

**THE EFFECT OF INCUBATION TEMPERATURE ON  
HATCHING SUCCESS AND HATCHLING SEX RATIOS  
OF LOGGERHEAD TURTLES (*CARETTA CARETTA*)  
IN KWAZULU-NATAL, SOUTH AFRICA**

by

Melissa K. Boonzaaier

Submitted in fulfilment of the academic requirement for the degree of Master in Science in  
the Department of Zoology at the Nelson Mandela Metropolitan University

January 2011

Supervisor: Dr Ronel Nel

Co-supervisor: Dr Alison Leslie

# DECLARATION

**Full name:** Melissa Kay Boonzaaier

**Student number:** 209091824

**Qualification:** Master in Science

**Declaration:**

In accordance with Rule G 4.6.3, I hereby declare that the above-mentioned dissertation is my own work and that it has not been previously been submitted for assessment to another University or for another qualification.

**Signature:** .....

**Date:** .....

## Summary

South Africa has an exceptional programme conserving and monitoring female loggerhead turtles nesting in KwaZulu-Natal. Finer scale research such as sex ratios of hatchlings produced or hatching and emergence success have not recently been conducted. Previous studies on these aspects date back two to three decades. These are critical population parameters as they provide an indication of the future success of the population. Sea turtles, like most other reptiles, do not possess heteromorphic chromosomes and the incubation temperature during the middle third of embryo development determines sex. This is called temperature-dependent sex determination (TSD). Incubation temperatures in sea turtle nests are entirely dependent on environmental conditions and hence structured by factors such as seasonal ambient and sand temperature, rainfall, shading by vegetation, dunes or developments, sand colour, depth of eggs etc. Metabolic heat generated by embryos also increases incubation temperature, especially towards the end of incubation. The aim of this study was to examine the effect of the thermal environment within the nests of loggerhead (*Caretta caretta*) hatchling sex ratios and hatching success. This was conducted in KwaZulu-Natal, South Africa. The results were also compared to the findings by Maxwell *et al.* 1988 in which the authors suggested a female-biased sex ratio for the peak nesting area. However, the present study focused on a 5 km stretch of beach which forms part of the high density nesting area for loggerhead turtles, was carried out over two nesting seasons (2008/9 and 2009/10). In December during the peak nesting season, temperature data loggers were deployed in the nests. Seven iButtons recorders were buried at nest depth along the beach to measure sand (*in situ*) temperature. The data loggers were retrieved in the hatching season (February/March) and nests were excavated post-hatching. Two methods were used to estimate hatchling sex ratios, namely (i) predicting sex ratios using sand and nest temperatures, and (ii) by determining the sex using gonadal histology. Mean incubation temperature (for 2008/9 season) was 29.4°C (n = 6), and the overall nest success (i.e. nests that successfully hatched > 20% of clutch) was high (94%, n = 110). In the second season (2009/10) mean incubation temperature was 30.0°C (n = 14) with a nest success of 88% (n = 223). Sand temperatures during the same time (2009/10) was 29.0°C which suggested metabolic heating contributed +1.0°C to the nest temperature. Even though sand temperatures were not recorded during the first season, nest temperature results suggested that the second season was marginally warmer, although both seasons had a mean nest temperature close to, or just warmer than the

theoretical pivotal temperature for loggerheads (29.0°C). Incubation temperature predicted a female-biased sex ratio of 85%. Histological examinations confirmed this result with an overall female bias of 79% (n = 134). The theoretical pivotal temperature estimated for this population (combining previous data and the current study) was 29.2°C. This is fractionally higher than the previous estimate of 29.0°C. Dedicated conservation regulations may provide a partial reason for the positive turtle population recovery especially during the last decade.

## Acknowledgements

Where do I begin? Two years ago, while completing my BScHons at Stellenbosch University, I would have never thought that I would have grown and gained this much knowledge, wisdom and scientific skills as I did in the past 2 years. There are so many people that I need to thank, who without nothing would have been possible.

I contacted Dr Ronel Nel two and a half years ago, almost desperate to do my MSc on sea turtles after I became fascinated with them during a herpetology module course. She responded enthusiastically with a great project and a bursary (which was indeed necessary!). Even though I was not keen to kill tiny cute hatchlings and knew absolutely nothing about histology, I thought it will be a great challenge! So, after these 2 years, loving my research project (with all its hassles!), receiving bursaries for both years and the opportunity to travel for the first time overseas to India for an international sea turtle symposium, I really want to thank Dr Nel for all her input, contribution, opinions (however “harsh” they may be) and pushing me to the limits. You never gave up on me, thank you for that.

We got Dr Alison Leslie on board as co-supervisor, a lecturer at Stellenbosch University who I always admired because of her love and interest for reptiles. Your input and contribution to this research project is also highly appreciated. Now, my family...

I want to thank my parents, Sonia and Richard, and my brother Wesley for always being interested in my studies. I know repeating what I do to other people, might not have been easy, but the fact that you tried to understand what I do and supported me through my tough times (especially my first few months of depression – being away from my family, starting anew in a different city and university), has been such a blessing. So, thank you for giving me the chance to spread my wings (even though Mom tried to keep me at home!) after spending 22 years under your roof and protecting me. Now I can truly say that I am more independent, emotionally stronger and “streetwise” than I used to be. Of course, I cannot forget to mention my extended family members. To “Ouma” Caroline, who REALLY never understood what I do and always asking: “So, what can you actually *do* with your degree?”, but nonetheless, you always said that you are proud of me. To Auntie Lorraine, my aunt in conservation, and Uncle Julian, my uncle the biology teacher and environmental enthusiast, who got me interested in just loving nature since I was young. Thank you for our “nature” chats and being enthusiastic about nature, and my research, as I am. Uncle André, my mentor uncle, thanks

for the intellectual talks and always encouraging me to further my studies. To all my cousins, other aunts and uncles not mentioned please know that all of you played a huge role in my success due to your encouragements, interest and support.

To all my “sisters”: Tracey (my “doido amiga”), who is now Dr Tracey-Leigh Abrahams, and her family, thanks for your all support in everything I do and always being there. Lauren “Lols” Saal and Natalie “Nats” Schoeman, my friends for many years, thank you for keeping me motivated and for your wise advice in just about everything (non-scientific that is!). Terry Reynolds, my dear friend, whom I have been studying BSc and BScHons with, is also completing her MSc this year (whoohoo!). You are my “nature” buddy, anything like camping, hiking, diving or any outdoor activity I know I can call you and you will be as excited as I am to do it! Jenny Tucek, thanks for being an amazing friend and colleague. Even though our “very honest” moments at times turn into heated conversations, I enjoyed the sampling trips with you and off-campus activities \*wink\*.

To my “brothers”: Thank you Marinus de Jager for your friendship through all the years when we started BSc together. Your love for nature, research and science has been fantastic, it is always great having a conversation with you, whether it be intellectual or just joking around over a beer (for you) and (maybe, if I am lucky) Amarula for me (haha). Leighton Mouton, friend, field ranger and general-knowledge-guy, thanks for everything and helping out in the lab when I needed someone and always being interested in, and excited about what I do.

To my “PE family”: I want to thank Daniel Bezuidenhout, Anneen van Rooyen, Juan Botha, Dane Uys and the rest of cell group of Harvest Christian Church, for their utmost support and love for me (even tolerating my jokes – haha!). If it was not for all you guys, I would have been a lost soul. You brought meaning into my life, and made my stay in PE unforgettable. Thank you for accepting me into your lives just the way I am and welcoming me with open arms. For all of you that visited me at the office for a chat over coffee, thanks for that! My days on campus have always been pleasurable especially when you came around! My office buddy and dear friend, Karien Bezuidenhout, thank you for your support, always caring about me (even though you do not say it, I know you love me!) and our intellectual talks. And also letting me know when I’m singing along to my music too loudly while we’re working (oops)...

I want to thank all the volunteers, and students, for collecting data in the field during the nesting and hatching seasons from 2008 - 10: Maria Combrink, Marié Botha, Christiaan

Conradie, Cornia Joubert, Marinus de Jager, Jenny Tucek, Lea Ezzy, Karien Bezuidenhout, Anje De Wet, Linda Harris and Wayne Brazier, and for those that came to help when I was not there. Without all your help and precision for data collection, my research project would not have been a success. A special thanks to Linda, for your GIS expertise and helping with the maps. Not to forget, the staff and postgrad students of NMMU Zoology Department. Thanks to Dr Gideon Rossouw and Dr Shirley Parker-Nance for your advice and input on histology. Your knowledge really got me through it. Thank you, Maggie, for always helping me when I needed you to. Thanks Piet for always willing to help out with technical problems. To all the postgrad students: Vishu Beeharry Panray, Veronica “Hermana” Chaidez, Cornia Joubert, Ryan Wasserman, Cherize Mattheus, Adrian Evans, Wesley De Klerk, Shaun Welman and all of you have made my time spent on campus (and off-campus) great!

Finally, a special thanks to Eric Mlaba and his team at Bhanga Nek. You were always so helpful when we “take over” each year, and whenever there’s a problem, you never hesitate to help us out. Thank you very much! I would also like to thank Ezemvelo KZN Wildlife management for their contribution towards the success of this project and the NRF for funding the research project.

# Table of Contents

Title page.....	i
Declaration.....	ii
Summary.....	iii
Acknowledgements.....	v
Table of contents.....	1
<b>Chapter 1: General introduction.....</b>	<b>3</b>
Introduction.....	3
<i>Sediment characteristics</i> .....	4
<i>Nest site characteristics</i> .....	4
<i>Nest depth</i> .....	5
<i>Turtle egg development</i> .....	5
<i>Hatching success and hatchling emergence</i> .....	6
<i>Temperature-dependent sex determination (TSD)</i> .....	7
<i>Effects of climate change on incubation temperatures</i> .....	8
<i>Problem identification: From a South African perspective</i> .....	9
Thesis outline.....	11
<b>Chapter 2: Study site.....</b>	<b>13</b>
Introduction.....	13
Study site.....	16
The South African long-term turtle monitoring programme.....	19
<i>Monitoring programme</i> .....	19
<i>Threats</i> .....	21
<i>Southernmost rookery</i> .....	22
<b>Chapter 3: Thermal incubation environment of loggerhead (<i>Caretta caretta</i>) nests in KwaZulu-Natal, South Africa.....</b>	<b>23</b>
Abstract.....	23
Introduction.....	24
<i>Factors affecting incubation temperature</i> .....	24
<i>Ex situ factors</i> .....	24
<i>Nest location</i> .....	25
<i>In situ factors</i> .....	26
<i>Effects of incubation temperature on hatching success</i> .....	26
Materials and methods.....	28
<i>Study site</i> .....	28
<i>Data logger calibration</i> .....	28
<i>Climatic data</i> .....	29
<i>Sand temperature</i> .....	29
<i>Incubation temperature</i> .....	31
<i>Nest monitoring</i> .....	31
<i>Statistical analyses</i> .....	32
Results.....	33
Discussion.....	47
Conclusion.....	53



<b>Chapter 4: Histological examinations to determine hatchling sex ratios of loggerhead turtles (<i>Caretta caretta</i>) in KwaZulu-Natal, South Africa .....</b>	<b>54</b>
Abstract .....	54
Introduction .....	55
Materials and Methods .....	57
<i>Study site</i> .....	57
<i>Field sampling</i> .....	57
<i>Histology</i> .....	58
<i>Histology – Refining procedures</i> .....	61
<i>Statistical analyses</i> .....	62
Results .....	62
Discussion .....	67
Conclusion .....	70
<b>Chapter 5: Predicting sex ratios using incubation temperature in loggerhead sea turtles (<i>Caretta caretta</i>) nesting in KwaZulu-Natal, South Africa .....</b>	<b>71</b>
Abstract .....	71
Introduction .....	72
Materials and methods .....	74
<i>Statistical analyses</i> .....	74
Results .....	75
Discussion .....	82
Conclusion .....	85
<b>Chapter 6: Conclusions: The implications of incubation temperature on TSD in the light of global warming and possible conservation strategies .....</b>	<b>86</b>
<b>Appendices .....</b>	<b>99</b>
Appendix A .....	99
Appendix B .....	103

# Chapter 1

## General introduction

### *Introduction*

Marine turtles belong to the class Reptilia (order: Chelonia) which is one of the oldest living reptile (and vertebrate) groups (Spotila, 2004). Originating from ancestral freshwater turtles which moved to sea, early turtles lived amongst the dinosaurs (Spotila, 2004). Modern sea turtles arose about 110 million years ago (Spotila, 2004). Four families of sea turtles were known from the Cretaceous Period, but only two lineages persisted through the Eocene (55-38 mya) (Bowen, 2003). Within these two families, namely Cheloniidae and Dermochelyidae, only seven extant species of sea turtles are found (Spotila, 2004). The family Cheloniidae constitute six of the extant species; this includes the green turtle (*Chelonia mydas*), loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), olive ridley (*Lepidochelys olivacea*), hawksbill (*Eretmochelys imbricata*) and finally the flatback (*Natator depressus*). The family Dermochelyidae has a single species, the leatherback turtle (*Dermochelys coriacea*) (Bowen, 2003; Spotila, 2004).

Sea turtles, along with sea snakes, are the only reptiles that live in the ocean making them unique in their biology and ecology among the reptiles. Individuals of both sea turtle families however have very similar reproductive strategies. Similarly to most reptiles, sea turtles are ectotherms but are the only reptiles, along with fresh water turtles, that change habitat to breed: females come to land to lay soft-shelled eggs (Spotila, 2004).

Female sea turtles excavate nest chambers on sandy beaches into which a large number of eggs (i.e. between 50-120 eggs) depending on the species are deposited. The environment dictates successful incubation of these eggs by regulating embryonic development (Miller, 1997; Godfrey *et al.*, 1997). Beach conditions, such as sediment characteristics, presence/absence of vegetation, and climate (including rainfall, air temperature and wind) are all factors that affects turtle embryo development (Wood and Bjorndal, 2000a). Unfavourable conditions such as extended rainfall periods, extreme heat, root infiltration by vegetation and insufficient moisture may inhibit embryo development or deformities (Ackerman, 1997), resulting in longer/shorter incubation periods (Broderick *et al.*, 2000; Houghton *et al.*, 2007)

and altered sex ratios (Hewavisenthi and Parmenter, 2000; Houghton *et al.*, 2007). The mechanism is that water-, gas- and heat exchange within the nest depends on these environmental conditions along with the dimensions of the nest cavity and nest depth (Carthy *et al.*, 2003). Each of the most important factors affecting embryo development will be reviewed.

### Sediment characteristics

Sand characteristics have been highlighted in the literature as critical in regulating the incubation environment. Water content, albedo (reflectivity), mean grain size, and thermal and hydraulic properties (i.e. how quickly heat and water move through the sand) are among the factors investigated (Carthy *et al.*, 2003). Light coloured sand (such as coral or high calcium sand) is cooler than dark sand (typically volcanic or high mineral contents) when exposed to the same intensity of radiation (Hanson *et al.*, 1998). Sand particle size determines how quickly thermal energy moves through the substratum (Carthy *et al.*, 2003). Heat moves easier through compact sand than through coarse, unconsolidated sand (Speakman *et al.*, 1998). Sediment sorting determines the exchange of water and gases among the eggs and the environment, with poorly sorted sand reducing gas exchange (Mortimer, 1990). Water concentration (saturation) and osmotic potential of the sand regulates the exchange of water among the eggs and the sand (Carthy *et al.*, 2003). As interstitial salinity increases, osmotic potential increases, hence retarding moisture uptake by the eggs, resulting in a high likelihood that the eggs will dehydrate (Carthy *et al.*, 2003).

### Nest site characteristics

Tidal range, beach slope, wave exposure and the placement of the nests also affects the incubation environment within the nest (Hamann *et al.*, 2003). If a turtle nest is located too close to the tidal zone, the nest may be inundated by high tides or storm surges reducing egg survival (Mrosovsky, 1983; Wood and Bjorndal, 2000a). The risk of failed development increases with frequency and period of inundation, which in turn is a function of drainage. In addition, nests closer to the tidal zone, should experience cooler temperatures. In contrast to nesting close to the sea, hatchlings from nests further upshore/inland may struggle with post-hatching orientation, thereby increasing the risk of predation, desiccation and heat stress (Mrosovsky, 1983; Hays and Speakman, 1993; Wood and Bjorndal, 2000a; Kamel and Mrosovsky, 2004; Kamel and Mrosovsky, 2005).

Nest site selection is the nonrandom placement of eggs within a particular area (Kamel and Mrosovsky, 2005), and tends to be species specific. Leatherbacks prefer to nest in areas of open sand (Whitmore and Dutton, 1985; Kamel and Mrosovsky, 2004) whereas hawksbill turtles tend to nest close to, or in the forest vegetation (Kamel and Mrosovsky, 2005). Green turtles prefer to lay their eggs in vegetated areas (Whitmore and Dutton, 1985) whereas loggerhead nests are located near vegetation (Hays and Speakman, 1993; Hays *et al.*, 1995; Kolbe and Janzen, 2002). It has been suggested that all these preferences may be correlated to sand temperature (see Kolbe and Janzen, 2002).

### Nest depth

Incubation temperature and temperature stability is affected by the depth at which a female buries her eggs (Carthy *et al.*, 2003). Both incubation temperature and temperature variation decrease with an increase in nest depth (Foley, 1998). Loggerhead nests showed greater diel temperature fluctuation than the deeper nests of green turtles nesting on the same beaches in eastern Mediterranean (Godley *et al.*, 2001a). Temperature variation for green turtles was measured, nest depth usually at about 70 cm, to be as little as 0.5°C for rookeries in Australia (Booth and Freeman, 2006), Ascension Island (Hays *et al.*, 1999; Godley *et al.*, 2002a) and the Mediterranean (Broderick *et al.*, 2000; Godley *et al.*, 2001a). In contrast, smaller-sized loggerhead turtles, had within-nest temperature variation > 0.5°C was recorded in South Africa (Maxwell *et al.*, 1988), the Mediterranean (Godley *et al.*, 2001a; Godley *et al.*, 2001b), Florida (Foley, 1998) and Australia (Maloney *et al.*, 1990; Chu *et al.*, 2008) where nest depth was about 50 cm.

### Turtle egg development

To examine the effects of temperature fluctuations on embryonic development, the authors incubated loggerhead eggs in the laboratory under different, but controlled conditions (Yntema and Mrosovsky, 1980). They found that the minimum temperature for successful development of loggerhead embryos was 25°C (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982). At this temperature embryo development was slow, but normal and the incubation period lasted for up to 13 weeks (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982). The maximum temperature for successful embryonic development was 34°C where development was rapid and completed in 6.5 weeks. This rapid development

increased the number of deformities (Yntema and Mrosovsky, 1982). Thus, incubation period and success varies inversely with temperature (Miller *et al.*, 2003).

Field-based studies that examined incubation temperatures and - duration concerning embryonic developmental rate, concurred with the laboratory experiments. Loggerheads nesting in Alagadi, in the Mediterranean, had incubation periods between 42 and 61 days (or 7 to 10 weeks) depending on temperature (Fig. 1.1) (Godley *et al.*, 2001a). Similar results were obtained for green turtles at Ascension Island (Godley *et al.*, 2002b).

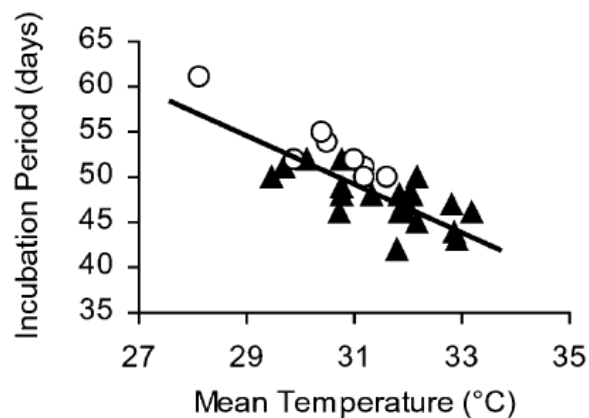


Fig. 1.1. The relationship between incubation duration and mean nest temperature of 23 loggerhead nests in Alagadi, northern Cyprus in the Mediterranean (from Godley *et al.* 2001).

#### Hatching success and hatchling emergence

Hatching success, i.e. the number of hatchlings that emerge from eggs relative to the clutch size, decreases with increasing incubation temperatures (Miller, 1999). This was the case with loggerheads in the Mediterranean (Miller, 1999). In Mon Repos, Australia, Chu *et al.* (2008) did not find a significant correlation between hatching success and incubation temperature, but rather with emergence success. The latter is defined as the number of hatchlings that successfully emerged from the actual nest chamber (Miller, 1999). Either way, hatchling production is negatively correlated with incubation temperature. Emergence success is generally lower than hatching success because deformed, weak and/or late hatchers tend to get stuck in the nest chamber without the assistance of the rest of the clutch through synchronous hatching (Miller, 1999).

Hatchlings use a series of environmental cues to initiate emergence from the nest. Most of these cues are temperature related (Hays *et al.*, 1992), but also include visual cues (Moran *et al.*, 1999; Drake and Spotila, 2002). Moran *et al.* (1999) examined thermal cues for hatchling emergence in loggerhead turtles. They demonstrated that hatchling emergence was controlled by a critical threshold temperature of 32.4°C. Hatchlings do not emerge until the sand temperature drops below this threshold temperature which is generally at/after sunset, and/or on cool, rainy and/or cloudy days (Moran *et al.*, 1999; Miller *et al.*, 2003).

Extreme temperatures (either too high or too low) can also induce physiological restraints, limiting hatchling emergence. Drake and Spotila (2002) conducted a study on the critical thermal maxima (CTM) for leatherback and olive ridley hatchlings on the Pacific coast of Costa Rica. Results from their study suggested that sand temperature closer to the surface is the crucial factor inducing thermal inactivation of hatchling emergence. Moreover, they found that hatchlings of both *Lepidochelys olivacea* and *Dermochelys coriacea* suffered from uncoordinated movements at induced temperatures of 35.7°C and 33.6°C, respectively. Nocturnal emergence is therefore beneficial for the survival of post-hatchlings because of the cooler conditions (Miller *et al.*, 1999). If hatchlings emerge during the day sand temperatures can be fatal and they are more vulnerable to predation (Moran *et al.*, 1999; Miller *et al.*, 2003).

#### Temperature-dependent sex determination (TSD)

In amniote vertebrates, such as birds and mammals, gender is genetically determined (Janzen, 1994). Unlike these taxa, turtles and many other reptiles do not possess heteromorphic sex chromosomes (Spotila, 2004; Valenzuela, 2004a). Instead, most reptilian embryos use egg incubation temperatures to determine the sex of individuals in a process called temperature-dependent sex determination (TSD) (Spotila, 2004; Valenzuela, 2004b). During the middle third of incubation, also referred to as the thermosensitive period (TSP), the temperature of the nest determines the sex of the hatchlings (Yntema and Mrosovsky, 1982; Broderick *et al.*, 2000; Spotila, 2004).

Temperature-dependent sex determination has been described in all sea turtle species: flatback (*Natator depressus*) (Hewavisenthi and Parmenter, 2000), Kemp's ridley (*Lepidochelys kempii*) (Carrasco *et al.*, 2000), olive ridley (*Lepidochelys olivacea*) (Wibbels *et al.*, 1998), hawksbills (*Eretmochelys imbricata*) (Mrosovsky *et al.*, 1992), leatherbacks

(*Dermochelys coriacea*) (Mrosovsky *et al.*, 1984), green turtles (*Chelonia mydas*) (Mrosovsky *et al.*, 1984; Kaska *et al.*, 1998) and loggerheads (*Caretta caretta*) (Maxwell *et al.*, 1988; Maloney *et al.*, 1990; Mrosovsky and Provancha, 1992). The pivotal temperature in sea turtles is the (mean) incubation temperature that yields a 1:1 sex ratio per clutch (Yntema and Mrosovsky, 1980; Alexander and Marais, 2007). Pivotal temperatures vary slightly between turtle populations. Green turtles from Costa Rica for example, have a pivotal temperature of 29.5°C, whereas in Suriname the pivotal temperature for green turtles is 28.8°C (Spotila, 2004).

