TEMPERATURE-DEPENDENT SEX DETERMINATION IN THE NILE CROCODILE, *Crocodylus niloticus*, IN THE OKAVANGO RIVER, BOTSWANA, AND THE EFFECT OF GLOBAL CLIMATE CHANGE

by

KRISTINE MACIEJEWSKI

Thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

Department of Conservation Ecology and Entomology
Faculty of AgriSciences
University of Stellenbosch

Supervisor: Dr. A. J. Leslie
December 2006

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Date: 23 November 2006

ABSTRACT

The Nile crocodile, *Crocodylus niloticus* exhibits temperature-dependent sex determination, where sex is determined by the incubation temperature of the egg prior to hatching. Laboratory incubation of the eggs indicated that the lower and upper pivotal temperature for the Nile crocodile in the Okavango River, Botswana was 31.4°C and 33.4°C respectively. Exclusively females were produced at a constant temperature of 30.0°C, 30.5°C and 34.0°C, 71% females were produced at 31.0°C and the majority males were produced at 31.5; 32.0; 32.5 and 33.0°C. *Crocodylus niloticus* in the Okavango Region therefore has a female-male-female pattern of temperature-dependent sex determination where females are produced at lower and higher incubation temperatures. Embryonic development, incubation period, hatching success and development rates were strongly temperature dependent. The location of nests plays an important role in determination of incubation temperature. At a distance of 6m from the river, soil temperature was at a maximum at a depth of 25cm. Breeding females choose nesting sites based on optimal soil temperatures. Along the Okavango River the average nest sites were 5.6m from the river, and the eggs were at an average depth of 24.5cm. Calculation of mean nest temperature during the thermosensitive period (sex determining period) of incubation for ten wild Nile crocodile nests indicated that the nests along the Okavango River are primarily female-biased. An increase in average air temperature due to Global Climate Change could possibly shift the population to a male-biased sex ratio, leading to eventual extinction of the Nile crocodile in the Okavango River.

OPSOMMING

Die Nylkrokodil, *Crocodylus niloticus,* vertoon temperatuur-afhanklike geslagsdeterminasie waar die geslag afhanklik is van die inkubasie-temperatuur van die eiers net voor hulle uitbroei. Laboratorium-inkubasie van die eiers het aangedui dat 31.4°C en 33.4°C, respektiewelik die beslissende lae en hoë temperatuur vir die Nylkrokodil in die Okavango Rivier, Botswana is. Uitsluitlik wyfies is geproduseer by 30.0°C, 30.5°C en 34.5°C, 71% wyfies is geproduseer by 31.0°C en 'n meerderheid mannetjies is geproduseer by 31.5°C, 32.0°C, 32.5°C en 33.0°C. *Crocodylus niloticus* in die Okavango gebied het dus n wyfie-mannetjie-wyfie-patroon van temperatuur-afhanklike geslagsdeterminasie waar wyfies geproduseer is by lae en hoë inkubasie-temperature. Embrionale ontwikkeling, inkubasie-tydperk, uitbroei-sukses en ontwikkelingstempos was sterk afhanklik van temperatuur. Die ligging van die nes speel 'n belangrike rol in die inkubasie-temperatuur van die eiers. Die temperatuur van die sand was die warmste by 'n afstand van 6m vanaf die rivier en by 'n diepte van 25cm. Telende wyfies kies nesgebiede gebaseerd op optimale sandtemperature. Langs die Okavango Rivier was die gemiddelde nes 5.6m vanaf die rivier en die eiers was 'n diepte van 24.5cm onder die grond. Berekening van gemiddelde nestemperature gedurende die termosensitieve tydperk van broei vir tien Nylkrokodilneste langs die Okavango Rivier, het 'n wyfie-gunstige neiging getoon. Die geslagsverhouding van die krokodilkleintjies is afhanklik van die inkubasie-temperatuur van die nes.; 'n verhooging in temperatuur, a.g.v. globale klimaatsveranderinge, sal die bevolking na 'n mannetjie-gunstige geslagsverhouding toe skuif en uiteindelik lei tot die uitsterwing van die Nylkrokodil in die Okavango Rivier.

Dedicated to the loving memory of my brother
Marc Maciejewski (1973 - 1993)

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisor Dr Alison Leslie, thank you for your immense help, encouragement and support throughout this project. Your love and passion for crocodiles was a great inspiration and set an example for me.

The Okavango Crocodile Research Team, without your teamwork, and dedication this project would not have been possible. Thank you for standing by me, encouraging me and helping me every step of the way.

Braam and Wilhemien, thank you for your love and much kindness you showed me throughout my stay in Botswana. I very much appreciate all your help. Annelise, a huge thank you for your help and use of the facilities and hatchlings at the Krokovongo Crocodile Farm.

I am indebted to Earthwatch, Inc., USA for providing not only financial support and equipment, but also for providing many pairs of helping hands. Thank you to the numerous volunteers who generously donated their time to assist with my fieldwork. Thank you to the National Research Foundation (NRF) for financial funding throughout my project.

Dr Eduard Hoffman at the Soil science department at the University of Stellenbosch. Thank you for assisting me with my soil moisture analysis. Thank you to Professor Daan Nel at the Department of Statistics at the University of Stellenbosch for aiding me in my statistical analysis.

Special thanks go to my family, for all your love, support and encouragement every step of the way. I would never have done it without you.

My boyfriend Brad, thank you for standing by me and supporting me, especially during the last few months when it was most needed. Thank you for all your love, strength and most of all, thank you for believing in me.

Lastly, I would like to thank our Heavenly Father. Thank you for creating this wonderful earth and giving me the opportunity to work and live in the Okavango Delta and work with one of God's most beautiful creatures.

TABLE OF CONTENTS

Page

CHAPTER 5 - CONCLUSIONS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Temperature control plays a significant role in the proper functioning of all individuals and abnormal variations can have detrimental consequences. Crocodiles are ectotherms and therefore rely on external heat sources to control their body temperatures (Lang, 1987). They are therefore vulnerable to environmental conditions, such as temperature change, which may induce behavioral changes or alter physiological performances (Taylor *et al.*, 2004).

The temperature at which the eggs are incubated plays a critical role in embryonic development and survival. Reptilian embryos are unable to develop successfully at extreme temperatures. Low temperatures may slow or arrest embryonic development and extreme high temperatures may increase embryonic abnormality and mortality (Lin *et al.*, 2003; Lang *et al.*, 1989). Incubation temperature affects the probability of embryos surviving (Lang *et al.*, 1989), the frequency of abnormalities among embryos and hatchlings (Webb *et al.*, 1983), body size at hatching (Hutton, 1987; Webb *et al.*, 1987), the weight of residual yolk at hatching (Webb *et al.*, 1987), hatchling pigmentation patterns (Deeming and Ferguson, 1989), post-hatching growth rates (Hutton, 1987; Joanen *et al.*, 1987) and post-hatching patterns of thermoregulation (Lang, 1987).

Vertebrates typically have a genetically fixed sex determination (GSD) in which the sex of the individual is determined at or before conception by sex chromosomes (Elf, 2003). In reptiles, however, the sex of the offspring is not determined by sex chromosomes, but is dependent on the environment it encounters during incubation. This is called environmental sex determination (ESD). While genotypic sex determination depends on genetic factors alone, environmental sex determination depends on post-fertilization environmental factors (Ciofi and Swingland, 1997). The most common form of ESD is temperature-dependent sex determination, also known as TSD (Janzen and Paukstis, 1991). TSD is a phenomenon in which the incubation temperature of the egg determines the sex of the offspring and this is restricted to certain groups of reptiles, such as crocodilians (alligators and crocodiles) [Hutton, 1987], chelonians (turtles and tortoises) [Hays *et al.*, 2003], some lizards (Gutzke and Crews, 1988) and tuataras (Ciofi and Swingland, 1997).

Thermal and hydric conditions within a nest influences the duration of incubation; hatchling size; yolk sac size, and sex of hatchlings in species with environmental sex determination

(Ferguson and Joanen, 1982). It has been shown that incubation temperature of certain reptilian eggs has a direct effect on determination of the sex ratio of the hatchlings.

All of the 11 species of crocodilians studied thus far show TSD (Ciofi and Swingland, 1997; Deeming and Ferguson, 1989) and it has become increasingly clear that this is a complex and variable phenomenon (Janzen and Paukstis, 1991). Shine (1999) states that TSD remains an 'evolutionary enigma' in reptiles, as many reptiles which have TSD are relatively long-lived organisms with delayed reproduction (Bowden *et al.*, 2000). The temperature that produces 50% of each sex under constant temperature incubation has been termed the pivotal temperature (Mrosovsky and Pieau, 1991). Pivotal temperatures vary with the thermal nesting environment and have become a 'benchmark' temperature to make comparisons between species (Mrosovsky and Pieau, 1991).

In several species of reptiles, temperature affects sex determination during particular stages in embryonic development when differentiation of gonads occurs. Preliminary temperature-shift experiments, designed to assess the temperature sensitive period (TSP) of gonadal differentiation were carried out by Ciofi and Swingland (1997). It is generally stated that thermosensitivity in TSD reptiles occurs during the middle third or half of incubation (Ciofi and Swingland, 1997). Crocodilian embryos are particularly sensitive to incubation temperatures during the first half of development (Webb *et al.*, 1987), which is the period that most squamate embryos are retained within the maternal oviducts (Shine, 1985). Squamates may thus be buffering their embryos against temperature extremes during the most sensitive stages of embryological development (Webb and Cooper-Preston, 1989). The remaining period of incubation is characterized by embryonic growth (Congdon *et al.*, 1995).

Sex ratios therefore seem to depend on the length of time eggs are exposed to a certain temperature regime. Moreover, temperature-shift experiments show that the duration of exposure, at the temperature required for typical male or female differentiation depends on the stage within TSP at which the temperature shift is initiated. The later the shift, the longer the exposure must be. This increase in time may reflect some modification of the gonads due to the temperature applied between the beginning of the TSP and the shift (Ciofi and Swingland, 1997).

During incubation the eggs produce their own heat, which is called metabolic heating. It has been found that metabolic heating of crocodilian eggs in the laboratory has been sufficiently strong to have elicited measures to depress rising incubator temperatures to maintain experimental conditions (Webb *et al.*, 1987). This only occurs later in the incubation period. Ewert and Nelson (2003) demonstrated that metabolic heating by eggs can influence the

outcome of temperature-dependent sex determination under carefully controlled laboratory conditions.

Three patterns of TSD have been described in chelonians, crocodilians and lizards:

1). Eggs incubated at constant, low temperatures produce only females, whereas those maintained at constant higher temperatures produce only males. Intermediate temperatures produce both sexes. This pattern is called the female-male pattern (FM) and is the most common pattern found in nature (Ewert and Nelson, 2003). This pattern is found in most species of lizards, including the skink, *Eulamprus tympanum* (Elf, 2003), and has been reported in crocodilians by Ferguson and Joanen (1982, 1983).

2). Females are produced at high incubation temperatures and males at low incubation temperatures. This pattern is called the male-female pattern (MF) and is common in many chelonians such as the painted turtle, *Chrysemys picta*, the red-eared slider turtle, *Trachemys scripta* (Elf, 2003) and sea turtles (Hays *et al.*, 2003).

3). Females are produced at low and high temperatures, with males produced at intermediate temperatures. This is called the female-male-female (FMF) pattern. This occurs in some species of chelonians, lizards and many species of crocodilians, such as *Alligator mississippiensis* (Deeming and Ferguson, 1989); *Caiman crocodylus* (Lang and Andrews, 1994); *Crocodylus johnstoni* (Deeming and Ferguson, 1989); *Crocodylus palustris* (Lang *et al.*, 1989) and *Crocodylus porosus* (Webb and Cooper-Preston, 1989). Leslie (1997) in her study of TSD in *Crocodylus niloticus* in the Greater St. Lucia Wetland Park, South Africa, found that the Nile crocodile also exhibits a FMF pattern.

Pattern one and two have a single pivotal temperature whereas pattern three has two pivotal temperatures at which ratios of males to females are produced (Elf, 2003). Within this pattern, two narrow transitional ranges of temperatures (TRT's) yield both males and females.

Crocodilians which actively maintain and defend breeding territories, show pattern three TSD almost exclusively. Pattern three is consistent with the production of females in suboptimal environments, males are produced at intermediate temperatures and females are produced at both extremes (Freedberg and Wade, 2004).

ESD is favoured over GSD when males do better than females in some environments and females do better than males in other environments. Charnov and Bull (1977) proposed a model where hatchling fitness varies most among environmental patches and between sexes, so that some patches provide optimum conditions for one sex but not the other. There are several ways in which an environment may be 'patchy'. For example: if an area has a high concentration of females and a low concentration of males, then an individual would have the greatest success in

being a male (Charnov and Bull, 1977). Webb *et al.* (1987) proposed post-hatching growth rate and body size as the most important fitness traits correlating with incubation temperature and offspring sex. For example: in *Alligator mississippiensis*, larger males are produced at higher temperature and a selective advantage has been reported for those individuals. They control larger harems of females and produce more spermatozoa for a longer time when compared to small males (Deeming and Ferguson, 1989). In this hypothesis, TSD would be favoured over GSD, as the latter would not guarantee that the eggs containing genotypic male embryos would be in optimum incubation conditions and therefore grow faster and into larger males. TSD on the other hand, allows the associations of sex with potential post-hatching growth-rate, as both are determined by incubation conditions (Ciofi and Swingland, 1997).

The presence of TSD in reptiles has important implications for their sex ratios, habitat requirements and reproductive success (Deeming and Ferguson, 1989). An evolutionary advantage of TSD is that incubation conditions determine the sex of a hatchling and its size (Ferguson and Joanen, 1982; Hutton, 1987; Deeming and Ferguson, 1989; Webb and Cooper-Preston, 1989). Incubation temperature influences sex, growth rate before and after hatching, metabolic rate, optimal thermoregulatory temperatures, plus sexual characteristics such as pigmentation pattern and intensity. The selective advantage is that large, fast growing animals will be males (Deeming and Ferguson, 1989).

Nest site selection combined with TSD may enable the female to control the sex of her offspring, and this may be the selective evolutionary advantage for TSD (Ferguson and Joanen, 1983). It is possible that TSD females can detect temperature differences and select preferred temperatures at which to lay their eggs (Bragg *et al.*, 2000). Some populations with TSD produce large proportions of unisexual clutches. In these species, TSD must reduce close inbreeding by largely precluding sib-matings, a result difficult to achieve with GSD (Ewert and Nelson, 2003), which might provide a selective advantage.

Global Climate Change

The International Panel on Climate Change (2001) defines climate as 'the average weather in terms of the mean and its variability over a certain time span and certain area.' Climate varies naturally on all time scales. Variations may result from radiative forcing but also from internal interactions between components of the climate system (IPCC, 2001). Man plays a large role in altering the natural variations of climate. The combustion of fossil fuels, emission of chlorofluorocarbons (CFCs) and land-use changes, such as deforestation, urbanization and agriculture all contribute to climate change (IPCC, 2001).

There has been a mean global warming of 0.4 to 0.8 °C of the atmosphere at the surface since the late 19th century (IPCC, 2001). This increase in temperature took place between 1910 and 1945 and since 1976. The warming rate between 1910 and 1945 is 0.17 °C per decade. The recent warming rate has a faster rate of warming over land compared to the oceans.

Sea levels have risen by 10 – 20 cm in the 20th century and there has been a general retreat in glaciers worldwide (IPCC, 2001). The occurrence of extreme weather events has changed in certain areas. In many regions of the world, there has been a disproportionate increase in heavy and extreme precipitation rates in areas where total precipitation has increased.

To understand, detect and predict the human influence on climate, it is important to understand the system that determines the climate of the Earth and the processes that lead to climate change. Numerous studies have been carried out to produce models, predicting the future status of the Earth in terms of climate change. It has been predicted that daily high temperature extremes will increase in frequency and the future increase in mean precipitation will very likely lead to an increase in variability (IPCC, 2001). The IPCC also predicts that the average temperature of the earth will increase between 1.4 °C and 5.8 °C over the next 100 years (Tonn, 2003).

Although Global Climate Change is a well-known complex phenomenon, the effects on biodiversity are poorly understood (Meynecke, 2004). Several studies have documented effects of changes in climate on several demographic characteristics of species. Saether *et al.*, (2000) found that many temperate bird species lay their eggs earlier in the year, due to warmer springs. Norway's national bird, the dipper has fluctuated in numbers with the change in the atmospheric pressure system, known as NAO (North Atlantic Oscillation) [Wuethrich, 2000]. In Europe, two-thirds of the butterfly species have shifted their ranges northward, by as much as 240 km. The shifting range caused the butterflies to move into fragmented landscapes, where they struggled to survive and so their numbers are declining (Penuelas and Filella, 2001).

In contrast to other amniote vertebrates, where the sex is determined genetically at conception, in many reptiles the sex is irreversibly determined by temperature experienced during the middle third of embryonic development (Janzen, 1994). The sex ratio of offspring in these taxa may be radically altered by as little as 1 °C shift in the incubation temperature.

Biased sex ratios are already known for reptiles with temperature-dependent sex determination. A general warming trend is already evident in sea turtle (*Chelonia mydas*) nests over the past 100 years at Ascension Island. In addition, findings so far, point to nest site selection by female reptiles for hatching success rather than sex ratio manipulation (Nelson *et al.*, 2004).

The way species with TSD respond to global warming or cooling has implications for the conservation of these animals (Janzen and Paukstis, 1991). The long-term survival of crocodiles is dependent on a sufficient range of incubation temperatures to ensure that both male and female hatchlings are produced. It is not known how skewed hatchling sex ratios affect the adult sex ratio. Crocodilians have long generation times and individuals can reproduce for many years. However, attention is required in the implications of TSD for the future survival of crocodiles under scenarios of global climate change.

Temperature-dependent sex determination of the Nile crocodile has been studied in various areas. Leslie (1997) carried out an ecological and physiological study of the threatened Nile crocodile population in Lake St. Lucia, South Africa. The influence of egg incubation temperature on embryonic survival, incubation time and hatchling sex was determined. Soil and nest temperatures were used to estimate hatchling sex ratios in wild crocodile nests. Swanepoel (1999) carried out a similar study in the Olifants River in the Kruger National Park, where the nesting ecology of the Nile crocodile was studied. Nest temperatures were recorded from wild crocodile nests and these temperatures were used to estimate hatchling sex ratios. Hutton (1987) and Kofron (1990) studied the Nile crocodile population in Lake Ngezi, Zimbabwe, where Nile crocodile eggs were incubated and population sex ratios were determined. A predominantly female-biased sex ratio was found in all of these studies, however, the pivotal temperature, varied. Lin *et al.,* (2003) suggested that reptiles differing in habitat use and/or distribution may have different optimal temperatures for developing embryos. Studying TSD of the Nile crocodile in the Okavango River, Botswana, allows us to determine whether a difference in optimal nest temperatures does exist and a comparison between various geographical distributions can then be made.

No previous TSD study in crocodiles has been carried out in the Okavango Region. The Okavango River hosts a large population of Nile crocodiles in their natural environment. The majority of crocodile nesting sites are concentrated in the Panhandle section of the river. The nesting ecology and incubation temperature of the Nile crocodile in the Panhandle will thus influence the sex ratios of the entire population of the Okavango Delta.

1.2 DESCRIPTION OF THE STUDY AREA

The Okavango Delta

The Okavango Delta is an inland delta situated in the north-western part of Botswana (Figure 1.1). The Okavango Delta System is declared as the World's largest RAMSAR site and is measured at approximately 68 640 km^2 (Kabii, 1997). The designated area includes the Okavango River, the entire Okavango Delta, Lake Ngami, and parts of the Kwando and Linyanti river systems that fall along the western boundary of Chobe National Park (Kabii, 1997).

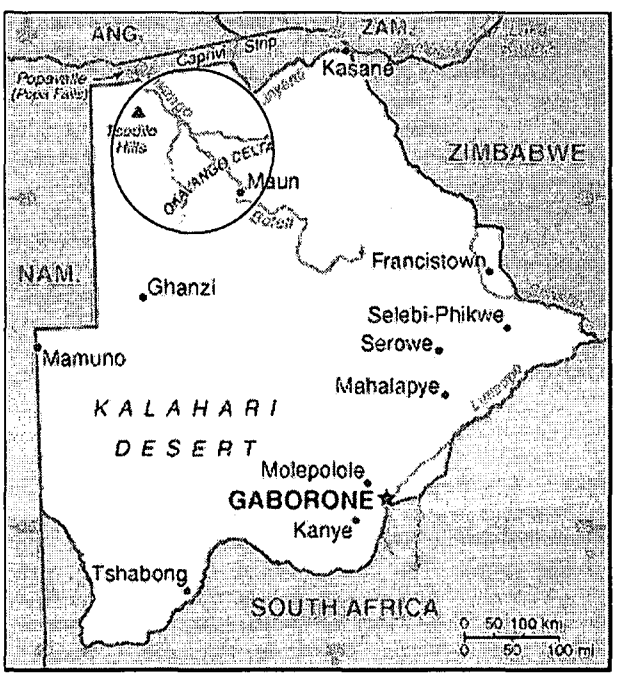


Figure 1.1 Map of the Okavango Delta, Botswana.

The Okavango Delta is hydrologically unique and is mainly sustained by rainfall in the Angolan highlands, which occurs during November and December. The rainwater converges to form the major tributaries, which merge into the Okavango River. The latter flows through the Caprivi Strip of Namibia and the Delta's panhandle region, and eventually fans out to form the Okavango Delta. The annual floods usually reach the seasonal swamps of the Delta in June-September (Mubyana *et al.*, 2003). The Okavango Delta is a unique ecosystem, providing high quality habitats for all species and is home to 5 000 types of insect, 3 000 plant species, 540 species of bird, 164 mammal species, 157 reptilian species, as well as countless micro-organisms (Mbaiwa, 2003). There are also an estimated 68 species of fish in the delta ecosystem, with the sharp-tooth catfish being endemic (Kabii, 1997).

The Nile crocodile is distributed throughout the Okavango Delta, although the majority of the breeding population is found in the Panhandle, where permanent water is available.

The Okavango River

The source of the Okavango Delta arises in the Angolan highlands where it travels towards Botswana as the Cuito and Cubango Rivers. These two rivers converge before the Angolan/Namibian border forming the Kavango River, which flows through the Caprivi Strip of Namibia. Sixty kilometers later the river enters Botswana and is known as the Okavango River. The river then flows approximately 100 km, as the crow flies, before it fans out forming the Okavango Delta. The upper reaches of the Okavango River and its adjacent floodplains fall between two parallel fault lines, which form a slight depression about 10 – 15 km in width (Gumbricht *et al.*, 2003). This permanent water body is known as the Panhandle. The Panhandle extends from Mohembo, (GPS: North 7 981 616 m, East 582 782 m, Projection: Universal Transverse Mercator) in the north to the area around the town of Seronga (GPS: North 7 917 727 m, East 648 145 m) in the south (Graham *et al.*, 1992). This single channel runs past the village of Shakawe from where it splits into the main channel and the Kgaolo Thaogo subsidiary channel (Figure 1.2). After Nxamasere the channel divides into two again, where the entrance to the Eastern Channel splits away from the main channel. The main stream runs southeastwards to Seronga, where it finally splits into the channels which form the base of the alluvial fan.

The vegetation of the area is dominated by papyrus (*Cyperus papyrus*), the slightly more elevated phragmites reeds (*Phragmites australis*) and thatching grass (*Miscanthus junceus*). These species facilitate in the distribution of water and regulate the distribution of sediment in the system. The vegetation regulates the system and prevents stagnation in a river that would otherwise be saline and sandy (McCarthy, 1992). The Panhandle is a permanent river and is thus an ideal breeding area for the Nile crocodile. It is approximately 200 km long and has an average width of 40 m. The seasonal swamp receives water that filters from the permanent swamp and the extent of this flooding varies each year between 4 000 and 8 000 km^2.

The Eastern Channel

The Eastern channel is unlike the upper panhandle, as it is not characterized by broad sweeping meanders, which indicates that most of the deposition of the Okavango River takes place in the upper reaches of the Panhandle (McCarthy, 1992). The Eastern Channel travels parallel to the main channel firstly as the Moremi, then the Phillipa Channel (Figure 1.2). The Eastern channel is approximately 28 km long.

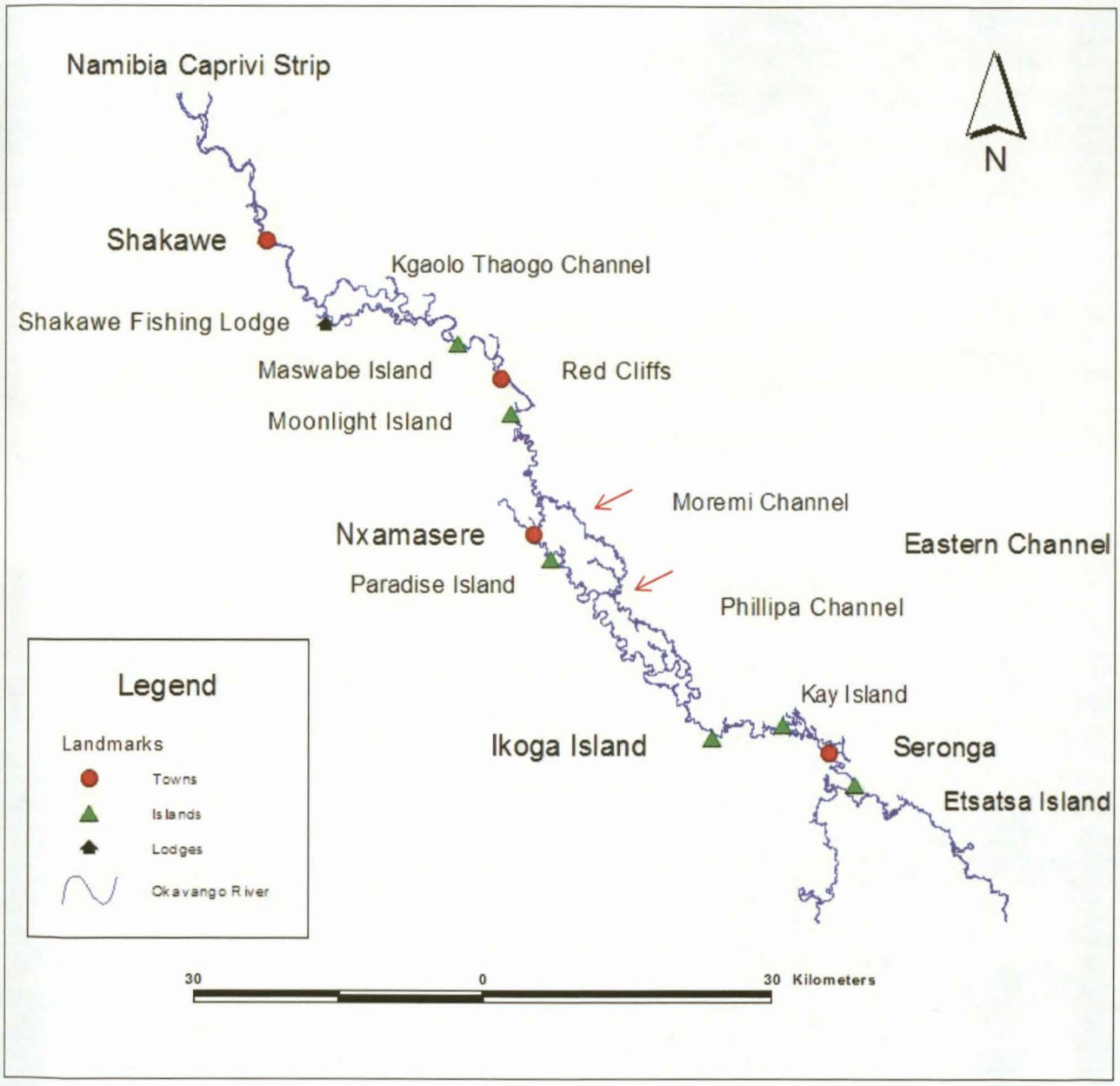


Figure 1.2 The Panhandle of the Okavango Delta, Botswana. The arrows indicate the study area in relation to the Okavango River.

1.3 DESCRIPTION OF THE STUDY ANIMAL

History and Evolution

All living crocodilians belong to the Family Crocodylidae, which occur in a broad band around the globe, in the tropics and subtropics of the Old and New World (Huchzermeyer, 2003). Crocodylidae consists of three subfamilies: the Crocodylinae (genera *Crocodylus* and *Osteolaemus*); the Alligatorinae (*Alligator, Caiman, Paleosuchus, and Melanosuchus*) and the Gavialinae (*Gavailus*), which can be distinguished by their different anatomical features, particularly of the skull and scale patterns of the skin (Taplin, 1984).

Distribution and Habitat

Crocodilians and Alligatorians are found between latitudes of Cancer (23.5° north) and Capricorn (23.5° south), in the rivers and lakes of South America, Africa, Asia and Australia. Only three species of Crocodylidae occur in Africa, with the Nile crocodile having the widest distribution. Its historical distribution includes the Nile River Delta and Mediterranean coast from Tunisia to Syria (Swanepoel, 1999). The African countries within its range include: Angola, Benin, Botswana, Burundi, Chad, Congo, Egypt, Ethiopia, Gabon, Gambia, Ivory Coast, Kenya, Liberia, Madagascar, Malawi, Mali, Mozambique, Nigeria, Rwanda, Somalia, South Africa, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe (Webb and Smith, 1987). Nile crocodiles have a wide habitat preference, which reflects their success and distribution as a species. They may be found in a variety of wetland habitats, including rivers, lakes, swamps and brackish water (Grenard, 1991). The Nile crocodile has a tolerance of saltwater (Leslie and Spotila, 2000) and therefore frequents coastal areas in west and southern Africa, and may also sometimes be found in very isolated bodies of water such as Lake Chula at the foot of Mount Kilimanjaro. In Mauritania small populations of Nile crocodiles have been found surviving in the extremely limited ephemeral wetlands of the Sahara desert (Lance, 2003).

Physical Characteristics

The Nile crocodile is a large crocodilian; averaging five meters in maximum adult length and which may in the past have reached lengths of up to seven meters (Leslie, 1997). Juvenile Nile crocodiles are dark olive in colour, with darker, often black crossbands on the tail and body. The crossbanding on the tail becomes fainter in adults. The abdomen is light in colour and the interior of the mouth is pale yellow. The adaptations that account for the success of the Nile crocodile are structural, physiological and behavioural (Pooley and Gans, 1976).

Nesting Ecology

Crocodilians, like most other reptiles, lay eggs. Nile crocodiles lay their eggs approximately three months after fertilization (Pooley and Gans, 1976). Some species of crocodilians lay their eggs in holes in open areas whereas other species construct mounds of vegetation (Magnusson *et al.*, 1985). The nesting behaviour of *C. niloticus* resembles that of *C. johnstoni* in which a hole-nest is constructed in dry soil or sand (Aulie and Kanui, 1995). Females excavate their nesting holes about two meters above the water line and between five and ten meters from the water's edge (Grenard, 1991). The Okavango crocodiles nest in relatively inaccessible areas, as the majority of observed sites are openings in thick stands of phragmites reeds, papyrus, or both (Blomberg, 1976, *pers. obs.*). The female crocodile digs a hole in the sand of a riverbank, in which the eggs are then deposited. The clutch varies in size and is directly proportional to the size of the female. The larger the female, the larger the eggs are in number as well as in mass (Pooley and Gans, 1976). The distance between the top layer of eggs and the nests' covering layer may be no more than 100 mm (Grenard, 1991). The female stays in close proximity to the nest protecting it. It is presumed that during the incubation period the female does not feed at all and she becomes quite inactive by the time the young begin to hatch. After approximately 90 days of incubation, the female is warned by a 'chirping' sound from below, which is the sound young crocodiles make at hatching time. This sound is loud enough to penetrate the overlying soil and can be heard as far as 20 m from the nest (Pooley and Gans, 1976). The hatchlings rupture the shell membrane by means of a special egg tooth on the tip of their snout, which is soon shed (Rose, 1950). The female excavates the nest working with her forelimbs and scraping and biting with her jaws. When the nest is opened the female picks up the hatchlings one by one until they are all in her mouth and she then carries them to the water.

The nests are vulnerable to predation and catastrophic events. They are usually elevated to avoid being flooded. Monitors (*Varanus niloticus*) are the chief cause of catastrophic mortality. Their predation accounts for many unhatched nests. It is sometimes difficult to determine whether a nest has hatched or whether it has been damaged by monitors. Monitor damage is often characterized by a shallow excavation, whereas hatched nests usually appear well excavated. Eggshells are usually scattered by monitors, while the shells of hatched nests tend to lie in the direction of the water (Blomberg, 1976). Hyenas and other carnivores may also dig out the eggs or pursue and devour the young hatchlings.

Crocodiles of the Okavango River

Crocodiles in the Okavango River system are a keystone species. Alcala and Dy-Liacco in 'Crocodiles and Alligators' (1989) describe a keystone species as "one that determines the structure of a community and if removed from a system the species diversity will decrease." The

Nile crocodile is the top aquatic predator and is responsible for controlling fish numbers in the system. The diets of larger crocodiles (1 250 – 3 250 mm in length) indicate that their main food source is fish, namely barbel (*Clarius* species) [Blomberg, 1976]. Crocodiles therefore control the numbers of predatory barbel, which are not as commercially exploited as the bream species. This in turn ensures stable numbers in all fish species.

The Okavango population of crocodiles have undergone three periods of commercial exploitation. Between 1957 and 1969 the Department of Wildlife and National Parks (DWNP) invited hide hunters to shoot an annual quota of 2 000 crocodiles per concessionaire. This 12 year period's harvest is said to have accounted for about 12 000 animals (Graham *et al.*, 1992). A further 940 crocodiles were shot for skins in 1974 and 1975 (Graham *et al.*, 1992). Murray-Hudson (1997) stated that after the commercial hunting period between 1957 and 1974, the crocodile population recovered under a decade of protection stipulated by the Botswana Government. From 1983 to 1988, 1 053 live crocodiles were caught from the wild and 14 000 eggs were collected by crocodile farmers (Murray-Hudson, 1997). This harvest resulted in a 50% decline in breeding females.

In 1987 the crocodile population was estimated at approximately 10 000 animals in the Panhandle region and throughout many of the wetland habitats, including permanent swamps, rivers, lagoons and seasonal swamps (Simbotwe, 1988). Graham *et al.* (1992) found that 99% of nesting sites were found in the Panhandle above the Delta between Shakawe and Seronga. Crocodile distribution, habitat preference and reproduction are closely linked to flood regimes and water levels as they affect food supplies, nest site location and availability, cover requirements and hatchling survival rates.

1.4 RESEARCH OBJECTIVES

1). To determine the influence of egg incubation temperature on embryonic survival and development, incubation period and hatchling sex ratios in laboratory experiments.

2). To determine the lower and upper pivotal temperature of the Nile crocodile in the Okavango River for comparative purposes.

4). To determine optimal nest site characteristics including nest site size, location and nest dimensions.

5). To record incubation temperatures in wild crocodile nests along the Okavango River and to estimate hatchling sex ratios.

6). To determine the possible effect of Global Climate Change on sex ratios of the Nile crocodile population in the Okavango River, Botswana.

1.5 SIGNIFICANCE AND EXPECTED OUTCOME OF STUDY

A better understanding of TSD may prove essential to the continued existence of a number of reptile species. Presently there are 30 species of lizards, 51 species of turtles and 18 species of crocodilians of threatened and endangered status as listed by the IUCN. Successful artificial incubation of eggs of these species must be undertaken with an understanding of the implications of TSD for conservation and management (Janzen and Paukstis, 1991). Hatchling sex ratios are of conservational significance since they may affect population sex ratios and could alter reproductive success of a population (Hanson *et al.*, 1998). The study of TSD will provide a better understanding of reptiles and improve our conservation efforts. Hays *et al.* (2003) in a study on sea turtles emphasized the importance of recording long-term nest temperatures to allow the impacts of Global Climate Change to be assessed, to allow informal decisions to be made with regards to management of reptiles.

