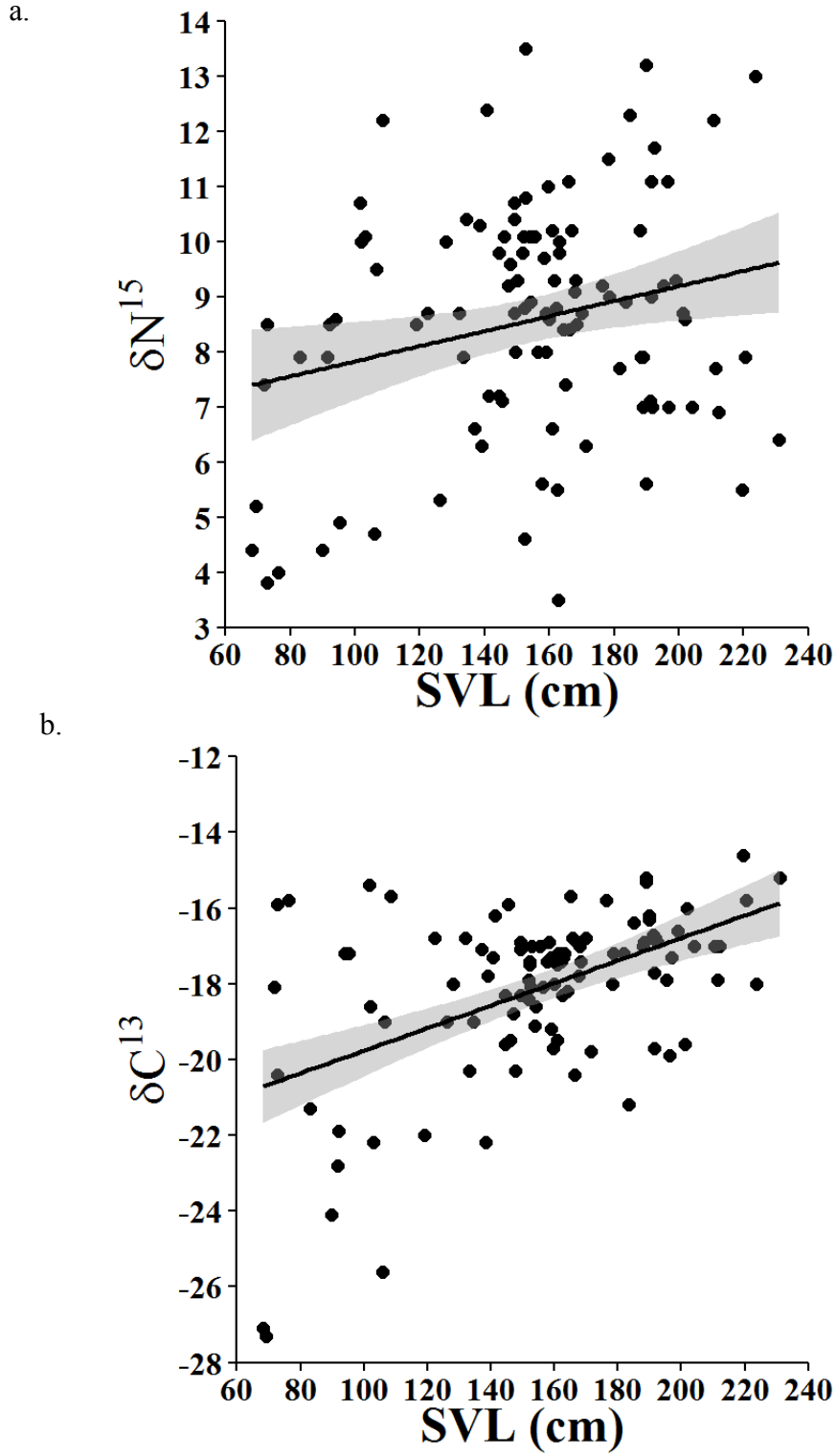
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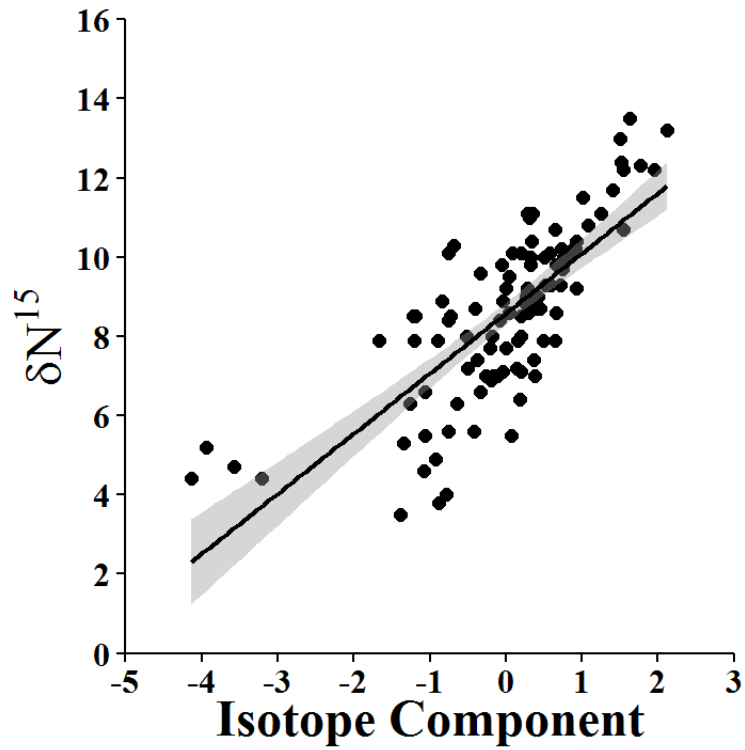