Generally, in sea turtles, lower incubation temperatures (< 29°C) result a majority of male hatchlings while higher temperatures (> 29°C) produce females (Marcovaldi *et al.*, 1997; Carthy *et al.*, 2003). These sex-specific temperatures are species specific and may differ between populations. In loggerhead populations from the Mon Repos rookery, in Queensland, Australia, male hatchlings were produced at 27.6°C and 100% females were produced above 30.9°C (Chu *et al.*, 2008). Loggerheads from southeastern USA, displayed 100% male production only at 29.5°C and 100% female clutches at 30.4°C (Spotila, 2004).

#### Effects of climate change on incubation temperatures

Even though marine turtles have survived multiple climate change events, it has been speculated that sea turtles and other reptiles displaying TSD, may not be able to adapt to the current, rapid rate of climate change (Janzen, 1994). Warming temperatures may result in female-bias hatchling production and so to the eventual detriment of individual populations, with possible extinction (Davenport, 1997; Matsuzawa *et al.*, 2002). Hays *et al.* (2003) examined the possible effects green turtles may endure due to climate change by reconstructing 150 years of incubation temperature data by using sand- and air temperature relationships of nesting beaches at Ascension Island. From these data, they suggested a general increase in sand temperature and resultantly increased nest temperatures, with an increased production of females (Hays *et al.*, 2003). Mazaris *et al.* (2006) examined the effect of climate change on loggerhead turtles on Zakynthos Island (Greece) using nesting data collected between 1984 and 2002. The authors concluded that average clutch size decreased with an increase in sea surface temperature (SST). Increased SST also resulted in earlier nesting and hatching (Mazaris *et al.*, 2008). The impacts of rising sea temperatures due to global climate change may thus have a significant influence on the demography and

reproductive output and success of these endangered species which in turn put significant constraints on conservation efforts (Janzen, 1994; Hays *et al.*, 2003). The picture is more complicated as increased rainfall may decrease sand temperature with the opposite overall effect, i.e. a male bias. However, this will also be detrimental to individual populations if it persists beyond specific seasons in the long term.

*Problem identification: From a South African perspective...*

Global climate change in addition to anthropogenic activities such as direct harvesting of eggs and adults, habitat destruction and incidental fishing (which include accidental by-catch and boat strikes) may harm already threatened sea turtle populations (Miller and Harley, 1996; Spotila, 2004; Alexander and Marais, 2007). Fortunately, much is being done globally, and specifically in South Africa, to conserve these migratory creatures (Musick, 1999). South Africa had an active and extensive turtle conservation and monitoring programme since 1963 (McAllister *et al.*, 1965).

Numerous studies have been conducted globally on the relationships between nest temperature, hatching success and hatchling sex ratios for various sea populations (Mrosovsky *et al.*, 1984; Chan and Liew, 1995; Kaska *et al.*, 1998; Öz *et al.*, 2004; Kaska *et al.*, 2006). However, these relationships are understudied for sea turtles nesting in South Africa and were previously conducted for one season only. Two sea turtle species, namely loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) turtles nest along the north-eastern coast in KwaZulu-Natal. This is likely to be the southern-most turtle rookery in the world (27°S), with turtles at the (lower) edge of their thermal distribution range.

To date, only one study from South Africa (Maxwell *et al.*, 1988) has been published on incubation temperatures and the effect thereof on sex ratios of loggerheads. Histological examinations were conducted on embryos sourced from nests of known incubation temperatures. The results from Maxwell *et al.*'s (1988) study revealed a male-bias with only seven out of 19 nests having 75% or more female hatchlings. All embryos were male when incubation temperature was on average below 28.5°C during the middle third of incubation, and average temperatures above 29.6°C produced 100% female clutches (Maxwell *et al.*, 1988).



Two decades since the above mentioned study, we question the representivity of the results of the current study, especially given the likelihood of small effects of increasing environmental temperature. In addition, better technology is available to monitor incubation temperature more accurately. Further, beach/dune systems are dynamic and these changes may affect the incubation environment. The beach has gone through a period of erosion (Harris, 2008) and has cut back into the adjacent sand dunes, and vegetation abundance has changed. Tourism has also increased somewhat in the area, especially since the completion of a national road to Mozambique. It has not yet been established as to whether any of these changes have affected incubation temperature in any way. The main focus of the present study is therefore to re-measure and compare the parameters measured by Maxwell *et al.* (1988) and to establish how current incubation temperatures control incubation- and hatching success for loggerheads nesting in South Africa.

The approach that was applied was to compare the estimated sex ratios from sand- and incubation temperature with the results of histological examinations. This will enlighten our understanding of the population demography of sea turtles found in South Africa. For example, in the study on nest temperature and sex ratios of loggerhead turtle hatchlings from the Fethiye beach rookery (Turkey), by Kaska *et al.* (2006), estimated hatchling sex ratios using nest temperatures and compared the results to actual sex ratio data from histological examinations of dead hatchlings and embryos from various nests. Using histological techniques the study confirmed a greater proportion of female hatchlings per clutch for the Fethiye study area. Thus, histological examinations provide more accurate estimate of the number of female hatchlings which may be more reliable than an estimated sex ratio from temperature values only. These results will also have direct implications for conservation. Furthermore, if the true sex ratios are close to the estimated sex ratios, future monitoring will only require the monitoring of incubation temperature rather than sacrificing hatchlings and conducting time-consuming histological examinations.

## ***Thesis outline***

The objective of this study was to investigate the effect of temperature on incubation success and sex ratios for *Caretta caretta* in northern KwaZulu-Natal. These findings were compared with the results found by Maxwell *et al.* (1988) to detect possible (small) changes in hatchling sex ratios of the species. The specific aims were to:

1. describe the thermal microhabitat of the nesting beach;
2. predict sex ratios by using incubation temperature;
3. confirm sex ratios of turtle hatchlings by using histological examinations and
4. estimate the theoretical pivotal temperature for this population

Chapter 2 is a description of the study site, including beach topography. It also describes the details with regards to the long-term monitoring and protection programme. In this study, we only focussed on the high-density nesting area which is a 5 km stretch of beach (out of > 150 km) of nesting beach along the KZN coastline.

In Chapter 3 we described the thermal incubation environment which is a first attempt to examine the thermal micro-environment along this high-density nesting beach. The main question concerned was how much the thermal gradient along the nesting beach effected the incubation environment of turtle eggs. Nest-, hatching- and emergence success are also discussed in this chapter in relation to temperature.

The aim of Chapter 4 was to estimate *Caretta caretta* hatchling sex ratios by examining gonads microscopically and describing the histological procedure used during the study. The staining procedure is described in some detail and sexing results are discussed.

The key question for Chapter 5 was to evaluate the effect of temperature on hatchling sex ratios from the high-density nesting beach. The chapter integrates the relationship between sand- and incubation temperature (Chapter 3), and the sex ratio results (Chapter 4). Sex ratios were predicted from nest incubation temperatures and histological examinations. The theoretical pivotal temperature for the loggerhead population was one of the key questions of this chapter. Temperature- sex ratio results were compared to a study conducted by Maxwell

*et al.* (1988). Implications of higher incubation temperatures, in the light of global warming, were discussed.

The thesis is concluded with Chapter 6, which reillustrates the main conclusions and highlight some future perspectives.

## Chapter 2

### Study site: Bhanga Nek, South Africa

#### *Introduction*

Sea turtle nesting in South Africa is restricted to the north coast of KwaZulu-Natal (> 150 km) (McAllister *et al.*, 1965; Hughes *et al.*, 1967; Nel, 2008), and takes place within the iSimangaliso Wetland Park, a UNESCO World Heritage Site. The nesting beaches are currently co-managed by Ezemvelo KZN Wildlife (EZEMVELO) and the iSimangaliso Wetland Authority, but were protected by the former Natal Parks Board in the years preceding 1999. The iSimangaliso Wetland Park, which was proclaimed as a World Heritage Site in 1999, contains a rich biodiversity, and many rare and threatened species (<http://www.isimangaliso.com/> accessed 31 December 2010). One of the main goals driving the world heritage site, in addition to conservation purposes, is promoting economic development of the adjacent region and empowering local communities, primarily through tourism (<http://www.isimangaliso.com/> accessed 31 December 2010).

The protected area stretches along the coastline and consists of two marine reserves, the St. Lucia Marine Reserve and the Maputaland Marine Reserve (both marine protected areas, or MPA's) that extend from Cape Vidal northwards to the southern border of Mozambican (Marine Living Resources Act, 1998) (Fig. 2.1). The MPA's extend three nautical miles seawards of the high water mark (Marine Living Resources Act, 1998).

Oceanographic conditions are regulated by the Agulhas current which flows along the continental shelf towards the southern tip of Africa (Schumann and Orren, 1980; Lutjeharms and Anson, 2001). In contrast to the cold Benguela current on the west coast of South Africa, the warm Agulhas current (Lutjeharms, 2001) reaches temperatures of up to 28°C in the summer months (Schumann and Orren, 1980). The Agulhas current is fast flowing with speeds ranging from 5.4 km/hr to 7.2 km/hr (Schumann and Orren, 1980; Lutjeharms, 2001) and seems to be important in sweeping adults (especially leatherbacks) and hatchlings in a southerly direction towards the tip of Africa (Baldwin *et al.*, 2003; Luschi *et al.*, 2006). Some of the warm water eddies spinning off into the colder Benguela current may disperse hatchlings even further (Luschi *et al.*, 2006).

The turtle nesting beaches along the South African coast are characterized by a narrow intertidal strip containing mainly silica sand (Hughes, 1996; Baldwin *et al.*, 2003). The coastline can be classified as medium to high energy (Baldwin *et al.*, 2003) due to the narrow continental shelf and is also characterized by small, asymmetric sandy bays *ca.* 5 km long (McAllister *et al.*, 1965; Hughes, 1996). A few beaches are situated within the bays as rocky headlands protect the southern ends of the bays (McAllister *et al.*, 1965). The dominant beach morphodynamic state within iSimangaliso Wetland Park is “intermediate beaches” with some coarse grained and steep reflective beaches (Harris, 2008), while back-beaches are bordered by high, secondary dunes which are stabilized by sub-tropical coastal dune forest (Botha, 2010). Mobile, wind-blown dunes characterize some stretches of the coast (McAllister *et al.*, 1965).

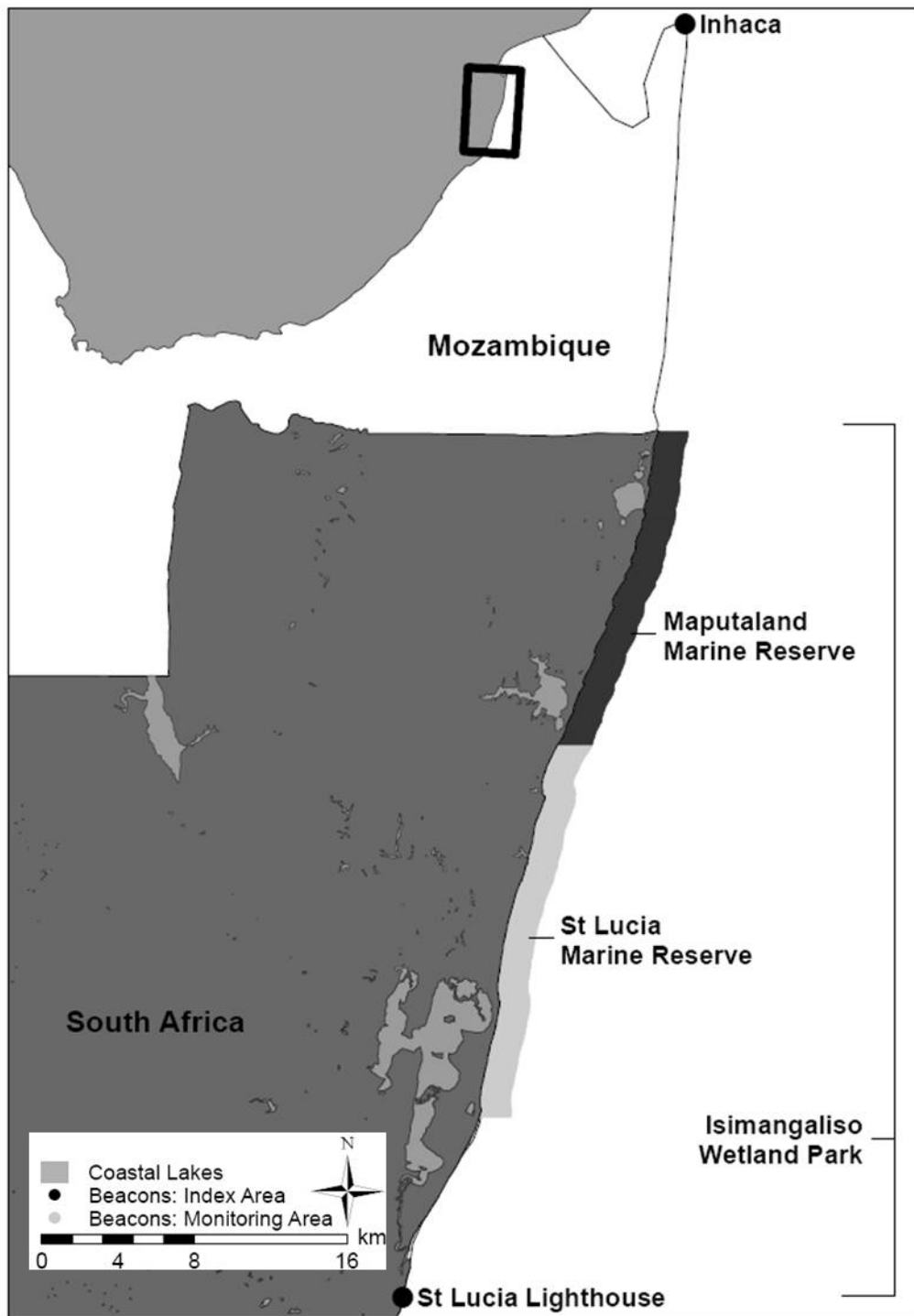


Figure 2.1. Map of the iSimangaliso Wetland Park, which include the St. Lucia Marine Reserve and Maputaland Marine Reserve, from Kosi Bay bordering Mozambique to Cape Vidal. The park includes approximately 65 km of coastline with both loggerhead and leatherback sea turtles nesting in the region.

## *Study site*

The monitored turtle nesting beach, which stretches from Bhanga Nek (0N) to Kosi Lake (32N), is considered an index beach for turtles of KZN (Fig. 2.2) (Nel, 2008). All beacons north of the Bhanga Nek research base (0N) are marked as north (N) and beacon numbers south of Bhanga Nek are marked as south (S) (Fig. 2.2). Due to the high density of turtles occurring in the northern section of the coast and the dynamic topography of the area, beacons are spaced 400 m from each other (Nel, 2008). These beacons were used as an index during the nesting season and to relocate nests during the hatching season. For the purpose of this study, Bhanga Nek beach, a 5 km stretch of beach which is the peak nesting area for loggerhead turtles (Nel, 2010), was examined. This high-density monitored area, from previously recorded nesting data, stretches from 0N (27°00'23.9"S 32°51'49.6"E) to 12N (26°58'06.5"S 32°52'27.3"E) (Fig. 2.3) (Nel, 2008).

The rookery is characterized by open sandy beaches backed by vegetated dunes and adjacent to reefs or rocky outcrops (Fig. 2.4a) (Hughes, 1974b; pers. obs.). The dunes at beacons 1N and 2N are the narrowest between the lake and the beach, and this section is regularly eroded during spring high tides (Botha, 2010). A camp site adjacent to beacons 2N and 3N was erected during 2009 and resulted in some artificial lighting (Botha, 2010). The beach-dune border northwards to beacon 10N is non-vegetated and uniform in width, but starts to widen as one proceeds towards beacon 12N (Botha, 2010). The beach at beacons 11N and 12N is characterized by non-vegetated, wind-blown dunes (Botha, 2010). Primarily *Ipomea* and *Scaevola* species are found covering the small, primary dunes between beacons 1N and 3N (Fig. 2.4b) (Botha, 2010). From beacon 3N to 5N the area is backed by established higher primary dunes with some alien *Cassurina* trees stabilizing the beach-dune border (Botha, 2010). No wind-blown dunes are apparent between beacons 5N to 10N (Botha, 2010). There are some inshore rocks protecting the beach at beacons 9N and 10N, which makes the beach inaccessible to turtles at low tide (Botha, 2010). During extreme spring low tides, rocks are visible from beacon 7N to 10N (Botha, 2010).

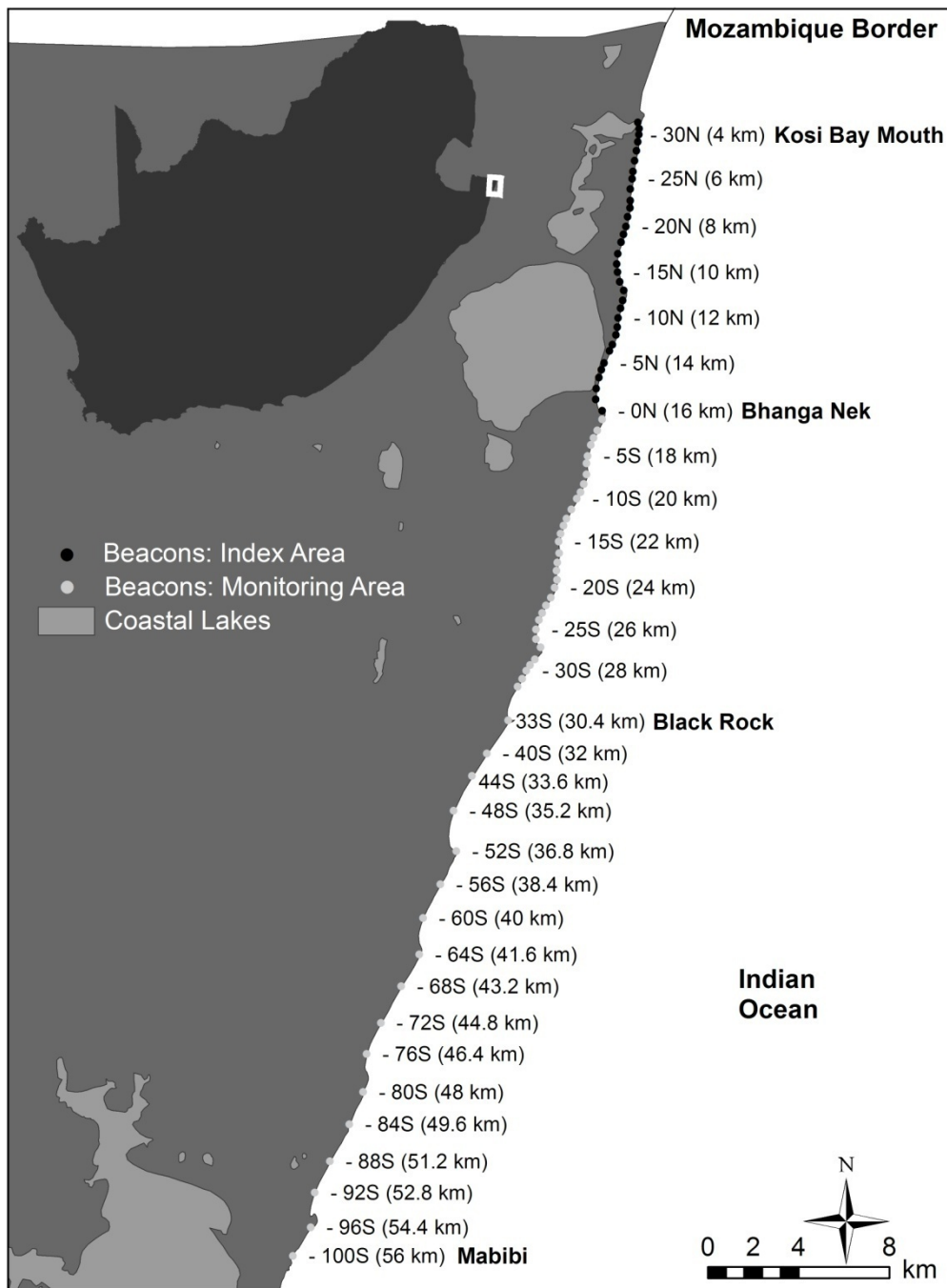


Figure 2.2. The turtle monitoring area extends from Kosi Lake mouth (32N) to Mabibi (100S).



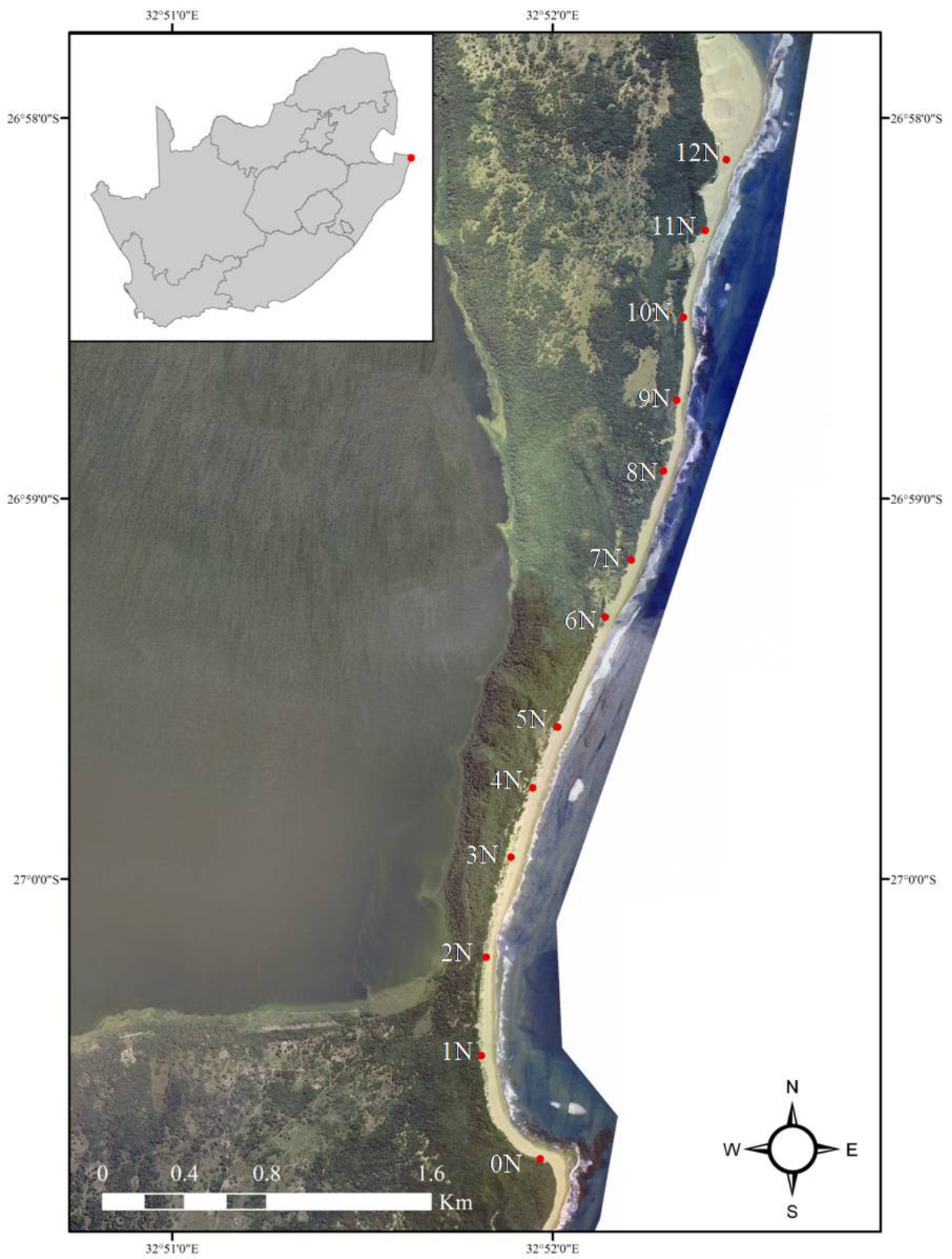


Figure 2.3. The high-density loggerhead nesting area showing beacons at 400 m intervals.