TSD in reptiles may not only be adaptive, but may also be able to evolve in the long term in response to skewed sex ratios caused by environmental perturbations, such as gradual long-term climate change (Janzen and Morjan, 2001).

The purpose of my study was to determine the influence of incubation temperature on the sex ratio of Nile crocodiles in the Okavango River, Botswana. It is hypothesized that Global Climate Change will affect incubation temperature and lead to a skewed sex ratio. To compensate for the rarer sex, this study determined at which temperature artificial incubation should take place. Crocodile farms are increasing in numbers in the Okavango Region. In Shakawe, a Crocodile Farm, Krokovongo opened in June 2005. The Department of Wildlife has issued the farm with a permit to collect crocodile eggs from the wild, with proviso that 5% of hatchlings are released back into the wild at the age of two years. This study determined at which temperature male and female hatchlings were produced.

Chapter 1 gives a background to the research topic and the literature on TSD was reviewed. The study area and study animal is described in detail and the significance of the study and expected outcomes is identified.

Chapter 2 focuses on the artificial incubation of wild Nile crocodile eggs at various incubation temperatures. It includes a detailed investigation into the influence of egg incubation temperature on embryonic survival, incubation period, hatching success and sex ratios. The temperature that produces a 1:1 sex ratio of males to females, known as the pivotal temperature was determined in this study and then compared to actual nest temperatures in Chapter 4.

Chapter 3 outlines climatological data including precipitation and air and soil temperatures recorded along the Okavango River. Soil temperatures were recorded at various depths at various distances from the river to determine the ideal nesting site with optimal soil temperature

to provide for a female-biased sex ratio. These results were combined with nest site characteristics and nest temperatures determined in Chapter 4 to predict the effect of global climate change on resultant sex ratios of Nile crocodiles.

Chapter 4 focuses on the details of Nile crocodile nests found along the Okavango River. Nest site characteristics were studied and the temperature of various nest sites was recorded and analysed. The incubation temperatures were used to predict the sex ratios of Nile crocodile nests.

In the final chapter (Chapter 5) the results from Chapters 2, 3 and 4 were combined. The pivotal temperature determined in Chapter 2 was incorporated with results from Chapter 4 to predict the sex ratio of wild crocodile hatchlings. Climatological data from Chapter 3 was combined with incubation temperatures recorded in Chapter 4 to make predictions on the possible effect that Global Climate Change may have on the sex ratio of Nile crocodile hatchlings. The optimal soil temperature determined in Chapter 3 was then combined with nest site characteristics to determine whether female crocodiles have the ability to choose preferred nesting sites. Based on pivotal temperatures, the resultant sex ratio of hatchlings and predicted climate change, conservation and management recommendations were made for both wildlife managers and crocodile farms.

1.6 REFERENCES

ALCALA, A.C. AND M.T.S. DY-LIACCO. 1989. Habitats. In: *Crocodiles and Alligators. Facts on File*. (Eds. Ross, C.A. and Garnett, S). Inc., New York. Pp. 136–155.AULIE, A. AND T.I. KANUI. 1995. Oxygen consumption of eggs and hatchlings of the Nile crocodile (*Crocodylus niloticus*). *Comparative Biochemistry and Physiology*. 112 A (1): 99–102.

BLOMBERG, G. E. D. 1976. Feeding and nesting ecology and habitat preferences of the Okavango Crocodiles. In: *Proceedings of the Symposium on the Okavango Delta and its future utilization*. Gaborone, Botswana.

BOWDEN, R. M., EWERT, M. A. AND C. E. NELSON. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. *Proceedings of the Royal Society*. London B 267: 1745–1749.

BRAGG, W. K., FAWCETT, J. D. AND J. B. BRAGG. 2000. Nest-site selection in two eublepharid gecko species with temperature-dependent sex determination and one with genotypic sex determination. *Biological Journal of the Linnean Society* 69: 319-332.

CHARNOV, E. L. AND J. J. BULL. 1977. When is sex environmentally determined? *Nature* 266: 828–830.

CIOFI, C. AND I. R. SWINGLAND. 1997. Environmental sex determination in reptiles. *Applied Animal Behaviour Science* 51: 251–265.

CONGDON, J. D., FISCHER, R. U. AND R. E. GATTEN, JNR. 1995. Effects of incubation temperatures on characteristics of hatchling American alligators. *Herpetologica* 51 (4): 497–504.

DEEMING, D. C. AND M. W. J. FERGUSON. 1989. The mechanism of temperature dependent sex determination in crocodilians: A hypothesis. *American Zoology*. 29: 973-985.

ELF, P. K. 2003. Yolk steroid hormones and sex determination in reptiles with TSD. *General and Comparative Endocrinology* 132: 349–355.

EWERT, M. A. AND C.E. NELSON. 2003. Metabolic heating of embryos and sex determination in the American alligator, *Alligator mississippiensis*. *Journal of Thermal Biology* 28: 159–165.

FERGUSON, M. W. AND T. JOANEN. 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature* 296: 850–852.

FERGUSON, M. W. AND T. JOANEN. 1983. Temperature-dependent Sex Determination in *Alligator mississippiensis*. *Journal of Zoology (London)* 200: 143–177.FREEDBERG, S. AND M. J. WADE. 2004. Male combat favours female-biased sex ratios under Environmental Sex Determination. *Animal Behaviour* 67: 177–181.

GRAHAM, A., SIMBOTWE, P. M. AND J. M. HUTTON. 1992. Monitoring of an exploited crocodile population on the Okavango River, Botswana. In: *The CITES Nile Crocodile Project.* (Eds. Hutton JM and Games I). Lausanne, Switzerland: Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Pp. 53–69.

GRENARD, S. 1991. Handbook of Alligators and Crocodiles. *Krieger Publishing Company.* Malabar, Florida. Pp. 107–139.

GUTZKE, W. H. N. AND D. CREWS. 1988. Embryonic temperature determines adult sexuality in a reptile. *Nature* 332: 832–834.

GUMBRICHT, T., WOLSKI, P., FROST, P. AND T. S. MCCARTHY. 2003. Forecasting the spatial extent of the annual flood in the Okavango Delta, Botswana. *Journal of Hydrology* 290: 178-191.

HANSON, J., WIBBELS, T. AND R. E. MARTIN. 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. *Canadian Journal of Zoology* 76: 1850–1861.

HAYS, G. C., BRODERICK, A. C., GLEN, F. AND B. J. GODLEY. 2003. Climate change and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Climate Biology* 9: 642–646.

HUCHZERMEYER, F. W. 2003. Crocodiles: Biology, Husbandry and Diseases. CABI Publishing. Onderstepoort Veterinary Institute, South Africa.

HUTTON, J. M.1987. Incubation temperatures, sex ratios and sex determination in a population of Nile crocodiles *Crocodylus niloticus*. *Journal of Zoology* 211: 143–155.

INTERNATIONAL PANEL ON CLIMATE CHANGE (IPCC) [online] 2001. Summary for Policymakers: A Report of Working Group I of the Intergovernmental Panel on Climate Change. Available from htpp://www.ipcc.ch.
Accessed 16 May 2005.

JANZEN, F. J. 1994. Climate change and TSD. *Proceedings of the National Academy of Sciences. USA*. 91: 7487–7490

JANZEN, F. J. AND G. L. PAUKSTIS. 1991. Environmental Sex Determination in reptiles: ecology, evolution, and experimental design. *The Quarterly Review of Biology* 66 (2): 149–179.

JANZEN, F. J. AND C. L. MORJAN. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour* 62: 73–82.

JOANEN, T., MCNEASE, L. AND M. W. J. FERGUSON. 1987. The effects of egg incubation temperature on post-hatching growth of American alligators. In: *Wildlife Management: Crocodiles and Alligators.* (Eds. G. J. Webb., Manolis, S. C. and P. J. Whitehead). Surrey Beatty and Sons, Sydney, Australia. Pp. 533-537.

KABII, T. 1997 [online]. Okavango – the world's largest Ramsar site. Ramsar Con vention Bureau. Switzerland.
Available from http://www.ramsar.org/w.n.okavango.htm
Accessed 31 May 2004.

KOFRON, C. F. 1990. The reproductive-cycle of the Nile Crocodile (*Crocodylus niloticus*). *Journal of Zoology* 221: 477–488.

LANCE, V. A. 2003. Alligator physiology and life history: The importance of temperature. *Experimental Gerontology* 38: 801–805.

LANG, J. W. 1987. Crocodilian thermal selection. In: *Wildlife Management: Crocodiles and Alligators.* (Eds. G. J. Webb., S. C. Manolis and P. J. Whitehead). Surrey Beatty and Sons. Sydney, Australia. Pp. 301–315.

LANG, J. W. AND H. ANDREWS. 1994. Temperature-dependent sex determination in Crocodilians. *Journal of Experimental Biology* 270: 28-44.

LANG, J. W., ANDREWS, H. AND R. WHITAKER. 1989. Sex determination and sex ratios in *Crocodylus palustris. American Zoology* 29: 935–952.

LESLIE, A. J. 1997. The ecology and physiology of the Nile crocodile, *Crocodylus niloticus* in Lake St. Lucia, Kwazulu-Natal, South Africa. Ph.D. Dissertation, Drexel University, USA. Pp. 5-10.

LESLIE, A. J. AND J. R. SPOTILA. 2000. Osmoregulation of the Nile crocodile, *Crocodylus niloticus* in Lake St. Lucia, KwaZulu-Natal, South Africa. *Comparative Biochemistry and Physiology* Part A 126 (2000): 351-365.

LIN, Z., XIANG, J., LUO, L. AND X. MA. 2003. Incubation temperature affects hatching success, embryonic expenditure of energy and hatchling phenotypes of a prolonged egg-retaining snake, *Deinagkistrodon acutus* (Viperidae). *Journal of Thermal Biology* 30: 289 – 297.

MAGNUSSON, W. E., ALBERTINA, P.L. AND R. M. SAMPAIO. 1985. Sources of heat for nests of *Paleasuchus trigonatus* and a review of Crocodilian nest temperatures. *Journal of Herpetology* 19 (2): 199–207.

MBAIWA, J. E. 2003. Enclave tourism and its socio-economic impacts in the Okavango Delta, Botswana. *Tourism Management* 26 (2): 157-172.

M^{C}CARTHY, T. S. 1992. Physical and biological processes controlling the Okavango Delta - a review of recent research. *Botswana Notes and Records*, 24: 57-86.

MEYNECKE, J. 2004. Effects of Global Climate Change on geographical distributions of vertebrates in north Queensland. *Ecological Modelling* 174: 347-357.

MROSOVSKY, N. AND C. PIEAU. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia* 12: 169–179.

MUBYANA, T., KRAH, M., OTLOGETSW, M. AND M. BONYONGO. 2003. Influence of seasonal flooding on soil total nitrogen, organic phosphorus and microbial populations in the Okavango Delta, Botswana. *Journal of Arid Environments* 54: 359–369.

MURRAY-HUDSON, M. 1997. Fauna of the Okavango River basin (Botswana sector). *Report. Botswana Maun: Permanent Okavango River Basin Commission.*

NELSON, N. J., THOMPSON, M. B., PLEDGER, S., KEALL, S. N. AND C. H DAUGHERTY. 2004. Do TSD, Sex ratios and nest characteristics influence the vulnerability of tuatara to Global Warming? *International Congress Series* 1275: 250–257

PENUELAS AND FILELLA. 2001. Responses to a warming world. *Science* 294: 793–795.

POOLEY, A. C. AND C. GANS. 1976. The Nile crocodile. *Scientific American* 234: 114–124.

ROSE, W. 1950. The Crocodile. In: *The Reptiles and Amphibians of Southern Africa*. Maskew Miller, Limited. Cape Town. Pp. 351–358.

SAETHER, B. E., TUFTO, J., JERSTAD, K., ROSTAD, O. W. AND J. E. SKATAN. 2000. Population dynamical consequences of climate change for a small temperate songbird. *Science* 287: 854–856.

SHINE, R. 1985. The evolution of viviparity in reptiles: An ecological analysis. In: *Biology of the Reptilia*. Volume 15. C. (Eds. Gans and F. Billett). John Wiley and Sons, New York. Pp. 605–694.

SHINE, R. 1999. Why is sex determined by nest temperature in many reptiles? *TREE* 14 (9): 186–189.

SIMBOTWE, M. 1988. Crocodile research and management. Kalahari Conservation Society *Kalahari Conservation Society library, Gaborone*. Newsletter No 21: 8-9.

SWANEPOEL, D. G. J. 1999. Movements, nesting and the effects of pollution on the Nile crocodile, *Crocodylus niloticus* in the Olifants River, Kruger National Park. MSc. Thesis, University of Natal, South Africa.

TAPLIN, L. 1984. Evolution and zoogeography of crocodilians: A new look at the ancient order. In: *Vertebrate Evolution and Zoogeography in Australasia*. (Eds. M. Archer and G. Clayton). Hesperian Press, Perth, Australia. Pp. 361–370.

TAYLOR, E. N., DALE, F. D. AND M. A. MALAWY. 2004. A comparison between point- and semi-continuous sampling for assessing body temperature in a free-ranging ectotherm. *Journal of Thermal Biology* 29: 91–96.

TONN, B. 2003. An equity first, risk-based framework for managing Global Climate Change. *Global Environmental Change* 13: 295–306.

WEBB, G. J. W. AND A. M. A. SMITH. 1987. A method for estimating residual yolk mass in hatchling crocodilians. *Herpetological Review* 18 (1):13.

WEBB, G. J. W., BUCKWORTH, R. AND S. C. MANOLIS. 1983. *Crocodylus johnstoni* in the McKinlay River Area, N. T. III. Growth, movement and the population age structure. *Australian Wildlife Research* 10: 383–401.

WEBB, G. J. W., MANOLIS, S. C., DEMPSEY, K. E. AND P. J. WHITEHEAD. 1987. Crocodilian eggs: A Functional Overview. In: *Wildlife Management: Crocodiles and Alligators*. (Eds. Webb, G. J. W., Manolis, S. C). Chipping Norton: Surrey Beatty and Sons Pty Ltd. Sydney, Australia. Pp.417-422.

WEBB, G. J. W. AND H. COOPER-PRESTON. 1989. Effects on incubation temperature on Crocodiles and the evolution of reptilian oviparity. *American Zoology* 29: 953–971.

WUETHRICH, B. 2000. How climate change alters rhythm of the wild. *Science* 287: 793–795.

CHAPTER 2

INCUBATION TEMPERATURE, EMBRYONIC SURVIVAL AND SEX RATIO OF THE NILE CROCODILE, *CROCODYLUS NILOTICUS*

2.1 INTRODUCTION

Incubation temperature has a profound effect on the development of embryonic reptiles (Leslie, 1997). The Nile crocodile, *Crocodylus niloticus* exhibits temperature-dependent sex determination (TSD), which is a phenomenon where the incubation temperature of the egg determines the sex of the individual. This is restricted to certain groups of reptiles, such as crocodilians (alligators and crocodiles) [Hutton, 1987], chelonians (turtles and tortoises) [Hays *et al.*, 2003], some lizards (Gutzke and Crews, 1988) and tuataras (Ciofi and Swingland, 1997). Incubation temperature not only determines the sex of hatchlings but also affects the probability of embryos surviving. Within the viable range of incubation temperatures the rate of differentiation and the rate of growth may vary, having a great effect on total incubation periods (Leslie, 1997).

Hutton (1987) and Leslie (1997) conducted studies on TSD in Nile crocodiles, concentrating on the influence of incubation temperatures on embryonic development. Leslie (1997) studied St. Lucia's Nile crocodile population in South Africa and found that embryonic survival, developmental rates and incubation periods at constant incubation temperature were clearly temperature dependent. This supported the findings of Hutton (1987) who studied the Nile crocodile population in Lake Ngezi in Zimbabwe. Embryonic survival decreased at higher incubation temperatures. Leslie (1997) also found that as incubation temperature increased, embryonic development in *C. niloticus* accelerated which in turn decreased the incubation period. The mean total incubation period for St. Lucia's Nile crocodiles was approximately 70 days. Hutton (1987) incubated Nile crocodile eggs from Lake Ngezi in Zimbabwe and reported that the shortest incubation period was 84 days and the longest incubation period recorded was 111 days.

Hutton (1987) and Leslie (1997) both found that the sex of *C. niloticus* hatchlings was determined by egg incubation temperature and a FMF (female-male-female) pattern existed. Hutton (1987) found that incubation temperature of 31.0 °C produced a majority of females and a range between 31.0 and 34.0 °C produced males. The FMF pattern of TSD has been documented in *C. niloticus, Alligator mississippiensis, Caiman crocodylus, Crocodylus johnstoni, Crocodylus porosus* and *Crocodylus palustris* (Lang, *pers. comm.*, 2002). Low incubation temperature (31.0 °C and below) produces exclusively females, whereas males resulted at

slightly higher temperatures (32.0 to 33.0 °C) and females again predominate when incubation temperatures are elevated further (34.0 and 35.0 °C) [Leslie, 1997].

The temperature that produces a 1:1 sex ratio of males to females under constant incubation temperature has been termed the pivotal temperature (Mrosovsky and Pieau, 1991). Pivotal temperature values vary with the thermal nesting environment and have become a 'benchmark' temperature for comparisons between species and between populations of the same species (Mrosovsky, 1994). In the study at St. Lucia, Leslie (1997) found that the lower and upper pivotal temperatures for the Nile crocodile were 31.7 °C and 34.5 °C respectively. Hutton (1987) did not incubate crocodile eggs above 34.0 °C; however he determined that the hatching success in Zimbabwe at 34.0 °C was 69%, in contrast to St. Lucia's Nile crocodiles where the mean hatching success over two nesting seasons at 34.0 °C was only 38%. The difference in hatching success and pivotal temperatures of the Nile crocodile studied in South Africa verses Zimbabwe, suggests that geographic variation may play a role in sex determination.

In several species of reptiles, incubation temperature affects sex determination during particular stages in embryonic development when differentiation of gonads occurs. Preliminary temperature-shift experiments, designed to assess the temperature sensitive period (TSP) of gonadal differentiation were carried out by Ciofi and Swingland (1997). It is generally stated that thermosensitivity in TSD reptiles occurs during the middle third or half of incubation (Ciofi and Swingland, 1997).

In this study *C. niloticus* eggs were collected from the Okavango River, Botswana and incubated at eight different constant temperatures. The aim of the experiment was to determine the effect that egg incubation temperature has on embryonic survival, incubation period, hatching success and hatchling sex ratios. I determined the upper and lower pivotal temperature in a controlled environment and these were then compared to incubation temperatures recorded in wild nests to estimate the sex ratio of the Nile crocodile hatchlings entering the Okavango River system (see Chapter 4).

2.2 METHODS AND MATERIALS

2.2.1 Laboratory Incubation of Eggs

Eight incubators, with dimensions of 70 x 70 x 50 cm (length x width x height) were constructed from 7.0 cm thick, closed-cell styrofoam sheets. A single plastic water tray was placed in each incubator and filled with six liters of water, to act as a humidifier and heat sink. A submersible aquarium heater (LifeTech Aquarium, China) was placed in each water tray to provide a heat source and an aquarium air pump with an air hose agitated the water, providing circulation and mixing. The aquarium heaters were all connected in unison to a 16 channel AC/DC temperature relay panel (Campbell Scientific, Inc., Logan, Utah), which was connected to a CR-10X datalogger (Campbell Scientific, Inc., Logan, Utah). Each of the eight incubators was set at a different air temperature, viz: 30.0 °C, 30.5 °C, 31.0 °C, 31.5 °C, 32.0 °C, 32.5 °C, 33.0 °C and 34.0 °C. The incubators were equilibrated for two days before adding the eggs. Humidity in the incubators was estimated to be between 80 - 100%. Water levels were checked weekly and when necessary, fresh water was added. Incubators remained closed, except during daily inspection to minimize temperature fluctuations. The internal temperature of the incubation room on the Krokovongo Crocodile Farm was maintained at a constant temperature of 23.0 °C, with the use of a 9 000 W air conditioner.

In total 212 eggs were collected from wild crocodile nests during the 2005/06 breeding season. Eggs were collected from five individual nests so as to avoid possible clutch effects. Clutch sizes ranged from 21 - 70 eggs per clutch. Eggs were collected within 24 hours of deposition, packed into a styrofoam box containing vermiculite and transported to the incubation room by boat. Each egg was weighed (± 0.1 g) and measured (length and width ± 0.1 cm) using a pesola spring scale and pair of calipers. Eggs were randomly divided among the incubators and placed side by side on a metal rack 10 cm above the water tray (Appendix 2.1A). No substrate was used in the experiment, as an 'open-air incubation' system was employed. A 24-gauge copper-constantan thermocouple (Cu-Cn) was calibrated with a Model BATT-12 thermocouple meter (Physitemp Inc., USA) and then positioned between the eggs in each incubator. The thermocouples were connected to a CR-10X datalogger, which was programmed to record the incubation temperature of each incubator every 15 minutes throughout the incubation period. Temperature readings were downloaded from the CR-10X datalogger onto a Hewlett Packard Laptop on a daily basis and adjustments were made to compensate for metabolic heating produced by embryos during the latter part of the incubation period. In order to determine total incubation time, the day following nocturnal deposition was designated as 'day 0'. The endpoint was the day hatchlings emerged from the eggs.

After 60 days of incubation, once all the eggs had passed the temperature-sensitive-period (TSP) the eggs were individually placed in bags made from mosquito netting and labelled in

accordance with the nests they were collected from (Appendix 2.1B). This ensured that when hatching took place, hatchlings were separated from one another until they were marked.

2.2.2 Hatchlings

Hatchlings were weighed (± 0.1 g) using a digital scale and morphometric body measurements taken (± 0.1 cm) immediately after hatching. The total length (TL – distance from the tip of the snout to the tip of the tail) and snout-to-vent length (SVL – distance from the tip of the snout to the posterior edge of the vent/cloaca) was measured (Appendix 2.2). Hatchlings were marked using a coded scute removal pattern (Appendix 2.3) according to the nests they were collected from and the temperature at which they were incubated. Hatchlings were held in an indoor pond at the Krokovongo Crocodile Farm at a temperature of 32.0 °C. Crocodile hatchlings have relatively short digestive tracts and therefore require frequent feeding of small amounts of food (Marais and Smith, 1992). They were fed pellets every other day *ad libitum* and were weighed on a monthly basis to monitor growth rates and general condition.

The hatchlings that died pipping the egg or post-hatching were placed in 70% formalin and preserved until they were dissected. Unhatched eggs were opened a week after hatching took place and if embryos were present, they were measured in length (± 0.1 cm) using a pair of calipers. Infertility/NSD (no sign of development) was assigned to the eggs that contained no signs of an embryo.

Incubation period, relative development rate and hatching success was analysed using computer software (Microsoft® Office Excel 2003, Microsoft Corporation, USA) and Statistica 7 (Statsoft, Inc., USA). Relative developmental rates were calculated by dividing the observed incubation period by the briefest observed period (100.5 days at 34.0 °C), and taking the inverse of this value (Georges, 1989). A one-way analysis of variance (ANOVA) was used to compare SVL, TL and hatchling mass between incubators. Significance was determined at the level $P < 0.05$ (two-tailed). A Kruskal Wallis Test (a non-parametric analysis) was used to confirm the significant difference of the ANOVA (if residuals are non-normally distributed). This was followed with either Bonferroni or Bootstrap (Efron and Tibshirani, 1993) multiple comparison procedures depending on the normality (or non-normality) of the residuals. A linear regression analysis was used to determine the relationship between incubation temperature, hatching success and incubation period.

2.2.3 External Sexing

Cloacal examination of the cliteropenis took place at 1, 2, 4 and 6 months of age without knowledge of the previous classification. The crocodile was restrained on its dorsum under a good light source. The cliteropenis was examined by separating the cloaca with a pair of blunt

forceps and applying manual pressure to the base of the cloaca opening (Appendix 2.4). Sexing criteria was based on Hutton (1987), Lang *et al.* (1989), Allsteadt and Lang (1995) and Leslie (1997). Sex was assigned based on the relative size, shape, texture and colour of the cliteropenis (Appendix 2.5).

2.2.4 Dissection

The animals that died pipping the egg were sexed macroscopically (Appendix 2.6). They were restrained under a good light source and the cliteropenis was examined and described as above. The body was opened by making a midventral incision from the base of the head to the vent to expose the gonadal-complex, including the kidneys, adrenal glands and mesonephric tissue. A dissecting microscope was used at a low power magnification and initial sexing was based on the presence or absence of an oviduct and the overall appearance of the gonad (Appendix 2.7). Sexing criteria was based on the findings of Leslie (1997) and Richardson *et al.* (2002).

2.3 RESULTS

2.3.1 Egg Morphometrics

Table 2.1 shows the average egg length, egg width, egg mass and clutch size per nest for the 2005/06 nesting season. A significant difference exists between egg length and egg mass (r^2 = 0.06, $P < 0.05$), and between egg width and egg mass (r^2 = 0.65, $P < 0.05$) among the five clutches (Nest 1 - 5).

The eggs from these clutches were randomly divided among the eight incubators to prevent any bias or clutch effects. Table 2.2 represents the morphometrics of eggs in each incubator once the eggs were randomly divided.

Table 2.1 Average egg dimensions (± 0.1 cm) determined per nest from wild Nile crocodile nests collected from the Okavango River, Botswana.

Nest	Length (cm)	Standard deviation (cm)	Width (cm)	Standard deviation (cm)	Mass (g)	Standard deviation (cm)	Clutch size
1	8.30	0.16	5.22	0.10	97.83	4.33	53
2	8.29	0.23	4.90	0.10	82.69	6.52	26
3	8.06	0.22	5.28	0.10	97.86	4.13	70
4	8.02	0.30	4.87	0.13	76.67	6.78	42
5	7.77	0.20	5.09	0.10	84.05	5.84	21
Average	8.09	0.22	5.07	0.10	87.82	5.52	42.40

Table 2.2 Average Nile crocodile egg dimensions (± 0.1 cm) determined per incubator. Eggs were incubated at the Krokovango Crocodile farm in the Okavango Delta, Botswana.

Incubator	Length (cm)	Standard deviation (cm)	Width (cm)	Standard deviation (cm)	Mass (g)	Standard deviation (g)
1	8.13	0.28	5.10	0.18	89.62	10.78
2	8.13	0.25	5.15	0.17	91.80	8.23
3	8.09	0.26	5.14	9.21	88.46	10.28
4	8.11	0.31	5.14	0.20	90.19	10.33
5	8.17	0.28	5.06	0.22	90.00	11.97
6	8.08	0.21	5.08	0.23	90.19	10.42
7	8.19	0.24	5.14	0.22	93.46	10.18
8	7.97	0.33	5.14	0.20	90.96	11.02
Average	8.11	0.27	5.12	1.33	90.59	10.40

A significant regression was found between egg mass and length, and between egg mass and width of the five clutches. Both regressions showed that an increase in egg mass resulted in an increase in either egg length or egg width. A significant relationship was also found between egg mass and egg width (r^2 = 0.65; $P < 0.05$) in comparison to egg mass and egg length (r^2 = 0.06, $P < 0.05$).

2.3.2 Hatchlings

A total of 149 hatchlings were weighed (± 0.1 g) and measured (snout-to-vent length (SVL) and total length (TL)) immediately after hatching. Averages were calculated for hatchlings from each individual nest (Table 2.3), and hatchlings per incubation temperature (Table 2.4). No hatchling data were available from Nest 4, as the entire clutch was infertile.

The average SVL for all four nests was 14.1 cm. Hatchlings from Nest 5 had the smallest SVL, TL and mass. Hatchlings from Nest 1 had the largest SVL and were the heaviest in mass (Table 2.3). The average hatchling TL for all four nests was 31.5 cm and the largest hatchlings were from Nest 3. Average hatchling mass from all four nests was 83.4 g. When comparing SVL of hatchlings a significant difference was found among incubators [F (7, 123) = 6.65, $P < 0.05$]. Hatchlings incubated at 30.0 °C (Incubator 1) had the shortest SVL and hatchlings incubated at 34.0 °C (Incubator 8) had the longest SVL. The residuals were not normally distributed (Appendix 2.8A) and a Kruskal Wallis Test was carried out to confirm ($P < 0.05$) the significant difference of hatchling SVL among incubators.

Table 2.3 Average Nile crocodile hatchling morphometric measurements from each of five nests. Eggs were collected from the wild in the Okavango River, Botswana.

Nest	SVL (cm)	Standard deviation (cm)	TL (cm)	Standard deviation (cm)	Mass (g)	Standard deviation (g)
1	14.24	0.20	31.66	1.12	86.81	3.34
2	14.08	1.49	31.49	1.12	79.66	3.21
3	14.16	0.69	31.67	1.83	84.47	7.94
5	13.94	0.35	31.31	2.23	78.79	3.37
Average	14.11	0.68	31.53	1.58	82.43	4.47

Table 2.4 Average Nile crocodile hatchling morphometric measurements from each incubator. Eggs were incubated at the Krokovongo Crocodile Farm in the Okavango Delta, Botswana.

Incubator	SVL (cm)	Standard deviation (cm)	TL (cm)	Standard deviation (cm)	Mass (g)	Standard deviation (g)
1	13.46	0.70	29.14	1.42	74.26	9.22
2	14.07	0.44	32.29	2.13	83.91	4.81
3	13.99	0.38	30.52	2.96	80.13	5.56
4	14.05	0.40	31.61	1.12	84.31	5.15
5	14.02	0.82	30.85	1.98	84.85	3.34
6	14.47	0.24	32.56	0.74	85.81	3.63
7	14.23	0.32	31.59	0.99	85.97	4.64
8	14.50	0.44	32.44	1.07	86.59	3.65
Average	14.10	0.47	31.38	1.55	83.23	5.00

A Bootstrap (Efron and Tibshirani, 1993) multiple comparisons procedure showed that hatchling SVL from incubator 1 was significantly different from all other incubators (Figure 2.1). The same holds true for hatchling TL. There was a significant difference among incubators [F (7, 123) = 6.38, $P < 0.05$]. Hatchlings incubated at 30.0 °C (Incubator 1) had the shortest TL and hatchlings incubated at 32.5 °C (Incubator 6) displayed the longest TL. A Bonferroni and Bootstrap (Efron and Tibshirani, 1993) multiple comparisons procedure confirmed that a significant difference exists in hatchling TL among incubators. Hatchling TL from Incubator 1 was different to the other incubators (Figure 2.2). The residuals were not normally distributed (Appendix 2.8B) and a Kruskal Wallis test was carried out to confirm ($P < 0.05$) the significant difference of the ANOVA.

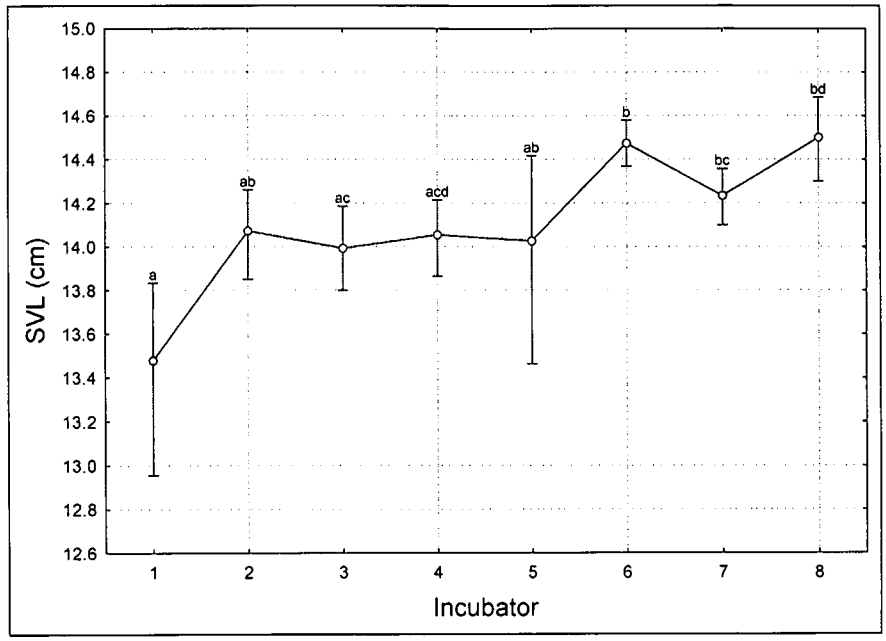


Figure 2.1 Bootstrap confidence intervals illustrating the differences in Nile crocodile hatchling SVL (snout-to-vent length) among incubators. Hatchlings were incubated at the Krokovongo Crocodile Farm in the Okavango Delta, Botswana.

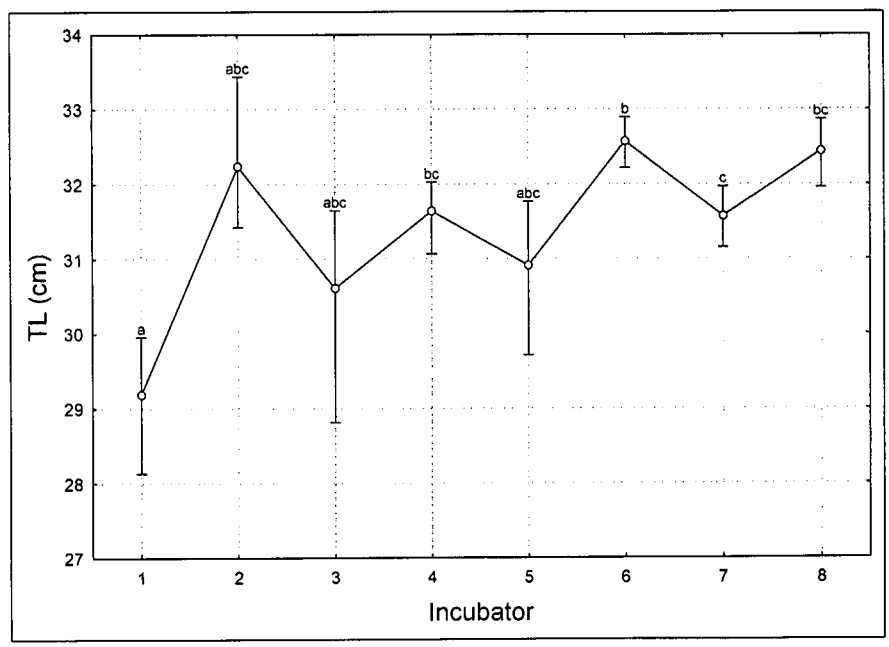


Figure 2.2 Bootstrap confidence intervals illustrating the differences in Nile crocodile hatchling TL (total length) among incubators.

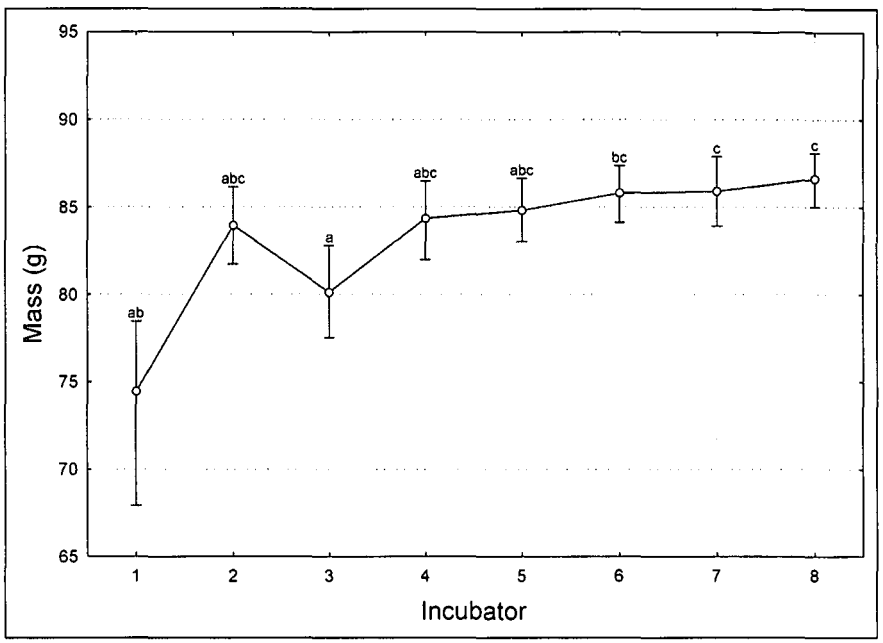


Figure 2.3 Bootstrap confidence intervals illustrating the differences in Nile crocodile hatchling mass among incubators.