**Figure 5.** Bi-plots of mean and standard error for  $\delta N^{15}$  and  $\delta C^{13}$  values by a.) sex, b.) capture site, c.) stage, and d.) year (2009-2012).

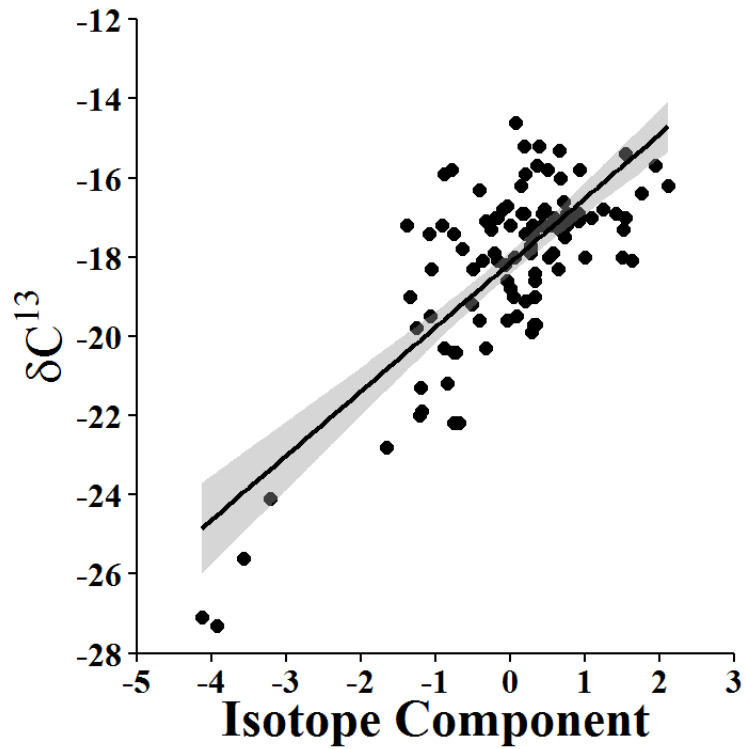


**Figure 6.** Scatter plots with 95% confidence intervals of the relationship of a.)  $\delta N^{15}$  and b.)  $\delta C^{13}$  to SVL in Nile crocodiles.