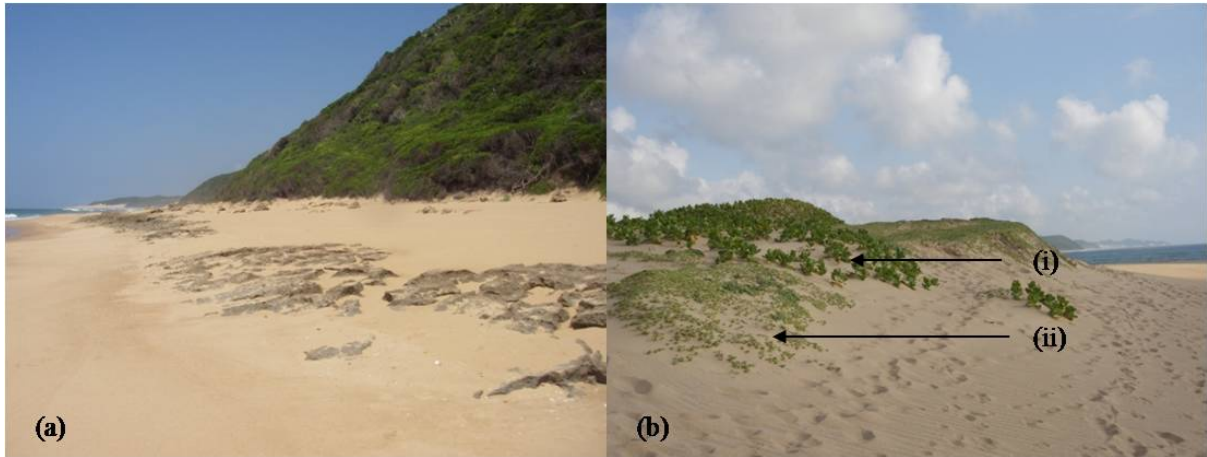


Figure 2.4. (a) Open sandy beach at low tide, sometimes adjacent to rocky outcrops where (b) vegetation consists mostly of (i) *Ipomea brasiliensis* and (ii) *Scaevola plumieri*. Photos: Leanne Ezzy

## ***The South African long-term turtle monitoring programme***

### **Monitoring programme**

Of the seven extant sea turtle species, two species nest in KwaZulu-Natal, namely loggerheads (*Caretta caretta*) and leatherbacks (*Dermochelys coriacea*) (Hughes, 1989). Their presence was documented as early as 1849, by Andrew Smith (Hughes, 1989; Baldwin *et al.*, 2003). The killing of sea turtles in South Africa was banned in 1916, but subsistence harvesting still continued as enforcement was negligible, and the numbers of turtles continued to decline (Hughes, 1989). In 1963 formal action was initiated to protect sea turtles, and the South African turtle monitoring and protection programme was started (Hughes, 1989; Hughes, 1996; Baldwin *et al.*, 2003), making this one of the longest running monitoring programmes for loggerheads and leatherbacks in the world (Wilson and Humphrey, 2004; Nel and Lawrence, 2007).

The monitoring programme takes place between October and March of each year as turtles nest in summer (Nel and Papillon, 2005). Reported data for loggerheads indicate a significant increase in the number of nesting females over the years, and leatherback female numbers seem stable with a possible marginal increase (Fig. 2.5) (Nel, 2008). This may be a positive recovery, the result of the protection programme and increased sampling effort combined with a good reproductive strategy (e.g. nest site fidelity) (Wilson and Humphrey, 2004; Nel, 2008). The exact drivers have not yet been established.

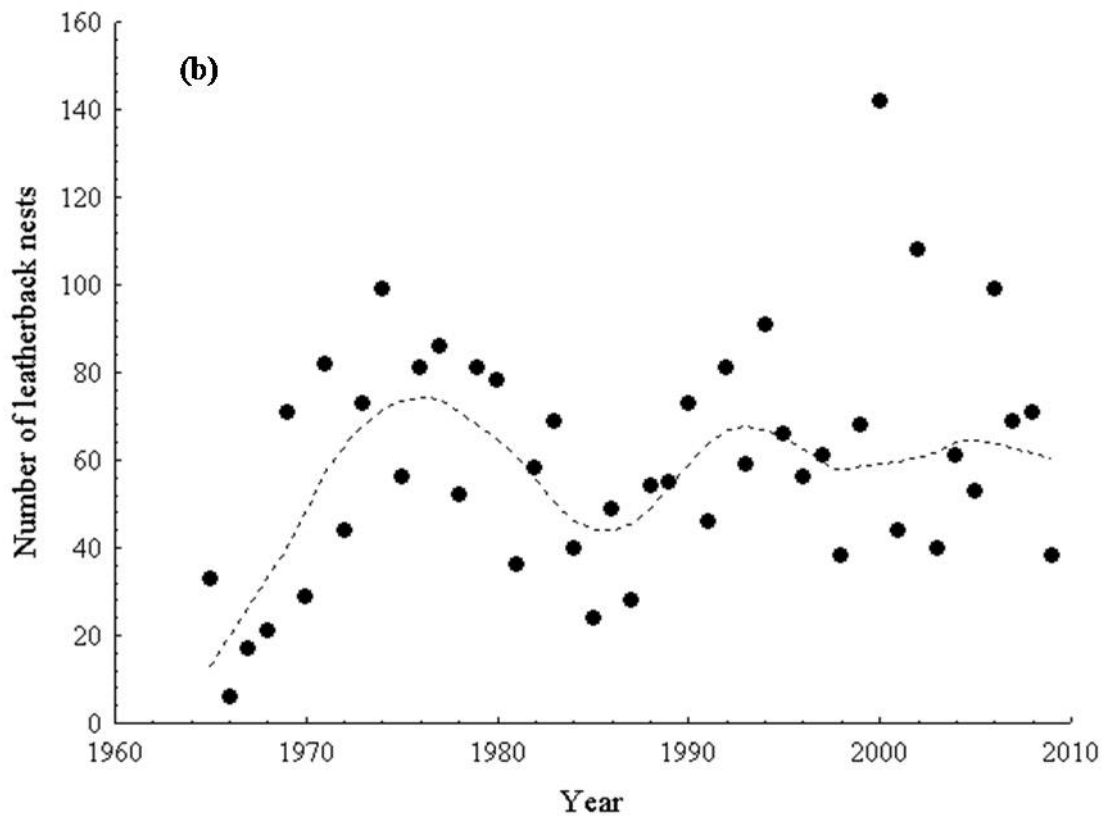
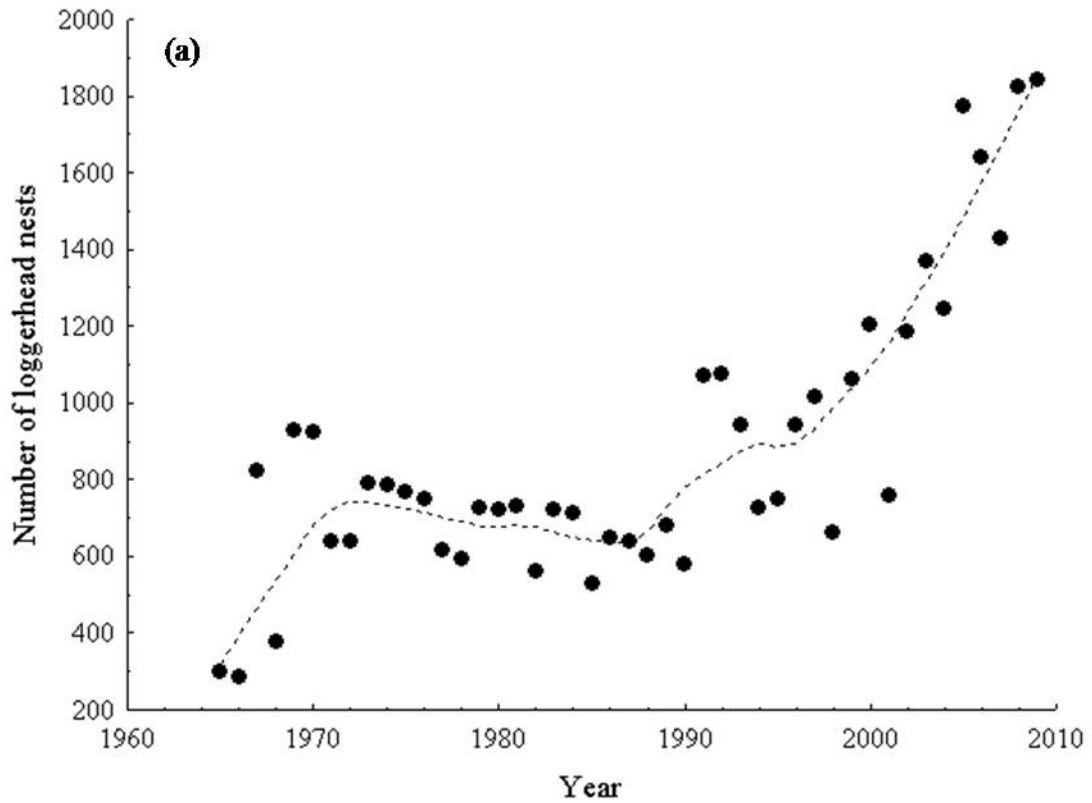


Figure 2.5. Along with increased sampling effort, the loggerhead populations (a) have increased whereas leatherbacks (b) show relatively stable numbers over the past 37 years of monitoring. Data obtained from Nel (2010) (lowess spline fit was used).

## Threats

Since public beach access is restricted and the nesting beaches monitored at night, the only remaining threats to turtles or their nests on these beaches are tidal inundation, beach erosion, nest predators and disease. Some of the predators include feral dogs, honey badgers (Fig. 2.6a), birds, ghost crabs, ants (Fig. 2.6b) and monitor lizards. Even though the protection programme for turtles is well-managed and killing of turtles is prohibited, there have been some incidents of turtle poaching, but they are scarce (E. Mlaba, pers. comm.).

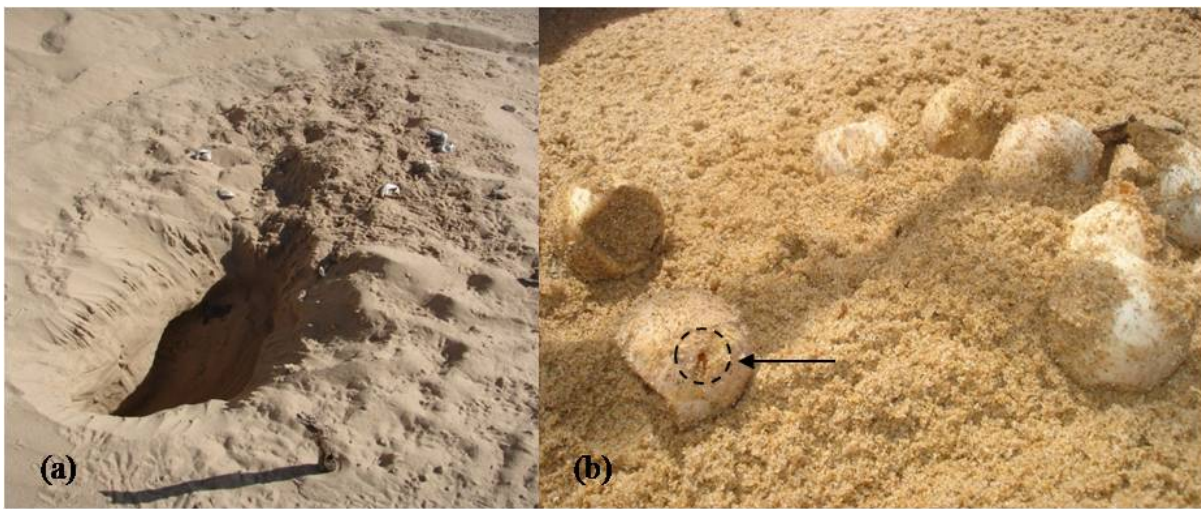


Figure 2.6. Nests can be destroyed by predators such as (a) honey badgers and (b) ants. Nests predated by ants and ghost crabs are usually discovered upon excavating the nests. Photos: Anje De Wet

The nesting beaches are remote (with the nearest rural town more than 10 km away) and very few local tourist developments exist. There is thus little artificial light shining onto the dunes that could confuse or disturb female turtles coming to nest (pers. obs.). However, during 2009 camp sites were erected next to the beach which created artificial light during both the nesting and hatching seasons. These camp sites were removed after the hatching season of 2010 (E. Mlaba, pers. comm.). During both hatching seasons (February/March from 2009-10) anglers were allowed to fish during the night where bright lights were used (pers. obs.). This may have confused emergent hatchlings as they are very sensitive to light, but no studies have been done in this area to establish whether confusion of hatchlings may happen on a broad scale.

### Southernmost rookery

Data on sand temperatures along the turtle nesting beaches in South Africa have not yet been published. The nesting beaches of South Africa host the southernmost loggerhead and leatherback rookeries (27°S) globally. Sand temperatures tend to decrease with increasing latitude (Carthy *et al.*, 2003), thus the assumption is that South Africa's nesting beaches may experience lower temperatures than other turtle rookeries, but are comparable (in latitude) to that of nesting beaches in Australia. The high-density loggerhead nesting area at Bhangha Nek, is thus an interesting study site to investigate the effect of temperature on parameters such as hatching success (i.e. the percentage of hatched eggs relative to the clutch size) as it represents two species at the edge of their distribution. Furthermore, clutches are naturally incubated and there is no/little human interference making it an ideal field "laboratory".

## Chapter 3

### Thermal incubation environment of loggerhead (*Caretta caretta*) nests in KwaZulu-Natal, South Africa

#### *Abstract*

Incubation temperatures in sea turtle nests are governed by climate and local environmental conditions such as shading (by vegetation, dunes or developments), sand colour, the depth of eggs etc. Metabolic heating adds to the temperature, especially towards the end of incubation. The purpose of this study was to determine the incubation temperature for loggerhead turtles (*Caretta caretta*) nesting in northern KwaZulu-Natal, South Africa. During two peak nesting seasons (i.e. December 2008 and 2009) a number of iButton temperature loggers were deployed in loggerhead nests as they were laid, along with a number of iButtons recorders buried at nest depth to measure *in situ* sand temperature, and retrieved during the hatching seasons (i.e. February/March 2009 and 2010). The mean incubation temperature (for the 2008/9 season) was  $29.6^{\circ}\text{C} \pm 1.27$  SD ( $n = 10$ ), and the overall nest success (i.e. the number of nests that hatched) was high at 94% ( $n = 110$ ). For the second season (2009/10), mean incubation temperature was  $29.9^{\circ}\text{C} \pm 1.36$  SD ( $n = 15$ ) with a nest success of 88% ( $n = 223$ ). *In situ* temperatures during the same time (2009/10) was  $29.0^{\circ}\text{C} \pm 1.11$  SD which suggest metabolic heating contributed *ca.*  $1.0^{\circ}\text{C}$  to the nest temperature. Even though *in situ* temperatures were not recorded in the first season, these results may suggest that the second season was marginally warmer. However, both seasons had a mean nest temperature close to, or slightly warmer than the theoretical pivotal temperature for loggerheads in KwaZulu-Natal ( $29.0^{\circ}\text{C}$ ).



## ***Introduction***

Female sea turtles excavate a nest constituting a body pit and egg chamber on sandy beaches and deposit a large clutch of eggs into the nest. After covering it up, she returns to the sea and the fate of the nest is entirely dependent on the environment. No parental care is given to the hatchlings (McGinley, 1989), thus nest site selection is pivotal for the successful incubation of a clutch. Nest site selection can be defined as the non-random placement of a clutch of eggs by an individual female turtle along a beach stretch to maximize her chances of nesting and successful incubation (Kamel and Mrosovsky, 2005). The thermal incubation conditions experienced by the turtle eggs are not constant and collectively determines turtle hatchlings' development and survival (Ackerman, 1997; Godfrey *et al.*, 1997; Carthy *et al.*, 2003). The thermal incubation environment is essentially determined by three suites of factors which are difficult to decouple. These are the external environmental (or *ex situ*) conditions, nest location and *in situ* conditions.

### *Factors affecting incubation temperature*

#### *Ex situ factors*

The majority of loggerhead nesting occurs in warm temperate to sub-tropical areas (Ross and Barwani, 1995). Nesting beach temperatures usually range between 24 - 33°C, and may even exceed this range for short periods (Limpus *et al.*, 1985). The ambient environment such as air temperature, rainfall, wind, shading by vegetation and dunes, seasonal temperature fluctuations (within seasons or between seasons) etc. directly influences the thermal incubation environment. These, generally warm sand temperatures can be lowered by heavy rainfall, for example storm surges (Mrosovsky, 1983), or tidal inundation (Carthy *et al.*, 2003). Precipitation resulted in lower, but non-lethal, incubation temperatures at leatherback turtle rookeries in Grenada, in the West Indies, which in turn, also resulted in male-producing conditions (Houghton *et al.*, 2007). Intra-seasonal temperature variation is more pronounced in turtle rookeries located at higher latitudes away from the tropics (Houghton *et al.*, 2007). Thus, if a female turtle laid her nest early in the season, the embryos may experience warmer temperatures towards the end of the season when temperatures becomes hotter. There may also be annual variation between seasons, for example where one season may be very hot and the following season much cooler.

### *Nest location*

Once a female selects a beach to deposit her eggs, she needs to choose where on the beach to dig the nest chamber. There are consequences to post nest site selection. Nests closer to the tidal zone may be washed away or eroded by high tides, which may be detrimental to the developing eggs (Whitmore and Dutton, 1985). However, if eggs are placed too far from the sea, the hatchlings are at a greater risk of predation (Blamires and Guinea, 1998) or unable to find the sea due to visual obstructions (Godfrey and Barreto, 1995). There are many physical factors a female takes into account when selecting a nesting site, with the most important factors being geomorphology of the nesting beach, bathymetry of the surf zone and dimensions of the beach (Eckert, 1987; Hays *et al.*, 1995; Kikukawa *et al.*, 1999; Miller *et al.*, 2003; Mazaris *et al.*, 2006).

Geomorphology of a nesting beach constitutes features of the coastline such as sheltered bays and rocky headlands (Botha, 2010). The bathymetry of the surf zone is closely linked to the coastal geomorphology since features in the surf zone include rocks, depth or slope and width of the surf zone (Botha, 2010). In general, sea turtles, especially leatherbacks, seem to prefer nesting areas that are obstacle or rock free (Eckert, 1987; Mortimer, 1995). This may allow turtles to avoid injury from rocks when coming ashore, especially at low tide, and the number of hatchlings predated along rocky shores or reefs may also decrease (Mortimer, 1995; Garmestani *et al.*, 2000). These factors do not affect the incubation environment, but rather exclude suitable high-shore habitat due to unfavourable low-shore conditions.

Once a female has come ashore, high-shore beach conditions such as shading by vegetation or dunes, distance from the sea and sand characteristics such as grain size, water potential etc. are important. Loggerheads in Greece (see Hays and Speakman, 1993), Brazil (Serafini *et al.*, 2009) and South Africa (Botha, 2010, unpublished data) tend to lay nests away from the high-water mark, and in close proximity to, and rarely behind, vegetation. Serafini *et al.* (2009) suggested that vegetation represents a visual cue for unfavourable nesting habitat. In contrast, 47% of loggerhead nests in Florida, were laid in the vegetation zone (Garmestani *et al.*, 2000). Vegetation may provide shade for some nests resulting in cooler incubation temperatures (Foley *et al.*, 2000). A disadvantage would be that the roots may penetrate the eggs and may result in low hatching success, but very little is known about the effect of root infiltration on hatch success (Serafini *et al.*, 2009).



### *In situ factors*

*In situ* factors that influence incubation temperature include nest depth (Foley, 1998), sand colour (albedo) (Hays *et al.*, 2001), grain size, water potential (i.e. the measure of water availability) (Mortimer, 1990) and moisture (McGehee, 1990). Incubation temperature, and the variation thereof decreases with nest depth (Foley, 1998). For example, loggerhead nests in the eastern Mediterranean showed greater diel fluctuations in temperature than the deeper nests of green turtles nesting on the same beaches (Godley *et al.*, 2001a). Lighter coloured sand (such as coral or high calcium sand) is cooler than darker coloured sand (typically volcanic or high mineral contents) exposed to the same intensity of radiation (Hanson *et al.*, 1998). Hays *et al.* (2001) found that green turtle nesting beaches with darker sand colour were hotter than lighter coloured beaches. Metabolic heating of the embryos has also been reported to increase nest temperature (e.g. Godfrey *et al.*, 1997) and the larger the clutch size, the more metabolic heat was produced (Broderick *et al.*, 2001).

### *Effects of incubation temperature on hatching success*

The minimum temperature for successful development of turtle embryos is 25°C as established through constant incubation temperature studies (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982). At this lower extreme embryo development is slow, but normal and the incubation period lasts for 13 weeks (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982). The maximum temperature for successful embryo development is 34°C. Here development is rapid with incubation as quick as 6.5 weeks, but with an enhanced risk of deformities (Yntema and Mrosovsky, 1982) and decrease in hatchling body size and mass (Booth and Astill, 2001; Burgess *et al.*, 2006).

Hatchling characteristics, such as gender, solely depend on incubation temperature, and this process is called temperature-dependent sex determination (TSD). For loggerheads, above the pivotal temperature (i.e. the temperature at which the sex ratio is 1:1 - 29°C) females are produced, and lower than the pivotal temperature produce mainly males. Sex is determined during the middle third of the incubation period and this window is called the thermal sensitive period (TSP). Therefore, higher temperatures may dramatically alter the phenotype and hatchling survival (Mrosovsky, 1980).

Embryos generate metabolic heat within the nest and simultaneous heat from the natural environment may create high sand temperatures that may inhibit physiological activity and/or even death or be vulnerable to predation (Ackerman, 1997). In constant incubation studies, sea turtle embryo development is impaired above or below the thermal tolerance range (TTR), which is 25-27 to 33-35°C (Ackerman, 1997). According to Yntema and Mrosovsky (1980) incubation temperatures near the lethal limit (< 25°C and > 34°C) result in decreases in hatching success.

Wood and Bjorndal (2000b) examined hatching success in loggerhead hatchlings with relation to temperature, moisture, salinity and slope, at a nesting beach in Florida. They found that hatching success was not correlated with temperature, moisture and conductivity while the relationship between hatching success and elevation was significant for some nests. In South Africa, hatching success was influenced mostly by predation (by ants, *Dorylus* spp.) and wind (Maxwell *et al.*, 1988). Some nests were exposed during north-easterly winds and predated by feral dogs or desiccated (Maxwell *et al.*, 1988).