A significant difference was found between hatchling mass and incubation temperature [F (7, 123) = 7.89, $P < 0.05$]. Hatchlings incubated at 30.0 °C (Incubator 1) were on average, significantly lighter when compared to hatchlings incubated at 34.0 °C (Incubator 8). The residuals were not normally distributed (Appendix 2.8C). A Kruskal Wallis comparison confirmed the significance ($P < 0.05$) and a Bootstrap (Efron and Tibshirami, 1993) multiple comparisons procedure (Figure 2.3) indicated that hatchling mass from incubators 1 and 3 were significantly different to hatchling mass from eggs incubated at the other temperatures.

The hatchlings were weighed at 1, 2, 4 and 6 months of age. Body mass dropped significantly one month after hatching (6.0 g difference between hatching and one month of age). There was a noticeable difference in mass between the eggs and hatchlings, suggesting that the yolk sac of the hatchlings retains a significant mass (Figure 2.4). Eggs were weighed when they were collected from the wild, 24 hours after deposition. On the day of hatching, yolk sacs had been absorbed by the hatchlings. A month later the yolk sacs were completely digested, which is reflected in the decrease in mass (Figure 2.4).

After one month of growth there was a significant increase in hatchling mass over time. Results show that the post-hatchling growth rate accelerated over time. The average difference in hatchling mass between one and two months of age was 14.2 g. The average difference between two and four months was 66.0 g, and the average mass difference between four and six months was 123.5 g (Figure 2.4).

Table 2.5 Average monthly mass (g) of Nile crocodile hatchlings per incubator, incubated at the Krokovongo Crocodile Farm in the Okavango Delta, Botswana.

		Incubator								
		1	2	3	4	5	6	7	8	Average
Mass (g)	Eggs	89.62	91.73	88.15	89.81	89.63	90.19	93.46	90.96	90.44
	Standard deviation	10.76	8.24	10.67	10.33	11.92	10.42	10.18	10.96	10.43
	At hatching	74.26	83.91	80.13	84.31	84.85	85.81	85.97	86.59	83.23
	Standard deviation	9.22	4.81	5.56	5.15	3.34	3.63	4.64	3.65	5.00
	1 month		75.87	74.22	77.62	73.54	80.47	77.11	81.79	77.23
	Standard deviation		4.57	4.71	9.71	3.50	16.86	7.57	13.55	8.64
	2 months		80.85	75.78	91.65	66.01	120.22	95.32	110.02	91.41
	Standard deviation		23.79	12.11	41.33	5.32	60.58	38.55	48.09	32.82
	4 months		137.37	94.84	152.79	70.94	228.18	182.92	234.84	157.41
	Standard deviation		83.87	42.82	106.48	18.36	131.05	125.90	142.64	93.02
	6 months		274.50	110.00	255.67	88.75	506.25	333.85	397.00	280.86
	Standard deviation		180.02	40.93	215.22	18.87	210.35	200.59	233.24	157.03

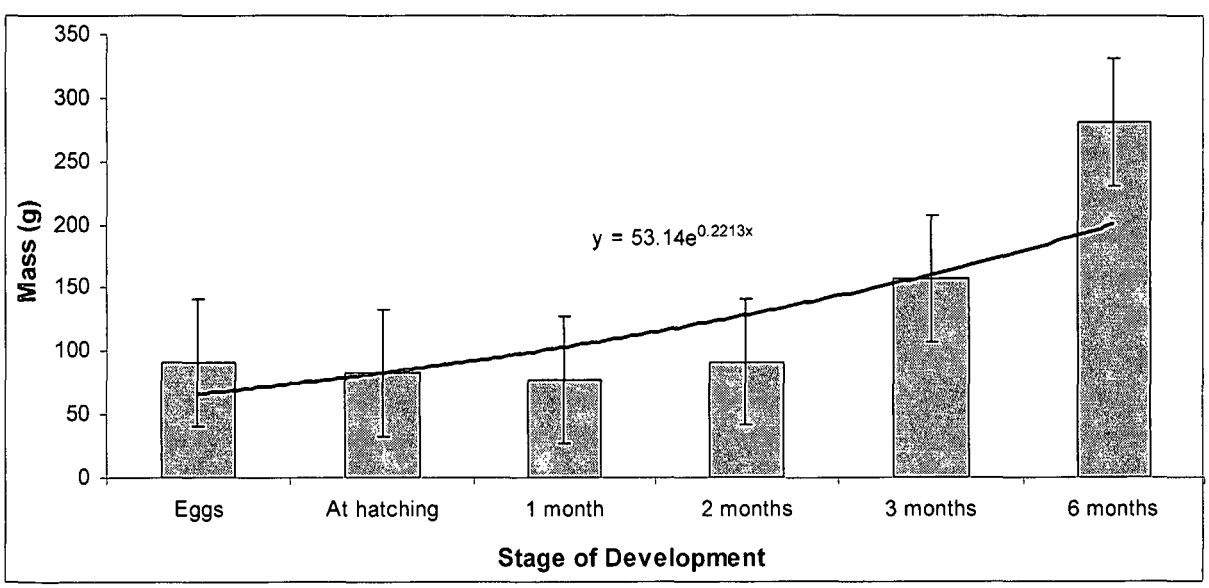


Figure 2.4 Average mass of the incubated Nile crocodile hatchlings at various stages of development.

No mass data were available from Incubator 1 as all the hatchlings from this incubator died within the first month of hatching.

2.3.3 Laboratory Incubation and Embryonic Survival

Incubation temperature significantly influenced the duration of incubation and embryonic survival of *C. niloticus* (Figure 2.5). A significant regression relationship was found between incubation temperature and incubation period (r^2 = 0.81, P < 0.05). Incubation temperature explained 81% of variation in incubation period (Figure 2.6). Incubation period decreased with increasing incubation temperature from 30.0 °C to 34.0 °C. The longest mean incubation period was 123 days at 30.0 °C and 31.0 °C, followed by 114 days at 30.5 °C, 109 days at 31.5 °C, 108 days at 32.0 °C, and 101.7 days at 33.0 °C. The shortest incubation period of 100.5 days was at 34.0 °C and 32.5 °C. The influence of temperature on incubation period can be explained by determining the rate of development. Relative development rates were slowest at the lowest incubation temperature (30.0 °C) and fastest at the highest incubation temperatures (34.0 °C) [Figure 2.5].

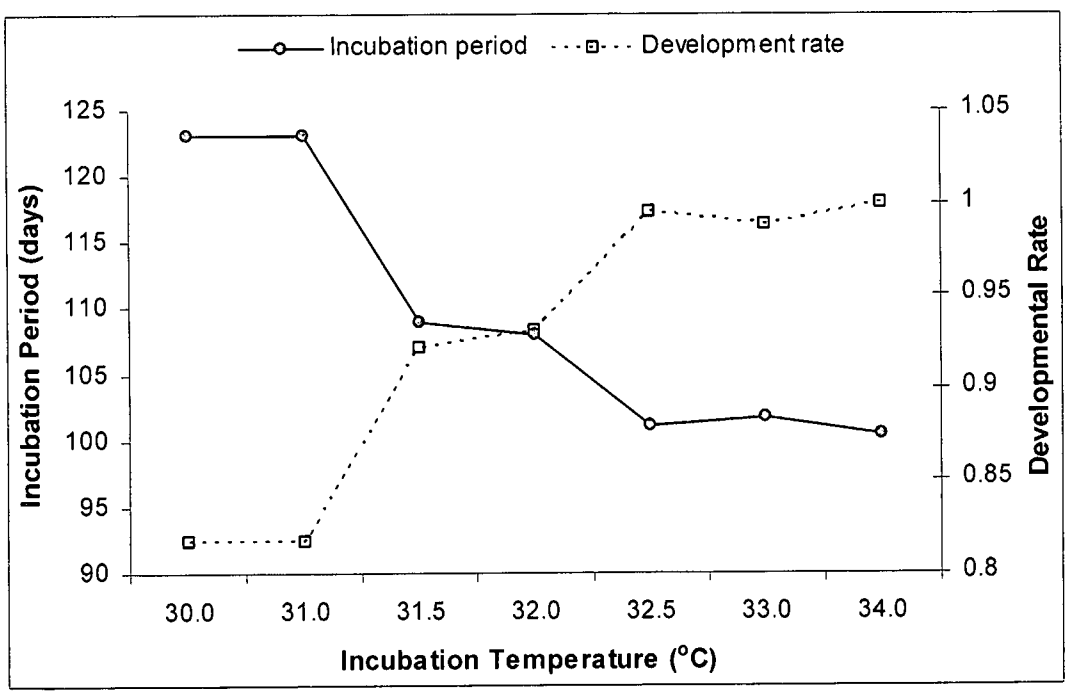


Figure 2.5 Dependence of the rate of embryogenesis on incubation temperature in *Crocodylus niloticus*, over a range of constant temperatures in a controlled constant temperature experiment. The solid line shows incubation period versus incubation temperature and the dotted line shows relative developmental rates versus incubation temperature.

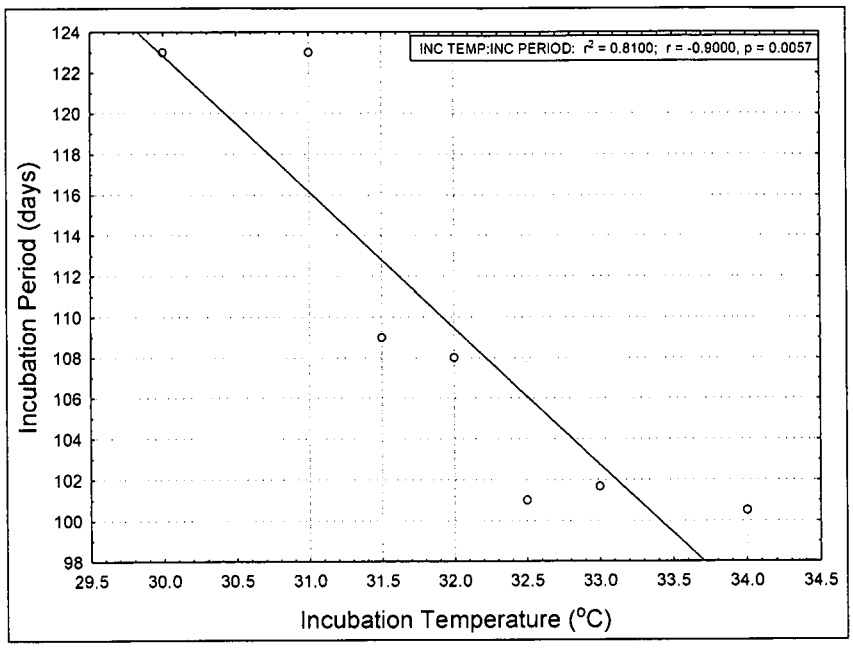


Figure 2.6 Regression between incubation period and incubation temperature of *Crocodylus niloticus* (r^2 = 0.81, r = -0.90, *P* < 0.05) (Estimated ŷ = 323.79 – 6.698*x).

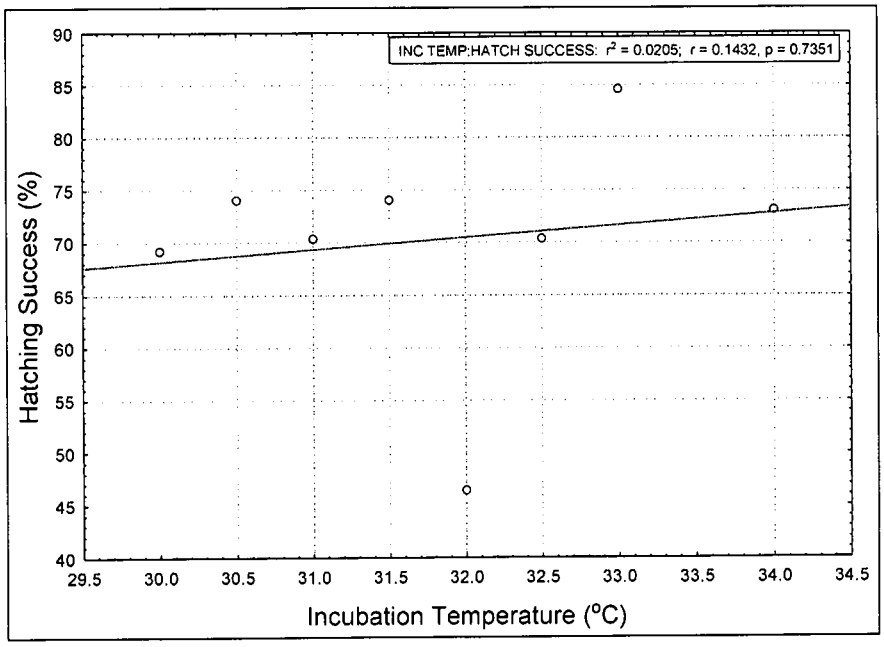


Figure 2.7 Regression between hatching success and incubation temperature of *Crocodylus niloticus* (r^2= 0.021, r=-0.14, *P* < 0.05) (Estimated ŷ = 33.51 + 1.16*x).

There was no significant regression relationship between hatching success and incubation temperature (r^2 = 0.002; P > 0.05) [Figure 2.7]. Hatchling success was highest at 33.0 °C (84.6%; n=23) and lowest at 32.0 °C (46.4%, n=28). There was a high percentage of infertile eggs present in Incubator 5 (32.0 °C).Within the first month all of the hatchlings from Incubator 1 (30.0 °C) died and so the mortality rate was 100% (Appendix 2.9). The mortality rate was the lowest in Incubator 8 (34.0 °C). This suggested that the probable lethal minimum constant incubation temperature for Nile crocodiles breeding in the Okavango River is 30.5 °C and the mean lethal maximum temperature is above 34.0 °C.

Infertility/NSD (no sign of development) was 26.4% (n=56). One of the five clutches collected (Nest 4) was 100% infertile. These eggs were laid by a small, young female (TL < 2.0 m). It can therefore be assumed that it was her first time of laying.

2.3.4 External Sexing

Hatchlings were sexed on four separate occasions, at the age of 1, 2, 4 and 6 months. Male or female sex was assigned by visually assessing the size, shape and colour of the cliteropenis. Sex identification by cloacal examination was highly unreliable in *C. niloticus* hatchlings from the Okavango River during the first four months of development. At six months of age morphological differences in the genitalia between males and females became more apparent as differences in the length and shape of the cliteropenis was more obvious. In males, the cliteropenis was larger and more elongated than in females (Appendix 2.5A). The base of the cliteropenis in the male was narrow, whereas in the female the base of the cliteropenis was typically squat and triangulated (Appendix 2.5B).

2.3.5 Dissection

In dissection the male and female gonads were similar in appearance (Appendix 2.7A and B). The gonads lay on the dorsal abdominal wall, overlying the true kidneys, known as the metanephros, on either side of the dorsal aorta and inferior vena cava. Gonads were closely associated with the yellow adrenal glands which were visible in the craniomedial half of the gonad, and mesonephric kidney. The gonads were long, spindle-shaped structures. In males, the gonad was smooth in texture. The thin walled Wolffian ducts emerged from the caudal pole of the gonad (Appendix 2.7A). The ducts ran caudally and entered the lateral walls of the cloaca. The oviducts were completely absent in males. In females, the gonads appeared more granular and translucent. Convoluted, bilateral Mullerian ducts were a conspicuous feature in females (Appendix 2.7B). The oviducts ran along the dorsolateral margins of the gonads and were attached to the gonads by a fibrous mesosalpinx mesentery. The oviducts were transparent and thick walled.

2.3.6 Pivotal Temperature

The sex of 149 crocodiles from the 2005/06 breeding season was determined using a combination of the two techniques described above (Appendix 2.9). The calculated lower pivotal temperature for the Okavango Delta's Nile crocodile was 31.4 °C with an upper pivotal temperature of 33.4 °C (Figure 2.8). Eggs incubated at 30.0 °C and 30.5 °C were 100% female, at 31.0 °C, 71% were female and hatchlings from, 31.5 °C, 32.0 °C, 32.5 °C and 33 °C were 55%, 67%, 81% and 72% male respectively. At 34.0 °C, 78% were female. Sample size from the 32.0 °C incubator was small (n=12) due to a low percentage hatching success. Females developed over the entire range of viable temperatures (30.0 °C to 34.0 °C). The Nile crocodile therefore has a female-male-female pattern of TSD, where female hatchlings were produced at lower and higher incubation temperatures (31.5 °C and 33.4 °C) and the majority male hatchlings were produced at intermediate incubation temperatures (32.0 – 33.0 °C).

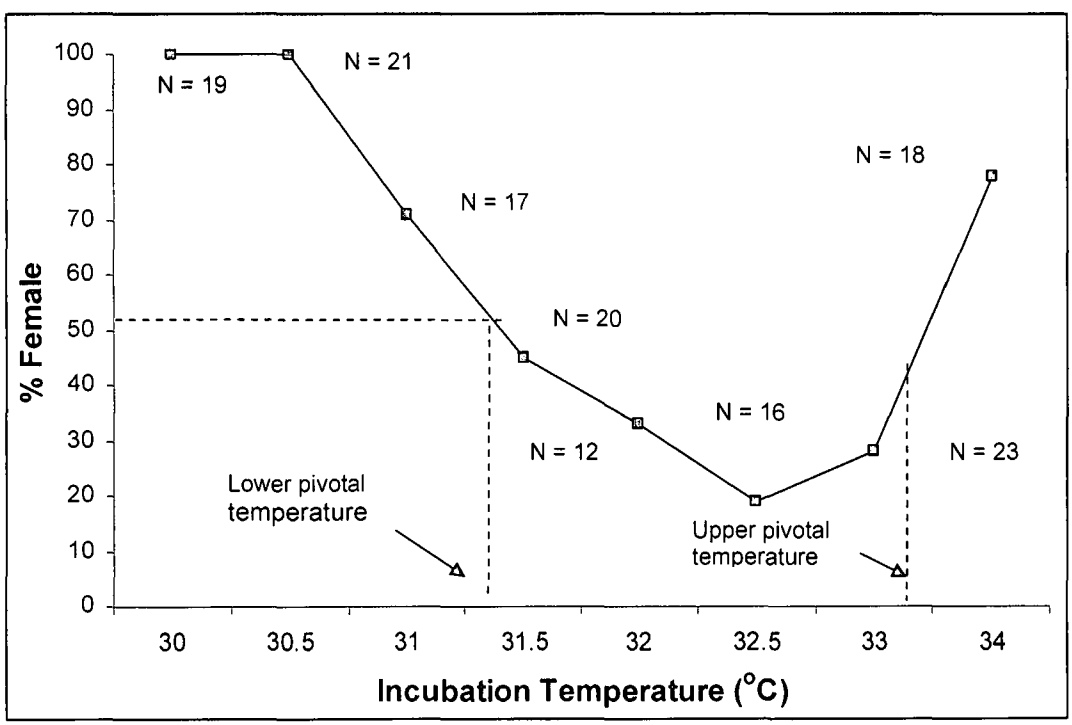


Figure 2.8 Effect of incubation temperature on sex determination in Nile crocodiles in the Okavango River. Eggs were incubated at constant temperatures at the Krokovongo Crocodile Farm. A female-male-female pattern of TSD occurred, with a lower pivotal temperature of 31.4 °C and an upper pivotal temperature of 33.4 °C.

2.4 DISCUSSION

Embryonic survival, developmental rates, incubation periods and sex ratio of the Nile crocodiles in the Okavango River were dependent on incubation temperature. There was no significant difference in the average length and width of *C. niloticus* eggs when compared to previous studies. Average egg length was 8.1 cm, which is slightly longer compared to the findings of Webb and Cooper-Preston (1989), where egg length was 7.8 ± 0.41 cm, and the findings of Swanepoel (1999), where the average egg length measured over two seasons was 7.7 cm. Average egg width (5.1 cm) of the five clutches of eggs used in the experiment was slightly greater than that of Webb and Cooper-Preston (1989) [width = 4.9 ± 0.24 cm]. Swanepoel (1999) reported the average width of eggs in the Olifants River to be 5.1 cm. The average mass of eggs collected in the Okavango River was 90.4 g, which was significantly lower than Webb and Cooper-Preston (1989) [109.2 ± 14.97 g] and Swanepoel (1999) [118.2 g]. Although the crocodile eggs collected from the Okavango River are larger in size compared to Nile crocodile eggs collected in the Olifants River (Swanepoel, 1999), they are smaller in mass at the initial stage of development. Pooley and Gans (1976) found that the size of the female is directly proportional to the size and mass of the eggs produced. This suggests that the average nesting female Nile crocodile in the Okavango River may be larger in size when compared to those in the Olifants River. However, further research is required to determine whether this relationship is significant.

There was a similar trend in the regression analysis between egg mass and length as well as egg mass and width. Both regressions showed that an increase in egg mass resulted in an increase in either egg width or length. This confirmed the findings of Swanepoel (1999) who found that egg mass was directly proportional to egg length and egg width.

The Okavango crocodiles had a mean clutch size of 42 eggs. This is low when compared to Nile crocodile nests in Zimbabwe (54 eggs per nest) [Hutton, 1987], but similar to the 47 eggs per nest for Umfolozi Game Reserve (Hartley, 1990). Female crocodile nests in the St. Lucia Estuary averaged 45 eggs (Pooley, 1969) and an average of 36 eggs per nest was found in the Olifants River in the Kruger National Park (Swanepoel, 1999). Clutch size may also be related to the size of the female.

It is important to remove eggs at an early stage as the embryonic disc, which is covered by a thin layer of albumin, still floats freely at the top of the yolk (Marais and Smith, 1992). If the egg is removed within the first 24 hours, the embryonic disc moves to the nearest position of the yolk without detrimental effects. After approximately 24 hours the disc attaches itself to the higher inner aspect of the shell membrane where it remains attached. Movement or turning at this stage may kill the embryo due to it being crushed by adjacent heavy yolk (Marais and Smith, 1992).

Previous studies have shown that incubation temperature directly affects body size of Nile crocodiles at hatching (Webb and Cooper-Preston, 1989; Hutton, 1987; Webb *et al.*, 1987 and Allsteadt and Lang, 1995). The size of an embryo at hatching depends largely on how much of the yolk has been converted to tissue and how much has been retained as residual yolk (Webb *et al.*, 1987). In this study total body length of hatchlings increased with incubation temperature. Hatchlings incubated at 30.0 °C (29.1 cm) were smaller in length when compared to hatchlings incubated at 34.0 °C (32.4 cm). There was a significant difference in hatchling SVL and TL at the different incubation temperatures. Hatchlings from Incubator 1 (30.0 °C) were significantly smaller in SVL and TL compared to hatchlings incubated at the higher temperatures. This suggests that an incubation temperature of 30.0 °C may be too low to ensure adequate growth among hatchlings. A smaller body size may be disadvantageous in terms of reproductive success, fitness and survival in the long term. Hutton (1987) found that incubation temperature had a significant effect on Nile crocodile hatchlings in Lake Ngezi in Zimbabwe. Hatchlings incubated at 31.0 °C were longer (30.8 cm) than those incubated at 28.0 °C (30.4 cm) and 34.0 °C (29.6 cm) respectively.

In *C. johnstoni*, low incubation temperature produces relatively heavy hatchlings with little residual yolk, whereas high incubation temperature produces relatively light hatchlings with a great amount of residual yolk (Webb *et al.*, 1987). In this study the opposite was found. A low incubation temperature produced relatively light hatchlings and higher incubation temperatures produced relatively heavy hatchlings. Mass is directly proportional to incubation temperature as there was a significant increase in hatchling mass with increasing incubation temperature.

There was a significant difference in the average mass of eggs compared to the hatchlings, and compared to the hatchlings at one month of age. The average mass of the Nile crocodiles at hatchling was 83.3 g, which is significantly greater than the hatchlings weighed by Aulie and Kanui (1995), where the mean mass of the hatchlings was 55.2 ± 2.4 g. The initial decline in mass from hatching to the age of one month could be related to the fact that the yolk sac is being absorbed. From two months of age hatchling mass increases. The growth rate accelerates over time, increasing from two to four months of age, with an even greater increase from four to six months of age. Kay (2004) found that *C. porosus* hatchlings grew allometrically, with TL doubling within a year and body mass increasing ten-fold. In this study it was found that post-hatching growth rates were affected by incubation temperature. The hatchlings incubated at 34.0 °C outgrew the hatchlings incubated at lower incubation temperatures. This supports the findings of Webb and Cooper-Preston (1989) who stated that in *C. porosus, C. niloticus* and *A. mississippiensis* incubation temperature exerts significant effects on post-hatching growth and survival. In 1987, Hutton found that *C. niloticus* hatchlings incubated at 34.0 °C outgrew those incubated at 28.0 °C and 31.0 °C within three months.

Embryonic survival, developmental rates and incubation periods at constant incubation temperature in the Okavango Nile crocodile were clearly temperature dependent. High embryonic survival occurred at constant incubation temperatures from 31.0 °C to 34.0 °C. Many other crocodilian species, including *A. mississippiensis* (Deeming and Ferguson, 1989); *Caiman crocodylus* (Lang and Andrews, 1994), *C. sinesis* (Chen, 1990), *C. porosus* and *C. johnstoni* (Webb *et al.*, 1987), have a high embryonic survival rate at constant incubation temperatures within this range.

Embryonic development in *C. niloticus*, as in most other crocodilian species, was accelerated as incubation temperature increased within the viable range, thus decreasing the incubation period. The underlying cause for this relationship is that metabolic processes proceed more rapidly at higher temperatures (Leslie, 1997). Relative development rate coefficients for the Nile crocodile in the Okavango River increased from 0.82 at 30.0 °C to 1.00 at 34.0 °C. In St. Lucia, Leslie (1997) found that the development rate for the Nile crocodiles initially increased from 0.85 at 31.0 °C to 1.00 at 33.0 °C, and then decreased to 0.98 at 34.0 °C and 0.87 at 35.0 °C. The initial stage of developmental rates of the Nile crocodile from the Okavango River followed the same trend as Nile crocodiles studied in St. Lucia, but there was a difference in development rates between incubation temperatures of 33.0 °C and 34.0 °C. This suggests that Nile crocodiles in the Okavango River may have an increased tolerance for higher incubation temperatures. It is recommended that in future studies, higher incubation temperatures should be used to determine at which temperature range the development rate starts to decline.

The incubation period for Nile crocodiles in this study was significantly longer in comparison to previous studies. At a constant temperature of 33.0 °C the mean incubation period was 102 days. This study determined that at constant incubation temperatures of 30.0 °C; 30.5 °C; 31.0 °C; 31.5 °C; 32.0 °C; 32.5 °C; 33.0 °C and 34.0 °C the incubation period was 123.0; 114.0; 123.0; 109.0; 108.0; 101.0; 101.7 and 100.5 days respectively. Leslie (1997) incubated Nile crocodile eggs at 31.0 °C; 31.5 °C; 32.0 °C; 32.5 °C; 33.0 °C; 34.0 °C and 35.0 °C and determined an incubation period of 82.2; 79.8; 77.3; 72.7; 69.9; 71.1 and 80.5 days respectively. Although there is a significant difference in the length of incubation period in comparison to the study by Leslie (1997), in this study incubation period decreases with increasing incubation temperature. Th ere was a significant relationship between incubation period and incubation temperature. Lin *et al.* (2005) found that within the range of temperatures yielding viable offspring, higher temperatures result in faster embryonic development and thus, a shortened period of incubation. Hutton (1987) incubated Nile crocodile eggs from Zimbabwe at 28.0 °C; 31.0 °C and 34.0 °C and reported that time from egg laying to hatching was shortest at 34.0 °C (84.4 days) and longest at 28.0 °C (111 days). Interpolation from these data indicated that eggs incubated at 33.0 °C would have a mean incubation period of approximately 86 days.

Mean total incubation period for *C. palustris, C. johnstoni, C. porosus, C. crocodylus* and *A. mississippiensis* at the same incubation temperature was 63.5; 67.4; 75.4; 71.2 and 63.4 days respectively. Head *et al.* (1987) states that higher incubation temperatures result in faster growth rates, shorter incubation periods, larger juvenile sizes and larger adult sizes. Lin *et al.* (2005) found that a prolonged incubation period at low temperatures in wild nests increases exposure of eggs to the effects of adverse biotic (increased microbial contamination) and abiotic factors (extreme thermal and hydric conditions) in the incubation environment, which potentially reduces hatching success.

The incubation period of Nile crocodile eggs in this study may have been affected by the bags the eggs were placed in during incubation. These bags were used to keep the eggs separated for when hatching took place. However, the bags may have retained additional moisture, thereby having a cooling effect on the eggs, thus prolonging the incubation period.

Among crocodilians, incubation temperature also affects the probability of embryos surviving (Webb and Smith, 1984; Gans and Billett, 1985; Lang *et al.*, 1989); the frequency of abnormalities among embryos and hatchlings (Webb *et al.*, 1983, Ferguson, 1985); body size at hatching (Hutton, 1987; Webb *et al.*, 1987, Allsteadt and Lang, 1995); the weight of residual yolk at hatching (Webb *et al.*, 1987; Allsteadt and Lang, 1995, Congdon *et al.*, 1995); hatchling pigmentation patterns (Deeming and Ferguson, 1989); post-hatching growth rates (Hutton, 1987 and Joanen *et al.*, 1987) and post-hatching patterns of thermoregulation (Lang, 1987). These effects are independent of sex (Webb *et al.*, 1987) and can be considered 'non-sexual' responses to incubation temperature. Further studies of these responses are required for the Nile crocodile.

In this experiment hatching success increased with incubation temperature. Percent success was highest at 33.0 °C and lowest at 32.0 °C. Leslie (1997) found the opposite to be true. Hatching success was highest at 31.0 °C in one year and in a second season percent hatching success was highest at 32.0 °C and lowest at 35.0 °C. In the St. Lucia Nile crocodile experiment, the mortality rate was greatest at incubation temperatures of 34.0 °C and 35.0 °C (Leslie, 1997). However in this study, embryonic mortality at the higher incubation temperatures was less than 15%. Post-growth mortality, however at the incubation temperature of 30.0 °C was 100%. The Nile crocodile population of the Okavango River is possibly better adapted to higher incubation temperatures compared to the population in St. Lucia, South Africa. This is appropriate in that the St. Lucia area represents the southernmost extent of the breeding range for the crocodiles in the world (Leslie, 1997).

The Nile crocodile hatchlings were sexed by visual examination of the cliteropenis and macroscopically to determine the lower and upper pivotal temperatures. Male or female sex was assigned by visually assessing the size, shape and colour of the cliteropenis. Sex is fully

determined at the time of hatching and is irreversible (Ferguson and Joanen, 1983). In crocodiles, marked sex differentiation in the genitalia has been observed in *C. porosus, C. johnstoni* (Webb and Smith, 1984), *C. niloticus* (Hutton, 1987), *C. palustris* (Lang *et al.*, 1989), *C. siamensis* and *C. moreletii* (Lang and Andrews, 1994). In *A. mississippiensis* sex identification by cloacal examination is highly reliable. Allsteadt and Lang (1994) found that the cliteropenis clearly differed between the sexes. In the males the cliteropenis is generally reddish in colour and in the females it is white. The cliteropenis in males is also more rounded whereas in the females it is less prominent.

In this study it was found that in males the cliteropenis was larger and more elongated than in females (Appendix 2.4). The glans penis appeared bi-lobed in the male, as described by Leslie (1997). The base of the cliteropenis was narrower and tubular in the male (Appendix 2.5), whereas in the female it was typically squat and triangular. As described by Webb *et al.,* (1983) the cliteropenis of *C. johnstoni* was larger in the male, elongated and tubular in shape. In addition, Hutton (1987) noted that the stem of the cliteropenis in *C. niloticus* was always dark in the males and pale in most females.

In dissection the male and female gonads were similar in appearance and position. The gonads lay on the dorsal abdominal wall, overlying the kidneys, on either side of the dorsal aorta and inferior vena cava. Richardson *et al.* (2002) describe the kidneys as paired, oval, elongated, lobulated structures lying on either side of the vertebral bodies. In males oviducts are completely absent and Richardson *et al.* (2002) found that it was difficult to see the vas deferens in male hatchlings. However, they may appear as thin straight tubes. In females, the gonads appear more granular and translucent and convoluted bilateral Mullerian ducts can be found (Leslie, 1997).

The sex of Nile crocodile hatchlings in the Okavango River was determined by incubation temperature and a FMF pattern of TSD occurred. This pattern was consistent with the TSD pattern studied in detail in five other species of crocodilians and proposed in an additional four species, for constant temperature incubation (Lang and Andrews, 1994). Leslie (1997) found a lower pivotal temperature of 31.7 °C and an upper pivotal temperature of 34.5 °C. Eggs incubated at 31.0 °C were 100% female, at 31.5 °C, 70% were female and those from 32.0 °C; 32.5 °C and 33.0 °C were 79.5%, 87.5% and 87.2% male respectively. Hutton (1987) also reported a FMF pattern in *C. niloticus* eggs incubated at 28.0 °C, 31.0 °C and 34.0 °C. He reported a pivotal temperature between 31.0 °C and 34.0 °C. In a second experiment, the eggs were incubated at 31.0 °C, 32.5 °C and 34.0 °C and the pivotal temperature was found to be between 31.0 °C and 32.5 °C. In this experiment the lower pivotal temperature of 31.4 °C fell within the FM pivotal temperature range of 31 - 32 °C for the five crocodilian species studied to

date, and the upper pivotal temperature of 33.4 °C was above the maximum male producing range of 32-33 °C for the same five species. For example, *A. mississippiensis* has a lower pivotal temperature of 31.8 °C and an upper pivotal temperature of 33.8 °C. The lower and upper pivotal temperature for *Caiman crocodylus*, *C. palustris*, *C. porosus* and *C. johnstoni* are 31.5 and 34.0 °C; 31.8 and 32.8 °C; 31.5 and 32.7 °C and 31.5 °C and 32.5 °C, respectively.

2.5 CONCLUSION

Incubation temperature has a direct effect on the development of embryonic reptiles. In the Okavango River an increase in incubation temperature resulted in an increase in hatchling body size and mass, development rate and hatching success. Embryonic development accelerated with increasing incubation temperatures, which resulted in a decrease in incubation period. Incubation temperature also influenced the sex ratio of *C. niloticus*. A female-male-female pattern of TSD exists with a lower and upper pivotal temperature of 31.4 °C and 33.4 °C respectively. Results show that the pivotal temperature for the Nile crocodile falls within the same range of temperature, regardless of geographical location (Botswana, South Africa and Zimbabwe).

The long-term survival of the Nile crocodile in the Okavango River is dependent on a sufficient range of incubation temperatures to ensure that both male and female hatchlings are produced. A change in temperature due to Global Climate Change will have a direct effect on the incubation of the Nile crocodile and affect the resultant sex ratio.

2.6 REFERENCES

ALLSTEADT, J. AND J. W. LANG. 1995. Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis. Herpetological* 51 (3): 314–325.

AULIE, A. AND T.I. KANUI. 1995. Oxygen consumption of eggs and hatchlings of the Nile crocodile (*Crocodylus niloticus*). *Comparative Biochemistry and Physiology* 112A (1): 99–102.

CHEN, B. 1990. The past and present situation of the Chinese alligator. *Asiatic Herpetological Research* 3: 129-136.

CIOFI, C. AND I. R. SWINGLAND. 1997. Environmental Sex Determination in reptiles. *Applied Animal Behavior Science* 51: 251–265.

CONGDON, J. D., FISHER, R. U. AND R. E. GATTEN, JR. 1995. Effects of incubation temperature on characteristics of hatchling American alligators. *Herpetologica* 51 (4): 497-504.

DEEMING, D. C. AND M. W. J. FERGUSON. 1989. The mechanism of temperature dependent sex determination in crocodilians: A hypothesis. *American Zoology* 29: 973-985.