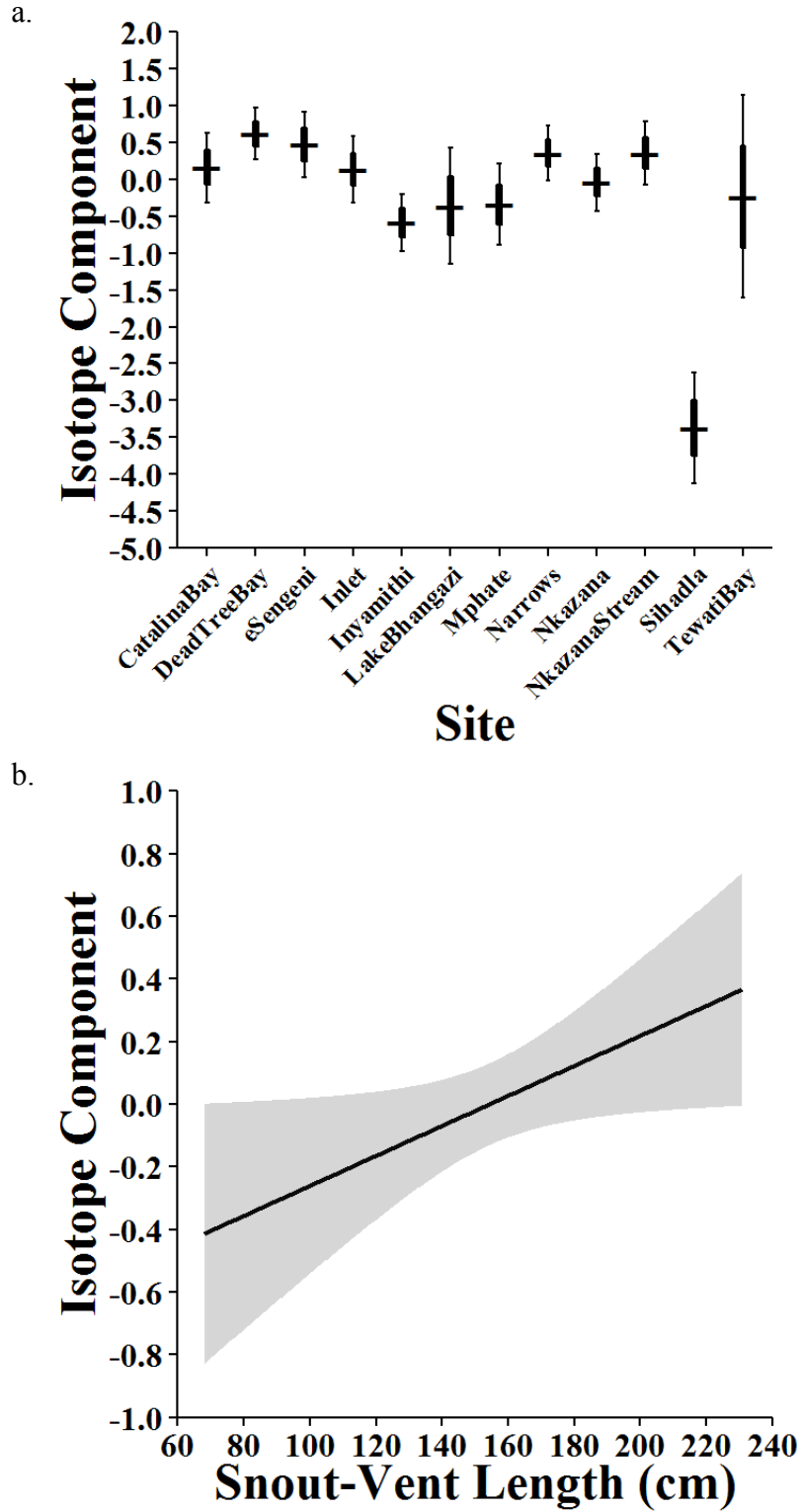
a.



b.



**Figure 7.** Scatter plots with 95% confidence intervals of the relationship of a.)  $\delta N^{15}$  and b.)  $\delta C^{13}$  to the isotope component (PC1) in Nile crocodiles.



**Figure 8.** The effects of a.) site (mean, bar = standard error, whiskers = 95% C.I.) and b.) SVL (mean line and 95% confidence limits) on the isotope component in Nile crocodiles.

## Appendix 1.

Data for Nile crocodiles (*C. niloticus*) captured in KwaZulu-Natal, South Africa for stable isotope analysis of diet.