Investigating the variation in sand and nest temperatures within rookeries is, therefore, pivotal for understanding how incubation temperatures may affect sea turtle populations especially in the light of changing climate and how this will affect sex ratios and populations (Booth and Freeman, 2006). Furthermore, information on the effect nest temperature may have on the loggerhead turtle population is lacking. To date, in South Africa, there has only been one published study concerning natural nest temperature regimes along the nesting beaches (Maxwell *et al.*, 1988). Maxwell *et al.* (1988) examined the effect of nest temperature on South African loggerhead hatchling sex ratios. The aim of the present study was to report data with sand and nest temperature profiles for the Bhanga Nek beach in Kosi Bay, including other abiotic influences i.e. rainfall, air temperature and wind (direction and strength) and to determine how these factors affect hatching success.

This study was conducted in the high-density nesting area for loggerheads, in Maputaland. In the present study, the influence of temperature profile along the nesting beach for *C. caretta* was examined in order to: (1) describe the thermal gradients within the nesting area using iButton data loggers at control sites; (2) measure incubation temperature and determine the biotic and abiotic factors causing changes in nest temperature, as well as (3) determine the

nest success and hatching success of various clutches. The hypotheses were that: (1) the control site situated farthest from the sea and from vegetation that could provide shade (i.e. beacon 12N) would experience the highest sand temperatures; whereas the control site that experienced frequent inundation by tides, with shading by high dune from mid-afternoon onwards (i.e. beacon 0N) would have the lowest sand temperatures. (2) Incubation temperatures were expected to be high ( $> 29^{\circ}\text{C}$ ) due to warm air- and sand temperatures, and (3) high nest success and high hatching success was expected as there was, along with strict protection and conservation regulations, a dramatic increase in loggerhead nesting numbers.

## ***Materials and methods***

### ***Study site***

This study focuses on a 5 km beach stretch of Bhanga Nek ( $27^{\circ}00'23.9''\text{S}32^{\circ}51'49.6''\text{E}$ ) in Kosi Bay, northern KwaZulu-Natal (or Maputaland), which is the peak nesting area for loggerhead turtles in the Western Indian Ocean. There are twelve beacons (400 m apart) along the nesting site (as discussed in Chapter 2).

### ***Data logger calibration***

The iButtons (Fairbridge Technologies,  $\pm 0.5^{\circ}\text{C}$  accuracy) could not be placed directly in the sand as they would disappear and corrode in the sand. The iButtons needed to be securely covered to protect them, and make them retrievable without affecting temperature recordings. Hence, the iButtons were tested in different containers before placing them in the field. The temperature iButtons ( $n = 15$ ) and moisture data loggers ( $n = 7$ ) were placed in an air-tight container to record temperature and relative humidity every hour for 60 hours. Some data loggers were placed in a ping-pong ball (to simulate eggs in the field), with holes (open) (temperature  $n = 7$ ; moisture  $n = 3$ ), and in ping-pong balls with mesh-covered holes (closed) (temperature  $n = 7$ ; moisture  $n = 3$ ), while two (one temperature- and moisture data logger) were not placed in ping-pong balls (control). The variance between the temperature data loggers (open and closed) and control data loggers was small ( $< 0.1^{\circ}\text{C}$ ). Hence, iButton data loggers placed in ping-pong balls do not significantly affect ambient temperature readings. But, the relative humidity (% RH) readings from the “closed” moisture buttons (variance  $<$

3% RH) were closer to the control than readings from the “open” moisture buttons (variance < 6% RH). Thus, the moisture buttons were placed in mesh-covered ping-pong balls to record nest temperature and % RH.

### Climatic data

Data for the first season (2008/9) were acquired from the South African Weather Station in Kosi Bay Nature Reserve (via Dr Scotty Kyle, from Ezemvelo KZN Wildlife), i.e. daily rainfall and the minimum- and maximum air temperatures (°C). Climatic data for the second season (2009/10) was recorded at the turtle research station (just on the other side of Kosi Lake, but on the main nesting beach). Weather observations of cloud cover (0%, 25%, 50%, 75% and 100%), rainfall (in mm), wind (Beaufort scale), air- and sand temperature ( $\pm 0.5^{\circ}\text{C}$ ) were recorded. Wind and rainfall were recorded three times a day (09:00, 15:00 and 21:00) whereas the iButtons recorded air- and sand temperatures every 30 minutes over 3.5 months. Data were processed in Statistica v9 to obtain a daily average values.

### Sand temperature

The correlation between ambient temperature and *in situ* sand temperatures was obtained by measuring both factors during the peak nesting season of 2009/10. Eight iButtons were placed (in a ping pong ball) in the sand at the base of every second beacon which was along the upper beach of the nesting area. iButtons were therefore placed 800 m apart (from beacon 0 - the research hut - to 12N) (Fig.3.1) and were planted at a depth of 60 cm (approximate nest depth for loggerhead turtles) at the dune base. Ambient temperature was measured at beacon 0N in the shade outside of the research station. The beach sections were mainly east facing (beacon 1 – 12N) whereas beacon 0N is north facing. The iButtons were set to record temperature every 30 minutes for 87 days, and started recording on 14 December 2009. In addition, the GPS coordinates, distance to vegetation and high-water mark were recorded at every iButton. The data loggers were retrieved after the last study nest hatched on 10 March 2010.

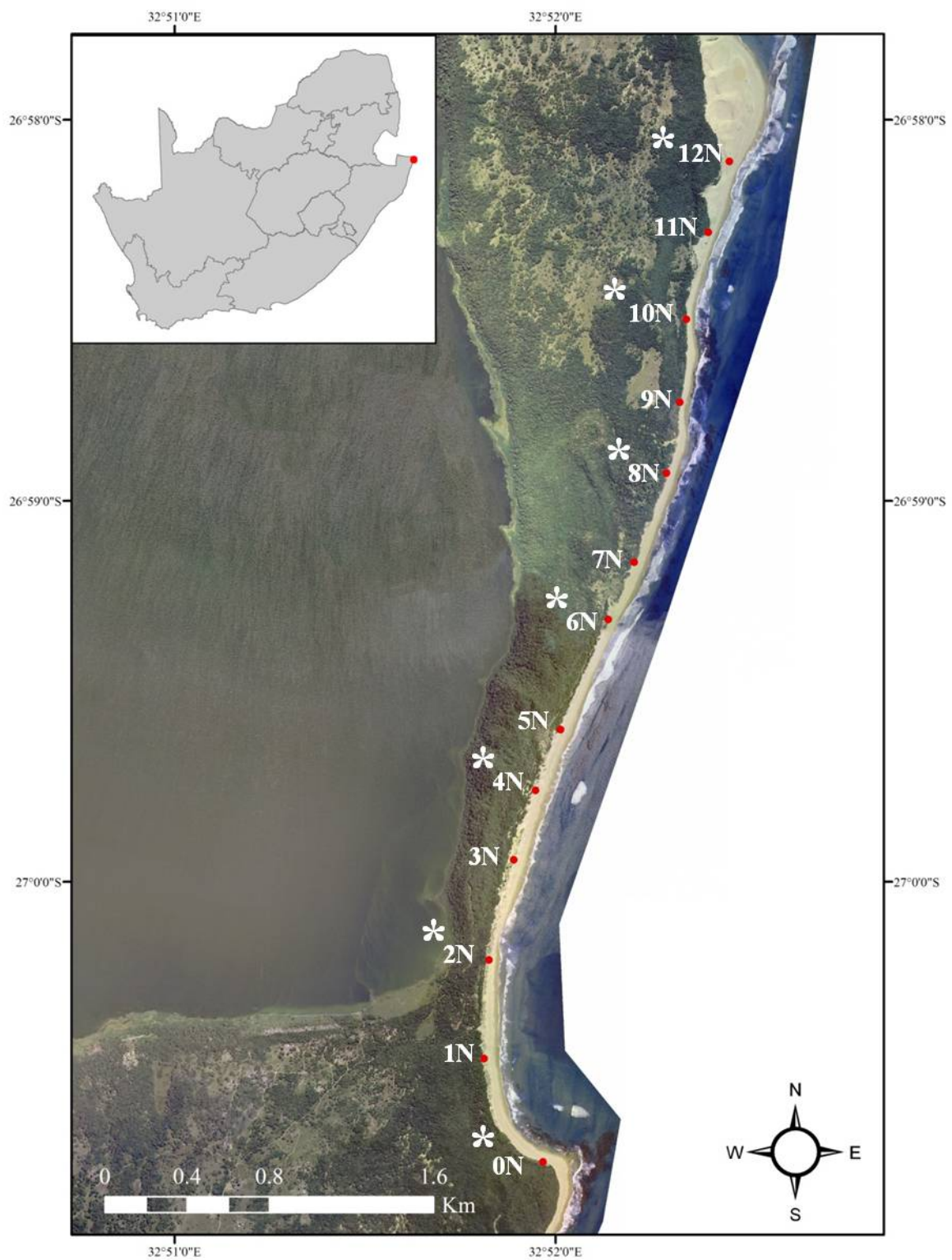


Figure 3.1. Map of the beacons with iButtons (marked with an \*) recording *in situ* (sand) temperature.

### Incubation temperature

iButton temperature loggers were deployed into nests of loggerhead females that were encountered while laying their eggs. Disturbance to the animal was minimised by waiting at a distance until the female laid a number of eggs, and was hence in a hormone “trans” tolerant of handling (Miller *et al.*, 2003). Thirty-five temperature loggers, of which eight were capable of additionally recording relative humidity (% RH), were placed opportunistically in nests as eggs were laid. The data loggers were placed when approximately half of the eggs were laid (*ca.* 50 cm) to record the core incubation temperature. The physical location of each nest was marked with a sub-surface nest marker which was attached to a piece of string, which in turn was secured to the nearest vegetation, and by recording the GPS coordinates.

Nests with iButtons were checked weekly to ensure that they were undisturbed (by people or natural predators) and that the strings were still visible after winds moved sand. As the hatching date approached, nests were checked daily to monitor signs of hatchling emergence or hatched nests (i.e. depression of sand surface) especially for marked nests. The end of the incubation period was defined as midnight of when the first hatchlings emerge. The nests were excavated four days after hatching to limit interference (Fig. 3.2a). Nests that did not appear to have hatched on the estimated due date were excavated eight days later.

### Nest monitoring

Hatching- and emergence success were determined by counting the number of hatched shells, unhatched eggs, and dead and live hatchlings found in the nest (Miller, 1999). Clutch size was defined as the sum of hatched and unhatched eggs, whereas hatching success was determined as the percentage of eggs that produced live hatchlings (Miller, 1999; Wood and Bjorndal, 2000b). Emergence success was the percentage of live hatchlings that successfully emerged to the surface, thus excluding dead (D) and live hatchlings (L) found in the nest (Miller, 1999) (Fig. 3.2b).

Developmental categories were assigned to the unhatched (whole) eggs found in the nest. The four categories included: (i) undeveloped (UD) – yolk, with no obvious embryo, (ii) unhatched (UH) – an egg with a tiny, but obvious embryo, (iii) unhatched full-term (UHT) – “large” nearly fully developed embryo and (iv) predated (P) – predated eggs (i.e. shredded

eggs by crabs or holes made ants) (Miller, 1999). A nest was considered as unsuccessful when the number of unhatched (whole) or predated eggs was more than 80% in relation to the clutch size. Nest success was scored (as a successful or unsuccessful nest) and then overall nest success was estimated for loggerheads in the high-density nesting area. Nest- and hatching success was not scored for nests that had been destroyed by predators, like feral dogs or honey badgers, since clutch size would be difficult to assess.

### Statistical analyses

Results are presented as means  $\pm$  standard deviations. Pearson Product-Moment correlation analyses were used to describe relationships between variables and one-way Analysis of Variance (ANOVA) was used to compare sand- and nest temperatures. Kruskal Wallis H-test was used to test for inter-seasonal differences for nest temperature. All analyses were conducted in Statistica v9. Statistical significance was set at p-value  $< 0.05$ .

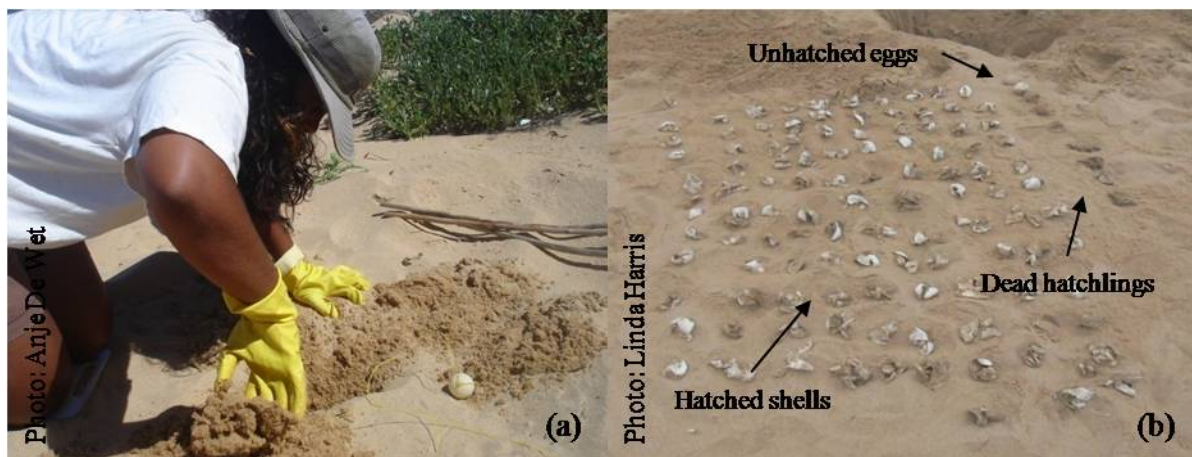


Figure 3.2. An example of a marked nest (a) being excavated four days after hatchling emergence, and separation of nest contents (b) into different stages.

## Results

### Climatic data

A one-way ANOVA detected no significant change in rainfall ( $F_{(1,177)} = 0.49$ ;  $p > 0.1$ ), minimum air temperature ( $F_{(1,177)} = 0.31$ ;  $p > 0.1$ ) and maximum air temperature ( $F_{(1,177)} = 1.50$ ;  $p > 0.1$ ) between the two seasons (2008/9 and 2009/10). The maximum temperatures measured were, however, slightly higher during the second season (Fig. 3.3). From this, the second season seemed drier and slightly hotter than the first season.

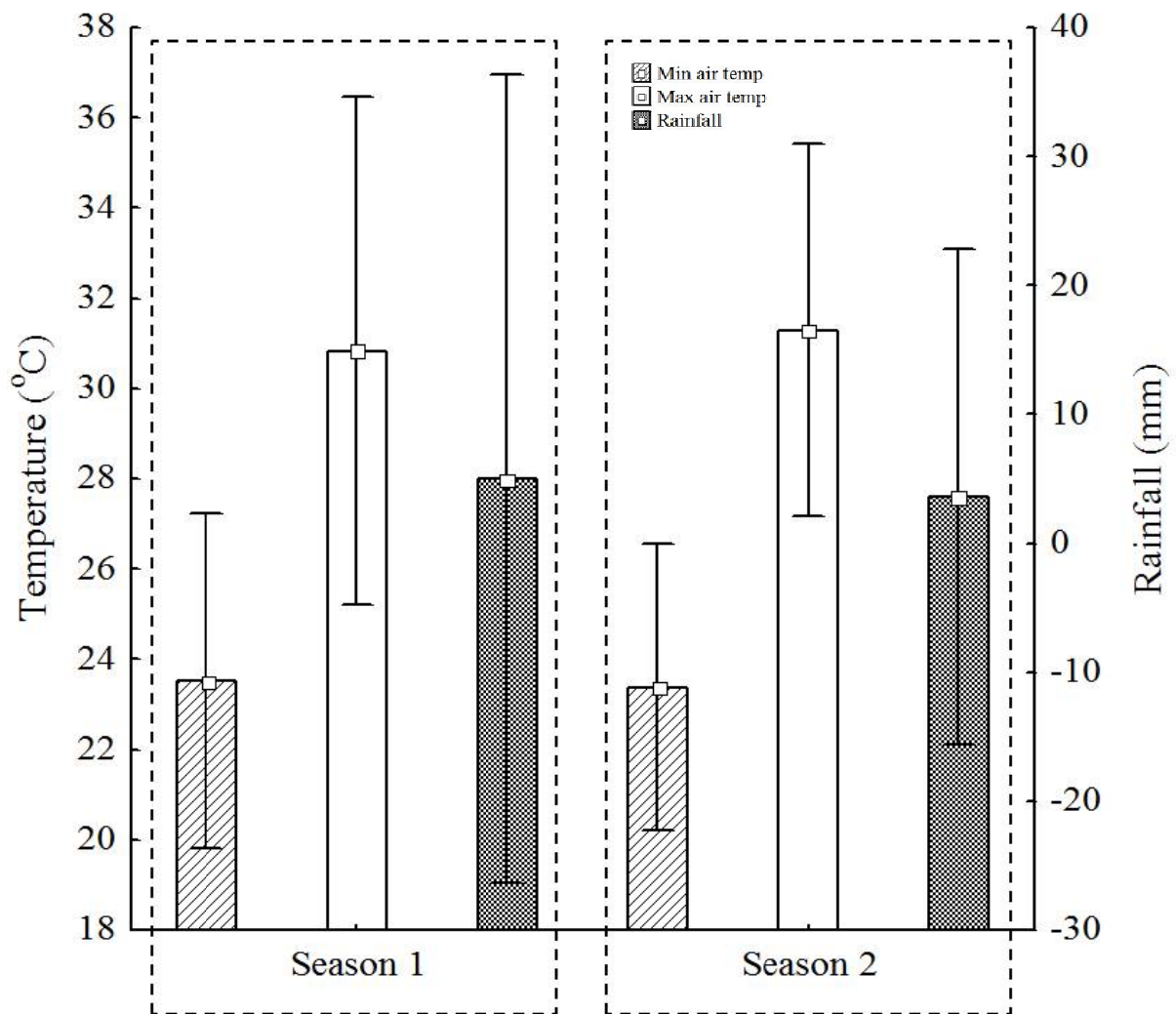


Figure 3.3. Basic climate data for two turtle nesting seasons; 2008/9 and 2009/10 with the average maximum (max)- and minimum (min) air temperatures measured per day and with daily average rainfall (in mm) and  $\pm$  SE bars.



### Sand temperature

Mean sand temperature across the sites was  $29.0^{\circ}\text{C} \pm 1.11$  SD. *In situ* temperatures varied significantly (one-way ANOVA  $F_{(5, 502)} = 13.6$ ) along the shore with the coolest temperatures experienced at beacon 0N ( $28.4^{\circ}\text{C} \pm 1.04$  SD) (which was north-facing) and the hottest temperatures recorded at beacon 12N ( $29.8^{\circ}\text{C} \pm 1.38$  SD) (Fig. 3.4) which was the farthest away from water ( $> 30$  m),  $> 39$  m away from the dune base and vegetation ( $> 40$  m) providing shade. However, little intra-beach variation in sand temperature was observed at the sites between beacon 0 and 12N (i.e. the extreme cool and warm site) where changes were  $\leq 0.2^{\circ}\text{C}$  (Table 3.1).

The mean daily sand temperature correlated positively with mean daily air temperature ( $p < 0.05$ ;  $r^2 = 0.38$ ). As the season progressed *in situ* sand- and air temperature evidently increased from early- (14 Dec 2009 – 12 Jan 2010), to mid- (13 Jan – 11Feb 2010) to late season (12 Feb – 10 Mar 2010). One-way ANOVA indicated a significant change in sand temperature from early- to mid-season ( $F_{(17,490)} = 12.2$ ,  $p = 0$ ; early season =  $28.4^{\circ}\text{C} \pm 1.21$  SD; mid-season =  $29.1^{\circ}\text{C} \pm 0.87$  SD; late season =  $29.4^{\circ}\text{C} \pm 1.05$  SD) as well as air temperature ( $F_{(17,490)} = 3.9$ ,  $p = 0$ ; early season =  $26.2^{\circ}\text{C} \pm 1.33$  SD; mid-season =  $27.0^{\circ}\text{C} \pm 1.02$  SD; late season =  $27.1^{\circ}\text{C} \pm 1.17$  SD), but no significant changes from mid- to late season.

Table 3.1. Mean-, minimum (min)-, and maximum (max) sand temperatures ( $^{\circ}\text{C}$ ) for the control sites recorded for 2009/10.

Beacon #	Orientation	Mean sand temp ( $^{\circ}\text{C}$ )	$\pm$ SD	Min temp ( $^{\circ}\text{C}$ )	Max temp ( $^{\circ}\text{C}$ )
0N	North	28.4	1.04	25.6	30.1
2N	East	-	-	-	-
4N	East	29.0	1.06	26.2	31.0
6N	East	28.9	1.00	25.9	30.6
8N	East	28.8	1.06	26.0	30.7
10N	East	29.0	1.14	25.8	30.9
12N*	East	29.8	1.38	27.0	31.7

\*The data logger at beacon 12N only recorded until 24 February 2010, whereas the others recorded from 14 December 2009 to 10 March 2010.

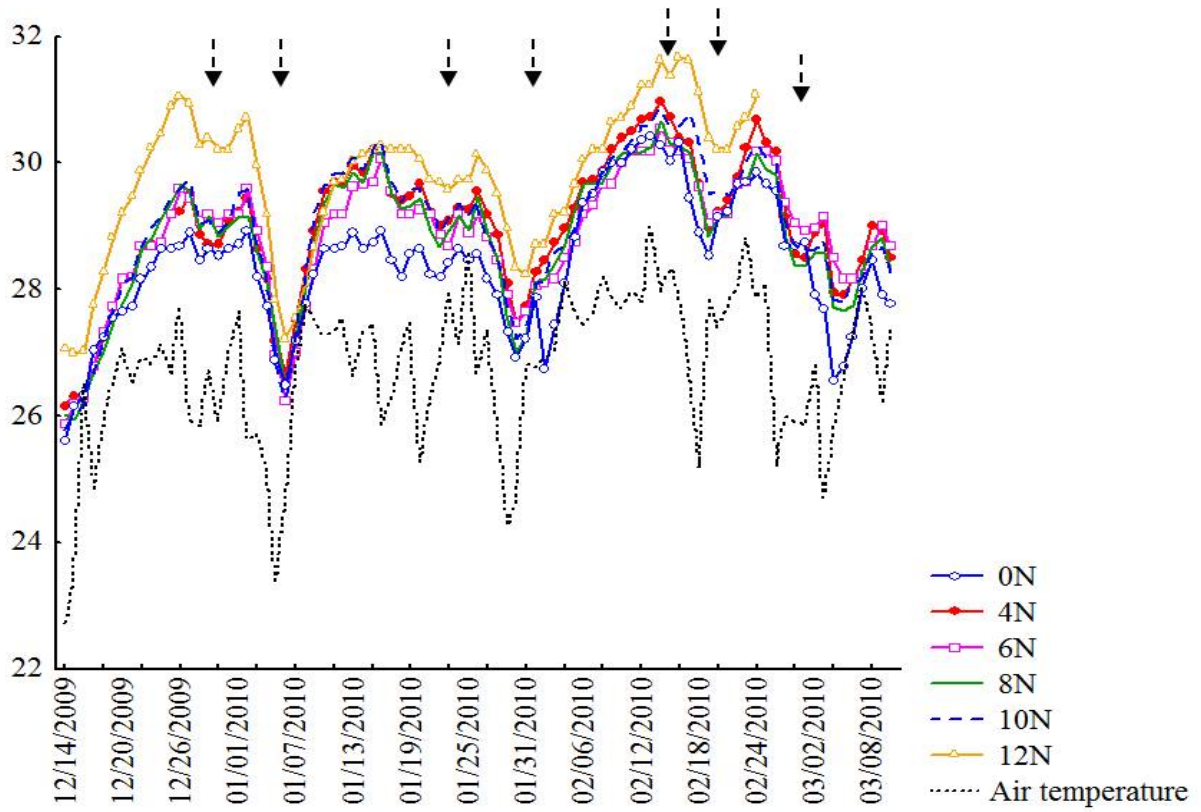


Figure 3.4. Daily average sand- and air temperatures measured throughout the peak nesting period (broken arrows indicate wet periods of significant daily rainfall, i.e. > 15 mm).