EFRON, B. AND R. TIBSHIRANI. 1993. An introduction to the Bootstrap. London. Chapman and Hall.

FERGUSON, M. W. J. 1985. Reproductive biology and embryology of the crocodilians. In: *Biology of the Reptilia*. Vol. 14. (Ed. Gans, C., Billett F. S. and P. F. A. Maderson). John Wiley and Sons, New York. Pp. 329-491.

FERGUSON, M. W. AND T. JOANEN. 1983. Temperature-dependent Sex Determination in *Alligator mississippiensis. Journal of Zoology, London* 200: 143–177.

GANS, C. AND F. S. BILLETT. 1985. Biology of the Reptilia. Vol 15. Wiley and Sons, New York.

GEORGES, A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologica* 81: 323–328.

GUTZKE, W. H. N. AND D. CREWS. 1988. Embryonic temperature determines adult sexuality in a reptile. *Nature* 332: 832–834.

HARTLEY, D. D. R. 1990. A survey of crocodile nests in Umfolozi Game Reserve. *Lammergeyer* 41: 1-12.

HAYS, G. C., BRODERICK, A. C., GLEN, F. AND B. J. GODLEY. 2003. Climate change and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Climate Biology* 9: 642–646.

HEAD, G. R., MAY, M. AND L. PENDLETON. 1987. Environmental determination of sex in reptiles. *Nature* 329: 198-199.

HUTTON, J. M. 1987. Incubation temperatures, sex ratios and sex determination in a population of Nile crocodiles (*Crocodylus niloticus). Journal of Zoology* 211: 143–155.

JOANEN, T., MCNEASE, L. AND M. W. J. FERGUSON. 1987. The effects of egg incubation temperature on post-hatching growth of American alligators. In: *Wildlife Management: Crocodiles and Alligators*. (Ed. Webb, G. J., Manolis, S. C. and P. J. Whitehead). Surrey Beatty and Sons, Sydney, Australia. Pp. 533-537.

KAY, W. R. 2004. Population ecology of *Crocodylus porosus* (Schneider 1801) in the Kimberley region of Western Australia. PhD. Dissertation. University of Queensland, Australia. Pp. 87-94.

LANG, J. W. 1987. Crocodilian thermal selection. In: *Wildlife Management: Crocodiles and Alligators.* (Ed. Webb, G. J., Manolis, S. C. and P. J. Whitehead). Surrey Beatty and Sons, Sydney, Australia. Pp. 301–315.

LANG, J. W. AND H. V. ANDREWS. 1994. Temperature-dependent sex determination in Crocodilians. *Journal of Experimental Biology* 270: 28-44.

LANG, J. W., ANDREWS, H. AND R. WHITAKER. 1989. Sex determination and sex ratios in *Crocodylus palustris*. *American Zoology* 29: 935–952.

LESLIE, A. J. 1997. The ecology and physiology of the Nile crocodile, *Crocodylus niloticus*, in Lake St. Lucia, Kwazulu-Natal, South Africa. Ph.D. Dissertation, Drexel University, USA. Pp. 110–200.

LIN, Z., XIANG, J., LUO, L. AND X. MA. 2005. Incubation temperature affects hatching success, embryonic expenditure of energy and hatchling phenotypes of a prolonged egg-retaining snake, *Deinagkistrodon acutus* (Viperidae). *Journal of Thermal Biology* 30: 289–297.

MARAIS, J. AND G. A. SMITH. 1992. Crocodile husbandry: The care of broodstock, eggs and hatchlings. In: *Conservation and Utilization of the Nile crocodile in Southern Africa. Handbook on Crocodile farming*. (Ed. Smith, G. A. and J. Marais). Pp. 63–78.

MROSOVSKY, N. 1994. Sex ratios of sea turtles. *Journal of Experimental Zoology* 270: 16-27.

MROSOVSKY, N. AND C. PIEAU. 1991. Transitional range of temperature, pivotal temperatures, and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia* 12: 169-179.

POOLEY, A. C. 1969. Preliminary studies on the breeding of the Nile crocodile, *Crocodylus niloticus,* in Zululand. *Lammergeyer* 10: 22-44.

POOLEY, A. C. AND C. GANS. 1976. The Nile crocodile. *Scientific American* 234: 114-124.

RICHARDSON, K. C., WEBB, G. J. W. AND S. C. MANOLIS. 2002. Getting inside-topographic dissection. Chapter 2. In: *Crocodiles: Inside Out. A Guide to the Crocodilians and their Functional Morphology.* Surrey Beatty and Sons. Sydney, Australia.

SWANEPOEL, D. G. J. 1999. Movements, nesting and the effects of pollution on the Nile crocodile, *Crocodylus niloticus,* in the Olifants River, Kruger National Park. MSc Thesis, University of Natal, South Africa.

WEBB, G. J. W., MANOLIS, S. C., DEMPSEY, K. E. AND P. J. WHITEHEAD. 1987. Crocodilian eggs: A Functional Overview. In: *Wildlife Management: Crocodiles and Alligators.* (Ed. Webb, G. J. W. and S. C. Manolis). Chipping Norton: Surrey Beatty and Sons. Pty. Ltd. Sydney, Australia. Pp.417-422.

WEBB, G. J. W. AND H. COOPER-PRESTON. 1989. Effects of incubation temperature on crocodiles and the evolution of reptilian oviparity. *American Zoology* 29: 953–971.

WEBB, G. J. W., BUCKWORTH, R. AND S. C. MANOLIS. 1983. *Crocodylus johnstoni* in the McKinlay River, N. T. V1. Nesting biology. *Australian Wildlife Research* 10: 607-637.

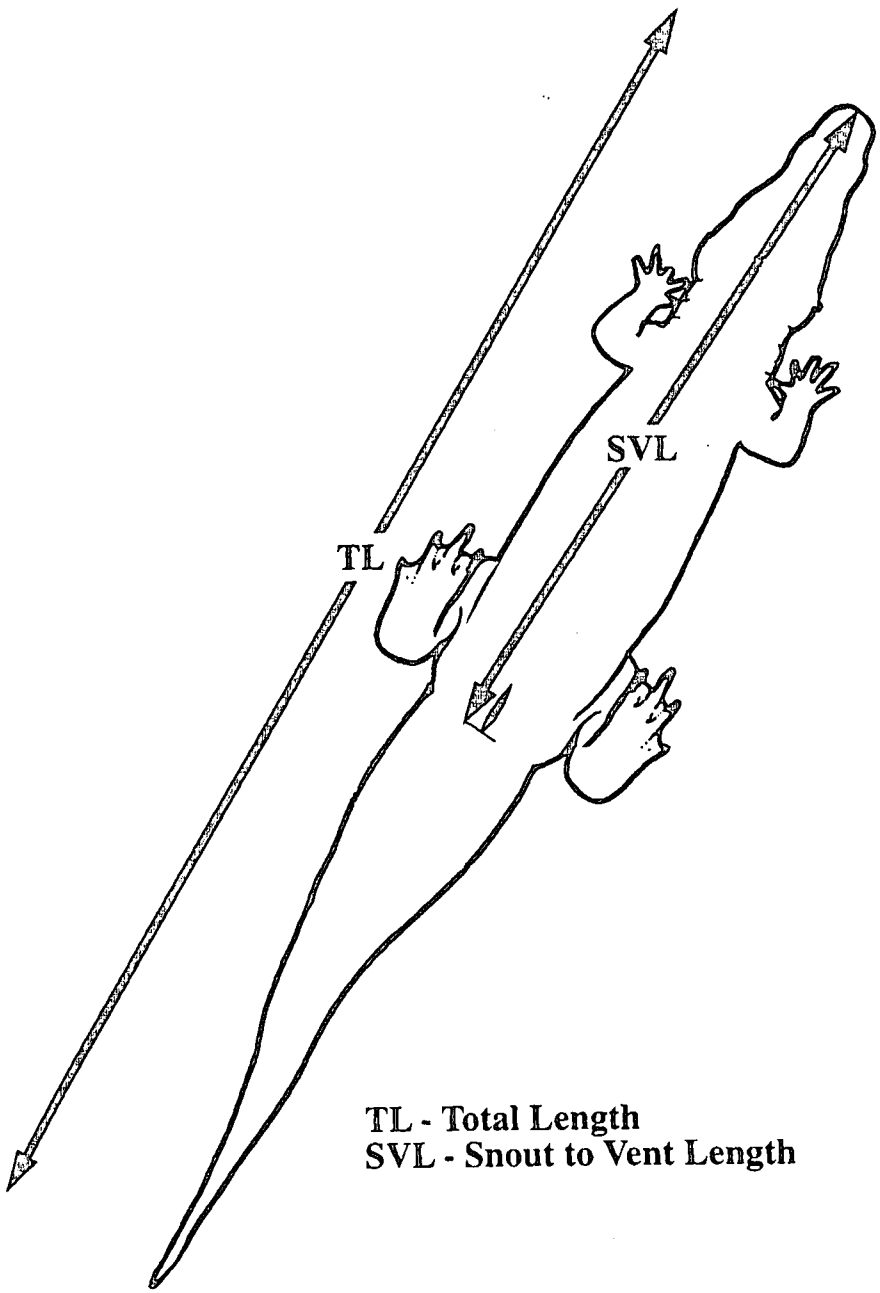
WEBB, G. J. W. AND A. M. A. SMITH. 1984. Sex ratio and survivorship in the Australian freshwater crocodile, *Crocodylus johnstoni*. In: *The Structure, Development and Evolution of Reptiles* (Ed. M. W. J. FERGUSON). Academic Press, London. Pp. 319-555.

Appendix 2.1 Photographs of the Nile crocodile eggs collected from wild nests in the Okavango River in the 2005/06 nesting season. (A) The crocodile eggs were marked according to the nest they were collected from and placed side by side on a metal rack 10 cm above a water tray in the incubator. (B) The eggs were individually packed into bags after the thermosensitive period and labelled to ensure that when hatching took place the hatchlings would be separated from one another. A 24-gauge copper-constantan thermocouple (Cu-Cn) wire was positioned between the eggs to record incubation temperature.

A

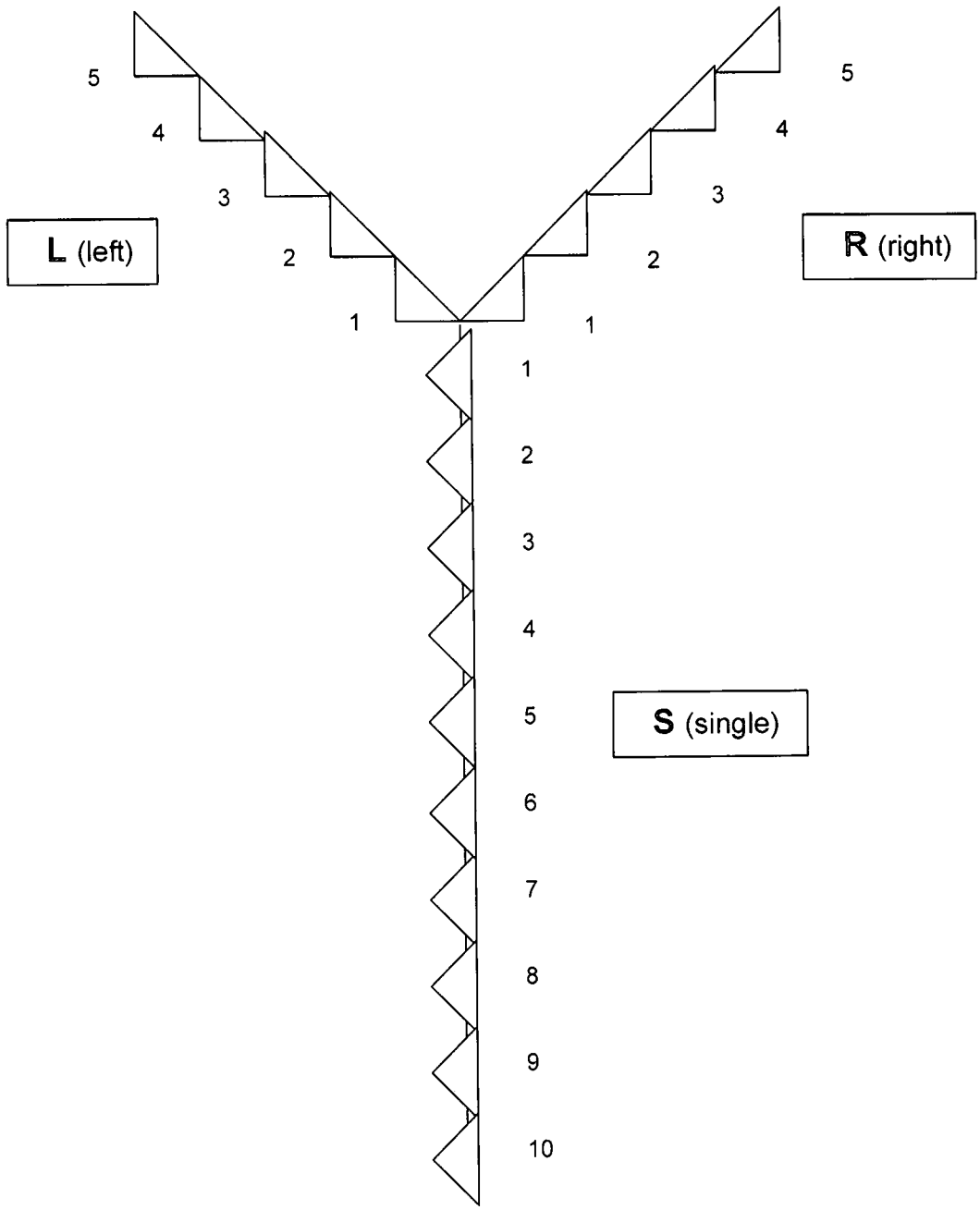
B

Appendix 2.2 Morphometric measurements were taken from the Nile crocodiles immediately after hatching. Eggs were collected from wild nests in the Okavango River in the 2005/06 nesting season. Measurements were recorded using a fiberglass tape measure. Total length (TL) was the measurement from the tip of the snout to the tip of the tail. Snout-to-vent length (SVL) was the distance from the tip of the snout to the posterior edge of the cloacal opening (Sketches from Leslie, 1997).



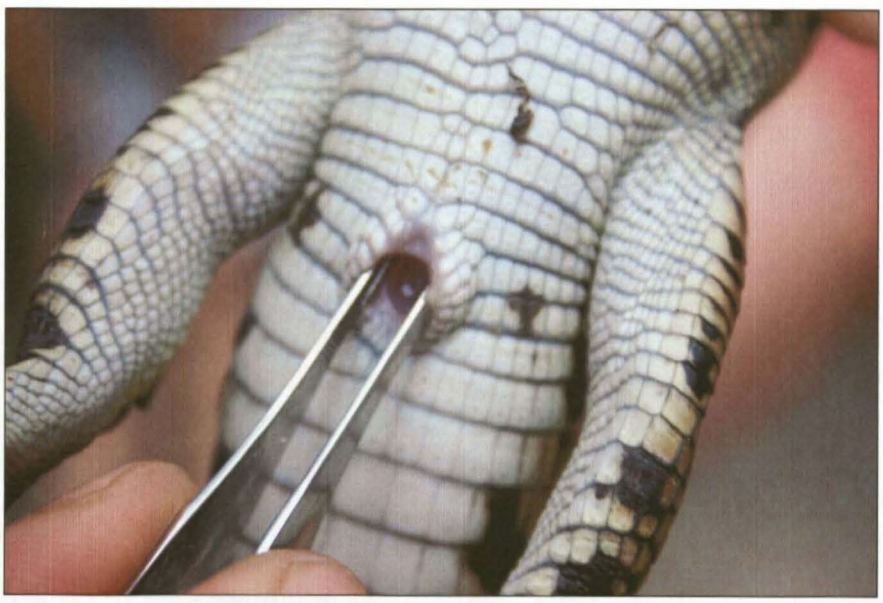
TL - Total Length
SVL - Snout to Vent Length

Appendix 2.3 The scute removal tagging system used to mark hatchlings. Eggs were collected from wild nests in the Okavango River in the 2005/06 nesting season. Scutes were removed immediately after hatching in a specific coded sequence using a pair of forceps and a scalpel. This system provided a permanent means of tagging the crocodiles. The removed scutes indicated which temperature the egg were incubated at (left), the clutch (right) and the number of the hatchling (single).



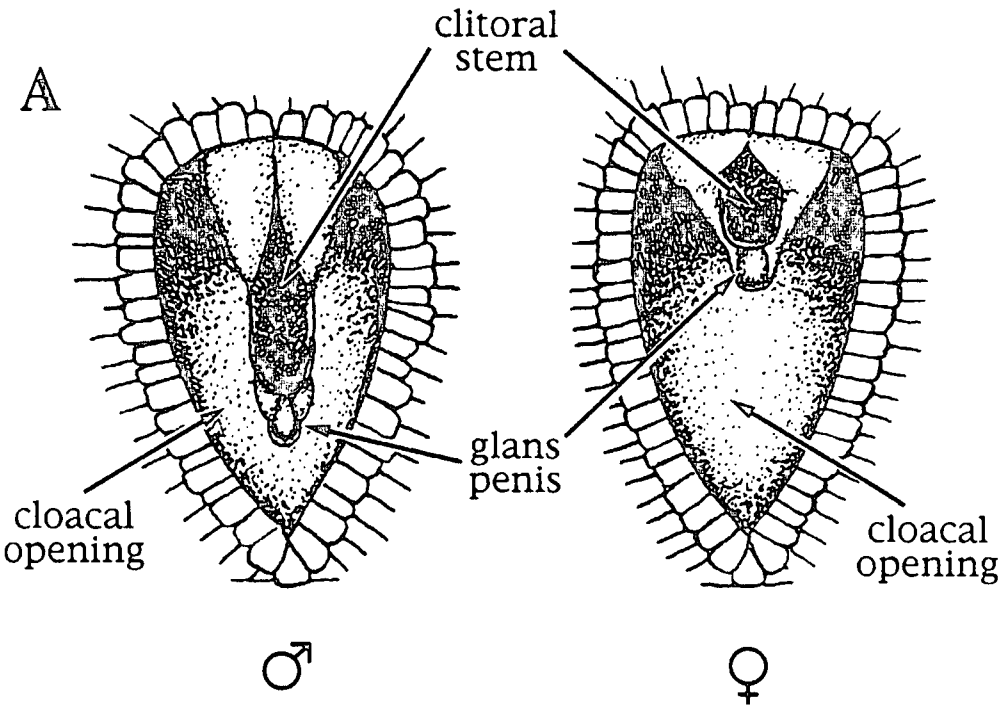
Appendix 2.4 Cloacal examination took place at 1, 2, 4 and 6 months of age. The crocodile was restrained on its dorsum and the cliteropenis was examined by separating the cloaca with a pair of blunt forceps and applying manual pressure to the base of the cloaca opening. Photographs A and B illustrate the sexing procedure.

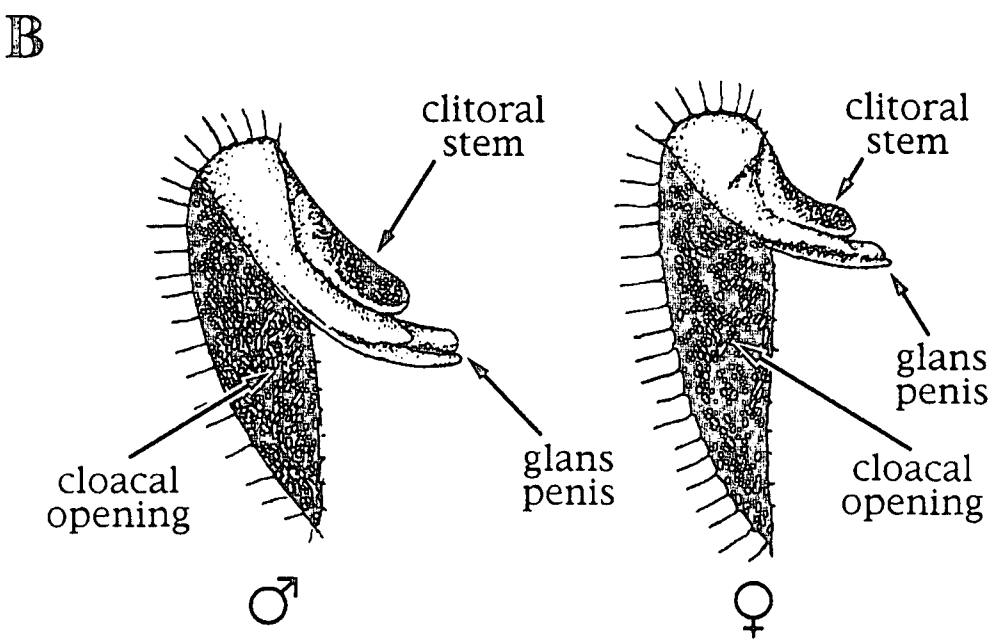
A



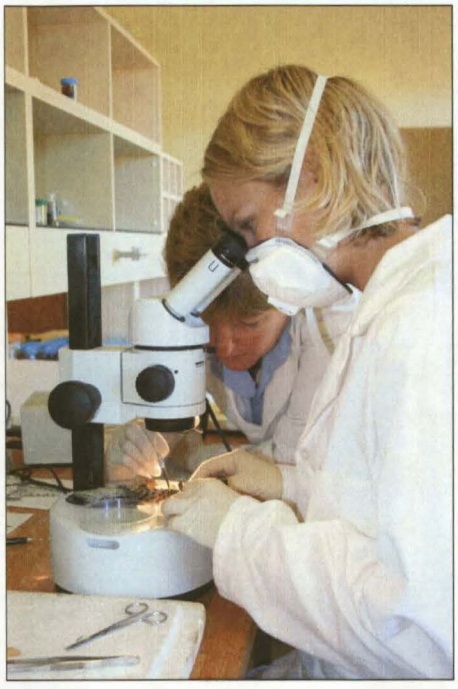
B

Appendix 2.5 Sexual Dimorphism of the cliteropenis of male and female Nile crocodile hatchling. (A) Ventral view of a male and female observed during cloacal examination. The cliteropenis in the male is usually larger; more elongated and had a narrower base than in the female. (B) Lateral view of male and female cliteropenis observed during cloacal examination. The glans penis was distinctly bi-lobed in the male but in the female this was not always visible (Sketches from Leslie, 1997).

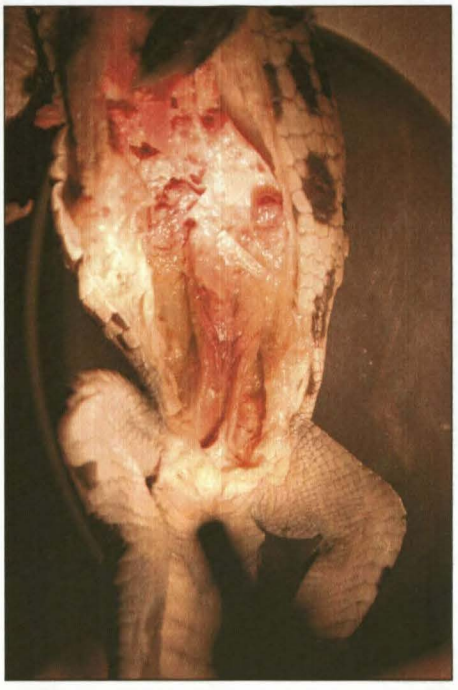




Appendix 2.6 (A-D). Dissection of the Nile crocodile hatchlings that died pipping the eggs. The body was opened by making a midventral incision from the base of the head to the vent to expose the gonadal-complex, including the kidneys, adrenal glands and mesonephric tissue. A dissecting microscope was used at a low power magnification and initial sexing was based on the presence or absence of an oviduct and the overall appearance of the gonad.

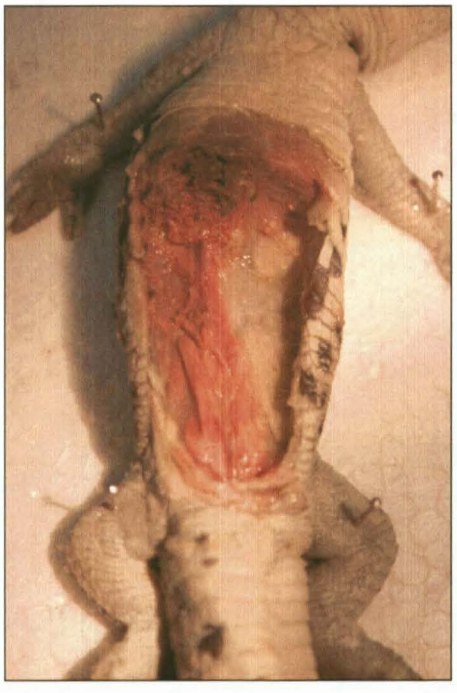


A



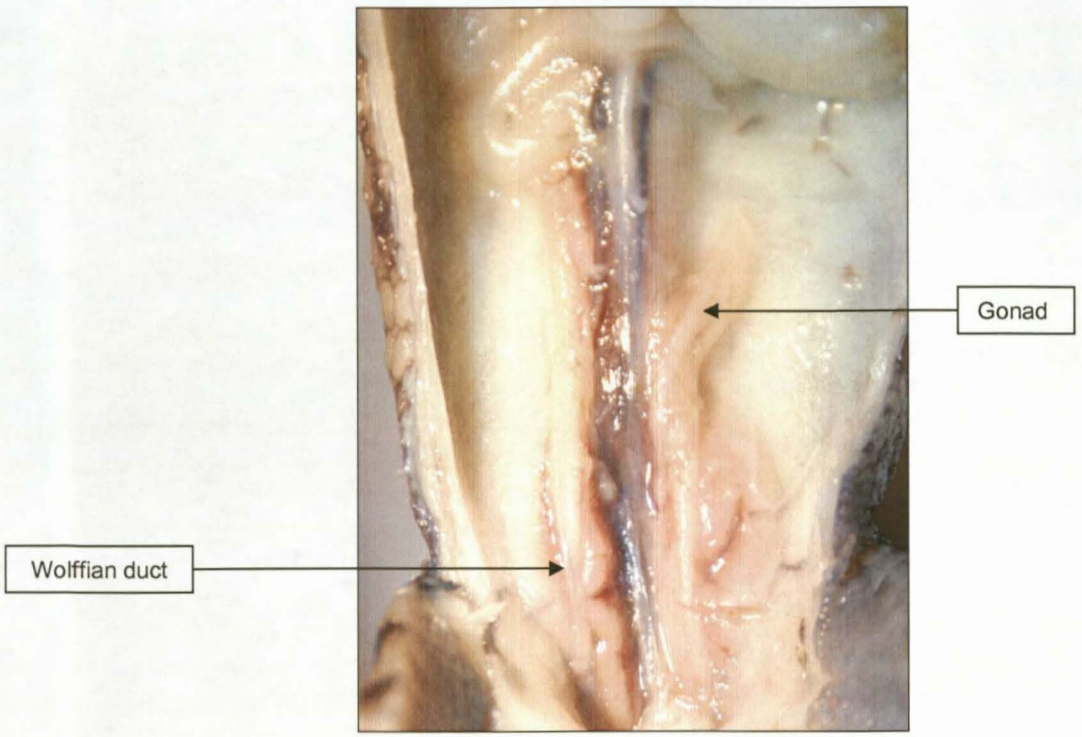
B

C

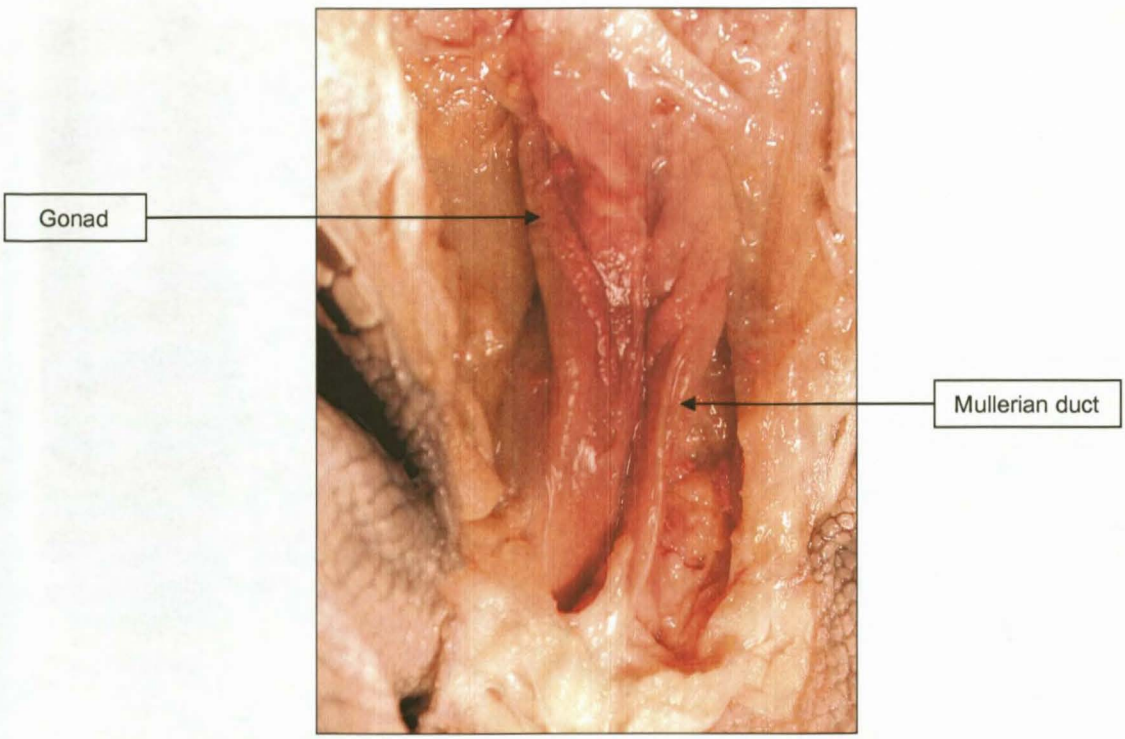


D

Appendix 2.7 Diagram of the macroscopic ventral view of (A) male and (B) female reproductive tracts at post-hatching in *Crocodylus niloticus*. Note the gonadal shape and Wolffian ducts in the male and the bilateral Mullerian ducts (oviducts) in the female. The gonads are closely associated with the adrenal glands and the mesonephric kidney.



A



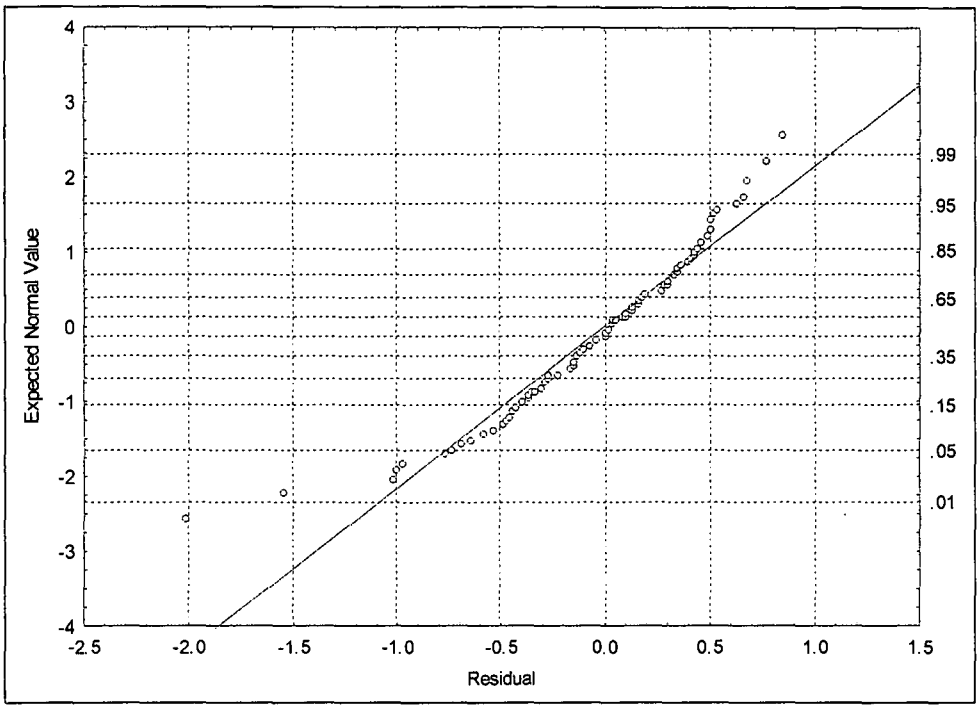
B

Appendix 2.8 Normal Probability Plot; Raw Residuals.

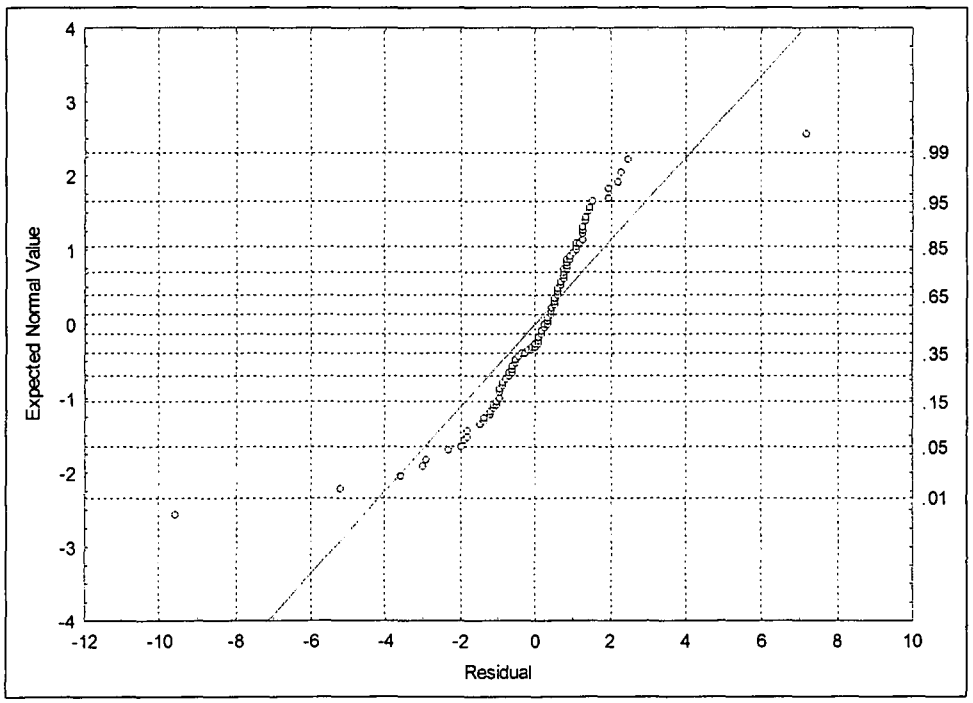
A Dependent variable is hatchling SVL (snout-to-vent length).

B Dependent variable is hatchling TL (total length). The residuals are not normally distributed.