SVL (mm)	Sex	Size class	Site	$\delta^{15}N$	$\delta^{13}C$	C/N ratio
1280	F	Sub-adult	Catalina Bay	10.0	-18.0	2.9
1700	F	Adult	Nkazana	8.7	-16.8	3.8
953	F	Sub-adult	Nkazana	4.9	-17.2	3.7
1494	F	Adult	Nkazana	8.0	-16.9	3.3
1370	F	Sub-adult	Nkazana	6.6	-17.1	2.8
1786	F	Adult	Catalina Bay	9.0	-17.2	4.0
1678	F	Adult	Dead Tree Bay	9.1	-17.8	3.2
1526	F	Adult	Dead Tree Bay	13.5	-18.1	5.5
1670	F	Adult	Dead Tree Bay	10.2	-16.9	4.6
1632	F	Adult	Dead Tree Bay	10.0	-17.2	4.3
1542	F	Adult	Dead Tree Bay	8.9	-18.6	4.1
1492	F	Adult	Dead Tree Bay	10.4	-17.1	5.3
1502	F	Adult	Dead Tree Bay	9.3	-17.0	4.2
1564	F	Adult	Dead Tree Bay	8.0	-18.1	3.1
1590	F	Adult	Nkazana	8.7	-17.3	3.9
1522	F	Adult	Nkazana	8.8	-17.5	3.9
940	F	Sub-adult	Dead Tree Bay	8.6	-17.2	3.5
718	F	Sub-adult	Narrows	7.4	-18.1	3.9
1472	F	Adult	Narrows	9.2	-18.8	4.0
1714	F	Adult	Nkazana	6.3	-19.8	4.8
1596	F	Adult	Nkazana	11.0	-19.7	7.4
1614	F	Adult	eSengeni	9.3	-17.2	4.0
1522	F	Adult	Nkazana	4.6	-17.4	1.6
1782	F	Adult	Nkazana	11.5	-18.0	4.1
1412	F	Adult	Nkazana	7.2	-16.2	3.2
1408	F	Adult	eSengeni	12.4	-17.3	5.4
1454	F	Adult	Nkazana	7.1	-15.9	2.7
1608	F	Adult	eSengeni	10.2	-17.5	3.3
1528	F	Adult	eSengeni	10.8	-17.0	4.3
1520	F	Adult	eSengeni	10.1	-17.9	3.9
1628	F	Adult	eSengeni	3.5	-17.2	1.3
1680	F	Adult	eSengeni	9.3	-17.0	2.5
1492	F	Adult	eSengeni	10.7	-18.3	4.0

1599	F	Adult	eSengeni	8.6	-18.0	4.0
1684	F	Adult	Narrows	8.5	-17.4	3.4
764	F	Sub-adult	Lake Bhangazi	4.0	-15.8	4.2
1642	F	Adult	Narrows	8.4	-18.2	3.4
1392	F	Adult	Mphate	6.3	-17.8	4.7
1610	F	Adult	Mphate	6.6	-19.5	2.3
1658	F	Adult	Mphate	11.1	-16.8	3.3
1650	F	Adult	Narrows	7.4	-15.7	3.5
2122	M	Adult	Mphate	6.9	-17.0	3.7
2196	M	Adult	Tewati Bay	5.5	-14.6	3.9
2310	M	Adult	Lake Bhangazi	6.4	-15.2	4.1
2106	M	Adult	Catalina Bay	12.2	-17.0	4.6
2115	M	Adult	Dead Tree Bay	7.7	-17.9	3.1
2206	M	Adult	Narrows	7.9	-15.8	4.0
2018	M	Adult	Nkazana Stream	8.6	-16.0	3.4
1850	M	Adult	Dead Tree Bay	12.3	-16.4	4.3
1630	M	Adult	Dead Tree Bay	9.8	-17.3	3.4
1900	M	Adult	Dead Tree Bay	13.2	-16.2	4.2
1970	M	Adult	Catalina Bay	7.0	-17.3	2.9
1900	M	Adult	Catalina Bay	5.6	-16.3	1.5
1990	M	Adult	Nkazana Stream	9.3	-16.6	3.9
1890	M	Adult	Catalina Bay	7.0	-15.2	4.2
1880	M	Adult	Nkazana Stream	10.2	-17.0	4.3
1882	M	Adult	Nkazana Stream	7.9	-16.9	3.4
2040	M	Adult	Nkazana Stream	7.0	-17.0	2.2
1918	M	Adult	Nkazana Stream	7.0	-16.8	3.7
1925	M	Adult	Nkazana Stream	11.7	-16.9	4.6
1890	M	Adult	Catalina Bay	7.9	-15.3	2.8
2012	M	Adult	Catalina Bay	8.7	-19.6	3.7
1914	M	Adult	Catalina Bay	11.1	-19.7	4.6
1818	M	Adult	Nkazana Stream	7.7	-17.2	3.4
1912	M	Adult	Narrows	7.1	-16.7	2.0
1764	M	Adult	Nkazana Stream	9.2	-15.8	3.7
1914	M	Adult	Dead Tree Bay	9.0	-17.7	3.1
1954	M	Adult	Dead Tree Bay	9.2	-17.9	3.1
1626	M	Adult	Mphate	5.5	-18.3	1.9
1582	M	Adult	Nkazana Stream	9.7	-16.9	4.0
1446	M	Adult	Narrows	7.2	-18.3	2.2
1622	M	Adult	Nkazana Stream	8.8	-17.4	4.4
1444	M	Adult	Mphate	9.8	-19.6	4.2
1224	M	Sub-adult	Narrows	8.7	-16.8	2.9