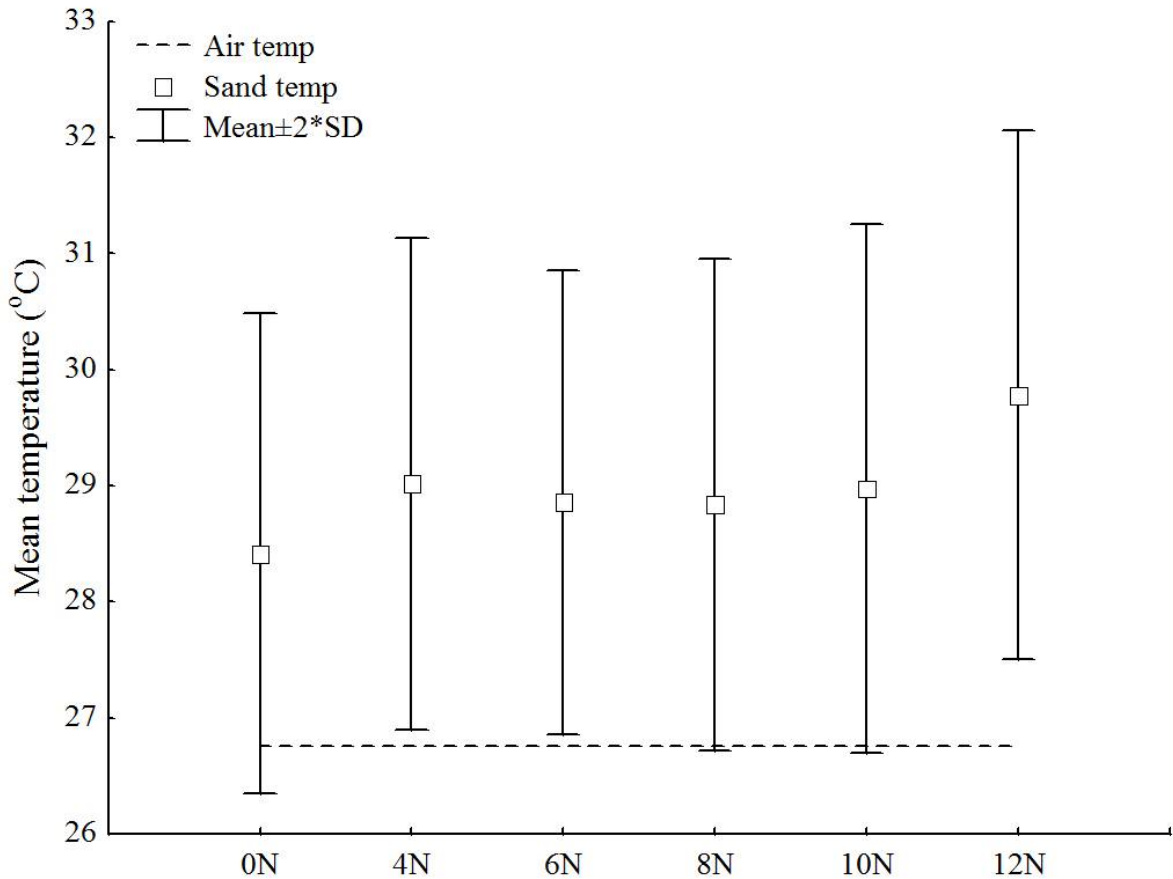


Figure 3.5. Mean sand ( $\pm$  SD) temperatures per site along the beach for the 2009/10 season. The mean air temperature for the 2009/10 season is indicated by the dotted line at 26.9°C.

The high sand temperatures at beacon 12N can be attributed to the greater distance from the sea and vegetation, whereas the orientation of beacon 0N is different - shading the beach in the afternoon (Fig. 3.5). One particular high tide resulted in tidal inundation of this same iButton. Where *in situ* temperatures are lower than the mean air temperature experienced at the study site, the differences may be attributed to tidal inundation.

### Incubation temperature

In total, 54 data loggers were placed in loggerhead nests over two nesting seasons (2008/9 n = 20; 2009/10 n = 34; Table 3.2; see Appendix B for nest information). During the first season, 11 of the 20 nests hatched (six with a known hatching date) while four nests did not hatch and five nests were not found. In the second season seven “moisture” iButtons that recorded relative humidity (% RH) and temperature (°C), in addition 27 temperature loggers, were deployed. Out of 34 temperature monitored nests, 18 nests hatched successfully (of

which the emergence date for two nests were missed; nest #10 had a hatching success < 20%, as only 20 hatchlings hatched from this clutch). Nine nests did not hatch at all (of which three were “moisture” logger nests), seven nests were not found again (irrespective of GPS coordinates) as nest markings were lost. Many unhatched clutches as seen in Appendix B, were predated before excavation, however, whether the predation (by ants and ghost crabs) took place before or after embryonic death cannot be concluded. These clutches were heavily decomposed that indicated the clutches died quite early during incubation, in addition the heat from the sand exacerbated the decomposing process.

Table 3.2. Summary of the number of iButton nests found, hatched nests with hatching success (HS) > 20%, hatching success < 20%, unhatched nests and the number of nests not found over two seasons.

	No. iButton nests	Nests found			Nests lost
		Hatched (HS > 20%)	Hatched (HS < 20%)	Not hatched	
Season 2008/9	20	11	1	4	5
Season 2009/10	34	16	1	9	7
<b>Total nests</b>	<b>54</b>	<b>27</b>	<b>2</b>	<b>13</b>	<b>12</b>

Mean nest temperatures of individual nests for the whole incubation period (IP) ranged from 24.0 – 34.9°C and showed an overall mean of 29.8°C ± 1.31 SD. During the TSP mean nest temperature was 29.9°C ± 1.09 SD and mean nest temperatures of individual nests ranged from 26.2 – 34.4°C (refer to Appendix B for nest data information). When hatching date could not be determined with certainty (due to strong winds, rain or some other disturbance of the nest tracks) (n = 7) the incubation period was taken as 60 days (average IP for loggerhead hatchlings) and if hatching success was lower than 20% (n = 2), nests were excluded from the incubation temperature statistical analyses.

Nest #9 (in the second season) experienced the highest mean nest temperature for both the whole IP and during the TSP. Hatching success for nest #9 was high (93%) and the nest parameters included nest depth (0.6 m), distance to the high-water mark (HWM) (56.0 m) and distance to vegetation (70.0 m). Nest #9 was located at 12N, the beacon at which the highest sand temperatures were recorded.

A one-way ANOVA showed no significant difference ( $p > 0.05$ ) between the nest temperatures for the two seasons. Inter-seasonal variation was relatively low suggesting that the nesting beach experienced a relatively stable thermal incubation environment, with little fluctuation within and between nesting seasons. As the season progressed (over both seasons), nests with an emergence date (excluding two nests with hatching success lower than 20%) became warmer (2008/9:  $n = 5$ ; 2009/10:  $n = 13$ ) (Fig. 3.6a). This coincides with the increasing ambient temperature from the beginning of the nesting season to the end of the study period. The incubation temperatures did not exceed critical thermal low ( $< 25^{\circ}\text{C}$ ) and high ( $> 34^{\circ}\text{C}$ ) incubation temperatures, except nest #9 that reached nest temperatures  $> 34^{\circ}\text{C}$ .

Two nests were excluded from the temperature data analysis, namely nest #12 (2008/10) and nest #10 (2009/10). Both nests had very low hatching success ( $< 20\%$ ). Nest #12 was predated by ghost crabs, whereas nest #10 was presumably predated by ants. These nests died earlier during incubation and were heavily decomposed at excavation. Mean nest temperature throughout the incubation period for nest #12 and #10 was  $29.3^{\circ}\text{C} \pm 1.60$  SD (range =  $25.8 - 32.5^{\circ}\text{C}$ ) and  $30.8^{\circ}\text{C} \pm 1.60$  SD (range =  $25.2 - 35.7^{\circ}\text{C}$ ), respectively. Nest #12 and #10 experienced mean nest temperatures during the TSP of  $29.3^{\circ}\text{C} \pm 1.51$  SD (range =  $26.1 - 31.2^{\circ}\text{C}$ ) and  $30.3^{\circ}\text{C} \pm 1.64$  SD (range =  $27.9 - 32.7^{\circ}\text{C}$ ). Temperature profiles for 18 nests are illustrated in Appendix A.

Sand temperature was recorded during the second nesting season (2009/10). Although air temperatures were acquired for both seasons, the second season of air temperatures were recorded every 30 minutes for daily means, while data obtained for the first season from Department of Environmental Affairs and Tourism (DEAT) was only maximum and minimum daily temperatures. Therefore, only sand-, nest- and air temperatures recorded during the second season were analysed.

Correlation analyses indicated strong positive correlations (Fig. 3.6b) ( $n = 13$ ;  $p = 0$ ) between mean nest temperature and mean sand temperature ( $r^2 = 0.63$ ), and a weaker, positive correlation between mean nest temperature and air temperature ( $r^2 = 0.24$ ), but no correlation was apparent with mean nest temperature and daily rainfall. Nest temperature correlated more strongly with sand temperature than with air temperature. From 14 December 2009 – 10 March 2010, mean nest temperatures, of nests with known hatching date ( $n = 13$ ), was  $30.0^{\circ}\text{C}$

$\pm 1.55$  SD (range = 25.8 – 34.9°C), mean sand temperature (n = 6) was 29.0°C  $\pm 0.99$  SD (range = 25.9 – 31.7°C) and mean air temperature was 26.9°C  $\pm 1.14$  SD (range = 22.7 – 29.1°C).

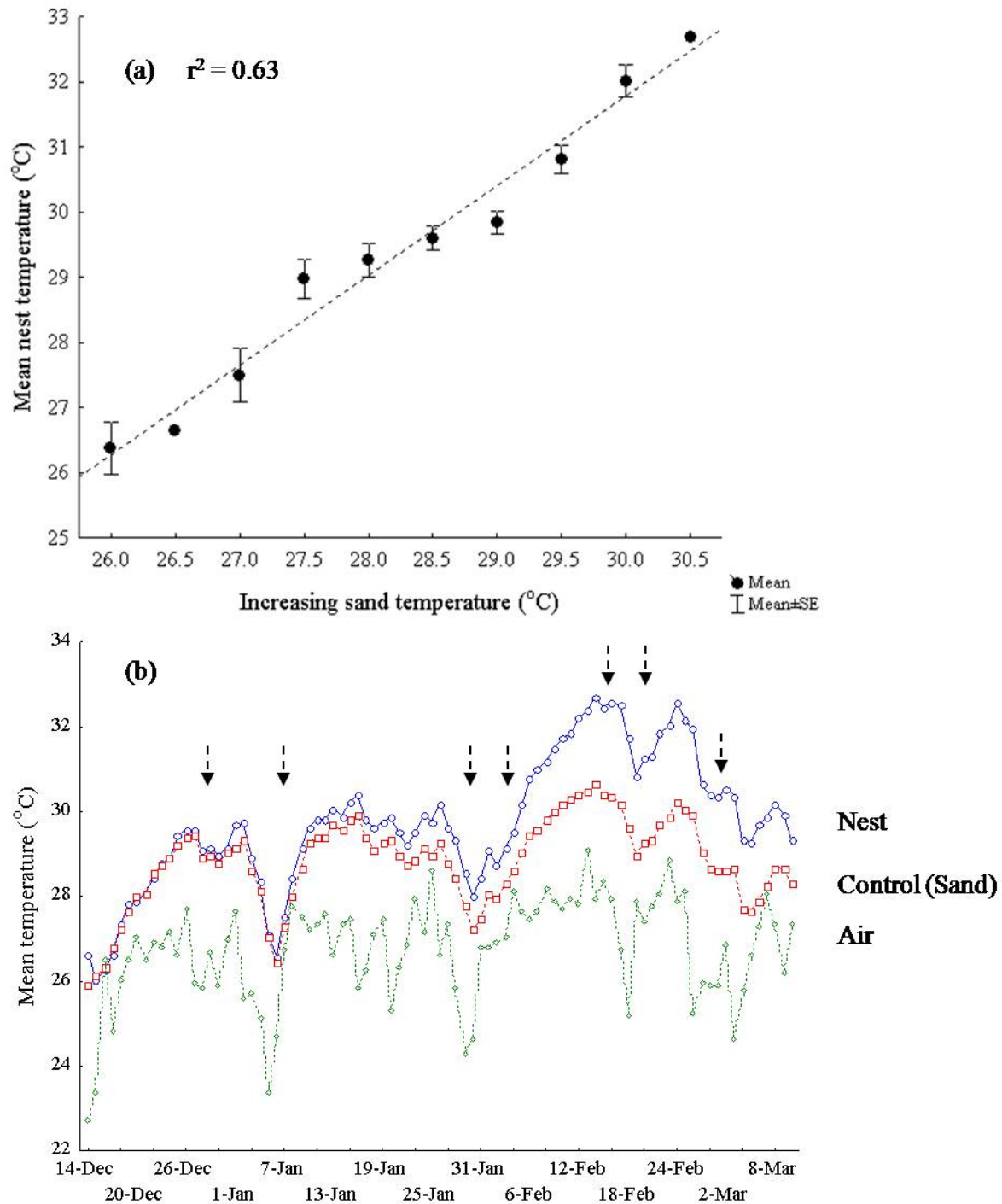


Figure 3.6. (a) There was a positive relationship between mean nest- and sand temperature ( $y = -6.171 + 1.246x$ ,  $n = 13$ ,  $r^2 = 0.63$ ). Standard error of means was calculated using pooled variance. (b) Graph of nest temperature (mean of 18 nests), sand (n = 6) and air temperature

over time (14 Dec – 10 Mar 2010) (dotted arrows indicate significant rainfall >15 mm events).

Only two moisture iButtons (out of seven) were retrieved from nests that hatched successfully, whereas three moisture iButton nests did not hatch and two were not found. From the two successfully hatched nests, the one moisture iButton indicated a relative humidity (% RH) of 100% for the duration of the incubation period. Water may have seeped through and inundated the data logger. Hence, only one moisture iButton (nest #17) had sufficient data. The % RH data retrieved showed a mean % RH of 94.56 (SD =  $\pm 1.09$ ; range = 91.17 – 96.81) during the TSP. Even though there was only one moisture monitored nest, % RH significantly correlated negatively with nest temperature ( $p < 0.05$ ;  $r^2 = 0.51$ ) and significantly correlated positively, but very weak, with the mean nest temperature during the TSP ( $p < 0.05$ ;  $r^2 = 0.23$ ). Thus, % RH decreases with increasing incubation temperature.

A stronger significant correlation was found between mean nest temperature and distance to the high-water mark ( $n = 20$ ;  $r^2 = 0.30$ ) (Fig. 3.7) than mean nest temperature during the thermal sensitive period (TSP) and distance to the high-water mark ( $r^2 = 0.26$ ).

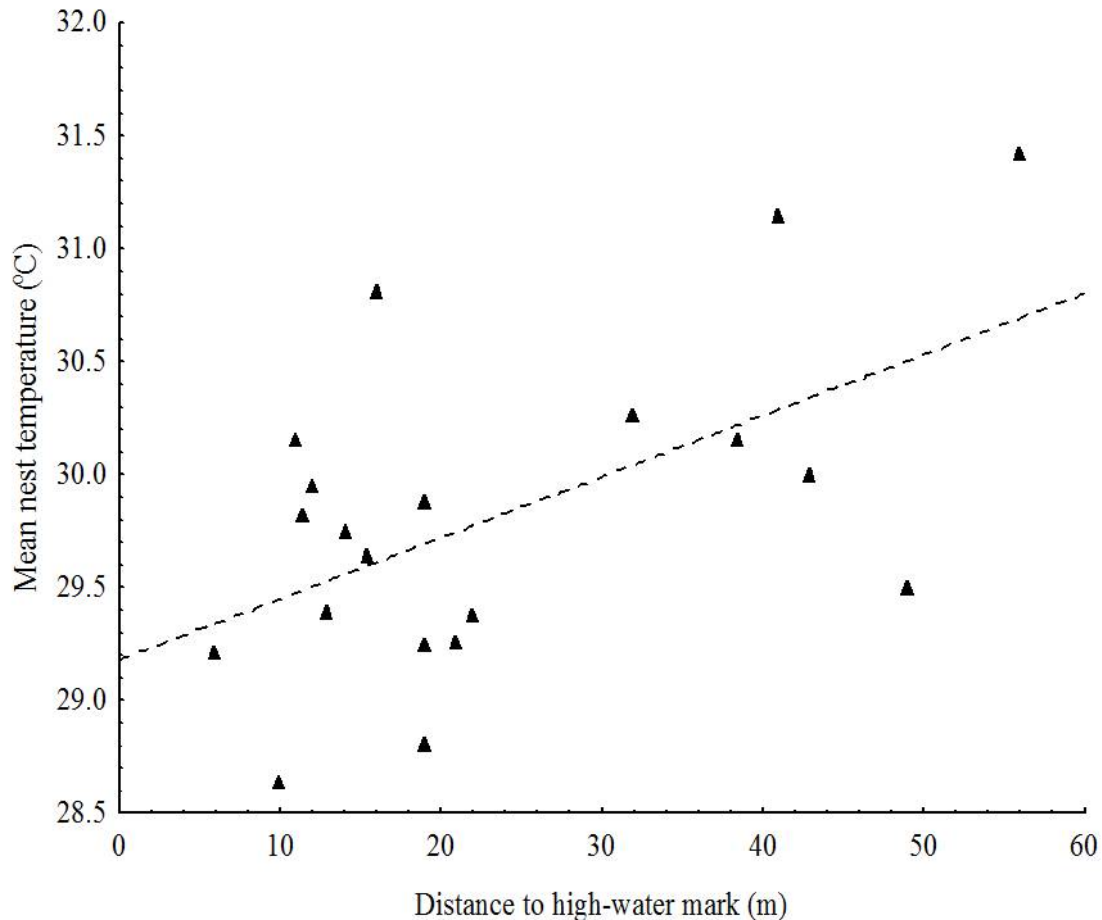


Figure 3.7. Positive relationship with the distance from high-water mark and nest temperatures ( $y = 29.1798 + 0.0271x$ ,  $n = 20$ ,  $r^2 = 0.30$ ).

Relationship between hatching success and incubation temperature

From 54 iButton nests, 29 hatched and showed an overall nest success of 68% (excluding lost nests). Heavily depredated nests, where hatching success was lower than 20% of the clutch, were excluded from hatching success analyses (2008/9: nest #12 had a hatching success = 9%; and in 2009/10 nest #10 had a hatching success = 18%). The overall hatching success and emergence success for this population (including the two clutches with hatching success < 20%) over these combined seasons are  $78\% \pm 23.29$  and  $77\% \pm 23.55$ , respectively ( $n = 29$ ).

Twenty study nests had usable temperature data with known incubation period. The mean hatching success was  $86\% (\pm 11.70 \text{ SD}; \text{range} = 53.52 - 98.61\%)$  and emergence success was  $85\% (\pm 13.13 \text{ SD}; \text{range} = 43.66 - 96.53\%)$ . Mean incubation temperature during the TSP



showed no significant correlations with hatching- and emergence success (Spearman Rank correlation,  $p > 0.05$ ). Clutch size, nest depth, distance to the high-water mark, distance to vegetation and elevation showed no significant correlations to hatching- and emergence success. In addition, a Kruskal-Wallis H-test indicated no significant difference (KW-H<sub>(1,20)</sub> = 0.17;  $p > 0.05$ ) between the two nesting seasons.

### Metabolic heating

The nest- and sand temperatures experienced regular fluctuations illustrated in Fig. 3.6a. Seasonal changes in sand temperatures at nest depth were described by the control data loggers (Fig. 3.6a) and were only recorded for the second season (2009/10). The data loggers in the nest equilibrated to the surrounding temperature after 4 hours. To examine the metabolic heating for the present study, metabolic heating within the nest was calculated by subtracting the sand temperature from the nest temperature from day 1 until day  $x$  of incubation (Godley *et al.*, 2001a).

Two of the 16 monitored nests, with known incubation period, were excluded from analysis to evaluate metabolic heating either because the suitable control data logger was not available (technical failure of control iButton at beacon 12N) or nests were laid before the control started with recording sand temperatures. Nest #10 had a low hatching success which implies lack of metabolic heating as the eggs did not develop further, but was included in Table 3.3. The mean contribution of metabolic heat to the nest throughout the IP was  $0.88 \pm 0.76^\circ\text{C}$ , with a range of  $-0.63$  and  $+2.85^\circ\text{C}$  (Table 3.3).

Table 3.3. The temperature (°C) measured for metabolic heating throughout the incubation period (IP), the first third IP, TSP and final third IP of study nests in the second season (2009/10). Metabolic heating was calculated by subtracting the sand temperature from the nest temperature from day 1 until day  $x$  of incubation.

Nest #	Metabolic heating for whole IP				First third IP				TSP				Final third IP			
	Mean	± SD	Min	Max	Mean	± SD	Min	Max	Mean	± SD	Min	Max	Mean	± SD	Min	Max
1	0.72	0.71	-1.01*	2.36	0.27	0.20	0.05	0.85	0.64	0.33	0.17	1.42	1.22	0.99	-1.01*	2.36
2	0.20	0.45	-0.50*	1.03	-0.21*	0.22	-0.50*	0.28	0.04	0.22	-0.19*	0.73	0.71	0.22	0.30	1.03
3	0.79	0.53	-0.24*	1.77	0.32	0.30	-0.15*	0.87	0.74	0.49	-0.24*	1.29	1.23	0.32	0.59	1.77
6	1.26	0.58	0.37	3.04	0.91	0.18	0.49	1.15	0.94	0.32	0.37	1.63	1.90	0.48	1.29	3.04
9	-	-	-	-	0.35	0.49	-0.48*	1.19	1.41	1.27	-0.95*	3.24	-	-	-	-
10	1.66	1.45	0.08	4.78	0.36	0.11	0.08	0.55	1.12	0.65	0.31	2.35	3.47	0.76	2.52	4.78
11	1.72	1.05	0.24	3.47	0.61	0.24	0.24	1.12	1.66	0.65	0.69	2.82	2.85	0.57	1.47	3.47
12	1.43	0.90	0.00	3.20	0.27	0.18	0.00	0.53	1.90	0.62	0.50	3.20	2.02	0.40	1.52	2.80
13	0.61	0.62	-0.83*	1.70	0.21	0.33	-0.36*	0.61	0.50	0.67	-0.83*	1.36	1.11	0.44	0.28	1.70
15	0.72	0.76	-0.47*	2.04	-0.08*	0.20	-0.47*	0.25	0.52	0.29	0.11	1.25	1.63	0.28	1.01	2.04
17	0.24	0.85	-1.01*	1.93	-0.63*	0.31	-1.01*	0.10	0.05	0.24	-0.56*	0.44	1.27	0.47	0.30	1.93
18	0.96	1.09	-0.13*	3.59	0.11	0.20	-0.11*	0.56	0.44	0.29	-0.13*	1.17	2.25	0.85	0.12	3.59
20	1.22	0.65	-0.68*	2.47	0.79	0.38	0.36	1.41	1.26	0.42	0.74	1.97	1.56	0.82	-0.68*	2.47
21	0.86	0.57	-0.22*	1.97	0.42	0.25	-0.22*	0.79	0.58	0.19	0.28	0.93	1.56	0.32	0.90	1.97

\*Sand temperature was higher than nest temperature, which implies cooling.