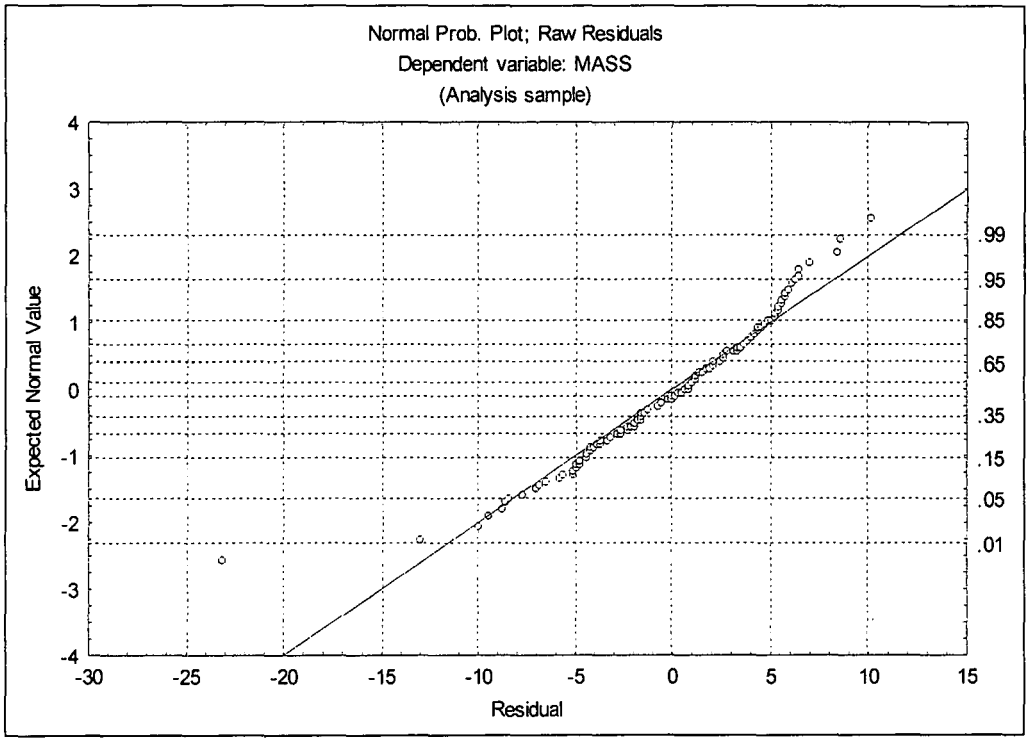
C Dependent variable is hatchling mass.



A



B



C

Appendix 2.9 Summary of sexing techniques and resultant sex ratios for 147 Nile Crocodiles used in the temperature-dependent sex determination study in the 2005/06 nesting season. Eggs were collected from five wild nests and incubated at one of eight constant temperatures (°C) at the Krokovongo Crocodile Farm in the Okavango Delta, Botswana.

				Macro Sex				Micro Sex	
Number	Incubator	Incubation Temperature (°C)	Scutes	Feb (1 month)	March (2 months)	May (4 months)	July (6 months)	Dissection	Concluded Sex
1	1	30.0	R1L1S1					F	F
2	1	30.0	R1L1S2					F	F
3	1	30.0	R1L1S3					F	F
4	1	30.0	R1L1S4					F	F
5	1	30.0	R1L1S5					F	F
6	1	30.0	R1L1S6					F	F
7	1	30.0	R2L1S1					F	F
8	1	30.0	R2L1S2					F	F
9	1	30.0	R2L1S3					F	F
10	1	30.0	R3L1S1					F	F
11	1	30.0	R3L1S2					F	F
12	1	30.0	R3L1S3					F	F
13	1	30.0	R3L1S4					F	F
14	1	30.0	R3L1S5					F	F
15	1	30.0	R3L1S6					F	F
16	1	30.0	R3L1S7					F	F
17	1	30.0	R3L1S8					F	F
18	1	30.0	R5L1S1					F	F
19	1	30.0	R5L1S3					F	F
20	2	30.5	R1L2S1	F	F	F	F		F
21	2	30.5	R1L2S2	F	F	M	F		F
22	2	30.5	R1L2S3	F	F	F	M		F
23	2	30.5	R1L2S4	F	F	M	F		F
24	2	30.5	R1L2S5	F	F	F	F		F
25	2	30.5	R1L2S6	F	F	F	F		F
26	2	30.5	R2L2S1	F	F	F	F		F
27	2	30.5	R2L2S2	F	F	F	F		F
28	2	30.5	R2L2S3	F	F	F	F		F
29	2	30.5	R2L2S4	F	F	F	F		F
30	2	30.5	R2L255					F	F

				Macro Sex				Micro Sex	
Number	Incubator	Incubation Temperature (°C)	Scutes	Feb (1 month)	March (2 months)	May (4 months)	July (6 months)	Dissection	Concluded Sex
31	2	30.5	R3L2S1	F	F	F			F
32	2	30.5	R3L2S2	F	F	F			F
33	2	30.5	R3L2S3					F	F
34	2	30.5	R3L2S4	F	F	F	F		F
35	2	30.5	R3L2S5	F	M	F	F		F
36	2	30.5	R3L2S6	F	F	F	M		F
37	2	30.5	R3L2S7	F	F	F	F		F
38	2	30.5	R4L2S4					F	F
39	2	30.5	R5L2S1					F	F
40	2	30.5	R5L2S2					F	F
41	3	31.0	R1L3S1	F	F	F	F		F
42	3	31.0	R1L3S2	F	F	M	F		F
43	3	31.0	R1L3S3	F	M	M	M		M
44	3	31.0	R1L3S5					F	F
45	3	31.0	R1L3S6	F	M	F	F		F
46	3	31.0	R2L3S1	F	F	F	F		F
47	3	31.0	R2L3S2					F	F
48	3	31.0	R2L3S3					M	M
49	3	31.0	R3L3S2	F	F	F	F		F
50	3	31.0	R3L3S3					F	F
51	3	31.0	R3L3S4	M	M	M	M		M
52	3	31.0	R3L3S5	F	M	F	F		F
53	3	31.0	R3L3S6					F	F
54	3	31.0	R3L3S7	M	F	M	M		M
55	3	31.0	R3L3S8	F	F	M	F		F
56	3	31.0	R3L3S9					F	F
57	3	31.0	R3L3S10					M	M
58	4	31.5	R1L4S1	M	M	M	M		M
59	4	31.5	R1L4S2	M	M	M	F		M

				Macro Sex				Micro Sex	
Number	Incubator	Incubation Temperature (°C)	Scutes	Feb (1 month)	March (2 months)	May (4 months)	July (6 months)	Dissection	Concluded Sex
60	4	31.5	R1L4S3	F	M	M	M		M
61	4	31.5	R1L4S4	F	F	F	F		F
62	4	31.5	R1L4S5	M	M	M	M		M
63	4	31.5	R1L4S7	M	M	M	F		M
64	4	31.5	R2L4S1					F	F
65	4	31.5	R2L4S2	F	F	F	F		F
66	4	31.5	R2L4S3	F	F	F	F		F
67	4	31.5	R3L4S1	F	M	F	F		F
68	4	31.5	R3L4S2	M	M	M	M		M
69	4	31.5	R3L4S3	M	F	F	F		F
70	4	31.5	R3L4S4	M	M	M	M		M
71	4	31.5	R3L4S5	M	M	M	M		M
72	4	31.5	R3L4S6	F	M	F	F		F
73	4	31.5	R3L4S7	F	M	F	F		F
74	4	31.5	R3L4S8	M	M	M	F		M
75	4	31.5	R3L4S9	M	M	M	M		M
76	4	31.5	R5L4S1					M	M
77	4	31.5	R5L4S2					F	F
78	5	32.0	R1L5S1	F	M	M	M		M
79	5	32.0	R1L5S2	F	M	M	M		M
80	5	32.0	R1L5S4	M	M	F	M		M
81	5	32.0	R1L5S5					F	F
82	5	32.0	R1L5S6	M	F	F	F		F
83	5	32.0	R1L5S7					M	M
84	5	32.0	R2L5S1	M	M	F	M		M
85	5	32.0	R2L5S2	F	M	M	M		M
86	5	32.0	R3L5S1					F	F
87	5	32.0	R3L5S2					M	M
88	5	32.0	R3L5S3	F	M	M	M		M
89	5	32.0	R3L5S5	F	F	F	F		F

				Macro Sex				Micro Sex	
Number	Incubator	Incubation Temperature (°C)	Scutes	Feb (1 month)	March (2 months)	May (4 months)	July (6 months)	Dissection	Concluded Sex
91	6	32.5	R1L6S3	F	M	M	M		M
93	6	32.5	R1L6S5	M	M	M	M		M
94	6	32.5	R1L6S6	M	F	F	F		F
95	6	32.5	R1L6S7	F	M	M	M		M
96	6	32.5	R2L6S1	F	M	M	M		M
97	6	32.5	R2L6S2	F	M	M	M		M
98	6	32.5	R2L6S3					M	M
99	6	32.5	R3L6S1	F	M	M	M		M
100	6	32.5	R3L6S3	M	M	F	M		M
101	6	32.5	R3L6S4	M	M	M	M		M
102	6	32.5	R3L6S5	M	F	F	F		F
103	6	32.5	R3L6S6	F	M	F	F		F
104	6	32.5	R3L6S7	M	M	F	M		M
105	6	32.5	R3L6S8	F	M	M	M		M
106	6	32.5	R5L6S2					M	M
107	7	33.0	R1L7S1	F	M	M	M		M
108	7	33.0	R1L7S2	F	M	M	M		M
109	7	33.0	R1L7S3	M	F	M	M		M
110	7	33.0	R1L7S4					F	F
111	7	33.0	R1L7S5	M	M	M	M		M
112	7	33.0	R1L7S6	M	F	M	M		M
113	7	33.0	R1L7S7	M	F		F		F
114	7	33.0	R2L7S1	F	M	F	F		F
115	7	33.0	R2L7S2	F	F	F	F		F
116	7	33.0	R2L7S3					M	M
117	7	33.0	R3L7S1	F	F	F	F		F
118	7	33.0	R3L7S2	M	M	F	M		M
119	7	33.0	R3L7S3	F	F	F	F		F
120	7	33.0	R3L7S4	F	F	F	F		F

				Macro Sex				Micro Sex	
Number	Incubator	Incubation Temperature (°C)	Scutes	Feb (1 month)	March (2 months)	May (4 months)	July (6 months)	Dissection	Concluded Sex
122	7	33.0	R3L7S6	F	F	F	F		F
123	7	33.0	R3L7S7	F	M	M	M		M
124	7	33.0	R3L7S8					M	M
125	7	33.0	R3L7S9					F	F
126	7	33.0	R4L7S3	M	M	M	M		M
127	7	33.0	R5L7S1	F	M	M	M		M
128	7	33.0	R5L7S2	M	M	M	M		M
129	7	33.0	R5L7S3	M	M	M	M		M
130	8	34.0	R1L8S1	F	M	M	M		M
131	8	34.0	R1L8S2	F	F	F	M		F
132	8	34.0	R1L8S3	F	F	F	F		F
133	8	34.0	R1L8S4	F	F	F	F		F
134	8	34.0	R1L8S5	F	F	F	F		F
135	8	34.0	R1L8S6	F	F	F	F		F
136	8	34.0	R1L8S7	F	F	F	F		F
137	8	34.0	R2L8S2	F	F	M	F		F
138	8	34.0	R2L8S3	F	F	M	F		F
139	8	34.0	R3L8S1	F	F	F	F		F
140	8	34.0	R3L8S2	F	M	M	M		M
141	8	34.0	R3L8S3	F	M	M	M		M
142	8	34.0	R3L8S5	F	F	F	F		F
143	8	34.0	R3L8S6	F	F	F	F		F
144	8	34.0	R3L8S7	F	M	M	M		M
145	8	34.0	R3L8S8	F	F	F	F		F
146	8	34.0	R3L8S9	F	F	F	M		F
147	8	34.0	R3L8S10	F	F	F	F		F

CHAPTER 3

SOIL TEMPERATURES AND SOIL MOISTURE OF POTENTIAL WILD CROCODILE NESTS ALONG THE OKAVANGO RIVER, BOTSWANA.

3.1 INTRODUCTION

Soil temperature and soil moisture play a significant role in sex determination in egg-laying (oviparous) reptiles exhibiting temperature-dependent sex determination. There is a close relationship between prevailing air temperature and soil temperature at nest depth, and temperature within nests (Godley *et al.*, 2001; Hays *et al.*, 2003). In any soil profile, heat is continually moving into or out of the soil and the thermal energy is being continually redistributed in the soil (Leslie, 1997).

Leslie (1997) recorded soil temperatures at various stations at potential nesting sites in the St. Lucia area, South Africa, and found that mean soil temperatures at a depth of 25 cm were higher than mean maximum air temperature during the same season. A depth of 25 cm was selected based on the average nest depth of wild crocodile nests. Based on biophysical principles, the lower atmospheric humidity in the dry year and reduced cloud cover permitted a high proportion of solar radiation to reach the ground, both in the visible and in the infra-red range. Temperature gradients directed heat into the soil. Part of the solar radiation was scattered and reflected from the ground, but the soil, which was warmed rapidly, became a radiator itself. Leslie (1997) found that precipitation has an effect, as it has a great cooling effect on soil temperatures at a depth of 25 cm.

Soil temperatures may differ between stations and Leslie (1997) found that this may be due to numerous factors including: sun verses shade (insolation) and distance from water and the angle of incline which influences rainfall runoff. There is a thermal lag at increased depth, which is proportional to the thermal conductivity, heat capacity and thermal diffusivity of the soil type (Rose, 1966).

Previous studies have suggested that a high proportion of the phenotypic variance in hatchling reptiles may have no genetic basis (Vleck, 1988) but depends on environmental factors alone. Shine *et al.* (1997) confirmed this finding, and demonstrated that thermal fluctuations within nests of a southeastern Australian skink (*Bassiana duperreyi*) and Australian water python (*Liasis fuscus*) can influence the reptile's hatchling phenotypes and developmental rates, independent of the mean incubation temperature.

Soil temperature is thus of utmost importance. Spatial factors, including sun verses shade, different depths and distances from the water have a biological effect on sex determination in

the Nile crocodile (Leslie, 1997). Previous studies have demonstrated that female Nile crocodiles nest at varying distances from the water in open, sunny areas (Leslie, 1997). This suggests that crocodiles may have the ability to choose optimal nest sites.

In this study, climatological factors including air temperature and precipitation were recorded along the Eastern Channel of the Okavango River. The effect that climate has on nest temperatures of the Nile crocodile was analysed by studying the temperature and moisture content of soil at potential nest sites in combination with air temperature and precipitation. Soil temperature and moisture were recorded at various distances from the river and at various depths to predict the 'optimal' positioning of nest sites along the river. These results were then compared to wild crocodile nest sites in order to determine if females were selecting optimal nesting sites (See Chapter 4).

3.2 METHODS AND MATERIALS

3.2.1 Climatological Data

Air temperature and precipitation was recorded throughout the entire 2004/05 breeding season. A Copper-Constantan (Co-Cn) thermocouple wire was used as a single temperature probe and placed in the shade at a height of 2 m at six potential nesting areas along the Eastern Channel of the Okavango River (Figure 3.1). The thermocouple wires were calibrated with a Model BATT-12 thermocouple meter (Physitemp Inc., USA) and connected to a CR-10X datalogger (Campbell Scientific, Inc., Logan, Utah) which was programmed to record air temperature every 15 minutes for the entire breeding season (135 days). A standard rain-gauge was set up at Station 1, situated in the middle of the Phillipa Channel of the Eastern Channel (Figure 3.1). The rain gauge was placed at a height of 1.5 m in a cleared area and was checked every four days and the average precipitation was determined. Microsoft® Office Excel 2003 (Microsoft Corporation, USA) was used to determine the relationship between precipitation and air temperature.

3.2.2 Soil Temperature

Six sites were chosen along the Eastern Channel of the Okavango River and allocated as 'stations.' Station localities were chosen based on the area available to the females for nesting. The stations were either previously used nesting sites, or they were potential nesting sites, that is, sites that posses nest characteristics as described in Chapter 4. At each station GPS co-ordinates were taken using a Magellan GPS 315 receiver. These co-ordinates were plotted on a map using Arcview GIS 3.2 (Environmental Systems Research Institute, Inc., USA) to illustrate the location of the stations within the Phillipa and Moremi Channels (Figure 3.1).

A thermal profile was constructed at each station to record soil temperature (Appendix 3.1). Each profile was 9 m long, with probes located 3 m (low-lying), 6 m (potential nesting site) and 9 m from the river. Each probe consisted of a wooden dowel stick with copper-constantan thermocouple (Cu-Cn) wires placed at 15, 25 and 40 cm depths. The thermocouple wires were calibrated with a Model BATT-12 thermocouple meter (Physitemp Inc., USA) and connected to a CR-10X datalogger (Campbell Scientific, Inc., Logan, Utah), which was buried underground and powered by a 12-volt car battery. The datalogger was programmed to record soil temperature every 15 minutes for the entire breeding season. The soil temperatures were recorded at four day intervals per station. Soil temperature was thus recorded at three distances from the river, at three different depths and at six different stations. Data were downloaded from the CR-10X datalogger every four days.

After 135 days, once all the eggs had hatched, the soil profiles were removed and the data were analysed using Microsoft® Office Excel 2003. Statistica 7.1 (Statsoft, Inc., USA) was used to run a repeated measures ANOVA with replication on the nine treatments, with three distances per treatment (3, 6 and 9 m), at three depths per distance (15, 25 and 40 cm) over a four month period. The dependent variable was the average temperature per day (Temp 1-23). Depth and distance were the categorical predictors and the within effects was 23 days. Significance was determined at the level of $P < 0.05$ (two tailed).

3.2.3 Soil Moisture

Soil moisture was measured at the six different stations along the Eastern Channel of the Okavango River during the 2004/05 breeding season. Soil moisture was measured using ECH_2O moisture probes (Decagon Devices, Washington, USA). The ECH_2O probe is a capacitance probe that measures dielectric permittivity of the soil, which is directly related to water content. The moisture probes were calibrated using the standard procedure for calibrating capacitance probes outlined by Campbell (2002). A soil sample was collected at a potential nesting site and packed into a container of known volume and wetted with a measured amount of water. An ECH_2O probe was inserted into the soil which was compacted by hand around the probe. Additional soil was then added so that the probe was buried at least 3 cm below the soil surface. The ECH_2O probe was connected to a portable hand-held readout device and 20 ml of water was added to the soil every 30 minutes and the millivolt reading and mass of the soil was recorded. The soil sample was then weighed and dried to determine the gravimetric water content. Convective oven-drying was used to dry the soil, where the soil was baked at 105 °C for 24 hours. Once the sample was completely dry the soil was again weighed to determine the dry weight. The volumetric water content was calculated as follows:

Volumetric water content (θ):

$$\theta = \frac{w\, P_b}{P_w}$$

was determined from the gravimetric water content (w):

$$w = \frac{M_w - M_m}{M_m}$$

where M_w is the mass of the wet soil sample and M_m is the mass of the dry soil sample. The bulk density P_b is:

$$P_b = \frac{M_m}{V_t}$$

where V_t is the total volume of the sample. The density of water P_w is 1 $mg.m^{-3}$.

At each station a soil moisture ECH_2O probe was buried alongside the 6 m temperature probe at a depth of 25 cm. The ECH_2O probe was inserted into the soil so the entire length of the probe was parallel to the soil surface with the width perpendicular. The ECH_2O probe was connected to a CR-10X datalogger, which was programmed to record soil moisture every 15 minutes for the entire breeding season. Soil moisture was recorded at four day intervals per station.

The data were downloaded from the CR-10X datalogger onto a Hewlett Packard Laptop every four days. The ECH_20 output values were expressed in millivolts, and used to determine the volumetric water content (in m^3m^{-3}) using the calculated calibration curve (Figure 3.7) [Appendix 3.2]. Microsoft® Office Excel 2003 (Microsoft Corporation, USA) was used to determine the relationship between precipitation and soil moisture content.

3.3 RESULTS

3.3.1 Climatological Data

The Phillipa Channel received a total rainfall of 131 mm between October 2004 and January 2005 (Figure 3.2). Mean monthly precipitation and air temperature was determined from averaging all the measures recorded every 15 minutes. The average monthly air temperature increased from October to January. January was the hottest, as well as the wettest month during the breeding season. Oviposition in October coincided with a period of minimum rainfall and therefore dry soil, and hatching in January coincided with a very hot and wet month when insect prey for hatchlings was plentiful.

Mean monthly precipitation was low in the 2004/05 breeding season and did not have a direct effect on air temperature (Figure 3.2). January experienced the highest rainfall with a monthly average of 14 mm. The total precipitation measured during January was 84 mm, which contributed more than 60% to the total rainfall recorded from October to January.

3.3.2 Soil Temperatures

The stations were selected along the Eastern Channel of the Okavango River. The total distance (along the river) between the northern and southernmost station (Station 2 and 6) was 24.1 km. Figure 3.1 illustrates the location of the six different stations within the Eastern Channel. Precipitation was recorded at Station 1.

The 2004/05 nesting season was a comparatively dry season and mean monthly soil temperature at a depth of 25 cm was lower than mean monthly air temperature (Figure 3.3). There was no significant difference between daily air temperature and soil temperature (P (T<=t) two-tail = 0.05, t Critical two-tail = 1.99). The minimum and maximum air and soil temperatures recorded were 22.07 °C, 37.99 °C and 24.84 °C, 31.95 °C respectively. Average soil and air temperatures followed the same pattern throughout the nesting season. Precipitation caused a decrease in soil and air temperature however, precipitation was low, thereby minimizing the effect on temperatures.

The average depth to the middle of crocodile nests in the wild was 25 cm, and the average distance from the bottom of the egg mass to the top of the egg mass was 20 cm. Nests therefore experienced soil temperatures that were intermediate between 15 and 35 cm. Soil temperature profiles from all six stations in the 2004/05 breeding season showed a similar pattern, with warmer temperatures at shallow depths and cooler temperatures at deeper depths (Figure 3.4).

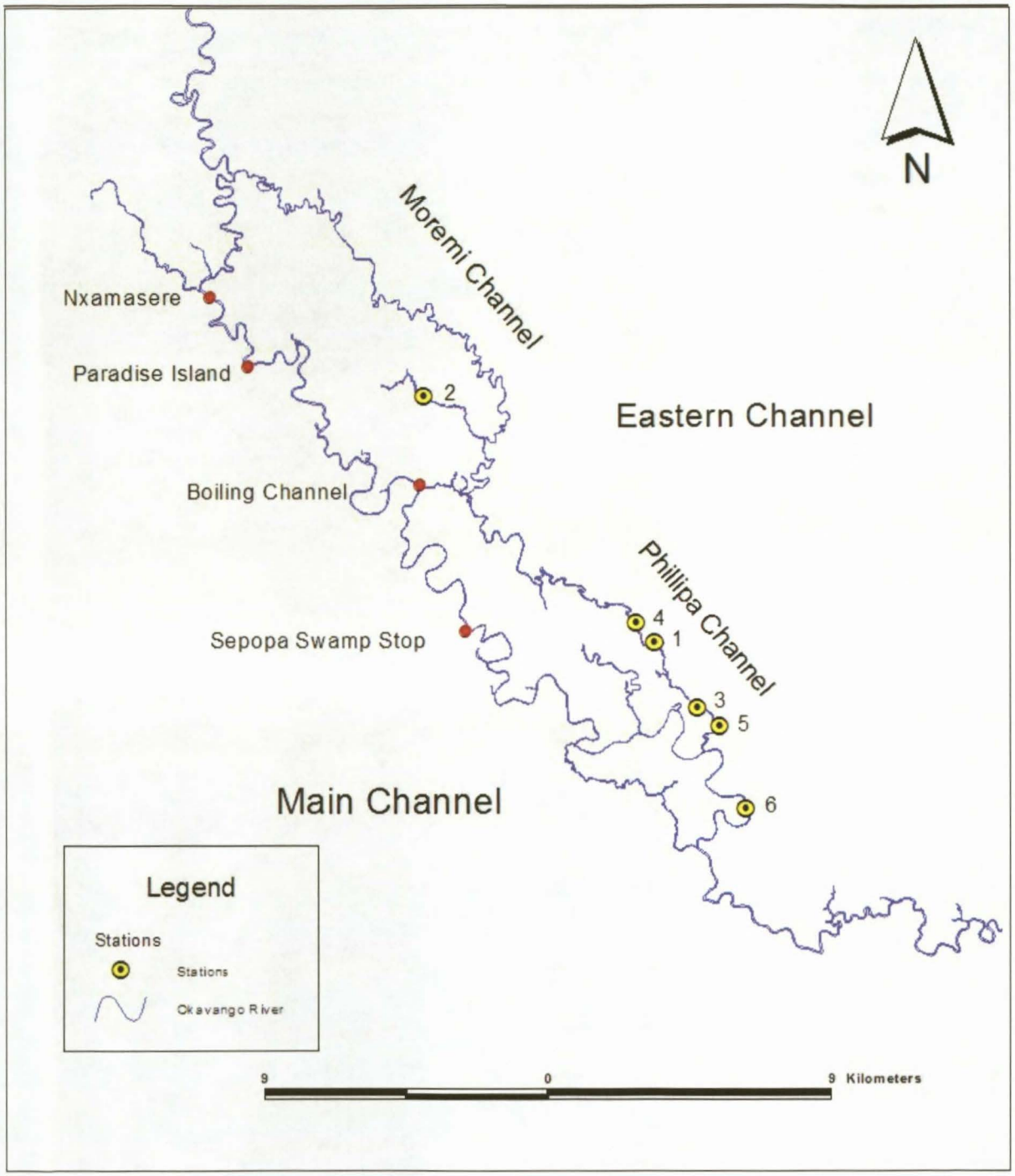


Figure 3.1 The Study Area in the Okavango Delta, Botswana. Six stations were selected in the Moremi and Phillipa Channel in the Eastern Channel of the Okavango River. Soil and air temperature was recorded at every station throughout the 2004/05 nesting season.

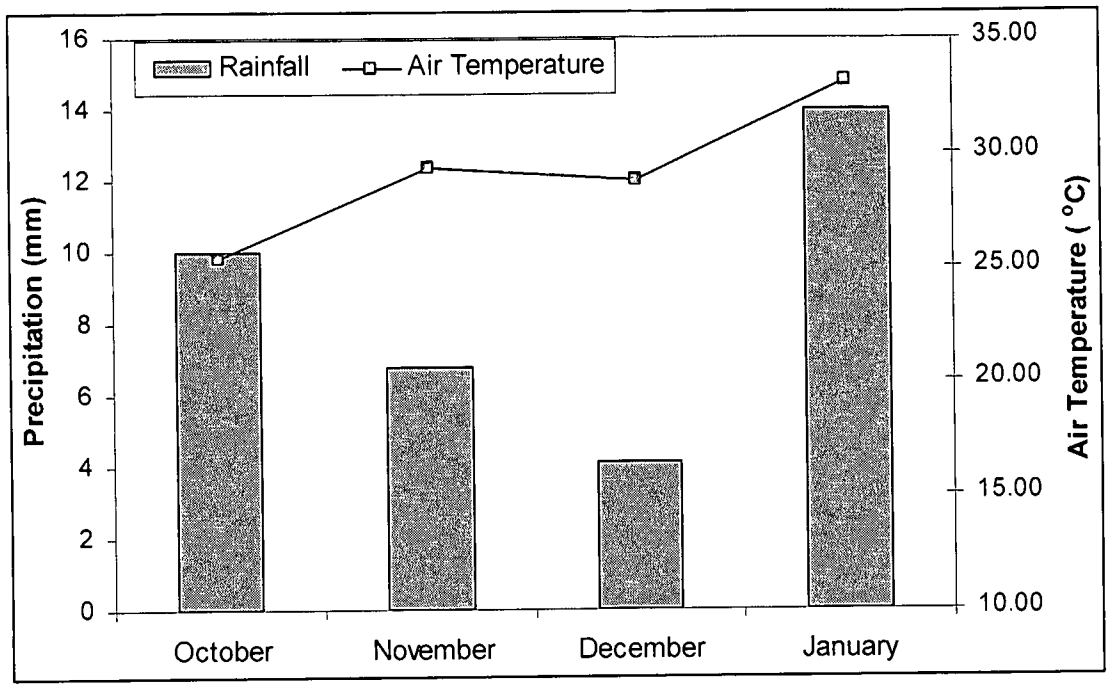


Figure 3.2 Mean monthly precipitation (mm) and air temperature (°C) recorded along the Phillipa Channel during the 2004/05 breeding season of *Crocodylus niloticus* in the Okavango River. (Air temperature was recorded every 15 minutes using a thermocouple probe placed at a height of 2 m at every station and the measurments were averaged per day).

The average soil temperature was determined by using the measures recorded from all six stations, depths and distances combined, and calculated as 27.1 °C for the entire breeding season. Depth and distance from water had statistically significant effects on soil temperature ($P < 0.05$, F = 13.07, MS = 97.7, df = 4) [Figure 3.5]. At a distance of 3 m from the riverbank, mean soil temperatures were 28.5 °C (range = 20.7 – 36.0 °C), 25.5 °C (range = 17.8 – 35.9 °C) and 27.4 °C (range = 19.1 – 31.9 °C) at 15, 25 and 40 cm depths. At a distance of 6 m from the riverbank the mean soil temperatures were 25.4 °C (range = 18.0 – 36.8 °C), 28.9 °C (range = 22.3 – 34.8 °C) and 25.5 °C (range = 17.3 – 35.9 °C) at the three depths respectively. At a distance of 9 m from the riverbank, where the soil has a high clay content, as opposed to sand, mean soil temperatures were 28.7 °C (range = 20.9 – 35.8 °C), 25.9 °C (range = 19.2 – 35.6 °C) and 27.9 °C (range = 20.3 – 33.3 °C) respectively at the three depths.

Depth of soil plays a significant role in soil temperature. At a depth of 15 cm, there is a significant difference in soil temperature with distance from water ($P < 0.05$), and a significant difference over time ($P < 0.05$). The average soil temperature at various distances from the river at a depth of 25 cm was 26.81 °C. At a depth of 15 and 40 cm, average soil temperature was the highest at a distance of 9 m from the riverbank.

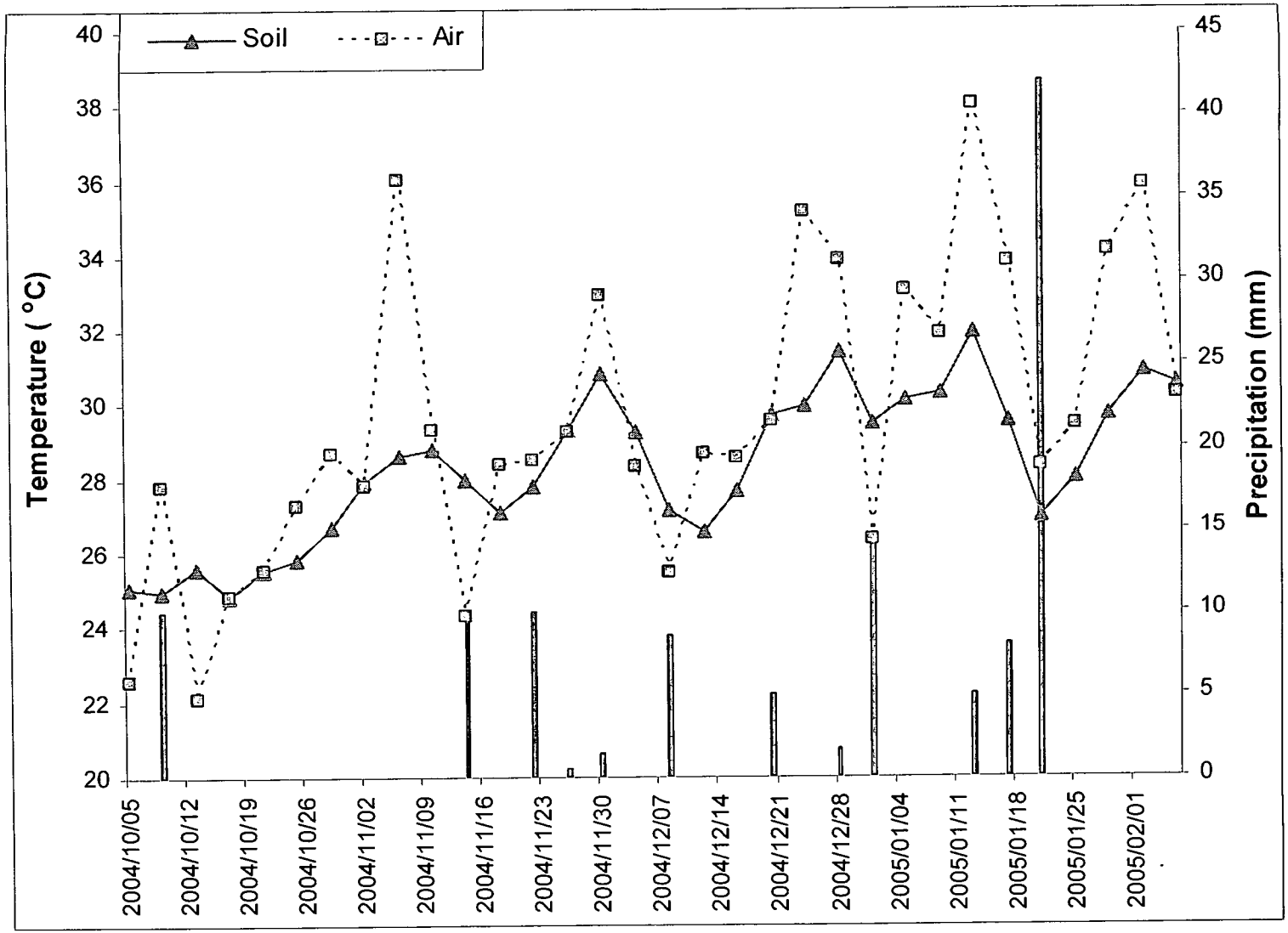


Figure 3.3 Period of nesting (October-February) for *Crocodylus niloticus* in the Okavango River in relation to ambient air and soil temperatures (°C) recorded in the Eastern Channel of the Okavango River (25 cm depth). Average air temperature (°C) and precipitation (mm) recorded on site every four days.

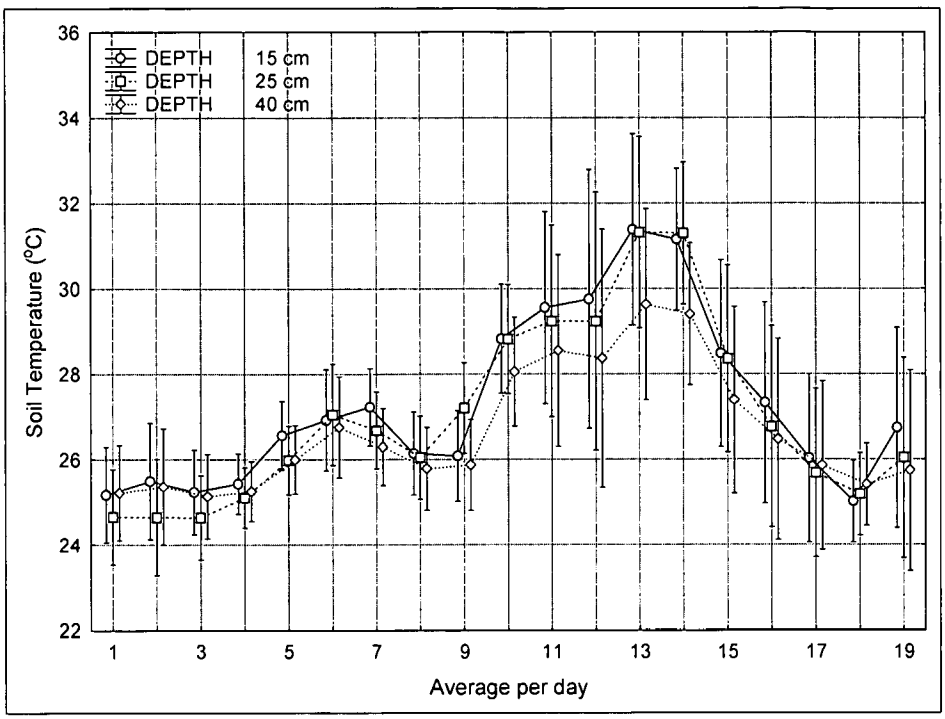


Figure 3.4 Average daily soil temperature (°C) at depths of 15, 25 and 40 cm recorded along the Okavango River, Botswana. (Current effect: F (36, 324) = 0.29, *P* > 0.05 Vertical bars denote 0.95 confidence intervals).