1066	M	Sub-adult	Narrows	9.5	-19.0	3.4
1020	M	Sub-adult	Narrows	10.0	-18.6	3.5
1085	M	Sub-adult	Narrows	12.2	-15.7	3.4
1016	M	Sub-adult	Narrows	10.7	-15.4	3.6
727	M	Sub-adult	Lake Bhangazi	3.8	-15.9	3.3
729	M	Sub-adult	Narrows	8.5	-20.4	3.3
1060	F	Sub-adult	Sihadla	4.7	-25.6	3.8
898	M	Sub-adult	Sihadla	4.4	-24.1	4.0
693	M	Sub-adult	Sihadla	5.2	-27.3	5.1
681	F	Sub-adult	Sihadla	4.4	-27.1	2.5
1591	F	Adult	Inlet	8.0	-19.2	3.5
1576	F	Adult	Inlet	5.6	-17.4	3.0
1492	F	Adult	Inlet	8.7	-16.9	3.8
1518	F	Adult	Inlet	9.8	-18.4	3.5
1461	F	Adult	Inlet	10.1	-19.5	3.8
2236	M	Adult	Inlet	13.0	-18.0	3.8
1836	M	Adult	Inlet	8.9	-21.2	2.3
1555	M	Adult	Inlet	10.1	-17.0	3.7
1321	M	Sub-adult	Inlet	8.7	-16.8	2.8
1478	F	Adult	Inyamithi	9.6	-20.3	4.3
920	F	Sub-adult	Inyamithi	8.5	-21.9	3.3
830	F	Sub-adult	Inyamithi	7.9	-21.3	2.4
1664	M	Adult	Inyamithi	8.4	-20.4	3.9
1190	M	Sub-adult	Inyamithi	8.5	-22.0	3.2
1262	M	Sub-adult	Inyamithi	5.3	-19.0	2.0
1344	M	Sub-adult	Inyamithi	10.4	-19.0	4.2
916	F	Sub-adult	Inyamithi	7.9	-22.8	3.6
1384	F	Sub-adult	Inyamithi	10.3	-22.2	4.5
1964	M	Adult	Inyamithi	11.1	-19.9	4.3
1540	F	Adult	Inyamithi	10.1	-19.1	4.1
1030	M	Sub-adult	Inyamithi	10.1	-22.2	4.4
1334	M	Sub-adult	Inyamithi	7.9	-20.3	2.7

## CHAPTER 6

### Conclusions and Future Research

It is hoped that this thesis will contribute positively to the proud research legacy of the Nile crocodile *Crocodylus niloticus* in southern Africa (including Cott 1961; Pooley 1982; Jacobsen 1984; Hutton 1987; Blake & Jacobsen 1992; Leslie 1997; Wallace & Leslie 2008; Ashton 2010; Botha et al. 2011; Champion 2011; Ferreira & Pienaar 2011; Combrink et al. 2011, 2013; Radloff et al. 2011; Woodborne et al. 2012; Calverley 2013; Lane et al. 2013; Combrink 2014; Myburgh et al. 2014). In many ways, our knowledge of this species reveals the paradoxes inherent to conservation biology: every new discovery uncovers questions that expose our ignorance, and every small victory reveals further the scope of the battlefield. In South Africa, the battle for the long-term survival of the Nile crocodile still has many years to be fought, and the outcome is uncertain. The threats to crocodiles in South Africa are a many-headed hydra, and represent in a microcosm what crocodylians are facing globally: anthropogenic alteration of waterbodies, uncontrolled and unsustainable water use, invasive vegetation, unsound land management practices, loss of nesting habitats, poaching in all its forms, and the unknown but salient effects of climate change. More recently, environmental pollution has emerged as an insidious threat to Nile crocodiles, highlighted by the pansteatitis outbreak and resultant deaths of hundreds of crocodiles from the Olifants, Letaba and Sabie Rivers in the Kruger National Park and local extinction of the Loskop Dam population.