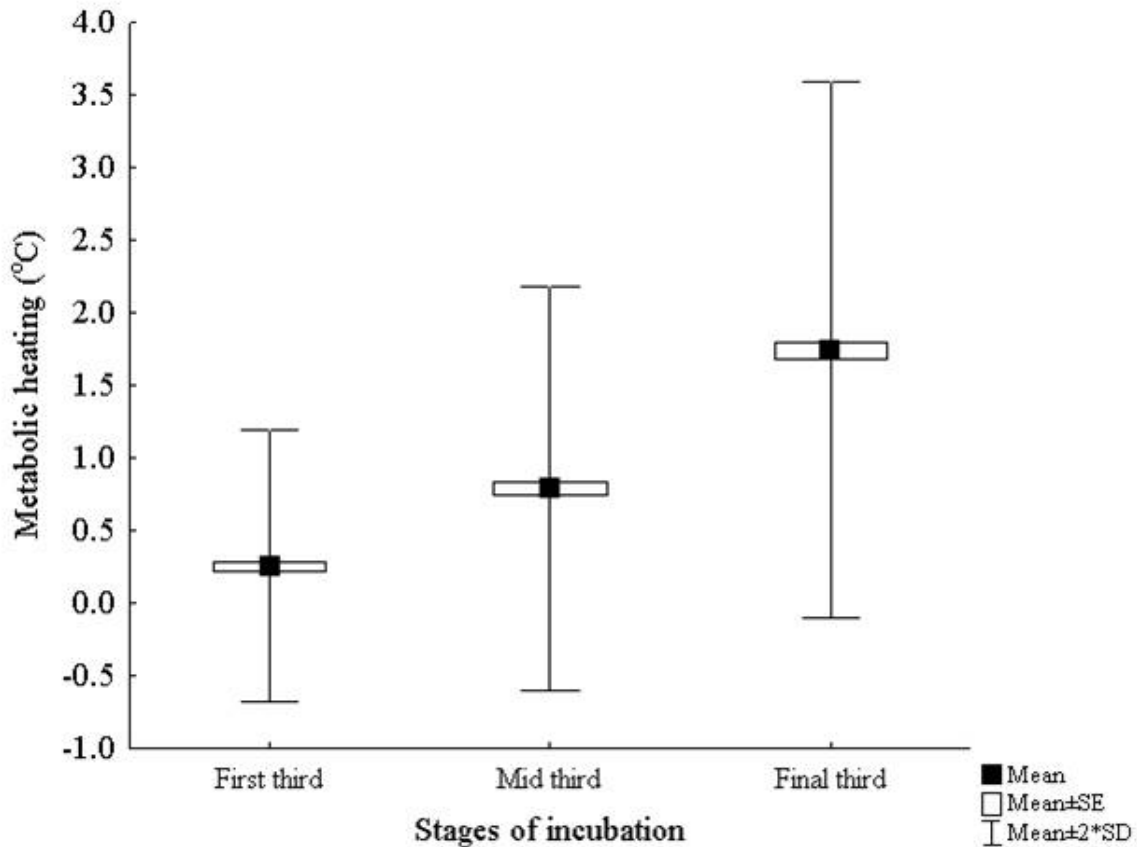


Figure 3.8. Mean ( $\pm$  SD) metabolic heating ( $^{\circ}\text{C}$ ) for three periods of incubation for 14 complete study nests showed a clear increase of metabolic heating within nests from early- to late incubation.

Metabolic heating varied significantly during the incubation period (Fig. 3.8). Analysis of variance (ANOVA) indicated a significant differences in the final third of incubation ( $F_{(2,35)} = 20.63$ ,  $p < 0.05$ ; mean first third =  $0.26^{\circ}\text{C} \pm 0.41$  SD; mean mid third =  $0.82^{\circ}\text{C} \pm 0.58$  SD; mean final third =  $1.61^{\circ}\text{C} \pm 0.76$  SD). Tukey HSD post-hoc analysis indicated significant differences ( $p < 0.05$ ) between all stages of incubation (first-, mid- and final third).

#### Relationship between nest temperature and incubation period (IP)

Overall, correlation analyses showed a weak negative correlation between the mean IP and mean nest temperature ( $y = 137.5186 - 2.6119x$ ;  $n = 18$ ;  $p = 0.0031$ ;  $r^2 = 0.39$ ) as well as with the mean IP and mean nest temperature during the TSP ( $y = 144.9876 - 2.8796x$ ;  $p = 0.011$ ;  $r^2 = 0.31$ ). When the two seasons were analysed separately, season 2008/9 ( $n = 5$ ) the IP did not significantly correlate to the mean nest temperatures. However, during the 2009/10 season ( $n$

= 13) the IP showed a strong negative correlation ( $p < 0.01$ ;  $r^2 = 0.86$ ) to the mean nest temperatures throughout the incubation period (Fig. 3.9a).

The incubation period was significantly shorter in the second season with a slight (but not significant) increase in temperature (ANOVA  $F_{(1,16)} = 5.61$ ,  $p = 0.03$ ). The mean incubation period was 59 days  $\pm$  2.63 the second season, as opposed to 63 days  $\pm$  4.09 for the first season (Fig. 3.9b). It may however be an artefact of the small sample size of the first season ( $n = 5$ ).

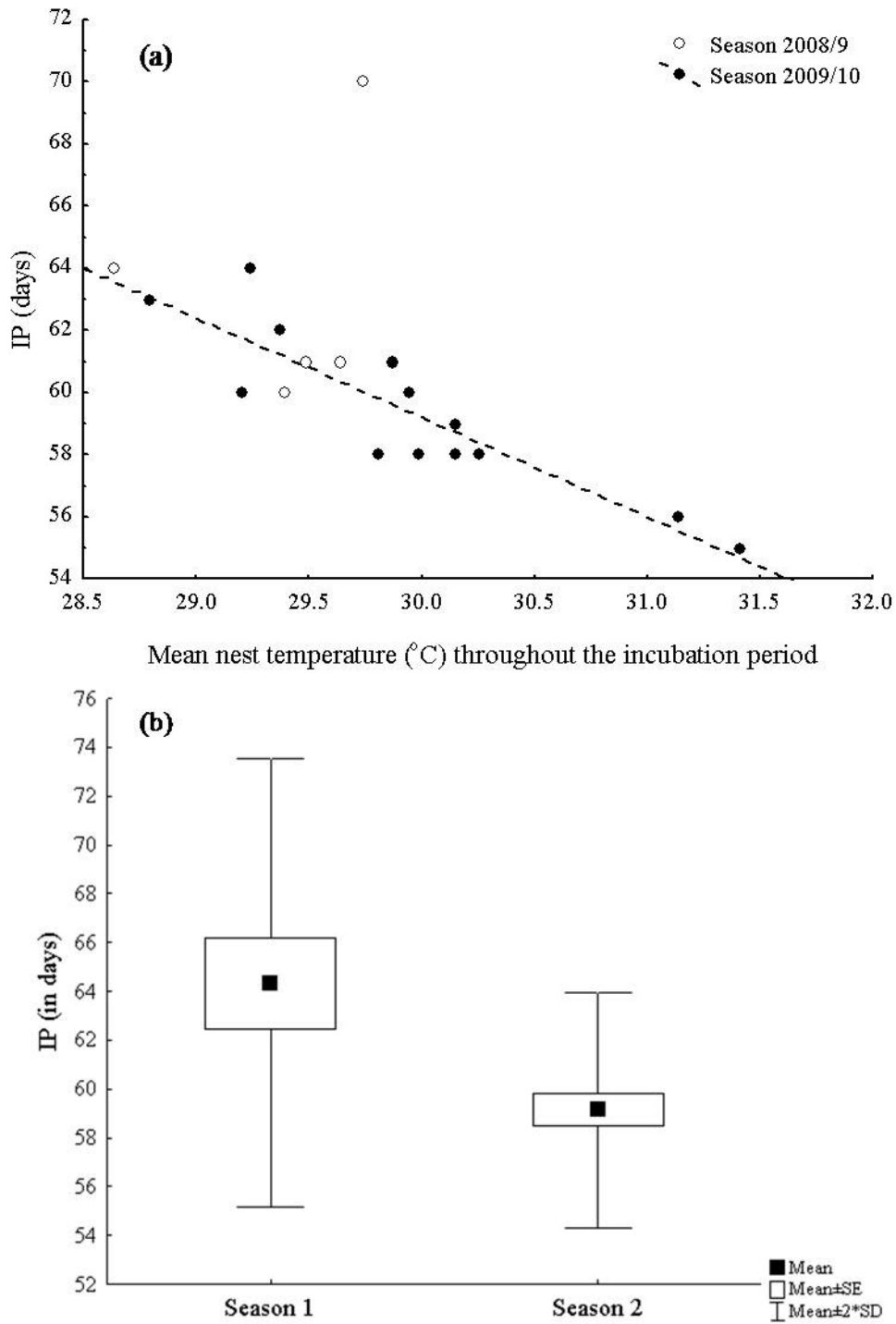


Figure 3.9. (a) The relationship ( $y = 137.5186 - 2.6119x$ ;  $n = 18$ ;  $p = 0.003$ ) between the IP (days) and the mean nest temperature for loggerheads for both seasons. (b) The mean incubation period for loggerheads nests for both seasons (2008/9 and 2009/10).

## ***Discussion***

South Africa hosts the southernmost loggerhead (and leatherback) rookery in the world (27°S) in Maputaland. Compared to other rookeries South Africa may experience lower incubation temperatures because sand temperatures decrease with increasing latitude (Carthy *et al.*, 2003). The main objectives of this chapter were to relate nest temperatures for loggerheads nesting in Maputaland to climatic and beach conditions, and to evaluate what the overall effect of nest temperature on hatching- and emergence success was.

Precipitation did not significantly correlate with daily nest- and sand temperature, however, it was evident that rain spells decreased nest- and sand temperatures, often followed by a temperature spike (even above critical thermal maximum temperatures usually after a rainy day). Houghton *et al.* (2007) examined the effect of protracted rainfall on leatherback nesting in Grenada Island in the Caribbean. They suggested that the overall effect of precipitation experienced by the clutches may result in male-producing conditions. Rainfall therefore can affect *in situ* temperatures which in turn affect incubation success. Similarly, the current study indicated a positive relationship between air- and sand temperatures, but there were no significant inter-seasonal changes (over two seasons). However, similarly to turtle nesting beaches in Australia (Booth and Astill, 2001; Hewavisenthi and Parmenter, 2002; Booth and Freeman, 2006), Japan (Matsuzawa *et al.*, 2002) and South America (López-Castro *et al.*, 2004) sand temperatures increased as the season progressed and became hotter later in the season.

Compared to other findings, sand temperature from the current study area coincided with other nesting beaches (ranging from 28 - 30°C) in Heron Island in Australia (Booth and Freeman, 2006) and Minabe rookery in Japan (Matsuzawa *et al.*, 2002) situated at lower latitudes. Diel fluctuations were observed in sand temperatures suggesting that the shallow depth (60 cm) at which the loggers were placed, which is also the average nest depth for loggerheads of the Maputaland region, is not sufficient to create a completely stable environment. Locally these temperatures were modified by beach conditions. The results indicated that sand temperature correlated with the distance to the dune base and nearest vegetation. Shading from both vegetation and the dunes may be the reason for the relationships. The sand temperatures at beacon 12N, which is a non-vegetated mobile dune

field, were dramatically higher than other sites. The sand temperatures were however still considered non-lethal ( $> 25^{\circ}\text{C}$  and  $< 34^{\circ}\text{C}$ ) for developing embryos.

In the southern hemisphere, north facing beaches have been suggested to experience warmer sand temperatures than beaches with other orientations (Booth and Freeman, 2006; Fuentes *et al.*, 2010). In this study, the only northern facing area (beacon 0N) experienced the lowest sand temperatures, while east facing beaches were warmer. This particular beach was backed by a high dune causing shade in the afternoon. The east-facing part of the beach was therefore exposed to the sun for longer. Furthermore, the iButton at beacon 0N was on occasion inundated by spring high tide contributing to the lower sand temperatures. The control (beacon) sites between the extreme sites (i.e. beacon 0N and 12N) had very little intra-beach variation which may suggest a stable thermal environment along the study site.

For the present study, moisture content within the clutch cannot be compared with hatching success as the sample size is not viable. Only one nest produced viable moisture data. Nonetheless, the results suggest that % RH decreased with increasing nest temperature. Sand moisture content was found to affect the hatching success of loggerhead hatchlings in Florida (McGehee, 1990). Hatching success was shown to decrease with increasing moisture content at 75% and 100% (McGehee, 1990). In contrast to the study conducted by McGehee (1990), the single clutch for this study showed a high moisture content throughout incubation with high hatching success (*ca.* % RH = 94.6%). However, this experiment needs to be repeated before any meaningful statements can be made. In southern Baja California (South America), olive ridley turtle (*L. olivacea*) nests showed high hatching success between 10 and 30 m from the high tide line where humidity was relatively low (relative surface humidity *ca.* 1%) accompanied by high sand temperatures ( $< 32^{\circ}\text{C}$ ) for this region (López-Castro *et al.*, 2004).

Mean incubation temperature was *ca.*  $30.0^{\circ}\text{C}$  with lower temperatures, but above the critical thermal minimum ( $< 25^{\circ}\text{C}$ ), experienced closer to the high-water mark. In Australia, green turtle (*C. mydas*) nest temperatures fluctuated around  $28^{\circ}\text{C}$  throughout the incubation period (Booth and Freeman, 2006). Incubation temperature was not affected by nest parameters including clutch size, nest depth and the distance from vegetation. Mean incubation temperature increased as the incubation period progressed due to general sand heating and

metabolic heating from embryos especially in the last middle third of incubation (Booth and Astill, 2001).

Two main processes affect changing nest temperatures, namely (1) climate (including daily and seasonal changes) and (2) metabolic heating (Godley *et al.*, 2001a). The results confirmed that nest temperature is a function of sand temperature (Fig. 3.6a) which, in turn, is a function of air temperature. During the first third of incubation, nest temperature followed sand temperature very strongly, as well as during the second trimester ( $0.82^{\circ}\text{C} \pm 0.58$ ). It was only in the third trimester of incubation that the incubation temperature rose significantly above sand temperature ( $1.61^{\circ}\text{C} \pm 0.76$ ) due to metabolic heating. Metabolic heating is produced by the embryos within the nest as development takes place, and it is clearly higher the more advanced the embryo development (Broderick *et al.*, 2001; Godley *et al.*, 2001a).

For this study, the magnitude of metabolic heating was high,  $< 3^{\circ}\text{C}$  (ranging between  $-0.63$  to  $+2.85^{\circ}\text{C}$ ), which is comparable with the results from Maxwell *et al.* (1988). Loggerhead populations in Alagadi, in the eastern Mediterranean, showed metabolic heating to range only from  $-0.4$  to  $+1.0^{\circ}\text{C}$  (Godley *et al.*, 2001a). This may be that loggerhead clutch sizes in Maputaland, as found in this study for two seasons (2008-10), are larger (mean clutch size =  $101 \pm 6.78$  SD) than compared to clutch sizes of loggerheads in Alagadi (mean clutch size =  $84.7$  eggs  $\pm 16.0$  SD) (Godley *et al.*, 2001a). The number of eggs laid in a clutch varies between clutches as well as between and within populations (Limpus, 1985; Dodd, 1988; van Buskirk and Crowder, 1994). In some studies, metabolic heating was significantly correlated to clutch size as larger clutches produced more heat than smaller clutches (Broderick *et al.*, 2001; Godley *et al.*, 2001a; Zbinden *et al.*, 2006). More so, Mrosovsky and Yntema (1980) also suggested that sex of hatchlings can be significantly affected by metabolic heat if the contribution is greater than  $1^{\circ}\text{C}$ . Care should be therefore be taken when predicting sex ratios based on sand temperatures as metabolic heat is not accounted for which may bias the outcome.

The incubation period was calculated from the oviposition date to the date of the first hatchling emerging. However, this may differ from the true incubation period, i.e. oviposition to hatching, by a few days (Godley *et al.*, 2001a). The estimated lag between pipping eggs to emerging above the substrate for loggerhead turtles (in the USA) is 4.1 days (Godfrey and



Mrosovsky, 1997). This estimate is not available for loggerhead turtles in South Africa as for many rookeries, but it was assumed to be similar. The estimated incubation time will thus be shorter than that calculated in most field-based studies (Kaska *et al.*, 1998; Godley *et al.*, 2001b).

It was clear that temperature influences the duration of incubation (Ackerman, 1997). In the current study, the relationship between mean nest temperature and the incubation period was significant and a better estimate than TSP ( $r^2$  of 0.39 versus 0.31). This is similar to studies on loggerheads in the Mediterranean (Kaska *et al.*, 1998; Godley *et al.*, 2001a; Godley *et al.*, 2001b; Rees and Margaritoulis, 2004). Even though the mean nest temperatures during the middle third of incubation are ultimately used to determine sex ratios, the incubation period is also an integration of the developmental rate throughout incubation (Godley *et al.*, 2001b).

It can be concluded that sand temperatures and, ultimately, incubation temperatures in Maputaland do not exceed the critical thermal limits for loggerhead turtle eggs and produce hatchlings. These results are similar to the findings of other studies (e.g. Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982) i.e. that clutches across the nesting beach normally experienced non-lethal temperatures. *Lepidochelys olivacea* embryos in Costa Rica showed low hatching success (< 2%) due to *in situ* incubation temperature exceeding the critical thermal maximum (> 35°C) (Valverde *et al.*, 2010). On the odd occasion during this study the nest temperature did exceed these thermal limits, but it did not persist and the effect on incubation success is unclear (e.g. nests #9 and 10, during the first and second season respectively). Nest #9 was laid at beacon 12N (the exposed beacon in open sand), which is known for the high sand temperatures. Nonetheless, hatching and emergence success of this nest was 97%, whereas nest #10 (with a higher maximum) had low hatching success of 18%. Metabolic heating from embryos may have spiked the incubation temperatures above the critical thermal limit. The possible lethal effect of metabolic heat within clutches should thus not be ignored.

The nest success (defined as nests that produce hatchlings, i.e. > 20% of clutch size) for the Bhangha Nek nesting beach was high during the study period of 2008-10 (*ca.* 89%; n = 363). The high nest success can in part be attributed to non-lethal sand temperatures which creates favourable incubation conditions, as well as the strict beach access and monitoring efforts

during the nesting season. The first season (2008/9) seems to have had a higher nest success, however, during the second season (2009/10), more nests were monitored, and the sampling effort was significantly higher and therefore more representative. Nests that were not marked, and did not hatch, cannot be taken into account for overall nest success of the high-density nesting area. However, the “opportunistic” nests (nests that were not marked, but discovered and excavated after hatching) were useful to assess the hatching- and emergence success of loggerhead hatchlings for this region.

The hatching success for the loggerhead hatchlings at Bhanga Nek beach, was high with a mean hatching success of 86%. This is typical of loggerhead clutches (National Research Council, 1990; Miller, 1999) and was slightly higher than, but coincides with, the 79% hatching success for the same region 36 years ago (Hughes, 1974a). Similar results were obtained in Australia (Chu *et al.*, 2008). But, unlike the studies by Chu *et al.* (2008) and Godley *et al.* (2001), hatching- and emergence success of the temperature monitored nests were not related to incubation temperature within the range obtained on these beaches. In addition, hatching success was not correlated to any physical parameters (e.g. nest depth, distance to vegetation etc.) investigated in this study. The conclusion is therefore that the beach conditions (both in terms of temperature and physical conditions) are well within the successful incubation range, and it is not one individual factor that is responsible for eggs/nests not to develop and hatch. Factors such as gas exchange or relative humidity (not successfully measured in this study) need to be further investigated.

The reason for the high number of unsuccessful clutches during the two seasons (2008-10) is not clear, but may be due to high spring tides that may have inundated the nests particularly between beacons 0 – 4N, which has a low back-beach area. Öz *et al.* (2001) showed similar results where only 19 of 85 nests hatched at Patara beach in the Mediterranean during year 2000. They suggested that nests experienced low hatch success mainly due to inundation.

The possibility that eggs from some of the unsuccessful clutches may not have been fertilized successfully is low. Loggerheads mostly lay fertile eggs with fertility typically above 80% (Miller, 1999; Miller *et al.*, 2003). Miller (1997) noted that distinguishing early embryonic death and intraoviducal death is difficult especially at the end of incubation (at 60 or more days). For this study, the conclusion is thus that the high number of unhatched eggs in some

clutches was due to unfavourable conditions, such as high temperatures, inundation, predation or desiccation rather than infertile eggs.

The degree of unsuccessful clutches may be higher than expected in Bhanga Nek. During the second season (2009/10) more nests were marked, monitored and assessed than during the first season (2008/9). However, we excavated more than 200 unmarked nests. In this case, failed clutches (of unmonitored nests) would have been missed, hence the degree of unsuccessful clutches might be higher than observed possibly due to unfavourable conditions.

Maxwell *et al.* (1988) did not publish sand temperature data, but TSP temperature data was published, and could be used for comparison. We found that the incubation temperature during the TSP increased from 22 years ago. From Maxwell *et al.* (1988), the mean incubation temperatures experienced during the TSP were *ca.* 28.8°C ± 1.15 SD (n = 17) while TSP temperatures, 22 years later, showed 29.8°C (n = 7). This data may suggest that there has been an increase in temperature over time, or that Maxwell *et al.* (1988) had a relatively cold season during which they monitored incubation.

Putting these results into context, sea turtles have overcome multiple climate changing events, but it has been suggested that sea turtles, and other reptiles displaying TSD, would not be able to adapt to the current fast changing climate (Janzen, 1994). Janzen (1994) used the TSD mechanism as a model to examine the biological effects of climate change in painted freshwater turtles (*Chrysema picta*) populations of North America. Their results, over a 5-year period, indicated unisexual nests each year and most of them were female-biased, but with the predicted increasing temperatures may result in the production of only females and may eventually lead to local extinction (Janzen, 1994).

In addition, Mazaris *et al.* (2008) examined the effect of climate change in loggerhead turtles in the Zakynthos Island by using data collected from 1984 to 2002. Loggerhead turtles from Laganas Bay in Zakynthos Island (Greece) showed a decline in the average clutch sizes as sea surface temperatures (SSTs) increased (Mazaris *et al.*, 2008). Increasing SSTs are also resulting in earlier sea turtle nesting and hatching seasons (Mazaris *et al.*, 2008). Thus, increasing temperatures due to the global warming effect may influence sea turtle populations over time.

Due to warmer thermal incubation conditions during the TSP, more female hatchlings are expected to have been produced at the high-density nesting area. Whether there are male-producing beaches within the > 120 km nesting area is not known. The study site is the high-density nesting beach, Bhanga Nek, and is ideal for examining sex ratios and hatching success in the natural nest regime due to its remoteness.