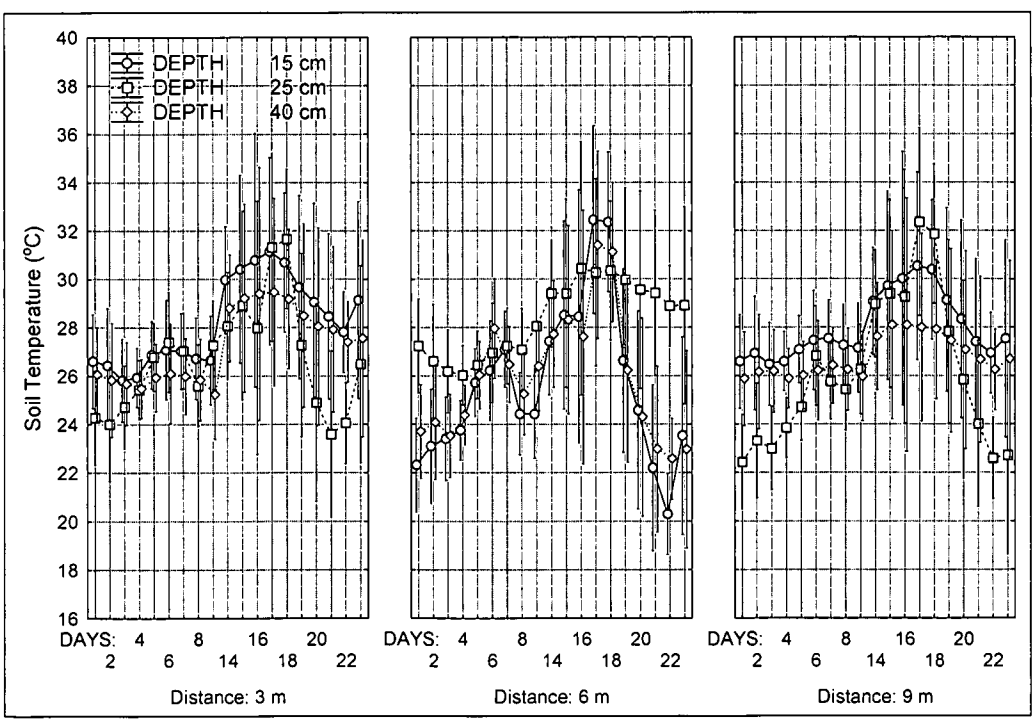


Figure 3.5 Average daily soil temperatures (°C) at various depths and distances from the Okavango River, Botswana. (Current effect F (72, 324) = 1.21, *P* > 0.05. Effective hypothesis decomposition. Vertical bars denote 0.95 confidence intervals).

Mean soil temperature was the highest at a distance of 6 m from the river, at a depth of 25 cm (28.34 °C) [Figure 3.6]. This could be regarded as the optimal nest site position to ensure adequate incubation temperature for crocodile eggs.

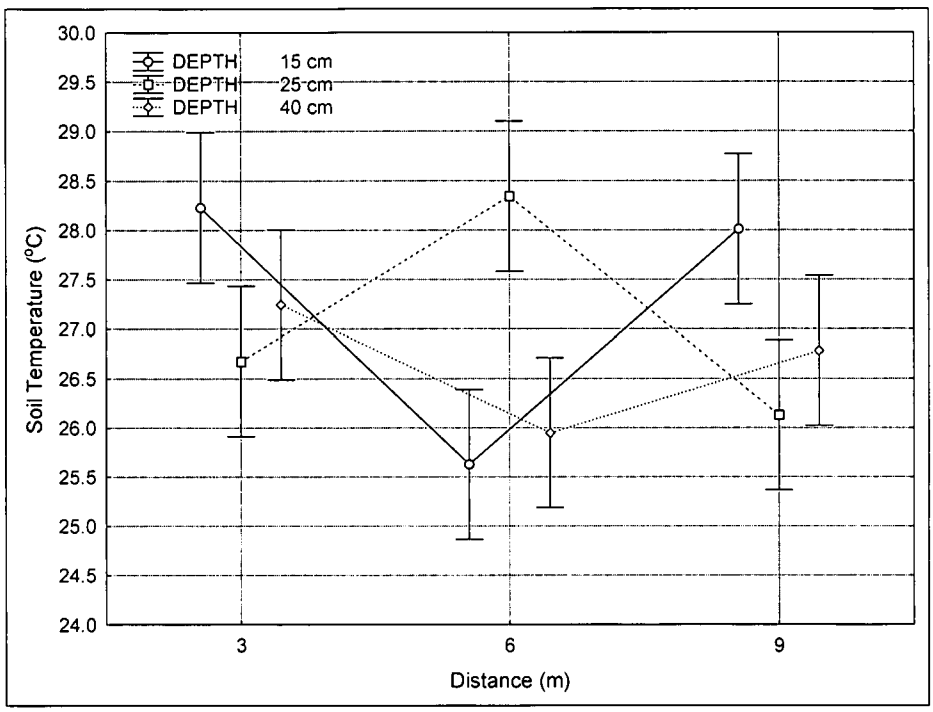


Figure 3.6 Average soil temperatures (°C) at various depths and distances from the Okavango River, Botswana. LS Means. (Current effect: F (4, 18) = 13.07, *P* < 0.05. Effective hypothesis decomposition. Vertical bars denote 0.95 confidence intervals).

3.3.3 Soil Moisture

The volumetric moisture content of the soil was determined and graphed with the average ECH_2O output values recorded at the six stations (Figure 3.7). The ECH_2O output values were significantly low which suggests that the moisture content of the soil was low. The volumetric water content was averaged per day and compared to precipitation measured (Figure 3.8). Rainfall did not have a significant effect on the volumetric water content. The average volumetric water content was 0.13 m^3m^{-3} with a range of 0.12 m^3m^{-3} and 0.14 m^3m^{-3}.

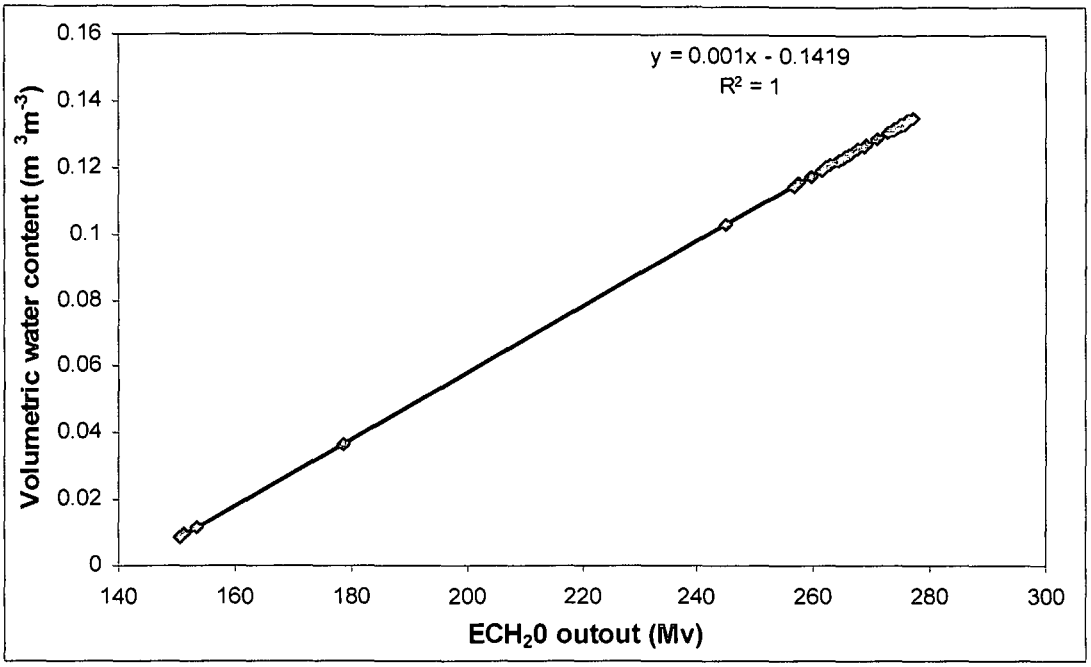


Figure 3.7 Volumetric Moisture Content (m^3m^{-3}) of soil determined along the Okavango River, Botswana during the 2004/05 nesting season.

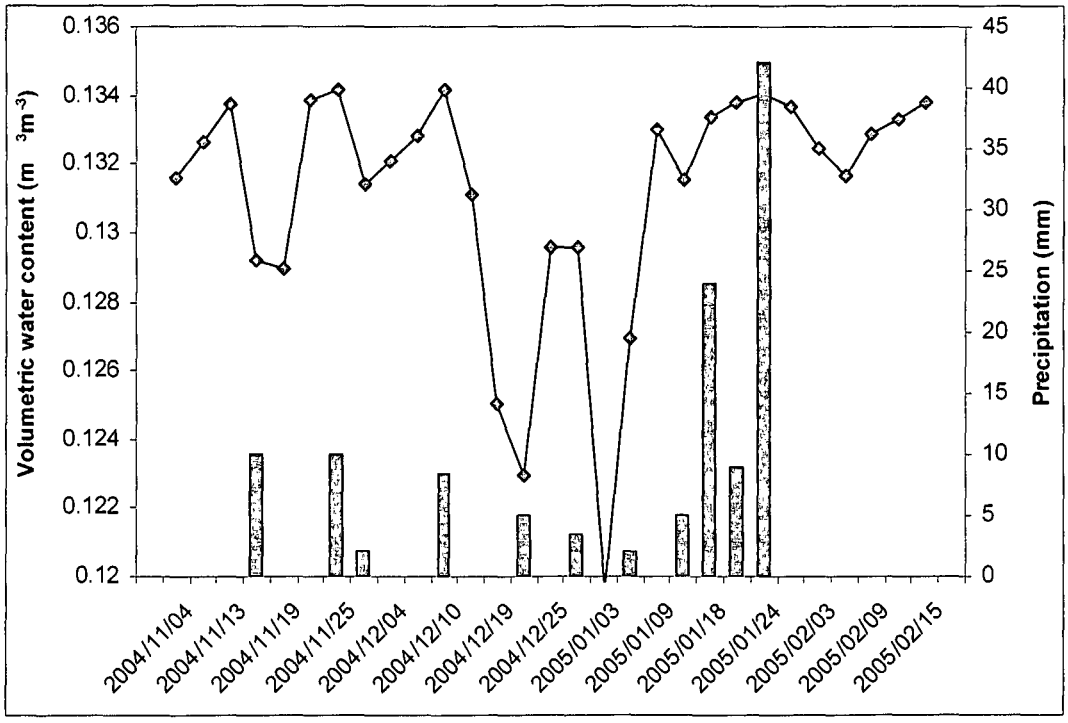


Figure 3.8 Average Volumetric Water Content (m^3m^{-3}) of soil (closed squares) determined along the Okavango River during the 2004/05 nesting season in relation to the average precipitation (bars) recorded in the Phillipa Channel.

3.4 DISCUSSION

In any soil profile, heat is continually moving into or out of the soil and the thermal energy is being continually redistributed in the soil (Leslie, 1997). In the dry 2004/05 nesting season, mean soil temperature at a depth of 25 cm along the Eastern Channel of the Okavango River was lower than the average air temperature during the same season. Precipitation was low and therefore did not play a significant role in decreasing average soil temperatures. It is evident that soil is a more stable medium than air, as soil temperature does not fluctuate as much as air temperature. Leslie (1997) found the opposite to be true, when she recorded temperatures at the Mpate River in St. Lucia, South Africa, where mean soil temperature was higher than the maximum air temperature. The difference in soil temperature between the Okavango Delta and St. Lucia may relate to the moisture content found in the air. Leslie (1997) recorded relative humidity in St. Lucia in 1994, 1995 and 1996 and found the average humidity to be around 70%. However, although the Okavango Delta exhibits a lower atmospheric humidity, the average soil temperature was lower than the average air temperature, which goes against the findings of Leslie (1997). Leslie (1997) found that based on biophysical principles, lower atmospheric humidity in the dry year in St. Lucia and reduced cloud cover, permitted a high proportion of solar radiation to reach the ground, both in the visible and in the infra-red range. Temperature gradients directed heat into the soil which warmed the soil and became a radiator of its own (Leslie, 1997).

From this study it is evident that precipitation does not have a significant effect on air and soil temperature. During the 2004/05 nesting season rainfall was low and so the effect on the average air and soil temperature was minimal. However, a comparison between a low and high rainfall season will allow for a more accurate analysis of the influence of rainfall on air and soil temperature. Leslie (1997) found that in St. Lucia precipitation plays an important role in cooling the soil temperature along the Mpate River.

When analysing soil moisture content we found that volumetric water content was very low. This suggests that the low rainfall during the 2004/05 nesting season had a significant influence on soil moisture content. However, the soil moisture probes were only placed at a depth of 25 cm. The soil may not be saturated at this depth, and thus may be a false representation of the effect of rainfall on soil moisture content. Future research is required where the soil moisture should be recorded at various depths. This will give a more accurate representation of the effect of precipitation on soil moisture.

A significant difference was found in soil temperature at various distances from the river. This supports the findings of Leslie (1997) that soil temperature differs between stations due to numerous factors including sun verses shade (insolation), distance from water and the angle of

incline which influences rainfall runoff. In this study, the average soil temperature was the greatest at a distance of 3 m from the river. Leslie (1997) found the opposite to be true; soil temperature was lowest at the profile lying closest to the river. The difference in soil temperature may be influenced by the soil type found along the Okavango River compared to St. Lucia. Rose (1966) found that at increased depth a thermal lag exists, which is proportional to the thermal conductivity, heat capacity and thermal diffusivity of the soil type. It is recommended that the soil type found in these two study areas are compared in terms of percentage organic matter, silt and clay content. The Okavango River consists primarily of fine to medium arenosol sands, called the Kalahari sands. The sands extend to a depth of at least 1 m and make up more than 70% of the body of the soil and less than 10% consists of clay and silt (Mendelsohn and el Obeid, 2004).

At a distance of 6 m from the Okavango River the average soil temperature for all three depths was 26.58 °C. This was low in comparison to the average soil temperature recorded at 7 m from the Mpate River in St Lucia (30.2 °C), which also had the highest temperature when compared to the other profiles. The reason that this soil profile experienced the lowest soil temperature was that the temperature readings were below 26 °C at depths of 15 and 40 cm. At a depth of 25 cm the average soil temperature was found to be the highest.

There was a significant difference in soil temperature at various depths. On average it was found that at a depth of 25 cm, which is the average depth of Nile crocodile nests, the soil temperature was 26.81 °C. This is low in comparison to the findings of Leslie (1997), who found the average soil temperature at 25 cm in St. Lucia to be 29.87 °C. The soil profile closest to the river (3 m) exhibited similar soil temperatures at various depths compared to the findings of Leslie (1997). At a distance of 3 m from the Okavango River the soil temperatures at 15, 25 and 40 cm depths were 28.51, 25.45 and 27.43 °C. Leslie (1997) recorded soil temperatures 4 m from the river in St. Lucia and found that soil temperatures were 29.0, 28.2 and 27.6 °C at the respective depths. At a distance of 6 m from the Okavango River the soil temperatures were recorded as 25.37; 28.87 and 25.49 °C at 15, 25 and 40 cm depths. In St. Lucia, at a distance of 7 m Leslie (1997) found the soil temperatures to be 30.6; 30.1 and 29.9 °C at the various depths. At a distance of 9 m from the Okavango River the soil temperatures were 28.01; 26.13 and 26.78 °C, which were low in comparison to soil temperatures recorded 10 m from the Mpate River in St. Lucia, where soil temperatures were 31.8; 31.3 and 30.6 °C at the three different depths.

Results from these soil profiles show that the average soil temperature recorded along the Okavango River is significantly lower in comparison to that of St. Lucia. The Okavango Delta is set in the semi-arid Kalahari (Mendelsohn and el Obeid, 2004) and therefore experiences

extreme temperature fluctuations, where soil temperature decreases at night, thereby decreasing the average soil temperature. The Nile crocodiles found in the Okavango River may have adapted to these low soil temperatures by prolonging their incubation period (further discussed in Chapter 5). During this time period soil temperatures were often below the lower pivotal temperature (Chapter 2) and therefore a predominantly female-biased sex ratio could be assumed. Soil temperatures recorded along the Okavango River show that warmer temperatures were found at shallower depths in the soil.

By looking at the range of soil temperatures at various distances from the river, and at various depths, it was found that soil temperature was at its maximum at a distance of 6 m from the river's edge, at a depth of 25 cm. This could be regarded as the 'optimal soil temperature' in terms of incubating crocodile eggs. Nest site characteristics are studied in detail in Chapter 4 and the relationship between nest site locality and soil temperatures is analyzed in Chapter 5. Shine and Harlow (1996) suggest that female crocodiles may be able to manipulate their phenotypes of their progeny indirectly, by inducing particular developmental pathways through selection of nest sites and thus, incubation conditions. Further research is required to determine whether female crocodiles choose optimal nest sites to ensure a female-biased sex ratio.

3.5 CONCLUSION

Soil plays an important role in temperature-dependent sex determination of the Nile crocodile. Air temperature raises the temperature of the soil, which conducts heat into the crocodile nest providing an incubation temperature which facilitates in the development and hatching of the eggs, as well as determining the sex ratio of the nest. Both temperature and moisture content of the soil are therefore of utmost importance. In this study we found that not only does air temperature influence soil temperature, but a great determining factor was the location of the nest in relation to the river. Soil temperature along the Okavango River varied according to distance from the river and depth below the soils surface. Soil temperature was greatest at a distance of 6 m from the river's edge at a depth of 25 cm. This could be regarded as an 'optimal nesting site'. Results have shown that the average soil temperature along the Okavango River is significantly lower in comparison to soil temperatures recorded in St. Lucia, South Africa.

3.6 REFERENCES

CAMPBELL, C. S. 2002. Calibrating ECH_2O soil moisture probes. Decagon Devices, Inc. Washington, USA.

GODLEY, B. J., BRODERICK, A. C., DOWNIE, J. R., GLEN, F., HOUGHTON, J. D., KIRKWOOD, I., REECE, S. AND G. C. HAYS. 2001. Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. *Journal of Experimental Marine Biology and Ecology* 263: 45–63.

HAYS, G.C., BRODERICK, A. C., GLEN, F. AND B. J. GODLEY. 2003. Climate change and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Climate Biology* 9: 642–646.

LESLIE, A. J. 1997. The ecology and physiology of the Nile crocodile, *Crocodylus niloticus* in Lake St. Lucia, Kwazulu-Natal, South Africa. Ph.D. Dissertation, Drexel University, USA.

MENDELSOHN, J. AND S. EL OBEID. 2004. Okavango River: The flow of a Lifeline. First edition. Struik Publishers, Cape Town.

ROSE, C. W. 1966. Agricultural Physics. Pergamon Press. Elmsford. New York.

SHINE, R. AND P. S. HARLOW. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77(6): 1808-1817.

SHINE, R., MADSEN, R. L., ELPHICK, M. J. AND P. S. HARLOW. 1997. The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* 78 (6): 1713–1721.

VLECK, D. 1988. Embryo water economy, egg size and hatchling viability in the lizard, *Sceloperous virgatus*. *American Zoology* 28: Pp.87A.

Appendix 3.1 Photographs of the transects used to measure the thermal profiles along the Eastern Channel of the Okavango River. (A) Thermal profiles constructed along the river in the Eastern Channel. (B) Copper-Constantan (Co-Cn) thermocouple wires were connected to a dowel stick at 15, 25 and 40 cm depths. (C) Connecting the Copper-Constantan (Co-Cn) thermocouple wires. (D) Covering the Copper-Constantan (Co-Cn) thermocouple wires with probes placed at 3 m (low-lying and closest to the river), 6 m (potential nesting site) and 9 m (furtherest away from the river). (E) Connecting the thermocouple wires to a CR-10X datalogger (Campbell Scientific, Inc., USA), powered by a 12 volt car battery. (F) Downloading temperature data from a CR-10X datalogger to a laptop. The datalogger was programmed to record soil temperature every 15 minutes for the duration of the breeding season.

A

B

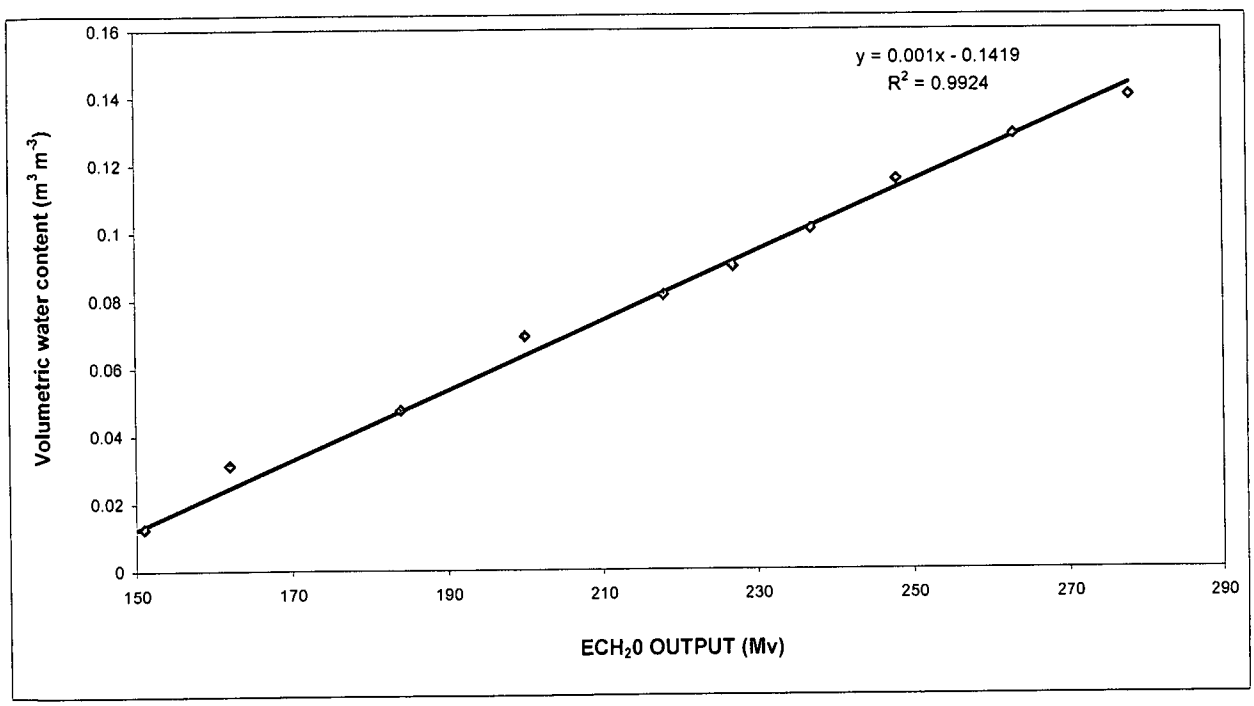
C

D

E

F

Appendix 3.2 The relationship between the ECH_2O output values (Mv) and the Volumetric Water Content (m^3m^{-3}) was plotted onto a graph using Microsoft Excel 2003 to obtain the equation y= 0.001x – 0.14



CHAPTER 4

NEST TEMPERATURE AND SEX RATIOS OF WILD CROCODILE NESTS IN THE OKAVANGO RIVER, BOTSWANA

4.1 INTRODUCTION

The nesting ecology of the Nile crocodile has been well documented over the past 40 years. Pooley (1962) recorded the breeding patterns of crocodiles in the Greater St. Lucia Estuary, South Africa. More recently Hutton (1984) and Leslie (1997) reported extensively on the nesting ecology of the Nile crocodile.

All crocodilians construct nests in which they incubate their eggs. Crocodilians construct two kinds of nests, vegetation mounds (alligators, caimans, most crocodiles including *Crocodylyus porosus, Crocodylus novaguineae* and *Tomistoma schlegelii*) and holes in the ground *(Crocodylus niloticus, Crocodylus acutus, Crocodylus intermedius, Crocodylus johnstoni, Crocodylus palustris* and *Gavialis gangeticus*) [Combrink, 2004]. Nile crocodiles lay their eggs in holes in open areas, where temperatures are maintained relatively stable by insolation and the moderating effect of the substrate (Leslie, 1997). Mound nests are affected by insolation and possible metabolic heat from the embryos (Webb *et al.*, 1983) but the majority of the heat is produced by rotting vegetation within the nests (LeBuff, 1957; Chabreck, 1973; Magnusson, 1979).

The sex ratio of hatchlings can be predicted by recording nest temperatures, especially during the thermosensitive period (TSP). The thermosensitive period is the period of incubation during which the sex of the crocodile is determined; this is usually the middle third of the incubation period (Webb *et al.*, 1987). The crucial temperature appears to be between 28.0 – 31.7 °C for females and 31.7 – 34.5 °C for males (Webb *et al.*, 1987; Leslie, 1997). Leslie (1997) found a female-biased sex ratio for Nile crocodiles in St. Lucia, South Africa, as the average incubation temperature for the majority of nests was below the experimentally determined lower pivotal temperature. Previous studies (Leslie, 1997) have shown that sex ratios in reptiles vary geographically and temporally both within and among years. It remains unknown, however, how hatchling sex ratios reflect or affect population sex ratios (Spotila *et al.*, 1987).

The sex of crocodilians is determined by incubation temperature, which in turn is influenced by the location of the nests (Campos, 1993; Ferguson and Joanen, 1983; Leslie, 1997 and Swanepoel, 1999). The location of the nest is determined by distance from water, height above water, incline of river bank to the nest, texture of the substrate and exposure of the nest to sunlight.

Exposure to sunlight is singled out as an important factor in determining the location of a nest (Hartley, 1990; Graham, 1968), although the availability of shade for the female plays just as important a role (Hutton, 1984; Leslie, 1997). Magnusson *et al.* (1985) found that clutch temperature was relatively stable and always much higher than nest surface and air temperatures.

The objective of this study was to locate and study wild Nile crocodile nests found along the Okavango River. Nest site characteristics were described and numerous nesting parameters were measured. Incubation temperature was recorded and the experimentally determined pivotal temperature (Chapter 2) was then used to predict sex ratios of hatchlings entering the Okavango system.

4.2 METHODS AND MATERIALS

4.2.1 Nest Site Characteristics

Riverbanks were patrolled by foot at the start of the nesting season and any sign of crocodile nesting activity was recorded. These signs included well-used 'slides' which is the path the female used to move between her nest and the river (Appendix 4.1A-C). The presence of trees in the Okavango gave an indication that a nest may be present. The Okavango River consists primarily of floating papyrus and reeds. Established trees indicated the presence of an embankment and therefore a suitable area for a hole-nester. At a potential nesting site test holes may be present or even the body print of a female, indicating the presence of a prospective nesting crocodile.

Once a potential nesting site was located a 4 mm diameter rod was used to probe the soil to determine the exact location of the eggs (Appendix 4.1D). The probing needs to be fairly firm to penetrate through the top layer of the compacted soil, but great care was taken to ensure the eggs were not damaged. The position of the nest was recorded using a handheld Magellan GPS 315 receiver and the data downloaded and mapped using ArcView GIS 3.2 (Environmental Systems Research Institute, Inc., USA).

A total of 14 nests were used in this experiment during the 2004/05 nesting season (Figure 4.1). At each site nest site characteristics were described including: nesting area and locality, estimated size of female and approximate date of laying, distance from water, height above water, substrate type, depth to first egg, depth to bottom of nest and hourly exposure of nest to sun or shade.

The distance of the nest from the water's edge was measured using a 30 m flexible tape measure. The distance was measured from the centre of the egg chamber to the nearest access channel and measurements were rounded to the nearest meter. Distance to water was crucial for the hatchlings as they were vulnerable to predation after hatching.

Height above water was measured with a wooden beam marked at 50 cm intervals. An observer would stand 20 m away and measure the height by keeping a spirit level at arms length and taking a reading from the beam.

Depth to the middle of the nest was determined by carefully removing an estimated half of the clutch of eggs and using a metal tape measure to measure the distance from the top layer of the soil to the middle of the nest.

The texture of the substrate was divided into three classes namely sand, soil and clay. Sand is defined as coarse loosely-packed river sand, soil is alluvial deposits on the flood plains adjacent to the streams and clay is defined as any substance that sticks to the metal rod used for probing (Swanepoel, 1999).

Exposure to sun was scored from 1 to 3, with 1 being less than three hours direct sunlight on the nest per day. Value 2 was an exposure of three to six hours sunshine per day and 3 represents more than six hours of direct sunlight per day.

The slope of the nests in relation to the river was determined by dividing the height of the nest by the horizontal distance of the nest from the river and determining the slope as a percentage.

Any vegetation was indicated by a value of 1 and the absence of any vegetation within a 10 m radius was indicated by a value of 0.

4.2.2 Nest Temperature

Once the eggs were located using the probe, an umbrella was set over the nest to protect the eggs from direct sunlight. Latex gloves were worn at all times. The sand that was removed from the top of the nest was placed in a container (Appendix 4.1 E-F) so as to limit the spread of the scent from the nest. The distance to the first egg (Appendix 4.1G) was measured and a sub-sample of eggs removed and placed in a separate container. A pencil mark indicated the top of the egg and care was taken not to turn the egg. Movement or turning at this stage may kill the embryo due to it being crushed by adjacent heavy yolk (Marais and Smith, 1992).

Each egg was removed, weighed (± 0.1 g) using a pasola scale and measured (length and width ± 0.1 mm) using digital calipers (Appendix 4.1H-I). Once approximately half the clutch of eggs was removed, a miniature temperature logger, (StowAway® Tidbit®, Onset Instruments, Massachusetts, USA) was placed within the nest at a depth of 20-25 cm (Appendix 4.1J). These waterproof units were small (30 x 41 x 17 mm) and had an operating temperature range of –20 °C to +50 °C. Each Tidbit was programmed to record nest temperature every 15 minutes for the duration of the incubation period (100 days).

Eggs were replaced in the nest and gently covered with a layer of sand. A large grid mesh (six gauge wire; 5 x 5 cm grid gaps, size: 50 x 50 cm) was placed over the nest and covered with fresh soil (Appendix 4.1K-L). The grid was used to protect the nest from predation by monitor lizards, mongoose and other predators.

Nests were monitored every four days to determine whether the female was still present and to ensure that the grid was still intact and the nest had not been predated. After approximately 75 days of incubation, after the thermosensitive period, the grids were removed, the nests uncovered and the Tidbits were retrieved. The Tidbits were coupled to an Optic Base Station™ (Onset Computer Corporation, Massachusetts, USA) and data were downloaded to a computer using BoxCar® Software (Version 3.7 for Windows. Onset Instruments, Massachusetts, USA). The nests were then covered with fresh soil and monitored weekly to determine the date

of hatching. It was essential to determine the hatching date so that the TSP could be estimated. Mean TSP nest temperature was determined by calculating the average nest temperature during the middle third of the incubation period. Mean and variance in incubation temperature was estimated as these indices best describe sex ratios in nature (Table 4.3).

Nest temperature was used to estimate sex ratios of hatchlings from wild nests based on the pivotal temperature determined by the artificial incubation of eggs (Chapter 2). A descriptive statistic package, Microsoft® Office Excel 2003 (Microsoft Corporation, USA) was used to analyse and compare nest temperatures. Average soil temperature, air temperature and precipitation were compared to nest temperatures for the entire breeding season.

The Statistica 7 (Statsoft, Inc., USA) software package designed for Windows was used for the descriptive statistics and regression analyses. A one-way analysis of variance (ANOVA) was used to compare TSP temperatures between nests. Significance was determined at the level of $P < 0.05$ (two-tailed). Bonferroni and Tukey's multiple comparison procedures were used to analyse the corresponding data, depending on the normality (or non-normality) of the residuals. Bonferroni is a post hoc test which is very conservative when a large number of group means are being compared. A non-parametric analysis, Kruskal-Wallis was used to confirm the significant difference of the ANOVA when residuals were non-normally distributed.

4.3 RESULTS

4.3.1 Nest Site Characteristics

Two of these nests (Nest 4 and 13) were predated at an early stage and therefore incubation temperatures were only recorded from 12 nests. Oviposition was well synchronized in females and the majority of nesting took place within a three to four week period, from approximately 13 September - 16 October 2004. Nest site characteristics varied between nesting areas thus influencing incubation temperatures and incubation periods (Table 4.1).

Distance to water was determined by topography and should be interpreted in conjunction with soil type, accessibility to deep water, height above water and vegetation (Swanepoel, 1999). Nests varied in terms of distance from water with a minimum distance of 1.5 m compared to a maximum distance of 10.9 m (Table 4.1). The mean distance from nests to water was 5.56 m which was not far when compared to previous studies (Swanepoel, 1999).

Height above water varied among nests but not to a great extent. The average height above water was 0.76 m (Table 4.1). Only two nests exceeded a height of 1 m above water. Females use a circular route to and from the nest and the angle of the slope, and to a lesser degree the distance from the waters edge, determines the amount of energy required to reach the nest (Pooley, 1969). Slope was calculated as a percentage by dividing the height difference of the nest above water by the horizontal distance of the nest from the river. The average slope of the nests was 23.11% (Table 4.1).

The average depth to the middle of the nests was 24.8 cm with a minimum to maximum range of 20 cm to 32 cm. Although the Nile crocodile was able to successfully nest in a large variety of substrate types in the Okavango region, raised banks and ready access to deep water is essential and the most commonly used soil type was course river sand (Table 4.1). Although there were scattered trees along the Okavango River, they were nowhere dense enough to be an influencing factor in either the placement of nests or prevention of nesting. The percentage direct sunlight on a nest was expressed as a value ranging from 1 to 3. The majority of the nests fell into category 3 indicating more than six hours of sunlight per day (Table 4.1). However, nests exposed to less than three hours of direct sunlight per day survived and hatched successfully.

Mean nest temperatures ranged from 25.29 °C to a maximum of 32.12 °C (Table 4.1). There was a correlation between nest temperature and exposure to sunlight. All nests exposed to more than six hours of direct sunlight per day had internal nest temperatures above 28.0 °C. Presence or absence of vegetation may also play a role in determination of incubation temperature.

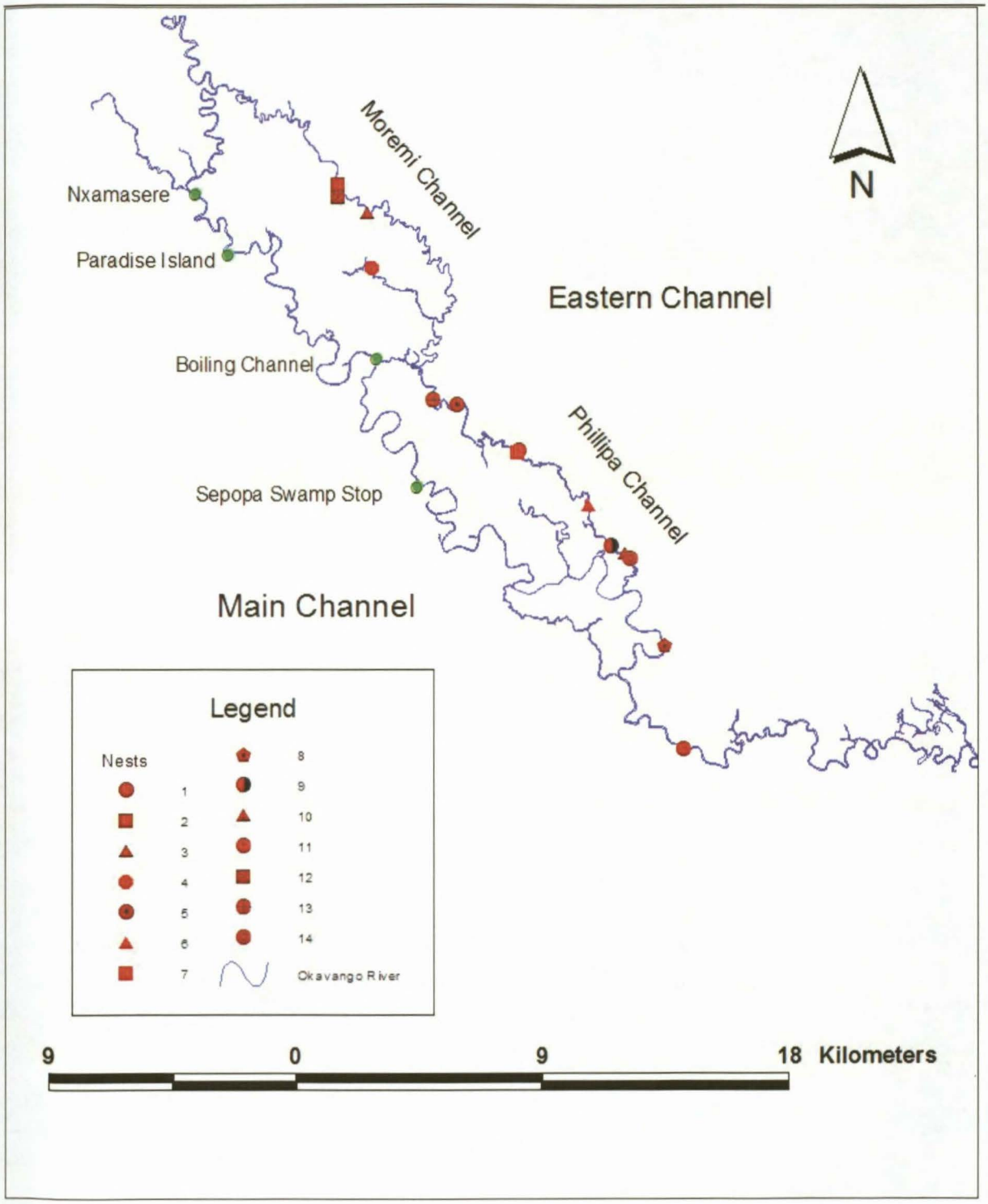


Figure 4.1 Map illustrating the position of the 14 Nile crocodile nest sites studied in the Moremi and Phillipa Channel of the Eastern Channel in the Okavango River, Botswana during the 2004/05 nesting season.