In northern KwaZulu-Natal (KZN), there were strong allometric relationships between measures of Nile crocodile body length (TL and SVL) and other physical attributes, with greater

variance in body form among larger crocodiles. The considerable individual variation found among all size classes for various morphometric relationships revealed a significant degree of morphological plasticity for crocodiles in KZN. This study adds to a growing body of literature that suggests there is an inherent high degree of variation between and among *C. niloticus* populations that probably exceeds that of all other crocodylian species (Hekkala et al. 2011, Nestler 2012). Additional morphometric and molecular data for *C. niloticus* across the species' range is urgently needed to delineate potential taxa synonymized under *C. niloticus* and to properly address the region-specific conservation challenges these crocodiles face (Shirley et al. 2014). The morphometric predictive models generated (Chapter 2) will assist future studies in assessing Nile crocodile body length from skulls found in the field, detecting ontogenetic shifts in morphology, and estimating crocodile biomass at population and regional scales. The even sex ratio found for Nile crocodiles in KZN will assist future management decisions regarding crocodile populations in the province. The sexual size dimorphism quantified in Chapter 2 is the most extensive examination of SSD for the species to date. Further research on the sex ratios and recruitment of subadult Nile crocodile are needed to assess the long term viability of remaining populations in KZN.

Our investigation of blood lead concentrations in free-ranging Nile crocodiles helps move forward our understanding of Nile crocodile ecotoxicology, and provides a quantitative baseline for future studies in both polluted and relatively pristine environments (Chapter 3). While it is likely adult Nile crocodiles may be able to tolerate elevated Pb body burdens, the effects of Pb pollution on reproduction and hatchling health, and therefore long-term population health, is still not clear. Experimental studies of Pb toxicosis in crocodylians are needed, and will help delineate dangerous levels of exposure for free-ranging animals. Routine field monitoring of Pb exposure

in Nile crocodiles is also needed, especially at Lake St Lucia where recreational fishing has introduced an unknown quantity of Pb into a critically important estuarine environment.

Stable isotope analysis of diet revealed a trophic niche width of 9.99‰ for Nile crocodile populations in KZN, the largest recorded to date among crocodylians and for large-bodied predators (Chapters 4 and 5). This quantitatively illustrates that the species is a generalist predator that feeds across the entirety of the food web. The high variances we found among  $\delta^{13}\text{C}$  and  $\delta\text{N}^{15}$  values suggests the Nile crocodile's ecological impact is probably even larger and more important than previously assumed. Crocodylian stable isotope ecology is still in its infancy, and future studies of  $\delta^{13}\text{C}$  and  $\delta\text{N}^{15}$  dynamics will significantly further our understanding of dietary differences among populations, ontogenetic dietary shifts, and food web structure. An experimental diet-switch study for sexually immature and adult Nile crocodile is needed to determine diet-tissue isotope discrimination values and tissue turnover times. Until these values are determined, assigning discrete trophic positions to free-ranging crocodiles will remain speculative.

From a broader research and management perspective, a South African national census for Nile crocodiles is urgently needed, along with a national conservation strategy that incorporates the findings of recent studies and addresses novel threats to the species such as environmental pollution. The conservation challenges crocodiles face in South Africa are inextricably linked to water availability, cleanliness and sustainability – essential human needs. The protection and persistence of the Nile crocodile is therefore a reflection not only of environmental responsibility, but of human dignity.

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## THESIS APPENDIX 1.

Publications not included in this thesis from Nile crocodile research in South Africa during the same research timeframe:

- Combrink X, **Warner JK**, Taylor RH, Downs CT (2014) Population size and distribution of Nile Crocodiles (*Crocodylus niloticus*) in the Lake St Lucia estuarine system, South Africa. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa
- Combrink X, **Warner JK**, Taylor RH, Downs CT (2014) Movements and activity of Nile Crocodiles (*Crocodylus niloticus*) in the Lake St Lucia estuarine system, South Africa. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa
- Combrink X, **Warner JK**, Taylor RH, Downs CT (2014) Home range and movements of Nile Crocodiles (*Crocodylus niloticus*) in the Lake St Lucia estuarine system, South Africa. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa
- Combrink X, **Warner JK**, Taylor RH, Downs CT (2014) An overview of Nile Crocodile (*Crocodylus niloticus*) nest abundance and distribution in the Lake St Lucia estuarine system, South Africa. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa
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