### ***Conclusion***

Bhanga Nek is the peak nesting area for loggerheads in KZN and the Western Indian Ocean, and by investigating the effect of temperature on hatching success of this stretch of beach can be considered representative for the population. This study reports the first thermal profile of the nesting beach and incubation environment for loggerhead clutches. From these results, the thermal gradient established along the nesting beach indicated high sand temperatures, but rarely do they exceed the critical thermal limits of > 34°C. Sand temperature should be used cautiously as a thermal baseline to determine effects of hatching success and sex ratios due to high metabolic heating in the nests, especially in the last trimester that may result in embryo death. Nest success (68%) and hatching success (86%) for loggerhead hatchlings in Bhanga Nek is high. This suggests that Bhanga Nek has optimum incubation temperatures, and as a result produces many successful hatchlings within the incubation environment. Incubation temperature did not affect hatching success or nest success. The surrounding sand temperatures form the thermal basis for embryo development and metabolic heating from embryos is negligible until the mid-third to the final third of incubation where metabolic heating becomes evident. However, there was uncertainty as to why some of the nests had low or no development as there were no obvious signs of predation, disturbance, infertility or deformity of eggs. Therefore, continued research is pivotal to understanding the effect of the thermal environment on the success and hatchling sex ratios of this population.

## Chapter 4

# Histological examinations to determine hatchling sex ratios of loggerhead turtles (*Caretta caretta*) in KwaZulu-Natal, South Africa

### *Abstract*

Sea turtles are like most reptiles in that they do not possess heteromorphic chromosomes, and despite the search for alternative methods to genetics, the most reliable method still for sexing hatchlings is through histological examinations of the gonadal complex. The present study examined the gonads of *Caretta caretta* hatchlings using standard histological methods described in Benabib and Mrosovsky (1990). The purpose of the project was to estimate hatchling sex ratios for the component of the Southwestern Indian Ocean loggerhead population nesting on the beaches in northern KZN, and to compare the results to a previous study. This histological study suggested a female-biased sex ratio for the peak nesting area, during the peak nesting/hatching season. Dead embryos and hatchlings were collected from nests during the hatching season in February and March over two seasons, preserved, and later processed. The results indicated an overall female bias (as was the case with Maxwell *et al.* 1988) with 79% of the hatchlings being female. The results between the two seasons were similar with the 2008/9 season producing 76% (n = 54) females and 2009/10 season producing 83% females (n = 82). The higher incubation temperatures resulted in shorter incubation periods in 2009/10, therefore favouring female production.

## ***Introduction***

Unlike humans, turtles and many other reptiles do not possess heteromorphic sex chromosomes and show no obvious genetic differences between sexes (Spotila, 2004; Valenzuela, 2004a). Instead, reptilian embryos use egg incubation temperatures to determine the sex of individuals in a process called temperature-dependent sex determination (TSD) (Spotila, 2004; Valenzuela, 2004b). Generally, in sea turtles, higher incubation temperatures ( $> 29^{\circ}\text{C}$ ) produce females, whereas lower incubation temperatures ( $< 29^{\circ}\text{C}$ ) produce males (Yntema and Mrosovsky, 1980; Marcovaldi *et al.*, 1997; Carthy *et al.*, 2003).

Sexing of turtle hatchlings, juveniles or sub-adults cannot be directly determined by morphological observations as sexual dimorphism is only evident at sexual maturity (Ceriani and Wyneken, 2008). Hence, obtaining reliable estimates of hatchling sex ratios is challenging. Much research has gone into non-invasive techniques to estimate sex ratios for hatchlings as it is an important parameter in managing populations. Early studies (Mrosovsky *et al.*, 1984; Mrosovsky and Benabib, 1990; Mrosovsky and Provanha, 1992) routinely used histology of hatchling gonads as a method of investigation, but because of endangered status of turtles histology was discouraged. Histological studies require euthanizing hatchlings. Alternative methods investigated included using incubation temperatures or beach temperatures (e.g. Booth and Freeman, 2006; Chu *et al.*, 2008), incubation duration (e.g. Marcovaldi *et al.*, 1997; Godley *et al.*, 2001b; Chu *et al.*, 2008) or hormonal assays. However, hormonal studies, using radioimmunoassays were unsuccessful in determining the sex of hatchlings ( $< 15$  cm) reliably because large volumes of blood are required and hormone concentrations are too low for sex discrimination (Owens *et al.*, 1978; Owens, 1999). More recent studies successfully used laparoscopy and external gonadal characteristics for determining sex (Wyneken *et al.*, 2007). Laparoscopy is a specialised surgical technique that relies on some grow-out of hatchlings to the age of approximately 6 months before it can be carried out successfully.

To date, histological studies (e.g. Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982; Mrosovsky and Benabib, 1990; Ceriani and Wyneken, 2008) have produced the most reliable results for sexual differentiation. Female gonads can be identified by tissue displaying poorly organized medulla typical of the ovaries, and having a well-developed

cortex lined with basophilic cells (Fig. 4.1a). In contrast, immature testis have seminiferous tubules and a poorly developed cortex (Fig. 4.1b) (Yntema and Mrosovsky, 1980; Mrosovsky and Benabib, 1990; Wyneken *et al.*, 2007).

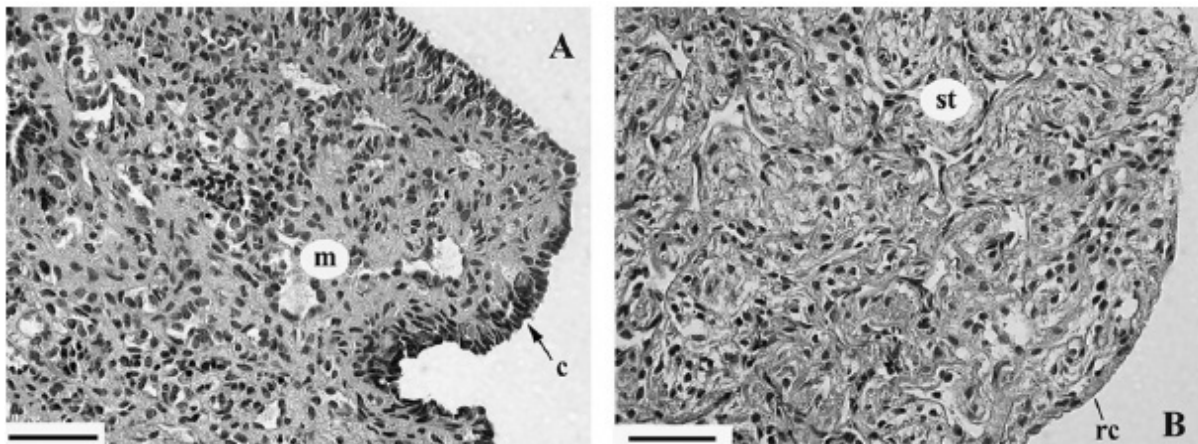


Figure 4.1. Photomicrographs of (A) an immature ovary, where the cortex (c) is well-defined and the medulla (m) is poorly organized, and (B) an immature testis, with a regressed cortex (rc) and seminiferous tubules (st), in *Caretta caretta* hatchlings. (Ceriani and Wyneken 2008)

Nesting trends for the loggerheads indicate a significant increase in the number of nesting females over the years, whereas leatherback female numbers are stable with a possible marginal increase (Nel, 2008). The positive recovery in loggerheads is a direct result of effective beach protection measures, but probably also a successful reproductive strategy (e.g. nest site fidelity) (Wilson and Humphrey, 2004; Nel, 2008). It is however uncertain as to the contribution the sex ratio has made to the recovery of loggerhead turtles in South Africa.

The study conducted by Maxwell *et al.* 1988, more than two decades ago, was for one season only. It is uncertain as to the current status of hatchling sex ratios for the South African component of the loggerhead population. Maxwell *et al.*'s (1988) study revealed a critical temperature of 28.5°C where nests with this incubation temperature or lower were all male-producing, and nests above 29.6°C were female producing. Males were therefore produced during the early part of the season and females from mid- to late season. The authors did however concede that the sex ratio across the population is presumably closer to a 1:1 ratio, given the number of nests laid during the mid- to late season (Maxwell *et al.*, 1988).

The objectives of this study were therefore to: (1) estimate sex ratios for the loggerheads over two seasons; (2) obtain a more representative sex ratio across the population by obtaining the sex ratio of hatchlings produced during the peak nesting and hatching season, for the peak nesting area; (3) to compare the results with that of Maxwell *et al.* (1988), and conclude on possible effects of global climate change; (4) possibly find evidence of sexual dimorphism (in size). It is hypothesized that the loggerhead population, given the current rate of recovery, is currently highly female-biased.

## ***Materials and Methods***

### ***Study site***

The study was focused on the 5 km beach stretch of Bhanga Nek (outlined in Chapter 2) that is the peak nesting area for loggerhead turtles in northern KwaZulu-Natal.

### ***Field sampling***

After the hatching season (February/March) a large number of nests were excavated, including some nests containing iButton temperature loggers. During the 2008/9 hatching season, dead embryos and dead hatchlings found during excavation, were frozen for later analysis. Dissections of the embryos/hatchlings were conducted in a laboratory after thawing the individuals overnight.

Additionally, during the 2009/10 hatching season, a number of hatchlings were euthanized from nests containing iButton recorders, by using an injection of sodium pentobarbital (2.5 ml per hatchling; 10 hatchlings per iButton nest, with the required animal ethics clearance obtained). Hatchlings were dissected at the study site and the gonads were preserved in 10% buffered formalin for four days, before preservation in a 70% ethanol solution. Straight- and curved carapace length and width measurements (in mm) were obtained prior to dissection to assess possible morphometric differences between sexes.





Figure 4.2. (a) Cages were placed over temperature monitored nests a few days before the estimated hatching date to ensure the capture of hatchlings (for histology). (b) Ten hatchlings per temperature monitored nest were euthanized for analysis.

### Histology

The present study used the method described in Mrosovsky and Benabib (1990). Experimentation with the histological procedures allowed for tailoring the methods to *C. caretta*. The histology was initiated by removing both the gonads and kidneys (Fig. 4.3 and 4.4a).

Histology is a step-wise process of dehydration of samples, infiltration and wax-mounting, sectioning, staining and mounting. Step 1 is the dehydration process, which is the removal of all water molecules from the (gonad) tissue by using a series of alcohol baths, i.e. 70% ethanol solution for 90 minutes, 95% ethanol solution for 90 minutes and finally a xylene bath for 15 minutes (Fig. 4.4b). This is followed (step 2) by a series of paraffin waxes used for the infiltration process (Fig. 4.4c). Tissue samples were placed in each (of three) waxes for one hour and dried before embedding the samples into a wax mould and finally mounting them onto wooden blocks (Fig. 4.4d & e) (Dr. G. Rossouw, pers. comm.).

Step 3 was the sectioning which was conducted using a microtome (Fig. 4.4f). Thin sections were cut (thickness = 10  $\mu\text{mm}$ , 5  $\mu\text{mm}$ , 3  $\mu\text{mm}$  or 1  $\mu\text{mm}$ ) and placed on albumenized glass slides by using a waterbath. The slides were dried overnight before the staining process (Fig. 4.4g). The stains used (in step 4) were Mayer's hematoxylin (lithium carbonate solution was used as the bluing reagent) and an alcoholic eosine solution. The stained sections were

mounted with cover slips by using DPX mountant (Fig. 4.4h) and finally (step 5) examined and sexed under a light microscope (Fig. 4.4i). Photomicrographs of the histological sections were taken by using *Motic Image Plus2.0*.

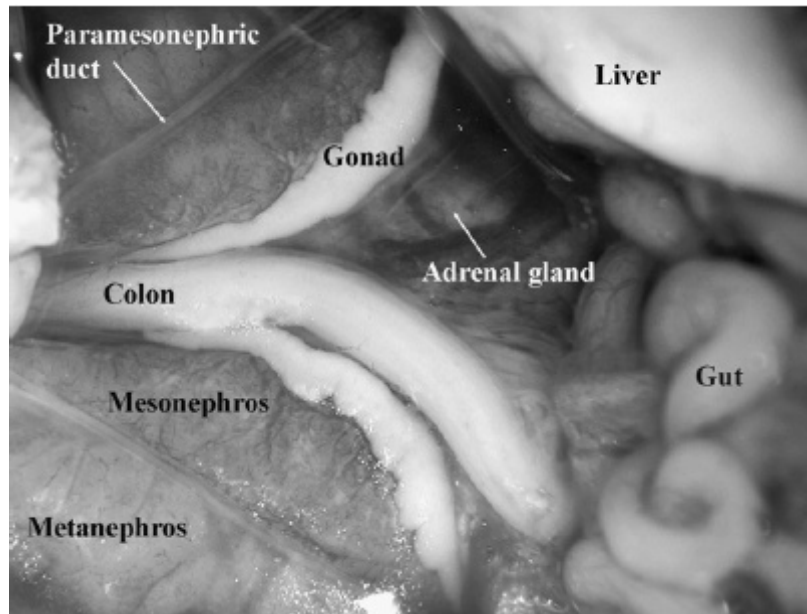
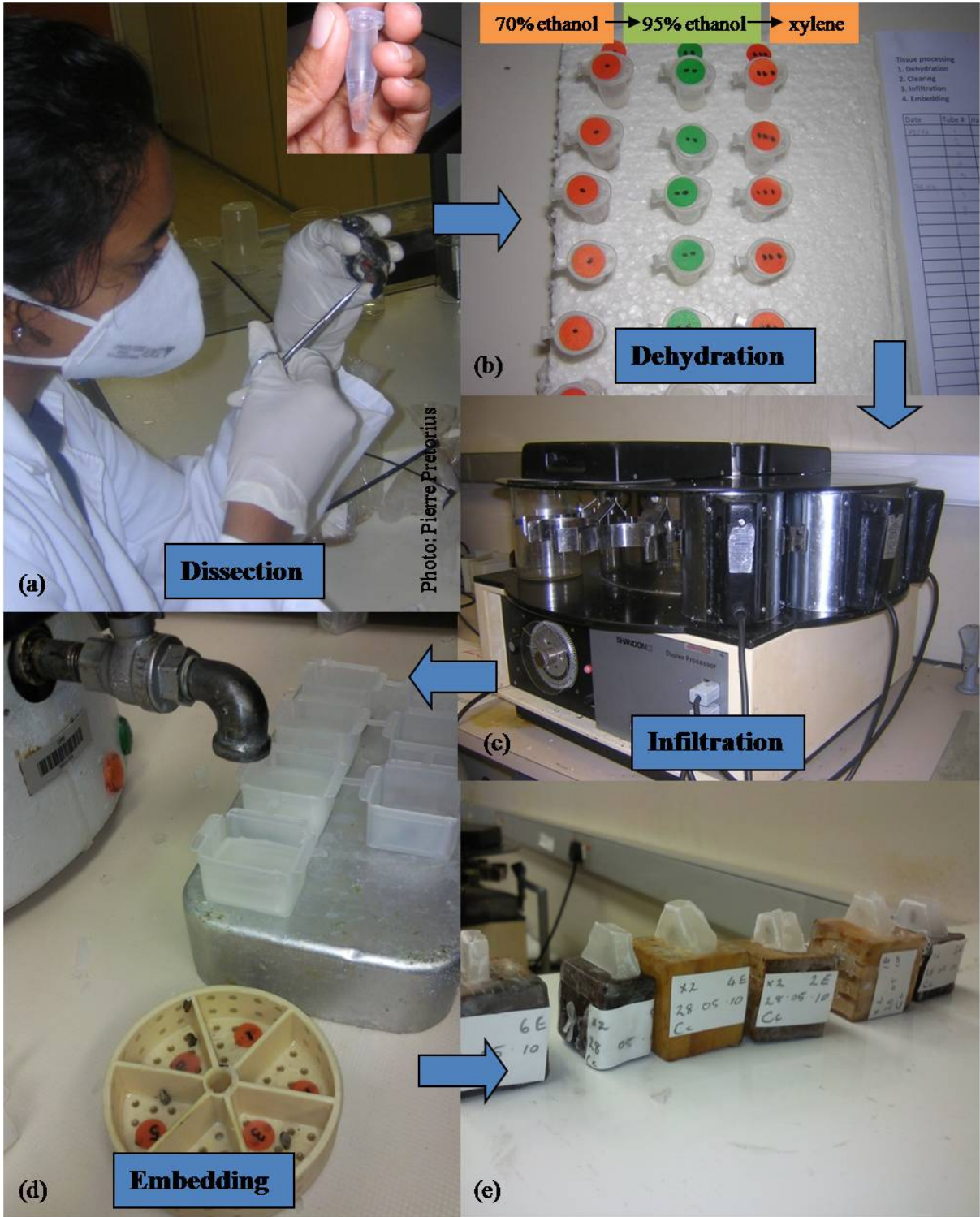


Figure 4.3. The ventral view of a sea turtle hatchling where the gonad is attached to the peritoneum overlying the kidney. Image from: Ceriani and Wyneken (2008).





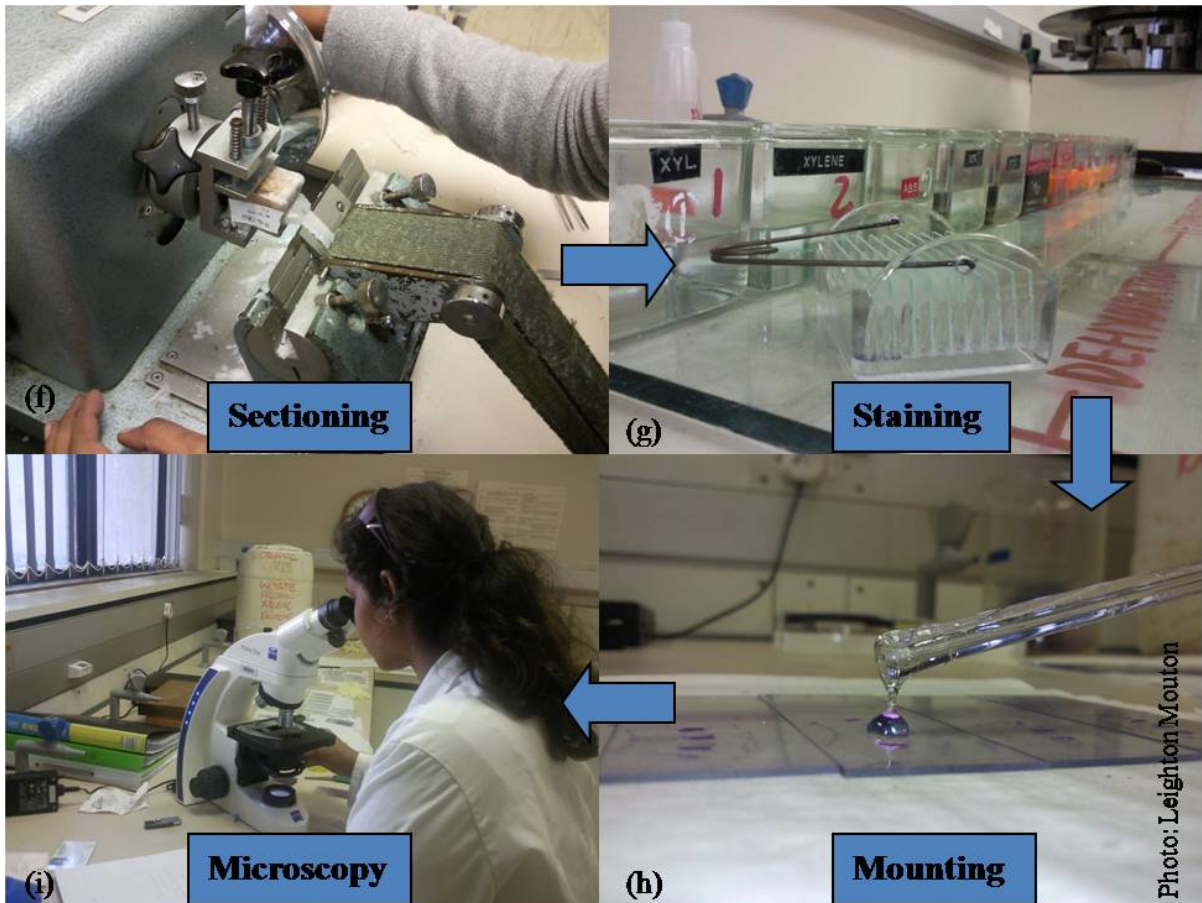


Figure 4.4. (a) – (h) Graphical description of the methods to prepare samples for histological examination.

#### Histology – Refining procedures

Initially four different tissue thicknesses were tested for appropriateness (10  $\mu\text{m}$ , 5  $\mu\text{m}$ , 3  $\mu\text{m}$  and 1  $\mu\text{m}$ ). At 10  $\mu\text{m}$  the tissue was too thick, and the stains appeared too dark for examination. At 1  $\mu\text{m}$  the tissue samples were cut poorly which resulted in breakage of the tissue. A tissue thickness of 5  $\mu\text{m}$  was sometimes useful and tissue sections showed sufficient staining, but the nuclei appeared black in colour. Even staining was obtained when the tissue was sectioned at 3  $\mu\text{m}$ . Both 5  $\mu\text{m}$  and 3  $\mu\text{m}$  sections were therefore used depending on the size or orientation of the tissue sample.

Initially slides were unclear and required experimentation with dehydration times. The first exposure to xylene removed the paraffin wax surrounding the tissue. The final xylene exposure reassured that all the wax and water molecules were removed from and around the tissue. If the xylene became turbid and creamy white, contamination occurred, i.e. the samples still had excess water molecules. This problem was overcome by extending the first

xylene stage to at least 5 minutes. Less than 3 minutes in the bluing solution (lithium carbonate solution) resulted in lightly coloured pink nuclei, while at 3+ minutes the nuclei were correctly stained a dark purple colour. The final procedure applied is summarised in Table 4.1.

### Statistical analyses

Chi-square and contingency tables were used to assess differences in the frequencies of females obtained per season and the ratio of males to females. Analysis of Variance (ANOVA) was used for seasonal variance between specimen length and width measurements. All data analyses were conducted in Statistica v9. Statistical significance was assumed at  $p\text{-value} \leq 0.05$ .

## **Results**

### Field sampling

During 2008/9, 11 out of 20 study nests hatched successfully (six with known hatching date). From the 11 hatched nests, dead hatchlings were opportunistically obtained from three nests. During 2009/10, 16 nests (out of 34 study nests) hatched successfully (the emergence date for two nests was missed; nest #10 had a hatching success  $< 20\%$ , and only 20 hatchlings hatched from this clutch and therefore none were euthanized). Hatchlings from five iButton nests were successfully caught and ten hatchlings per nest were randomly selected for euthanization. Hatchlings and embryos of unmonitored (opportunistic) nests were also collected for analysis during both seasons.

During the first season of this study (2008/9) 64 hatchlings were usable (in terms of size and not being in advanced decomposition). Only hatchlings/tissues were used from nests with two or more individuals, which brings the number of hatchlings for the first season (2008/9) to 52. It seemed unreasonable to obtain a sex ratio for a nest from a single individual. During the second season, 172 embryos/hatchlings were collected, including the euthanized hatchlings. Of these 38 embryos/hatchlings were discarded due to decomposition or because the embryos were too immature. The final sample size for the 2009/10 season was 85 individuals.

### *Sex determination and sex ratio data*

To estimate hatchling sex ratios, 137 paired gonads were examined (2009: n = 52; 2010: n = 85). Unknown sexes (i.e. tissue samples were too poorly sectioned; n = 3) were not included in the statistical analyses.

Female tissue was identified, where the germinal epithelium or cortex was evident, forming the outer layer of the ovary (Fig. 4.5a). The medulla was homogenous and the germinal epithelium extended into the medulla associated with surface grooves (Yntema and Mrosovsky, 1980). Females were easily identified due to the well-defined germinal epithelium.