Table 4.1 Nest Site Characteristics of 14 wild Nile crocodile (*Crocodylus niloticus*) nests monitored in the Eastern Channel of the Okavango River, Botswana during the 2004/05 nesting season.

Nest	GPS (E)	GPS (N)	Distance to water (m)	Height above water (m)	Slope (%)	Average depth of nest (cm)	Substrate type	Exposure to sunlight	Vegetation	Mean Nest Temperature (°C)
1	629020	7930809	8.60	0.40	4.65	25.30	sand	3	1	29.21 (24.19 - 32.30)
2	622453	7940451	4.00	0.80	20.00	26.00	sand	3	0	32.12 (27.72 - 35.26)
3	623542	7939409	10.00	0.50	5.00	32.00	sand	2	2	25.84 (21.47 - 29.45)
4	623695	7937408	3.40	0.60	17.65	28.00	sand	3	1	predated
5	626778	7932466	8.00	0.40	5.00	27.00	sand	3	1	29.90 (25.87 - 34.05)
6	631566	7928808	2.00	0.97	48.50	20.00	sand	3	1	28.25 (24.86 - 32.17)
7	629016	7930774	5.70	0.47	8.25	31.00	sand	3	2	28.59 (24.36 - 31.36)
8	634320	7923745	3.40	0.70	20.59	26.00	sand	3	1	28.92 (24.33 - 33.26)
9	632394	7927354	2.50	1.00	40.00	20.00	sand	3	2	25.29 (21.98 - 28.63)
10	632886	7927081	3.00	0.90	30.00	20.00	sand	2	1	28.07 (23.73 - 31.71)
11	635010	7920014	10.00	0.70	7.00	20.00	sand	3	2	32.01 (25.28 - 37.98)
12	622456	7939992	10.90	0.20	1.83	26.00	sand	2	1	27.74 (24.29 - 30.94)
13	625917	7932657	4.80	1.80	37.50	21.00	sand	3	2	predated
14	633075	7926906	1.50	1.20	80.00	24.50	sand	2	1	27.38 (23.70 - 30.42)
Mean			5.56	0.76	23.11	24.77		2.57	1.29	**28.69**
Standard deviation			3.29	0.41	23.45	4.08				**2.16**

The majority of nest sites in this study exhibited a low percentage of vegetation cover within 10 m of the nest (Table 4.1).

4.3.2 Nest Incubation Temperatures

There was a monthly increase in mean incubation temperature from October to November in all of the nests studied (Table 4.2). This reflected the increase in air temperature between October and November (Figure 4.2). Average nest temperatures for October, November, December and January were 27.5; 29.5; 29.4 and 30.8 °C respectively. From November to December there was a decrease in mean nest temperature in Nest 2, 5, 6 and 8. However during the same time period there was an increase in mean incubation temperature and in Nest 7, 10, 11, 12 and 14. This can be correlated to a decrease in air temperature which was related to the rain that fell in November, thus decreasing the air temperature.

Time of nesting had a major effect on incubation temperature and therefore, on incubation period and the resultant sex ratios of hatchlings. For example: an early nest, Nest 2, which was laid in late September, differed in thermosensitive period to Nest 14, which was laid in late October (Figure 4.3).

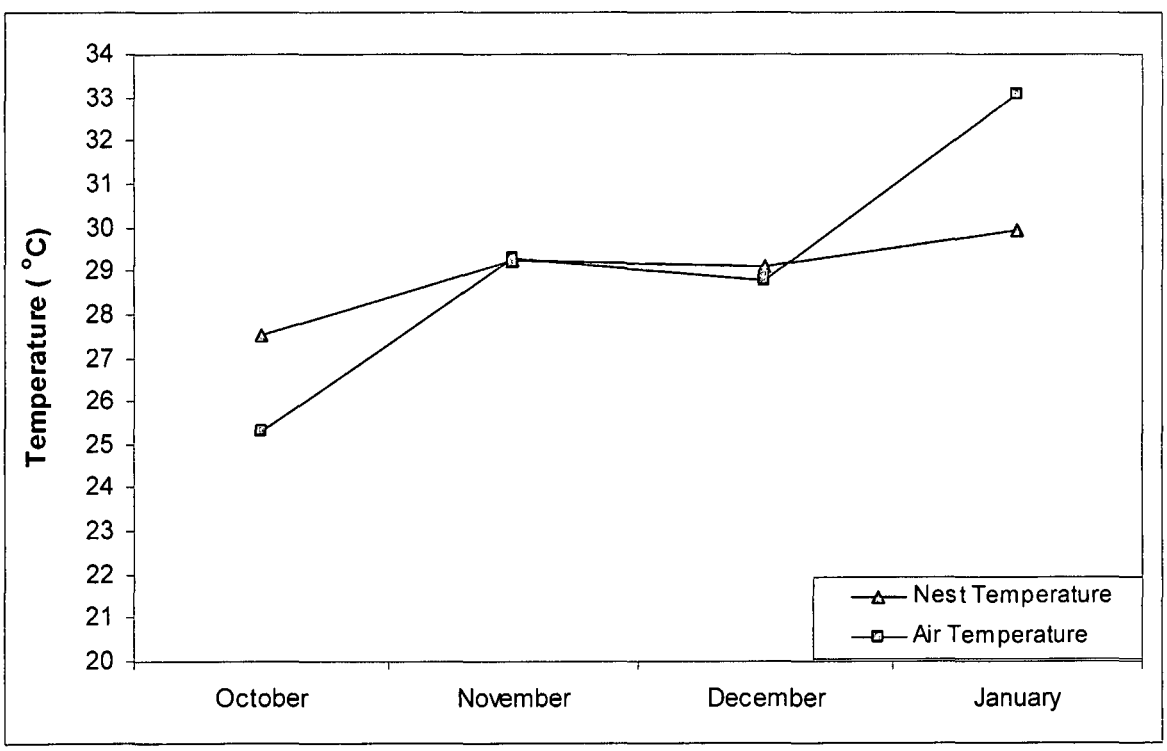


Figure 4.2 Graph illustrating the relationship between mean monthly nest temperature (°C) of wild Nile crocodile nests and mean monthly air temperature (°C) during the incubation period in the Okavango River, Botswana.

Table 4.2 Mean monthly nest temperatures recorded from wild *Crocodylus niloticus* nests in the Eastern Channel of the Okavango River, Botswana during the incubation period (± standard deviation with range in parenthesis).

Nest	Nest Temperature (°C)			
	October	November	December	January
1	27.57 ± 1.99 (24.19-31.95)	29.69 ± 1.18 (26.09 - 32.22)		
2	29.78 ± 2.01 (22.67 - 34.59)	32.31 ± 1.50 (27.71 - 35.83)	32.29 ± 1.99 (27.53 - 35.62)	
3	25.85 ± 2.04 (21.47 - 31.13)			
4	Predated early			
5	27.16 ± 2.02 (22.12 - 31.13)	29.92 ± 1.58 (25.37 - 34.05)	29.19 ± 1.83 (25.35 - 32.47)	
6	26.46 ± 1.44 (23.12 - 29.50)	28.25 ± 1.36 (24.86 - 32.17)	27.83 ± 1.97 (23.81 - 33.74)	
7	27.21 ± 1.22 (24.13 - 29.47)	28.84 ± 0.91 (26.39 - 31.36)	29.32 ± 1.92 (24.64 - 32.72)	
8	29.14 ± 1.50 (25.71 - 32.28)	29.61 ± 1.56 (25.19 - 33.26)	27.01 ± 1.66 (23.81 - 31.13)	
9	25.37 ± 1.36 (22.32 - 28.45)			
10	26.84 ± 1.00 (24.77 - 29.05)	27.55 ± 1.09 (24.59 - 30.55)	28.23 ± 1.85 (23.73 - 32.09)	30.84 ± 1.00 (29.24 - 32.48)
11	30.23 ± 1.10 (28.11 - 32.43)	31.22 ± 1.68 (26.68 - 35.85)	33.31 ± 3.17 (25.28 - 38.19)	
12	27.23 ± 0.85 (25.50 - 29.25)	27.74 ± 1.30 (24.29 - 30.94)	27.77 ± 1.44 (24.46 - 30.37)	
13	Predated early			
14		27.22 ± 0.92 (24.56 - 29.87)	27.15 ± 1.54 (23.70 - 30.42)	29.07 ± 0.91 (27.33 - 30.80)

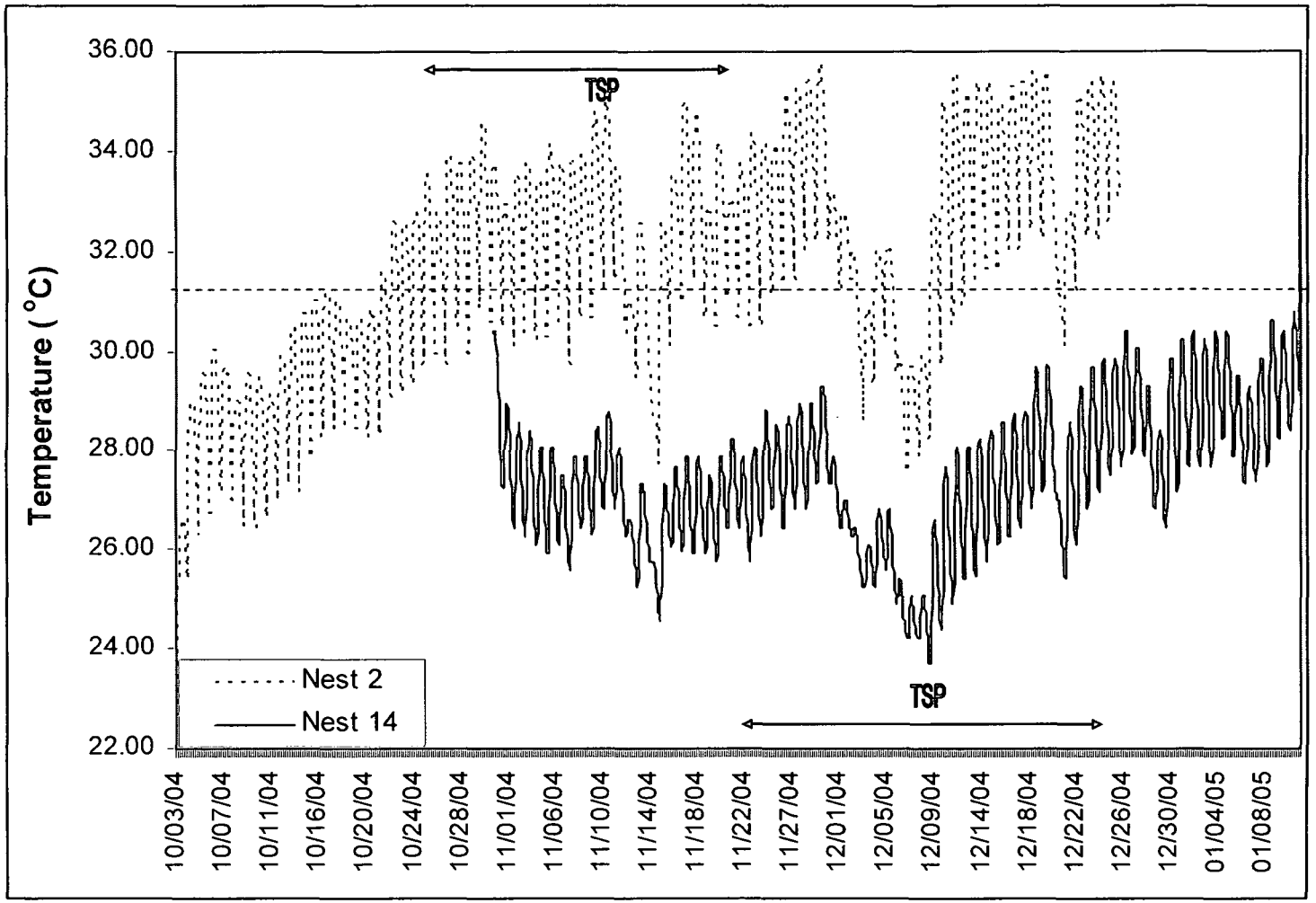


Figure 4.3 Thermal characteristics of two wild Nile crocodile nests studied along the Eastern Channel of the Okavango River in the 2004/05 nesting season. The graph illustrates a variation in both the means and the diel ranges of incubation temperature and the effects of seasonality (time of nesting) on the thermosensitive period. The lines with arrows denote the thermosensitive period for the two nests. The dashed line represents the lower pivotal temperature of 31.4 °C.

The early nest (Nest 2) was also oviposited in the upper reaches of the Moremi Channel, whereas the late nest was laid much lower south in the Phillipa Channel. They were approximately 24 km apart, as the crow flies. Incubation temperature in the early nest was initially low (26.6 °C) but increased to 31.19 °C through day 14. By day 40 of incubation, minimum and maximum temperatures had increased to 24.8 °C and 33.1 °C. Rain on day 37-39, 41, 43-44, 48-49 and 55 resulted in a decrease in the incubation temperature to a minimum of 27.8 °C on two occasions prior to hatching. The thermosensitive period (middle third of incubation) for this nest was from approximately 27 October – 26 November 2004. During this time period the incubation temperature was well above the lower pivotal temperature (daily mean = 32.1 °C, mean daily minima = 27.7 °C, mean daily maxima = 35.2 °C) for the Nile crocodile in the Okavango Delta (Chapter 2) and therefore this early season nest probably produced the majority male hatchlings. The incubation period for Nest 2 was 90 days.

In the late nest (Nest 14), temperatures were initially low, ranging from 25.7 °C to 28.7 °C through day 12 of incubation and remained between 26.1 °C and 29.3 °C through day 30 of incubation. Rain on day 9-11, 13, 15-16, 20-21, 23, 27, 31, 39, 51, 59 and 63 resulted in a decrease in nest temperature to a low of 23.7 °C prior to hatching. The thermosensitive period for this late nest was estimated from approximately 28 November 2004 – 3 January 2005. The incubation period for this nest was 109 days, which was longer than the average incubation period previously documented for the Nile crocodile (Swanepoel, 1999; Leslie, 1997). During this time period the incubation temperature was below the lower pivotal temperature (daily mean = 27.4 °C; mean daily minima = 23.7 °C; mean daily maxima = 30.4 °C) and therefore this late nest probably produced the majority female hatchlings.

The date of laying therefore plays a critical role in determining the sex ratio of nests. We estimated that two nests (Nest 2 and 11) produced the majority males and eight nests (Nests 1, 5, 6, 7, 8, 10, 12 and 14) produced 100% females based on pivotal temperatures determined in Chapter 2 of this study. The TSP of Nest 2 and 11 fell within the month of November. Nest 2 and 11 both experienced a mean daily TSP fluctuation that exceeded 3.4 °C. Mean daily fluctuations (the difference between daily minima and maxima temperatures) for the TSP of all 12 nests was 2.8 °C, with mean daily temperatures within nests fluctuating as little as 1.4 °C and as much as 3.64 °C. The mean daily TSP temperature fluctuation was the greatest in Nest 2 and 11 in comparison to the other nests, and these were the two nests that probably produced the majority male hatchlings (Table 4.3).

Table 4.3 Thermosensitive period temperatures (°C) and estimated sex ratios for ten Nile crocodile nests, laid from September to October 2004, in the Eastern Channel of the Okavango River, Botswana during the 2004/05 nesting season.

Nest	Date Laid	TSP Date	Mean TSP nest temperature range (°C)	Mean daily TSP temperature fluctuation (°C)	Expected sex ratio
1	13 September 2004	13 Oct – 12 Nov	29.14 (24.19 – 32.33)	2.62	100% female
2	27 September 2004	27 Oct – 26 Nov	32.12 (27.71 – 35.21)	3.44	majority male
5	01 October 2004	31 Oct – 30 Nov	29.90 (25.87 – 34.05)	2.86	100% female
6	02 October 2004	1 Nov – 1 Dec	28.25 (24.86 – 32.17)	2.73	100 % female
7	06 October 2004	11 Nov – 11 Dec	28.59 (24.36 – 31.36)	1.40	100% female
8	09 October 2004	8 Nov – 8 Dec	28.92 (24.33 – 33.26)	3.35	100 % female
10	14 October 2004	20 Nov – 27 Dec	28.07 (23.73 – 31.71)	2.53	100% female
11	06 October 2004	13 Nov – 21 Dec	32.01 (25.28 – 37.98)	3.64	majority male
12	02 October 2004	1 Nov – 1 Dec	27.74 (24.29 – 30.94)	2.86	100% female
14	23 October 2004	28 Nov – 3 Jan	27.38 (23.70 – 30.42)	2.32	100% female

The 2004/05 nesting season therefore produced a strongly female biased sex ratio of hatchlings entering the Okavango system.

An analysis of TSP temperatures from the nests in the 2004/05 nesting season allowed a comparison of thermosensitive periods between nests (Figure 4.4 A-D). Egg laying occurred over a six week period and as a result the period of sex determination varied between nests. One nest was laid early in the nesting season, eight in the middle and one in the latter part of the season. Nest temperatures during the TSP ranged from 27.4 °C to 32.1 °C. The thermosensitive periods were compared and the nests that fell within the same TSP were compared. Nest 1, 2 and 5 exhibited a TSP that ranged from 13 October to 30 November 2004 (Figure 4.4A). Nest 6, 8 and 12 experienced a TSP between 1 November and 8 December 2004 (Figure 4.4B). Nest 7 and 11 were grouped together as the TSP of these two nests fell between 11 November and 21 December 2004 (Figure 4.4C). Nest 10 and 14 portrayed a TSP range from 20 November 2004 to 3 January 2005 (Figure 4.4D).

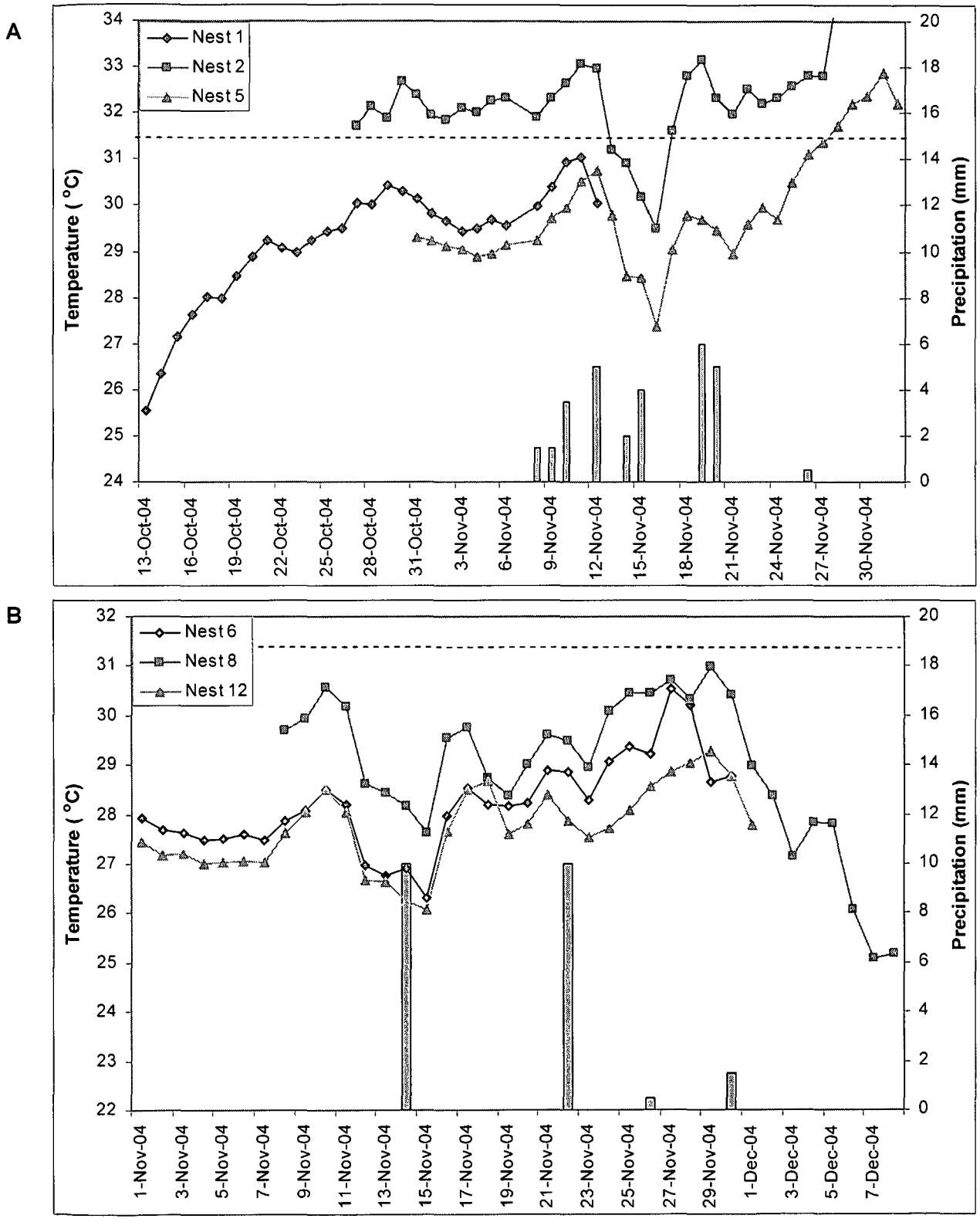
Rainfall in the 2004/05 season had an effect on nest temperature. Nest 1, 2 and 5 showed an increase in temperature at the onset of the TSP, only to be reduced by rains on day 26-28, 30, 32-33, 38-39 and 44 (Figure 4.4A). However the TSP temperature of Nest 2 was still well above the lower pivotal temperature, probably producing the majority male hatchlings.

Nest 1 and 5 were well below the lower pivotal temperature resulting in an estimated female-biased sex ratio. Nest 1 and 5 showed similar TSP temperatures (29.14 °C and 29.90 °C), which could be supported by the fact that they were only 2.7 km apart. Temperatures during the TSP of Nests 6, 8 and 12 (28.25 °C, 28.92 °C and 27.74 °C) [Figure 4.4B] which were laid later in the season, increased initially but were affected by rain on day 14, 22, 26 and 30.

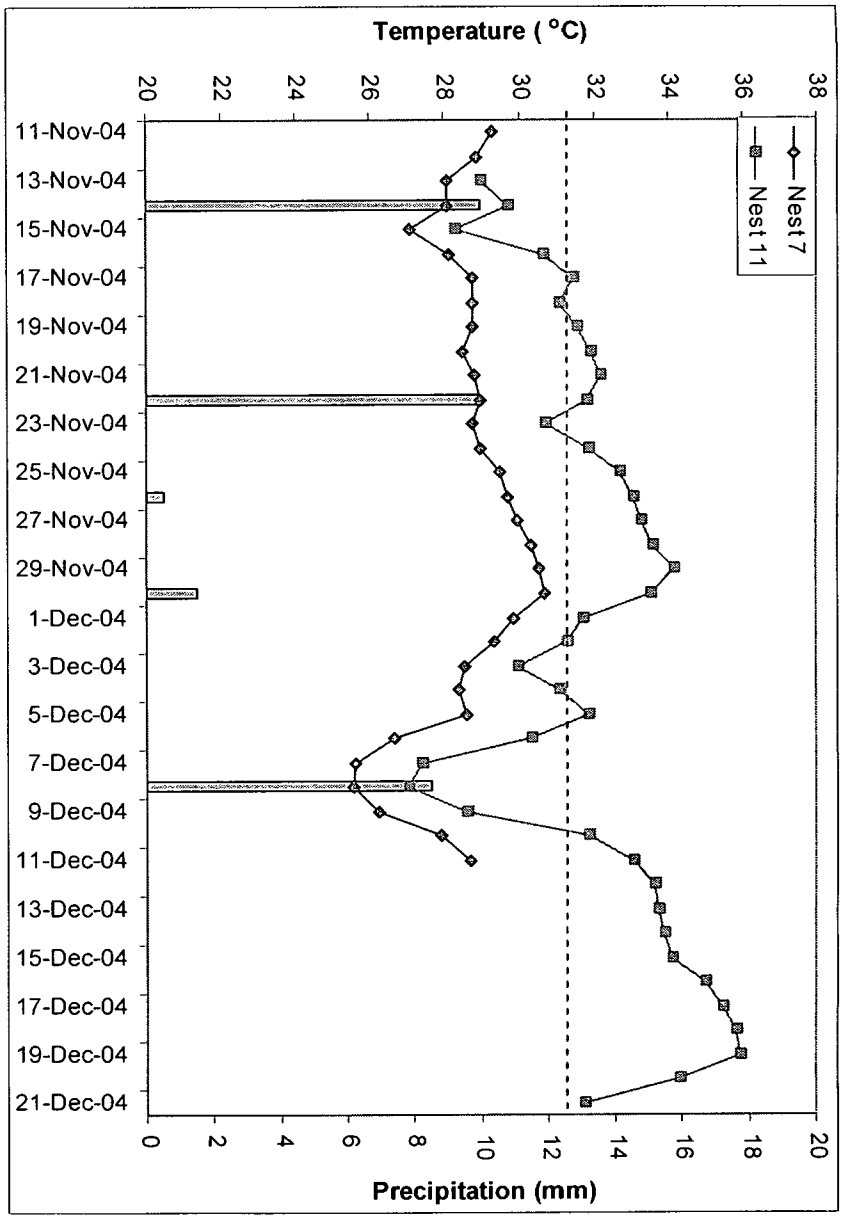
Nest 6, 8 and 12 temperatures were well below the lower pivotal temperature and therefore possibly produced 100% female hatchlings. Rain on day 30 caused a reduction in nest temperatures at the end of the thermosensitive period. Nest 7 and 11 (Figure 4.4C) which were laid in the middle of the nesting season were also affected by rain. Rain on days 4, 12, 16, 20 and 28 accounted for low initial temperatures during the thermosensitive period. Nest temperatures increased but again decreased due to rain on day 22. Rain on day 30 resulted in a severe decrease in temperature, but they then increased at a steady rate. The TSP for Nest 11 (32.01 °C) was well above the lower pivotal temperature and therefore probably produced the majority male hatchlings. The TSP temperature of Nest 7 (28.59 °C) was below the lower pivotal temperature resulting in an estimated female-biased sex ratio.

Nest 10 and 14 showed an increase in temperature in the initial stages of the TSP, only to be reduced by good rains on days 3, 7, 11 and 19 of the TSP (Figure 4.4D). Nest 10 and 14 (28.07 °C and 27.38 °C) then increased in temperature until days 31, 39 and 43 when it rained.

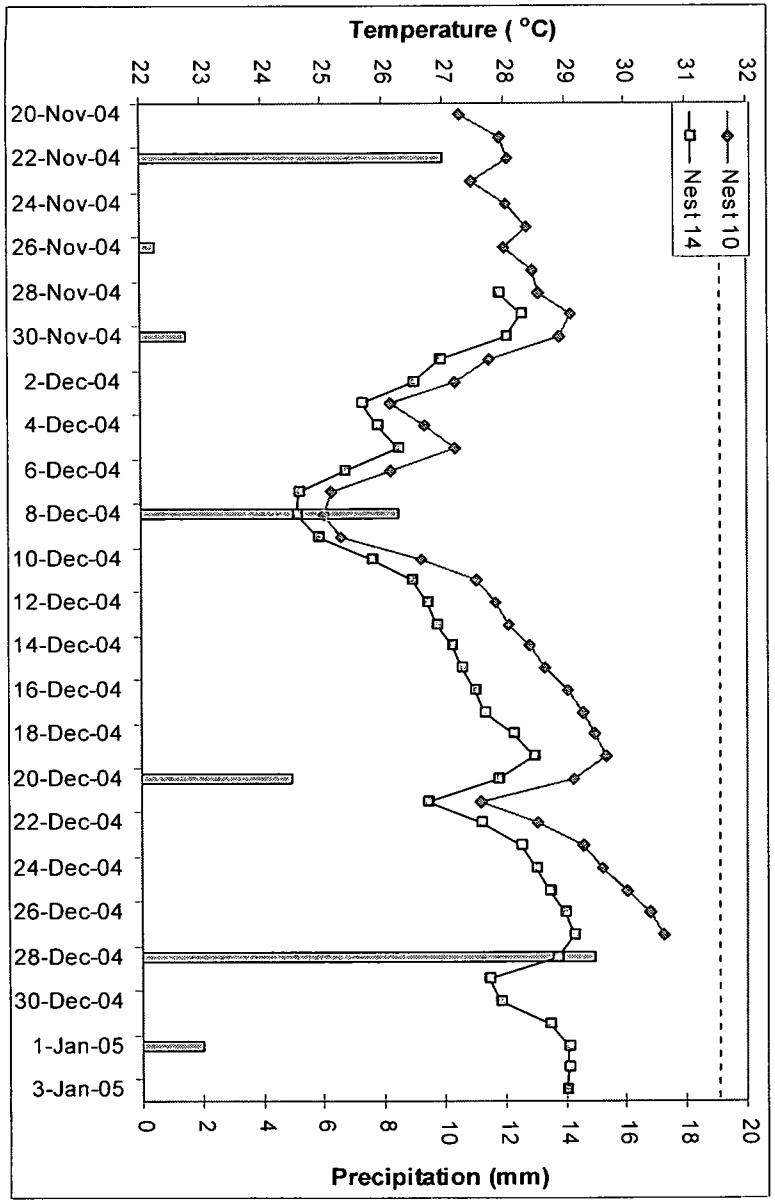
Figure 4.4 A-D The daily mean nest temperatures (°C) for *Crocodylus niloticus* with similar thermosensitive periods in the Eastern Channel of the Okavango River combined with daily mean precipitation (mm). The dashed line denotes the calculated lower pivotal temperature for the Okavango's Nile crocodile.



C



D



Temperatures in both these nests never reached the pivotal temperature and therefore produced 100% females. Nest 14 had the lowest mean temperature during the TSP. Not only was this nest laid late in the nesting season but rain reduced temperatures on three occasions during the TSP. As a result this nest produced 100% female hatchlings.

Results of a one-way analysis of variance (ANOVA) indicated that there was a significant difference in nest temperatures during the thermosensitive period of incubation of Nile crocodile eggs ($P < 0.05$, MS = 98.3, F =51.8, df = 9). The degree of significance varies among nests (Figure 4.5). Nest 2 and 11 were the only two nests with an average TSP temperature of 32.0 °C, which can be correlated with Table 4.3 that shows that these two nests are predicted to have male biased sex ratio.

A Bonferroni and Tukey test ($P < 0.05$, MS = 1.897, df = 321) [Appendix 4.2] confirms that a significant difference exists in nest temperatures of the ten nests studied during the thermosensitive period of the incubation period. The nest temperatures from Nest 2 and 11 were significantly different to the other nests which confirmed that these two nests produced the majority male hatchlings whereas the other eight nests were predicted to produce primarily female hatchlings.

The residuals from the ANOVA showed that nest temperatures are not normally distributed therefore a non-parametric test, Kruskal-Wallis was used, which confirmed that a significant difference was found in nest temperatures between the nests ($P < 0.05$, H (9, N=331)= 185.08) [Appendix 4.4].

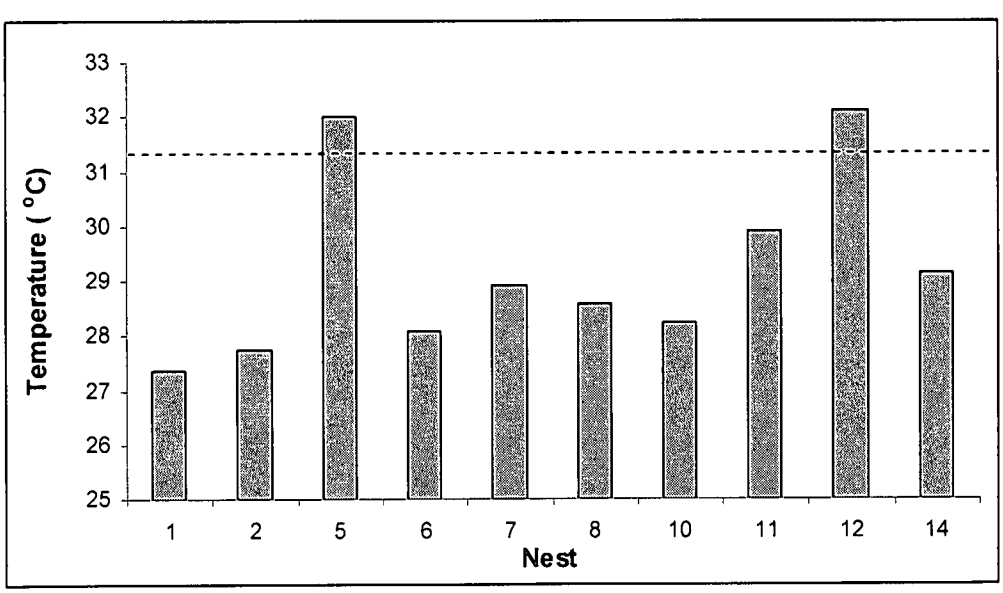


Figure 4.5 Mean TSP temperature (°C) for wild Nile crocodile nests. (Current effect: F (9, 321) = 51.81, $P < 0.05$). The dashed line represents the lower pivotal temperature.

4.4 DISCUSSION

4.4.1 Nest Site Characteristics

The average distance to water was found to be 5.6 m (min = 1.5 m, max = 10.9 m), which is low in comparison to previous studies. In the Kruger National Park it was found that distance to and height above water were not important factors in nest location (Swanepoel, 1999). Swanepoel (1999) found that the mean distance to water was 18.9 m. Pooley (1969) found that distance from the nests to water in Kwazulu-Natal varied from 15.0 to 50.0 m. Hartley (1990) found that, despite a large variation ranging from 1.0 m to greater than 80.0 m, the majority of nests were approximately 6.0 m from water in the Umfolozi Game Reserve. In 1984, Hutton noted the mean distance of nests from water to be approximately 6.0 m for the Ngezi population in Zimbabwe. Kofron (1989) indicated an initial range of 2.5 m to 10.0 m from the water when laid, but rising water levels later submerged all the nests for that specific season and the eggs subsequently drowned. Hutton (1984) found that flooding of nests during incubation accounts for a large number of nest mortalities.

In Chapter 3 in this study, it was determined that at a depth of 25.0 cm the soil temperature was highest at a distance of 6.0 m from the river. At this distance the soil temperature was regarded as the 'optimal' temperature required for incubation of crocodile eggs. This indicates that distance to water is an important factor in terms of temperature-dependent sex determination. Close proximity to water may also increase survival chances of a nest as it enables female crocodiles to tend to their nests immediately thereby deterring predation. Campos (1993) found that predation is one of the main causes of mortality of crocodilian eggs.

The average height of nest sites above water in the Okavango River was found to be 0.76 m, which is significantly lower than previously determined in other study areas. Swanepoel (1999) found the maximum height above water before the flood was 14.0 m and after the flood it was 25.0 m. In 1990, Hartley determined the mean height of nests above water to be 4.5 m and in three nests he recorded heights of 15.0 m above water level. Swanepoel (1999) determined that height of nest sites above the river plays a large role in contributing to the amount of energy expended by the female crocodile to move between her nest and the river. In the Okavango system female crocodiles do not utilize excessive energy when moving between the river and the nest therefore decreasing the chances of predation.

The incline of the bank to the nest was also used by Swanepoel (1999) as an indication of the amount of energy used by the female in reaching the nest site. Pooley (1969) found that females tend to use a circular trail to and from the nest and the inclination will partly determine the accessibility of the nest. Swanepoel (1999) calculated slope, or inclination, as a cotan function of height over distance and converted the values into degrees. The mean slope in 1994,

1995, 1996, 1997, and 1998 was calculated as 20.3; 21.0; 25.1; 21.8 and 24.2° respectively. Combrink (2004) studied Nile crocodile nest sites along Lake Sibaya, in KwaZulu-Natal and found the nesting sites sloped upwards with an approximate angle of 40°. In my study average slope of nest sites from the river was 23.11%, which again shows that female crocodiles have easy access to nests, which may deter predators.

The average depth of the nests studied in the Eastern Channel of the Okavango River was 24.8 cm, which is similar to findings along the Olifants River in the Kruger National Park (Swanepoel, 1999), where the mean nest depth was 25.0 cm. In KwaZulu-Natal, Hartley (1990) found an average depth of 20.3 cm with 42.0 cm as the deepest and 15.0 cm as the shallowest. In Zimbabwe the average depth of crocodile nests was 16.4 cm (Hutton, 1984). Leslie (1997) found the average depth of the crocodile nests in St. Lucia to be 25.0 cm. The depth of the nest plays an important role in temperature determination during incubation of the eggs. In Chapter 3 of this study it was found that soil temperature was the highest at a depth of 25.0 cm. At this depth the eggs were incubated at the desired temperature required for development and determination of sex of the embryos.

The predominant soil type in the Okavango River was found to be sand. In the Olifants River, Swanepoel (1999) found the soil type to be predominately coarse river sand bordered by fine silt on flood plains away from the main stream or covered by a thin layer of alluvial silt closer to the water. In 1989, Kofron found that all nests were on sand ridges, 82% in coarse sand, 8% in coarse sand with gravel and 10% in fine sandy soil. Sand is a good conductor of heat and from these results it can be concluded that coarse to fine river sand, in its pure form, provides the desired temperature required for the incubation of crocodile eggs.

Hutton (1984) singled out exposure to direct sunlight as one of the most important factors in choosing a nesting site, besides access to water. Not only was sunlight required to maintain a suitable incubation temperature but it also influenced the sex determination of embryos (Leslie, 1997). Kofron (1989) found that 97% of the nests during the 1984 season were initially in direct sunlight and Swanepoel (1999) found that along the Olifants River, 56% were exposed to more than six hours of direct sunlight and 36% of nests were exposed to three to six hours of direct sunlight per day. Along the Okavango River the greatest percentage of vegetation consisted of papyrus (*Cyperus papyrus*), phragmites reeds (*Phragmites australis*) and thatching grass (*Miscanthus junceus*) therefore the majority of nests (71%) were exposed to more than six hours direct sunlight per day. Shade for the female is important as she needs to take refuge (Pooley, 1969). If no shade is available in the immediate vicinity, the female has to leave the nest unguarded and thus increase the risk of predation. The Eastern Channel of the Okavango River was found to be well suited as a nesting terrain because of the sparse vegetation cover. The

majority of nests fell within the score 3 category, where the nests were exposed to more than six hours of sunlight per day. Leslie (2001) found that direct sunlight contributed to internal soil temperature, as much as a 6.0 °C difference at a depth of 25.0 cm was found.

This study shows that female crocodiles may have the ability to choose an optimal location for nests so as to avoid predation and ensure that eggs are incubated at the optimal incubation temperature. Janzen (1994) found that female western painted turtles (*Chrysemys picta*) choose the thermal environment of nests, thereby selecting the sex ratio of their offspring. Shine and Harlow (1996) suggest that mothers may be able to manipulate the phenotypes of their progeny indirectly, by inducing particular developmental pathways through selection of nest sites, and thus incubation conditions. In St. Lucia, Leslie (1997) found that Nile crocodiles selected specific oviposition sites two to three weeks prior to oviposition. They chose open, sunny areas which were in close proximity to a fresh water source, clearly avoiding shaded or partly shaded sites.

4.4.2 Nest Temperatures

There was a monthly increase in mean nest temperature along the Okavango River in the 2004/05 nesting season. The average monthly nest temperatures from October to January were 27.5; 29.5; 29.4 and 30.8 °C. Leslie (1997) recorded nest temperatures along Lake St. Lucia in South Africa between November and February in the 1994/95; 1995/96 and 1996/97 seasons and also found a mean monthly increase in temperature. Average nest temperatures from November to February throughout the three seasons were 22.6; 27.7; 30.1 and 30.9 °C.

The average nest temperature along the Okavango River throughout the incubation period was 29.3 °C, which is similar to the findings of Leslie (1997). The average nest temperatures for 1994/95; 1995/96 and 1996/97 were 28.8 °C; 28.3 °C and 31.0 °C respectively. The results from the Okavango River and St. Lucia show a general increasing trend in nest temperature throughout the incubation period. The nest temperature reaches a peak within the last month of incubation, which in the Okavango Delta, is January. This increase in temperature was due to metabolic heating produced within the clutch of eggs. In previous studies Modha (1976) and Webb *et al.* (1983) also suggested that metabolic heat contributes significantly to nest temperatures.

Due to the fact that there was a significant increase in nest (and air) temperature throughout the incubation period, time of nesting plays a large role in the determination of hatchling sex. Two nests, one laid early in the nesting season and one laid later in the season, were focused on, and they differed significantly. The early nest (Nest 2) had an average nest temperature of 32.1 °C, and therefore it can be estimated that this nest produced the majority male hatchlings based on the lower pivotal temperature determined in Chapter 2 of this study.

The late nest (Nest 14) had an average nest temperature of 27.4 °C, which was well below the lower pivotal temperature and therefore probably produced the majority female hatchlings. Leslie (1997) compared nest temperatures of wild Nile crocodile nests in St. Lucia and found that an early nest had an average nest temperature of 26.4 °C, and a later nest had a much higher average temperature of 32.8 °C. The early nest was estimated to produce the majority female hatchlings and the late nest was predicted to produce primarily males. However, results obtained from temperatures from the early and late nests along the Okavango River, suggested that there was a decrease in nest temperature throughout incubation period. The thermosensitive period of the late nest (Nest 14) was from 28 November to 3 January, which coincided with a period of high rainfall. The exact date of the thermosensitive period (TSP) was of utmost importance, as it was the period at which sex was determined (Webb *et al.*, 1987). The TSP date was determined by calculating the middle third of the incubation period and the nests were grouped together based on the TSP dates. It was found that nests that were grouped together based on TSP dates were predicted to produce similar sex ratios.

4.4.3 Sex Ratio of Wild Nests

Mean nest temperatures were used to estimate sex ratios of hatchlings entering the Okavango River. Temperature was recorded from a total of ten nests in which two were predicted to produce the majority male hatchlings and eight of the nests were predicted to produce 100% female hatchlings, based on the pivotal temperatures determined in Chapter 2 of this study. According to these results there was a possibility of a female-biased hatchling sex ratio in the Okavango River in the 2004/05 nesting season. This can be compared to the findings of Leslie (1997), in St. Lucia South Africa, where out of eight nests, one nest was predicted to produce more than 90% males, three nests were predicted to be more than 50% females and the remaining four nests produced 100% female hatchlings. In 1999, Swanepoel recorded nest temperatures from wild crocodile nests along the Olifants River and also found a female-biased sex ratio.

The only two nests predicted to produce the majority male hatchlings were Nest 2 and Nest 11. The thermosensitive period of these two nests was from 27 October – 26 November 2004, and 13 November – 21 December 2004. The TSP range overlapped from 13 – 26 November 2004. When comparing these results to Figure 3.2 (Chapter 3), it was found that during this period air and soil temperature was at a maximum, with the exception of actual hatching time. During the latter part of the season, high rainfall resulted in reduced nest temperatures, thereby producing a greater percentage of female hatchlings from nests later in the season.

4.5 CONCLUSION

Nest site characteristics of 14 wild crocodile nests along the Okavango River were studied and distance to river, depth of nest and exposure to direct sunlight were determined as important factors in nest site location. Results suggested that female Nile crocodiles may be choosing sites in which to lay their eggs where predation was low and soil temperature was at a maximum. The incubation temperature of the eggs was determined by the location of the nest site along the Okavango River, which in turn determined the sex ratio of the hatchlings.

There was a mean monthly increase in nest temperature throughout the incubation period. Nest temperature was found to be the highest during the last month of incubation, which was primarily due to metabolic heating. Date at which the eggs were laid played a critical role in the determination of the sex of hatchlings within a nest. The temperature of ten nests were recorded along the Okavango River in which two were predicted to produce majority male hatchlings and eight nests were predicted to produce 100% female hatchlings. The sex ratios of nests along the Okavango River were thus predicted to be female-biased.

4.6 REFERENCES

CAMPOS, Z. 1993. Effect of habitat on survival of eggs and sex ratio of hatchlings of *Caiman crocodilus yacare* in the Pantanal, Brazil. *Journal of Herpetology* 27(2): 127-132.

CHABRECK, R. H. 1973. Temperature variations in nests of the American alligator. *Herpetologica* 29: 48–51.

COMBRINK, A. S. 2004. Population status of *Crocodylus niloticus* (Nile crocodile) at Lake Sibaya, Republic of South Africa. MSc. Thesis, University of Kwazulu-Natal, Pietermaritzburg, South Africa.

FERGUSON, M. W. AND T. JOANEN. 1983. Temperature-dependent Sex Determination in *Alligator mississippiensis*. *Journal of Zoology, London* 200: 143–177.

GRAHAM, A. 1968. The Lake Rudolf Crocodile (*Crocodylus niloticus*) Laurenti population. Report to the Kenya Game Department, Nairobi, Kenya. Pp.145.

HARTLEY, D. D. R. 1990. A survey of crocodile nests in Umfolozi Game Reserve. *Lammergeyer* 41: 1-12.

HUTTON, J. M. 1984. The population ecology of the Nile crocodile (*Crocodylus niloticus*) laurenti, 1768, at Ngezi, Zimbabwe. PhD Dissertation, University of Zimbabwe.

JANZEN, F. 1994. Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology* 75(6): 1593-1599.

KOFRON, C. P. 1989. Nesting ecology of the Nile crocodile (*Crocodylus niloticus*). *African Journal of Ecology* 27: 335–341.

LEBUFF, C. R. 1957. Observations on captive and wild North American Crocodilians. *Herpetologica* 13: 25-28.

LESLIE, A. J. 1997. The ecology and physiology of the Nile crocodile, *Crocodylus niloticus* in Lake St. Lucia, Kwazulu-Natal, South Africa. Ph.D Dissertation, Drexel University, USA.

MAGNUSSON, W. E. 1979. Maintenance of temperature of crocodile nests. *Journal of Herpetol*ogy 13: 439-443.

MAGNUSSON, W. E., LIMA, A. P. AND R. M. SAMPAIO. 1985. Sources of heat for nests of *Paleosuchus trigonatus* and a review of crocodilian nest temperatures. *Journal of Herpetology* 19 (2): 199-207.

MARAIS, J. AND G. A. SMITH. 1992. Crocodile Husbandry: The care of broodstock, eggs and hatchlings. In: *Conservation and utilization of the Nile crocodile in Southern Africa. Handbook on Crocodile farming*. (Ed. Smith, G. A. and J. Marais). Pp. 63–78.

MODHA, M. L. 1976. The ecology of the Nile crocodile (*Crocodylus niloticus* Laurentii) on Central Island, Lake Rudolf. *East African Wildlife Journal* 5: 74-95.

POOLEY, A. C. 1962. The Nile crocodile: notes on the incubation period and growth rate of juveniles. *Lammergeyer* 2: 1: 1-55.

POOLEY, A.C. 1969. Preliminary studies on the breeding on the Nile crocodile *Crocodylus niloticus* in Zululand. *Lammergeyer* 10: 22-44.

SHINE, R. AND P.S. HARLOW. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77(6): 1808-1817.

SPOTILA, J. R., STANDORA, E. A., MORREALE, S. J. AND G. J. RUIZ. 1987. Temperature-dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. *Herpetologica* 43: 74-81.

SWANEPOEL, D. G. J. 1999. Movements, nesting and the effects of pollution on the Nile crocodile *Crocodylus niloticus* in the Olifants River, Kruger National Park. MSc. Thesis, University of Natal, South Africa.

WEBB, G. J. W., SACK, G. C., BUCKWORTH, R. AND S. C. MANOLIS. 1983. An examination of *Crocodylus porosus* nests in two Northern Australian freshwater swamps, with an analysis of embryo mortality. *Australian Wildlife Research* 10: 571-605.

WEBB, G. J. W., MANOLIS, S. C., DEMPSEY, K. E. AND P. J. WHITEHEAD. 1987. Crocodilian eggs: A functional overview. In: *Wildlife Management: Crocodiles and Alligators*. (Eds. Webb, G. J. W. and S. C. Manolis). Chipping Norton. Surrey Beatty and Sons Pty Ltd. Sydney, Australia. Pp.417-422.

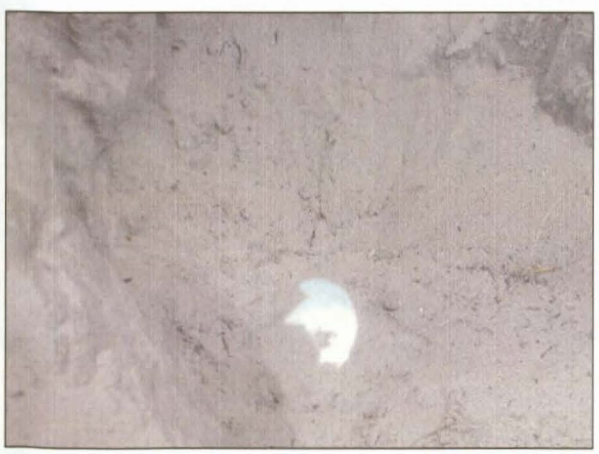
Appendix 4.1 Photographs of the field work carried out to study the nest site characteristics and record nest temperature of wild Nile crocodile nests along the Okavango River. (A) The typical 'slide' made by the female crocodile which was the path used to move between the nest and the river. (B) A nest site characterized by an open sandy area, surrounded by vegetation. (C) The body print of a female crocodile on a nest site. (D) Probing the soil with a 4 mm diameter rod to determine the exact location of the eggs. (E) Uncovering the nest to expose the eggs. (F) The eggs are packed closely together in a hole in the sand. (G) Measuring the depth to the first egg using a metal tape measure. (H) The mass of the eggs was determined by weighing a sub-sample of eggs using a spring scale (± 0.1 g). (I) A sub-sample of eggs from each nest were measured in length and width (± 0.1 mm) using a pair of calipers. (J) A miniature temperature logger, StowAway® Tidbit® (Onset Instruments, Massachusetts, USA) was placed within the nest and programmed to record nest temperature every 15 minutes for the duration of the incubation period (90 days). (K) A wire grid mesh (50 x 50 cm) was placed over the nest to prevent predation attempts.

A

B

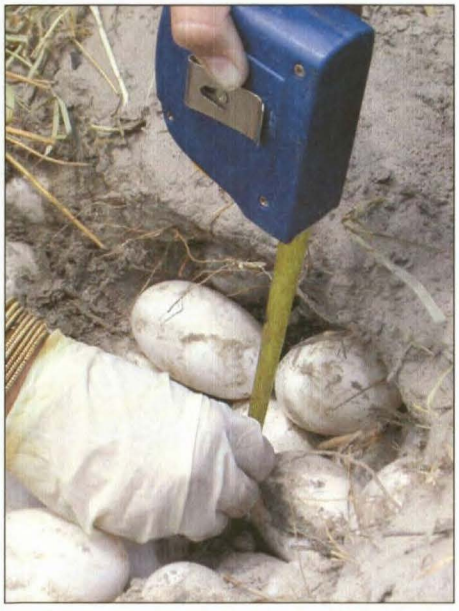
C

D



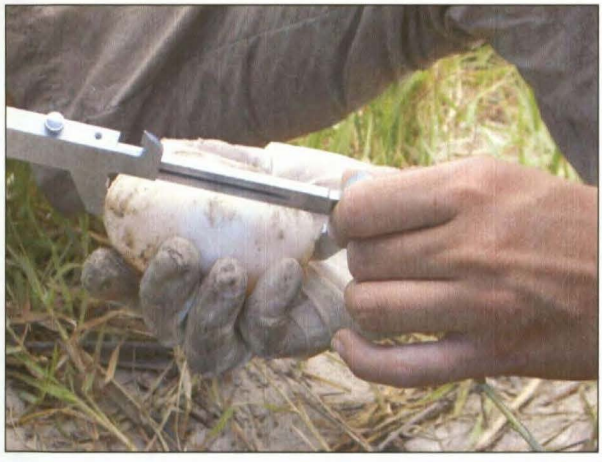
E

F



G

H



I

J

K

L

Appendix 4.2 Summary of the Tukey Test of Nest Temperatures recorded from 10 wild Nile crocodile Nests along the Eastern Channel of the Okavango River, Botswana. Approximate Probabilities for Post hoc Error. Between MS= 1.897, df = 321.

Nest	1	2	5	6	7	8	10	11	12	14
1		**0.000012**	0.706295	0.083764	0.675669	0.995289	**0.014218**	**0.000012**	**0.000677**	**0.000013**
2	**0.000012**		**0.000012**	**0.000012**	**0.000012**	**0.000012**	**0.000012**	1.000000	**0.000012**	**0.000012**
5	0.706295	**0.000012**		**0.000074**	**0.007195**	0.142654	**0.000014**	**0.000012**	**0.000012**	**0.000012**
6	0.083764	**0.000012**	**0.000074**		0.983832	0.558681	0.999997	**0.000012**	0.953810	0.241882
7	0.675669	**0.000012**	**0.007195**	0.983832		0.994587	0.865396	**0.000012**	0.307214	**0.006857**
8	0.995289	**0.000012**	0.142654	0.558681	0.994587		0.234142	**0.000012**	**0.024836**	**0.000098**
10	**0.014218**	**0.000012**	**0.000014**	0.999997	0.865396	0.234142		**0.000012**	0.993042	0.386194
11	**0.000012**	1.000000	**0.000012**	**0.000012**	**0.000012**	**0.000012**	**0.000012**		**0.000012**	**0.000012**
12	**0.000677**	**0.000012**	**0.000012**	0.953810	0.307214	**0.024836**	0.993042	**0.000012**		0.973000
14	**0.000013**	**0.000012**	**0.000012**	0.241882	**0.006857**	**0.000098**	0.386194	**0.000012**	0.973000	

Appendix 4.3 Multiple Comparisons, Kruskal Wallis Test of Nest Temperatures recorded from 10 wild Nile crocodile Nests along the Eastern Channel of the Okavango River, Botswana. H (9, N = 331) = 185.08, $P < 0.05$

Nest	1	2	5	6	7	8	10	11	12	14
1		**4.445868**	1.163888	2.943290	1.640326	0.793621	3.035534	**3.693084**	**4.096834**	**4.606961**
2	**4.445868**		**3.281979**	**7.352566**	**6.086194**	**5.239488**	**7.701470**	0.999962	**8.542702**	**9.272898**
5	1.163888	**3.281979**		**4.097599**	2.804215	1.957509	**4.257035**	2.464487	**5.260722**	**5.828462**
6	2.943290	**7.352566**	**4.097599**		1.316465	2.156202	0.078367	**6.763285**	1.119824	1.478832
7	1.640326	**6.086194**	2.804215	1.316465		0.846706	1.314013	**5.424608**	2.456508	2.885440
8	0.793621	5.239488	1.957509	2.156202	0.846706		2.202630	**4.530828**	**3.303213**	**3.774057**
10	3.035534	7.701470	**4.257035**	0.078367	1.314013	2.202630		**7.121597**	1.264091	1.657765
11	**3.693084**	0.999962	2.464487	**6.763285**	**5.424608**	**4.530828**	**7.121597**		**8.017691**	**8.790092**
12	**4.096834**	8.542702	**5.260722**	1.119824	2.456508	**3.303213**	1.264091	**8.017691**		0.307336
14	**4.606961**	9.272898	**5.828462**	1.478832	2.885440	**3.774057**	1.657765	**8.790092**	0.307336	**4.606961**

CHAPTER 5

CONCLUSIONS

The study of TSD of the Nile crocodile in the Okavango River, Botswana was carried out from June 2004 – June 2006. The objectives of this study were:

1). To determine the influence of egg incubation temperature on embryonic survival and development, incubation period and hatchling sex ratios in laboratory experiments.
2). To determine the lower and upper pivotal temperature of the Nile crocodile in the Okavango River for comparative purposes.
4). To determine optimal nest site characteristics including nest site size, location and nest dimensions.
5). To record incubation temperatures in wild crocodile nests along the Okavango River and to estimate hatchling sex ratios.
6). To determine the possible effect of Global Climate Change on sex ratios of the Nile crocodile population in the Okavango River, Botswana.

5.1 INCUBATION TEMPERATURE OF THE NILE CROCODILE

In this study it was found that incubation temperature of crocodile nests had a profound effect on the development of Nile crocodile hatchlings. The Nile crocodile exhibits temperature-dependent sex determination and thus incubation of the eggs was temperature dependent. An increase in egg incubation temperature resulted in an increase in hatchling body size in terms of SVL (snout-to-vent length), TL (total length) and body mass. It was found that incubation temperature had a significant influence on the duration of incubation and embryonic survival of *Crocodylus niloticus*. An increase in incubation temperature resulted in a decrease in incubation period. The influence of temperature on incubation period can be determined by the rate of development. Relative development rates were slowest at the lower incubation temperatures and fastest at the higher incubation temperatures.

The incubation period of *C. niloticus* in the Okavango River was found to be longer on average when compared to previous studies. This could be due to low soil temperatures which were recorded in this study. Nile crocodile eggs in the Okavango River may require a longer incubation period to reach the desired temperature required for development.

Two different techniques were employed to sex the hatchlings incubated at various constant temperatures. An incubation temperature below and including 31.4 °C and 33.4 °C

produced the majority female hatchlings, and the range between 31.5 °C and 33.0 °C produced the majority male hatchlings. *C. niloticus* in the Okavango River therefore had a female-male-female pattern of temperature-dependent sex determination, where female hatchlings were produced at lower and higher incubation temperatures and males were produced at intermediate incubation temperatures. The Nile crocodile in the Okavango River therefore has a lower and upper pivotal temperature of 31.4 °C and 33.4 °C respectively which can be used as a 'benchmark' temperature to make comparisons of this species between areas. Leslie (1997) determined the upper and lower pivotal temperature in St. Lucia, South Africa (the southernmost extent of the breeding range of the Nile crocodile) as 31.7 °C and 34.5 °C. Hutton (1987) found that incubation temperature of Nile crocodile eggs in Lake Ngezi, Zimbabwe, produced the majority females at an incubation temperature of 31.0 °C and a range between 31.0 °C and 34.0 °C produced primarily male hatchlings. Compared to previous studies *C. niloticus* in the Okavango River therefore had a narrower range of male-producing temperatures.

5.2 SOIL TEMPERATURE AND SOIL MOISTURE OF WILD CROCODILE NESTS

Temperature and moisture content of soil played an important role in temperature-dependent sex determination of the Nile crocodile. Air temperature warmed up the soil, which conducted heat into the crocodile nest providing an incubation temperature for the nest which facilitated in the development of the eggs, as well as determining the sex ratio of hatchlings from the nest. Results from this study show that average soil temperatures along the Okavango River were significantly lower in comparison to the average soil temperatures recorded in St. Lucia, South Africa. The Okavango Delta is set in the semi-arid Kalahari (Mendelsohn and el Obeid, 2004) and therefore experiences extreme fluctuations in day and night air temperatures, which affected the average soil temperatures.

Not only did air temperature influence the temperature of the soil, but a great determining factor was the location of the nest in relation to the river. Soil temperature along the Okavango River varied according to distance from the river and soil depth. Soil temperature was highest (28.3 °C) at a distance of 6.0 m from the river and at a depth of 25.0 cm. This falls below the lower pivotal temperature for Nile crocodiles in the Okavango River and could be regarded as an 'optimal nesting site', as this would ensure a female-biased sex ratio. Nest sites were found at an average distance of 5.2 m from the river and eggs were found at an average depth of 24.8 cm. This showed that the location of a nest in relation to the river plays a large role in determination of the sex ratio of the nest and female crocodiles may have the ability to choose optimal nest sites which allows them to 'control' the sex ratio of the nest.

5.3 NEST TEMPERATURE AND SEX RATIOS OF WILD CROCODILE NESTS

An investigation of nest sites along the Okavango River showed that differences in nest site characteristics were apparent, which influenced incubation temperature and incubation periods. Exposure of nest sites to direct sunlight, distance of nests from the river and depth of nests were found to be primary determining factors in nest site location. Distance of nests from the river and height of nests above the river were used to determine the slope of nests, which was an indication of the amount of energy expended by the female crocodile in reaching her nest. The entire study region was found to be well suited as a nesting area because of the sparse vegetation cover. Results from this study suggested that female crocodiles may be choosing nest sites where predation is low and soil temperature was at a maximum. This supports the findings of Shine and Harlow (1996) who found that in an oviparous lizard the mothers may be able to manipulate their progeny indirectly, by inducing particular pathways through selection of nest sites, and thus incubation conditions.

There was a mean monthly increase in nest temperature along the Okavango River in the 2004/05 nesting season. The average nest temperature recorded from wild nests throughout the incubation period was 29.3 °C, with the highest temperature occurring during the last month of incubation. Nest temperatures recorded from each nest were compared to the pivotal temperatures determined in this study and 80% of the nests studied were predicted to have produced the majority female hatchlings and 20% were expected to have produced the majority male hatchlings. This suggests that Nile crocodile nests in the Okavango Region produced a female-biased sex ratio. Nile crocodile nests in St. Lucia (Leslie, 1997) and in the Olifants River in the Kruger National Park (Swanepoel, 1999) were also found to be female-biased.

Due to the fact that there was a significant increase in nest and air temperature throughout the incubation period, time of nesting plays an important role in the determination of hatchling sex. The thermosensitive periods of an early and late nest were compared and they differed substantially. The early nest was predicted to produce the majority of female hatchlings and the late nest was predicted to produce the majority male hatchlings.

5.4 GLOBAL CLIMATE CHANGE

The Intergovernmental Panel on Climate Change (IPCC, 2001) states that there has been a mean global warming of 0.4 °C to 0.8 °C of the atmosphere at the surface since the late 19th century. The IPCC also predicts that the average temperature of the earth will increase between 1.4 °C and 5.8 °C over the next 100 years (Tonn, 2003). This will have a great effect on the incubation temperature of the Nile crocodile, which will affect the sex ratio of the population. The average air and soil temperature determined during the 2004/05 nesting season was 21.2 °C

and 20.9 °C, whereas the average nest temperature was 29.3 °C. An increase in air temperatures between 1.4 °C and 5.8 °C would result in an average air temperature of 22.6 °C to 27.0 °C. In Chapter 3 of this study it was shown that air temperature influences the temperature of the soil, which in turn influences the incubation temperature of crocodile nests. An increase in the average soil temperature may thus cause the average incubation temperature of nests to exceed the lower pivotal temperature of 31.4 °C. This may shift the female-biased sex ratio of hatchlings to the majority male and lead to a skewed sex ratio of Nile crocodiles in the Okavango River.

An increase in incubation temperature will not only affect the sex ratio of hatchlings but will also affect the probability of embryos surviving, body size at hatching (Hutton, 1987; Webb *et al.,* 1987), the weight of residual yolk at hatching (Webb *et al.*, 1987), hatchling pigmentation patterns (Deeming and Ferguson, 1989), post-hatching growth rates (Hutton, 1987; Joanen *et al.*, 1987) and post-hatching patterns of thermoregulation (Lang, 1987).

Nelson *et al.* (2004) states that reptiles with TSD have previously survived extreme climate change. Extreme climatic changes have occurred in the geological recent past, similar in magnitude to the predicted rise (Janzen, 1994). Extinction was relatively minimal for reptiles with TSD, most species shifted their geographical ranges southward in response of advancing glaciers. However, the short time frame and scale of global temperature increase predicted may result in sex ratio imbalances that will threaten population persistence (Nelson *et al.*, 2004).

Although the propensity to produce offspring of a given sex has a high heritability under constant laboratory conditions, the likelihood that this trait would evolve rapidly enough to keep pace with the rate of climatic warming is slim (Janzen, 1994). The strength of selection would be greater and the intensity of selection would be extraordinarily large, requiring a shift in the mean threshold temperature over the period of changing climatic temperatures (Janzen, 1994). Such a rapid rate of adaptive thermal evolution would be difficult to achieve in organisms with short generation time, much less in long-lived species such as crocodiles.

Species with narrow transitional ranges and long generation times, which indicates limited potential to respond to rapid changes in climate, have four alternatives to global climate change: (i) to modify their geographical range, (ii) to convert to genetic sex determination, (iii) to modify their nesting behaviour, or (iv) to go extinct (Nelson *et al.*, 2004).

In order to modify their geographical range, crocodiles need to move to a cooler environment, where an increase in air temperature will not have a great effect on incubation temperature of the nests and therefore not influence the sex ratio of the hatchlings. In this case this would not be possible, as the Okavango River is a closed system and crocodiles are restricted to the area.

The possibility for crocodiles to convert to genetic sex determination is unlikely, because the sex of crocodiles is not determined by sex chromosomes, but is dependent on the environment the eggs encounter during incubation.

For crocodiles to modify their nesting behaviour the females must be able to 'control' the sex ratio of their offspring and this 'control' must be heritable (Janzen and Morjan, 2001). The Okavango female crocodiles may have the ability to choose optimal nest sites and therefore 'control' the sex ratio of their offspring. However, this trait may have a very low heritability, as it does in a lizard with TSD and would clearly fail in thermally extreme years in this population (Janzen, 1994). Further research is required to determine the degree of heritability of this trait. It is also recommended that nesting sites of breeding females in the Okavango River should be monitored closely over a number of years to determine the relationship between the female's choice of nest site and the global increase in temperature.

Biased sex ratios are already known for reptiles with temperature-dependent sex determination. A general warming trend is already evident in sea turtles (*Chelonia mydas*) nests over the past 100 years at Ascension Island. In addition, findings so far, point to nest site selection by female reptiles for hatching success rather than sex ratio manipulation (Nelson *et al.*, 2004). The way species with TSD respond to global warming has implications for the conservation of these animals (Janzen and Paukstis, 1991). The long-term survival of crocodiles is dependent on a sufficient range of incubation temperatures to ensure that both male and female hatchlings are produced. However, it is not known how skewed hatchling sex ratios will affect the adult population sex ratio. Crocodiles have long generation times and individuals can reproduce for many years. Attention is required in the implications of TSD for the future survival of crocodiles under scenarios of Global Climate Change. In short, warming temperatures might lead to a population of only male hatchlings and so to extinction of the Nile crocodile in the Okavango River, Botswana.

5.5 RECOMMENDATIONS FOR CROCODILE FARMS

The results from this study will aid management of crocodile farms in Botswana and elsewhere in Southern Africa. The lower and upper pivotal temperature for incubation of Nile crocodiles in the Okavango River was determined in constant temperature experiments. Crocodile farms should incubate eggs at a temperature within the range of 30.0 °C to 31.4 °C to produce female hatchlings and incubate their eggs between 32.0 °C and 33.0 °C to produce the majority male hatchlings. Eggs should be incubated for a period of approximately 100 days, this will allow for determination of sex and the hatchlings would have undergone complete development.

A better understanding of TSD and successful artificial incubation of Nile crocodile eggs is important for conservation and management of the species. Crocodile farms need to produce primarily male hatchlings as they grow faster and are larger in size. Therefore, knowledge of the incubation temperature required to produce male hatchlings would be important for a re-stocking programme. It has been shown that climate has an effect on the incubation temperature of crocodile nests in the wild and therefore Global Climate Change will affect the sex ratio of the hatchlings. To compensate for the rarer sex it is recommended that crocodile farms incubate a sub-sample of their eggs below 32.0 °C to ensure female hatchlings are produced and can be released into the wild to ensure the adult population in the Okavango River does not become male-biased.

5.6 REFERENCES

DEEMING, D. C. AND M. W. J. FERGUSON. 1989. The mechanism of temperature dependent sex determination in crocodilians: A hypothesis. *American Zoology*. 29: 973-985.

HUTTON, J. M.1987. Incubation temperatures, sex ratios and sex determination in a population of Nile crocodiles, *Crocodylus niloticus*. *Journal of Zoology* 211: 143–155.

INTERNATIONAL PANEL ON CLIMATE CHANGE (IPCC). [online] 2001. Summary for Policymakers: A Report of Working Group I of the Intergovernmental Panel on Climate Change. Available from htpp://www.ipcc.ch.
Accessed 16 May 2005.

JANZEN, F. J. 1994. Climate change and TSD. *Proceedings of the National Academy of Sciences. USA*. 91: 7487–7490

JANZEN, F. J. AND C. L. MORJAN. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour* 62: 73–82.

JANZEN, F. J. AND G. L. PAUKSTIS. 1991. Environmental Sex Determination in reptiles: ecology, evolution, and experimental design. *The Quarterly Review of Biology* 66 (2): 149–179.

JOANEN, T., MCNEASE, L. AND M. W. J. FERGUSON. 1987. The effects of egg incubation temperature on post-hatching growth of American alligators. In: *Wildlife Management: Crocodiles and Alligators*. (Ed. Webb, G. J., Manolis, S. C. and P. J. Whitehead). Surrey Beatty and Sons. Sydney, Australia. Pp. 533-537.

LANG, J. W. 1987. Crocodilian thermal selection. In: *Wildlife Management: Crocodiles and Alligators*. (Eds. Webb, G. J., Manolis, S. C. and P. J. Whitehead). Surrey Beatty and Sons, Sydney, Australia. Pp. 301–315.

LESLIE, A. J. 1997. The ecology and physiology of the Nile crocodile, *Crocodylus niloticus* in Lake St. Lucia, Kwazulu-Natal, South Africa. Ph.D. Dissertation, Drexel University, USA. Pp. 5–10.

MENDELSOHN, J. AND S. EL OBEID. 2004. Okavango River: The flow of a lifeline. First edition. Struik Publishers, Cape Town.

NELSON, N. J., THOMPSON, M. B., PLEDGER, S., KEALL, S. N. AND C. H DAUGHERTY. 2004. Do TSD, sex ratios and nest characteristics influence the vulnerability of tuatara to Global Warming? *International Congress Series* 1275: 250–257.

SHINE, R. AND P. S. HARLOW. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77(6): 1808-1817.

SWANEPOEL, D. G. J. 1999. Movements, nesting and the effects of pollution on the Nile crocodile, *Crocodylus niloticus* in the Olifants River, Kruger National Park. MSc.Thesis, University of Natal, South Africa.

TONN, B. 2003. An equity first, risk-based framework for managing Global Climate Change. *Global Environmental Change* 13: 295 – 306.

WEBB, G. J. W., MANOLIS, S. C., DEMPSEY, K. E. AND P. J. WHITEHEAD. 1987. Crocodilian eggs: A Functional Overview. In: *Wildlife Management: Crocodiles and Alligators*. (Eds. Webb, G. J. W. and S. C. Manolis). Chipping Norton: Surrey Beatty and Sons Pty Ltd. Sydney, Australia. Pp. 417-422.