In the males, simple squamous epithelium was observed in the immature testis (Yntema and Mrosovsky, 1980). In addition, seminiferous tubules formed by primary cords were observed (Yntema and Mrosovsky, 1980). Similarly to the study conducted by Yntema and Mrosovsky (1980) complete resorption of the oviduct, in the male, was not observed (Fig.4.4b). The gonadal characteristics that distinguish males from females concurs with other studies (Yntema and Mrosovsky, 1980; Mrosovsky and Benabib, 1990; Ceriani and Wyneken, 2008).

Table 4.1. The exposure times (in minutes) for correct staining of gonad samples. To remove excess alcohols or stain solution, running tap water (H<sub>2</sub>O) was used for the time indicated.

Paraffin wax removal		Rehydration			Staining						Dehydration					
Xylene		Alcohol solutions			Stains						Alcohol solutions				Xylene	
I	II	100%	95%	70%	H <sub>2</sub> O	Hematoxylin	H <sub>2</sub> O	Bluing	H <sub>2</sub> O	Eosin	70%	95%	Abs I	Abs II	I	II
5.00	5.00	3.00	2.00	2.00	4.00	1.30	4.00	3.00	4.00	2.30	2 dips	2 dips	3.00	3.00	3.00	3.00

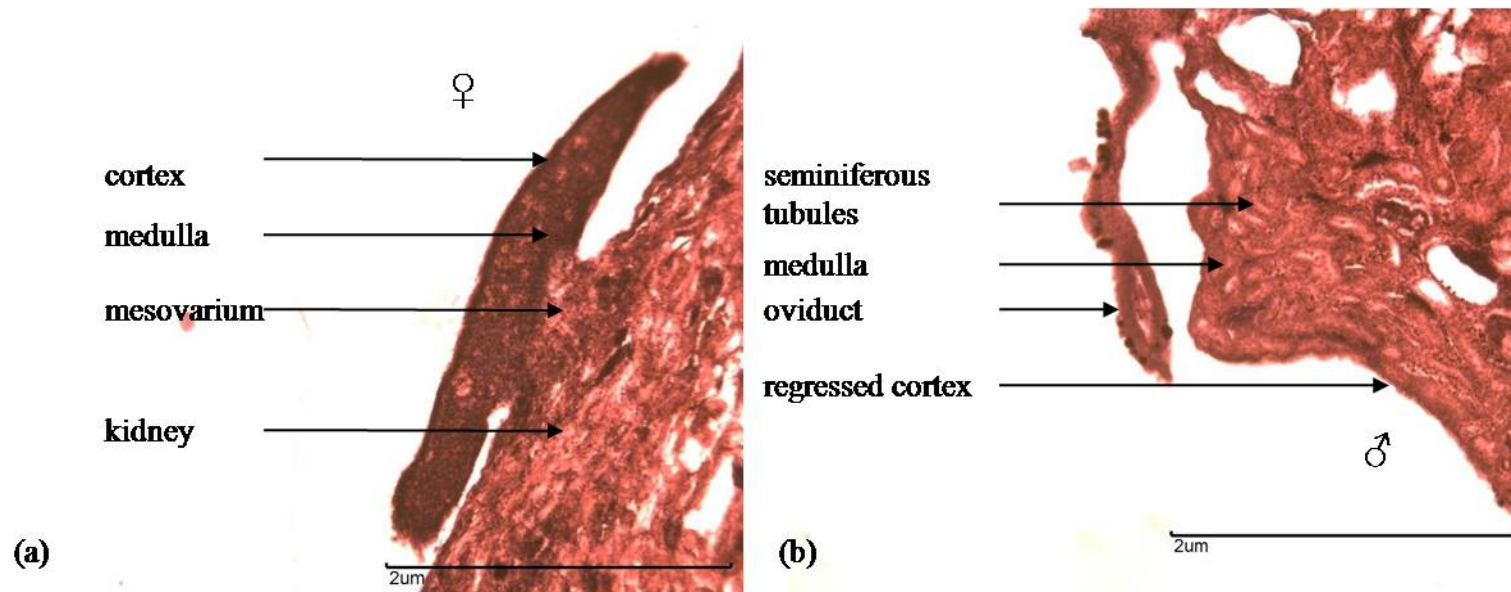


Figure 4.5. (a) The immature ovary of *C. caretta* distinguished by the thick cortex or germinal epithelium and unorganized medulla (magnification 10x/0.25). (b) The immature testis was distinguished by the seminiferous tubules and regressed cortex (magnification 10x/0.25). The non-regressed oviduct of the male hatchling can be seen from this photomicrograph.

The overall percentage females for both seasons was 79% (n = 134). First season's samples were 71% female (15 nests; n = 51; Table 4.2) whereas the second season's samples were 82% females (9 nests; n = 83; Table 4.2).

Table 4.2. The percentage females found per nest for unmonitored nests (Temperature monitored- (\*)) and moisture/temperature nests (\*\*)) for both seasons; three individuals with unknown sexes were excluded).

<b>Nest #</b>	<b>No individuals (n)</b>	<b>No female</b>	<b>% female</b>
<b>First season 2008/9</b>			
18	3	2	67
28	2	2	100
44	3	2	67
46	2	2	100
74	18	14	74
76	3	2	67
86	2	2	100
96	6	4	67
106	2	1	50
109	2	1	100
114	3	1	33
130	2	1	50
17*	3	2	67
	51	36	71%
<b>Second season 2009/10</b>			
33	3	3	100
40	21	16	76
97	6	5	83
6*	9	7	78
12*	10	10	100
13*	10	8	80
17**	5	3	60
18**	9	7	78
21*	10	9	90
	83	68	82%
<b>Total</b>	<b>134</b>	<b>106</b>	<b>79%</b>

Due to some small sample sizes the sex ratio was reanalysed using nests with 5 or more samples. The overall sex ratio (as % female) was still high at 78% which is a significant female bias ( $X^2 = 22.375$ ;  $p = 0.0075$ ;  $df = 9$ ). The hatchling sex ratio for the population was therefore highly female bias. There was no significant difference in the number of females produced between seasons ( $X^2 = 3.612$ ;  $p = 0.057$ ;  $df = 1$ ).



### Morphometrics

Statistical comparisons (using ANOVA) were conducted using hatchling size. A significant inter-seasonal variation was apparent for straight carapace measurements ( $F_{(2,58)} = 31.6$ ;  $p < 0.01$ ) in hatchlings ( $n = 61$ ), however, this may be due to a sampling bias as only dead individuals were collected during the first season (also a small sample size), while healthy individuals were euthanized during the second season. The second season's specimens were larger than the first season. However, female hatchlings seemed larger (mean SCL = 43.2 mm  $\pm$  2.76 SD; mean SCW = 34.5 mm  $\pm$  2.16 SD) than male hatchlings (mean SCL = 42.9 mm  $\pm$  4.01 SD; mean SCW = 33.9 mm  $\pm$  3.88 SD) (Table 4.3), but the difference was not significant.

Table 4.3. The straight carapace length (SCL) and width (SCW) measurements recorded for the female (F) and male (M) hatchlings.

<b>Sex</b>	<b>n</b>	<b>SCL (mm)</b>	<b><math>\pm</math> SD</b>	<b>SCW (mm)</b>	<b><math>\pm</math> SD</b>
<b>Season 2008/9</b>					
M	2	36.9	6.22	27.3	4.10
F	5	37.6	4.03	30.6	1.65
<b>Season 2009/10</b>					
M	8	44.3	1.59	35.6	1.17
F	46	43.8	1.75	34.9	1.78

## ***Discussion***

Histology has been suggested to be the most reliable method for sexing of hatchlings. In this chapter, the histological procedures applied to *C. caretta* gonads were discussed, sex ratios determined and possible sexual dimorphism, in straight carapace measurements of hatchlings, was examined. By comparing these sex ratio results to a study conducted 22 years ago (Maxwell *et al.*, 1988), the changes were documented and from the results, conservation strategies may be altered to adapt hatchling sex ratio production accordingly.

The main characteristic to identify sex in turtle hatchlings through histology was described by Yntema and Mrosovsky (1980). In this study, histological sections showed the well-defined germinal epithelium and homogenous medulla that was indicative of females, while the seminiferous tubules and poorly defined cortex was indicative of males, which coincides with previous studies (Ceriani and Wyneken, 2008). The non-regressed oviduct was clearly seen in one male. The complete resorption of oviducts in newly hatched (and even hatchlings reared for a 4.5 – 5 weeks) hatchlings may still be present, suggesting that oviducts had no clear sex distinguishing characteristic (Yntema and Mrosovsky, 1980).

The overall percentage of females was 79% (n = 134) over two seasons. The loggerhead population of Maputaland, in the high-density nesting area, therefore seems to be producing a large majority of female hatchlings. The higher number of females in the second season (82% versus 71%) suggests higher incubation temperatures and shorter incubation periods were experienced, however no conclusive statements can be made as more seasonal data and larger sample sizes are needed.

In this study, we found no sexual dimorphism using straight carapace and width measurements between female and male hatchlings. Hatchlings seemed to have been smaller during the first season than the second season. However, this may be due to a sampling bias as only dead (possibly weak) individuals were collected during the first season (also resulting in a small sample size), while healthy individuals were euthanized during the second season. Differences between sexes are evident only in mature individuals (Kamezaki, 2003). Adult males tend to be larger than the adult females, but the relationship between straight carapace

length and width in both sexes are rather similar (Kamezaki, 2003). Thus, there were no significant external features to distinguish between sexes.

Maxwell *et al.* (1988) suggested an overall equal sex ratio (1:1) due to male-production in the early season and female-bias during the late season, as the sand progressively becomes hotter towards the end of the season. However, their study was conducted in one season only. In the present study, the overall sex ratio was highly female-biased (79%) over two seasons. A female-biased sex ratio is consistent with loggerhead rookeries globally such as Brazil (Marcovaldi *et al.*, 1997), USA (Hanson *et al.*, 1998), Australia (Chu *et al.*, 2008), Greece (Rees and Margaritoulis, 2004), Turkey (Öz *et al.*, 2004; Kaska *et al.*, 2006) and in the Mediterranean (Casale *et al.*, 2006).

Kaska *et al.* (2006) described sex ratios for four beaches in Turkey which showed varied sex ratio production. Long-shore thermal variation may thus result in a combination of sex ratios (male-biased, female-biased or equal sex ratios). The lower density nesting beaches in KZN may possibly be male-producing beaches, but further investigation is needed to confirm this. Bhangha Nek beach is the peak nesting site for loggerheads and seems to produce primarily female hatchlings, but the rest of the nesting beaches may be producing males, hence the return of hundreds of nesting females – thus, naturally maintaining a viable loggerhead population.

In the “short-term”, female-biased sex ratios may be an advantage for any turtle species. Loggerheads become sexually mature between 12 -37 years of age (Bjorndal *et al.*, 2001). The effects of skewed sex ratios boosting or depressing population numbers may only be observed after some time. For loggerheads in South Africa, the effects of a skewed female sex ratio may be part of the explanation for the numbers of females dramatically increasing, especially in the last decade (Nel, 2010). Protection has been in place for nearly five decades, but the number of nesting females has really escalated in the last 15 years (Nel, 2010).

The negative effects of skewed sex ratios may also only be observed after many years, when population numbers decrease. Over a 5-year period, Janzen (1994) investigated the possible effects of climate change and noted unisexual nests each year (predominantly female-biased), but with predicted increasing ambient temperatures may result in the production of only females and may eventually lead to extinction (Janzen, 1994; Hulin *et al.*, 2009).

Population bottlenecks or severe reductions in population size, in a relatively short time, may result in large reductions in genetic variation (Storfer, 1996). The homing by turtle females suggests that each nesting colony is demographically independent, with a distinct sex ratio, age class structure, survivorship and other demographic characteristics (Bowen, 2003). Considering the two extreme scenarios: (1) if males were eliminated, the nesting population would still continue to produce offspring, since females will be inseminated in foraging areas or migratory channels by males from other populations (Bowen, 2003; Bowen and Karl, 2007). In contrast, (2) if females were eliminated, the nesting population could become extinct because the natal homing behaviour of the females defines the breeding population, regardless of male behaviour (Bowen, 2003; Bowen and Karl, 2007). Due to male-mediated nuclear gene flow that mediates disease resistance, environmental responses and other traits relevant to survival, small nesting populations in, for example southeastern USA (North Carolina, South Carolina, Georgia and Florida) will not succumb to population bottlenecks (Pearce, 2001; Bowen and Karl, 2007).

The South African loggerhead population has been found to be genetically distinct (mtDNA) along with three other loggerhead rookeries in the Indian-Pacific basin (Bowen *et al.*, 1994). The feeding grounds for South African loggerheads include Tanzania, Madagascar, Kenya and Mozambique (Bowen *et al.*, 1994), and may promote male-mediated gene flow from the other overlapping populations thus maintaining a viable population. However, the vulnerability for South African loggerheads to possible loss of genetic diversity needs to be assessed.

Investigating hatchling sex ratios is an important parameter for population studies, especially in the light of global climate changes (Hulin *et al.*, 2009). To counter the affect of feminization of hatchlings, some management programs involve hatching males in temperature-controlled incubators for later release to help equalize the sex ratios (Storfer, 1996). If the population collapsed due to human encroachment or natural phenomena, re-establishing the population may take many years (Bowen *et al.*, 1994). Focussing on assessing the ability of TSD species to adapt to changing environments, may increase accuracy of predicting the responses to different climate change scenarios for these species (Hulin *et al.*, 2009).

## ***Conclusion***

In this study, histological procedures were adapted to give the best results for hatchling gonads. The learning curve for this study was to delegate proper exposure times for staining the tissue samples, thus to provide guidelines for continuing research in this field specifically for *C. caretta* hatchlings. A previous study (Maxwell *et al.* 1988) conducted histological examinations of hatchling gonads and suggested female-biased sex ratios for the study area in the future. The overall percentage of females found for the current study was 79% (over two seasons). Highly female-biased sex ratios may suggest higher incubation temperatures, which coincide with studies globally, that may provide empirical evidence for global warming. Unfortunately, the historical study (Maxwell *et al.* 1988) was conducted for one season only and it is uncertain as to the representativity of that season. However, multiple seasons of data and larger sample sizes may be needed to establish whether the population is under threat as a result of the heavily skewed female sex ratios. Heavily skewed female ratios may be advantageous for a recovering population, but the production of unisexual nests may threaten the population. As a result, population bottlenecks may occur and ultimately extinction if genetic diversity is lost. The lower density nesting beaches may be producing predominantly male hatchlings and should be examined in the future. Moreover, multiple seasons of histological data may suggest altering current conservation strategies, for example the possible implementation of hatcheries to increase male hatchling production. The status of loggerhead turtle populations in South Africa also needs to be re-evaluated.

## Chapter 5

### **Predicting sex ratios using incubation temperature in loggerhead sea turtles (*Caretta caretta*) nesting in KwaZulu-Natal, South Africa**

#### ***Abstract***

The incubation temperatures in sea turtle nests are highly dependent on environmental conditions and are hence influenced by factors including shading from vegetation, dunes or developments, seasonal temperature fluctuations, sand colour, rain, depth of eggs etc. The purpose of this study was to examine the effect of thermal conditions within the nests on loggerhead (*Caretta caretta*) hatchling sex ratios in KwaZulu-Natal, South Africa. During the peak nesting season, temperature data loggers were deployed in 54 nests, along with measuring sand- and air temperature for the duration of incubation period. Nests were excavated after hatching took place and data loggers were retrieved. Two methods were used to estimate population sex ratios, namely predicting sex ratios using sand and nest temperatures, and secondly, hatchling sex ratios determined by histological examinations of the gonads. Incubation temperature predicted an 85% female sex ratio, but histological examinations reported female-biased sex ratios of *ca.* 79% (n = 134) for both seasons (2008-10). Small sample size of hatchlings used for histological examination may have limited the results. Future research in this field may need a larger sample size, and investigating other (low density nesting) beaches may reveal male-producing conditions due to large back dunes that may give shading.

## ***Introduction***

Incubation temperatures experienced by the eggs determines the turtle hatchlings' development, sex ratio and survival (Ackerman, 1997; Godfrey *et al.*, 1997; Carthy *et al.*, 2003). These conditions are in turn affected by the ambient environment such as air temperature, rainfall, wind, shading etc. Nesting beach temperatures usually range between 24 - 33°C, even though short periods exceeding this range may occur occasionally (Limpus *et al.*, 1985). Sand temperatures can be lowered by heavy rainfall, for example storm surges (Mrosovsky, 1983), or inundation (Carthy *et al.*, 2003) by tides. For example, precipitation patterns resulted in low, but non-lethal, incubation temperatures at leatherback turtle rookeries in Grenada, West Indies, which in turn, also resulted in male-producing conditions (Houghton *et al.*, 2007). Intra-seasonal thermal variation is more pronounced in turtle rookeries located at higher latitudes away from the tropics (Houghton *et al.*, 2007). Nests early in the season may experience cooler temperatures than those towards the end of the season when temperatures become hotter. There may also be annual variation between seasons. For example one season may experience very hot temperatures whereas the following season may be cooler or wetter (Mrosovsky and Provancha, 1992). Map turtles in Wisconsin, USA, showed 72% females (Vogt and Bull, 1984) and in a cooler year 55% females were produced (Bull and Charnov, 1988).

Field-based studies that examined the effect of incubation temperatures and incubation duration, mirrored the results of laboratory experiments to a certain extent. For example, loggerhead populations in Alagadi, in the Mediterranean, indicated that incubation duration negatively correlated with mean nest temperatures (Godley *et al.*, 2001a). These, and other field-based studies, reflected laboratory results indicated that incubation period decreased with increasing nest temperature. In addition to the influence of temperature on the incubation period, the effect of temperature is also evident in determining sex for most reptilian embryos (Miller and Harley, 1996).

The influence of temperature on sex ratios in reptiles has been well documented in most lizards (Harlow, 2004), crocodylians (Wang and Andrews, 1994) and chelonians (Standora and Spotila, 1985). Unlike humans, turtles and many other reptiles do not possess heteromorphic sex chromosomes and show no consistent genetic differences between sexes (Spotila, 2004; Valenzuela, 2004a). Instead, reptilian embryos use egg incubation

temperatures to determine the sex of individuals in a process called temperature-dependent sex determination (TSD) (Spotila, 2004; Valenzuela, 2004b).

Temperature-dependent sex determination has been described in all sea turtle species: flatback (*Natator depressus*) (Hewavisenthi and Parmenter, 2000), Kemp's ridley (*Lepidochelys kempii*) (Carrasco *et al.*, 2000), olive ridley (*Lepidochelys olivacea*) (Wibbels *et al.*, 1998), hawksbills (*Eretmochelys imbricata*) (Mrosovsky *et al.*, 1992), leatherbacks (*Dermochelys coriacea*) (Mrosovsky *et al.*, 1984), green turtles (*Chelonia mydas*) (Mrosovsky *et al.*, 1984; Kaska *et al.*, 1998) and loggerheads (*Caretta caretta*) (Maxwell *et al.*, 1988; Maloney *et al.*, 1990; Mrosovsky and Provancha, 1992). For loggerheads, incubation temperatures above the pivotal temperature (i.e. the temperature at which a 1:1 sex ratio is produced, *ca.* 29°C) produce primarily female hatchlings, whereas incubation temperature below the pivotal temperature produce primarily male hatchlings (Spotila, 2004). However, this may differ between populations. In loggerhead populations from the Mon Repos rookery in Australia, it was found that only males were produced below 26.6°C whereas only females were produced above 31.4°C (Georges *et al.*, 1994), but populations in the southeastern region of America, displayed male production at 29.5°C and females at 30.4°C (Spotila, 2004). Sex is determined during the middle third of the incubation period and this window is called the thermal sensitive period (TSP).

Sexing of hatchlings cannot be directly determined by morphological observations (Mrosovsky and Benabib, 1990; Wyneken *et al.*, 2007). There are four ways to determine the sex of hatchlings and therefore to estimate the sex ratios of a population under study, by (1) predicting sex ratios from known incubation temperatures, which includes the constant temperature equivalent (CTE method) (Rees and Margaritoulis, 2004; Chu *et al.*, 2008), (2) using gonadal characteristics for sexual differentiation (Wyneken *et al.*, 2007; Ceriani and Wyneken, 2008), (3) radioimmunoassays (Owens *et al.*, 1978) and (4) sexual differentiation using histological examinations of the gonads (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982). Previous studies (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982; Mrosovsky and Benabib, 1990) have shown that histological examinations of the gonads give the most reliable results for sexual differentiation. Even though sex ratio predictions have been conducted in most well-studied turtle rookeries globally, South Africa (even though we have one of the longest running programmes) lacks field-based research studies on the two nesting sea turtle species, namely loggerheads and leatherbacks.



Comparing estimated sex ratios with the results of histological examinations will, therefore enlighten our understanding of the sea turtle reproduction biology in South Africa. All hatchlings were male when temperatures were below 28.5°C during the mean TSP, and with temperatures above 29.6°C all hatchlings were female (Maxwell *et al.*, 1988). Maxwell *et al.*'s (1988) study revealed male-biased results for the hatchlings during the early season, but suggested female-biased sex ratios for the high-density region during the peak nesting period. His final conclusion was a balanced 1:1 sex ratio for the population. However, the accuracy of the methods used by Maxwell *et al.* (1988) is out-dated with regard to technology. Furthermore, it is uncertain what the current sex ratios are, given the possible minor temperature shift (of less than 1°C) in the last 22 years ago.

Thus, the main questions of the present study, were to: (i) establish how current incubation temperatures determine loggerhead hatchling sex ratios in South Africa, and (ii) see if incubation temperature can be used to predict sex ratios for loggerhead turtles. Further we aimed to estimate the pivotal temperature from the temperature data and corresponding sex ratios, and compared the findings to that of Maxwell *et al.* (1988) and other studies.

## ***Materials and methods***

This study integrated the data obtained in chapter three and four hence all the field and laboratory methods apply.

### **Statistical analyses**

Temperature and sex ratio data results are presented as means  $\pm$  standard deviations. Pearson Product-Moment correlation analyses were used to describe relationships between variables and Analysis of Variance (ANOVA) was used to compare sand- and nest temperatures. All data analyses were conducted in Statistica v9. Statistical significance was set at a p-value  $\leq$  0.05. Sex ratios were plotted against incubation temperature, by using CurveExpert v1.4, and the pivotal temperature was estimated by using the sex ratio value at 50%.

## ***Results***

### ***Intra-nest temperatures***

In Table 5.1 the hatching data for the 27 hatched nests (2008/9: n = 11; 2009/10: n = 16) are summarised. Mean nest temperatures of individual nests for the whole incubation period (IP) ranged from 24.0 – 34.9°C and showed an overall mean of 29.8°C ± 1.31 SD. During the TSP of complete nests (i.e. nests with a lay- and emergence date) mean nest temperature was 29.9°C ± 1.09 SD and mean nest temperature of individual nests ranged from 26.2 – 34.4°C (2008/9: n = 10; 2009/10: n = 12).

Nest #9 experienced the highest mean nest temperature for the whole IP and during the TSP of all the temperature monitored nests (see Table 5.1). Hatching success for nest #9 was high (93%) and the nest parameters include nest depth (0.6 m), distance to the high-water mark (HWM) (56.0 m) and distance to the vegetation (70.0 m). Nest #9 was located at 12N, the

Table 5.1. Descriptive data for the 27 hatched loggerhead nests for which temperature data was obtained over two seasons. Data include deposition date, incubation period (IP in days, where applicable), clutch size (number of eggs), temperature data for the whole IP and the TSP.

Nest #	Lay date	IP (days)	Clutch size (# eggs)	Total IP				TSP			
				Mean	± SD	Min	Max	Mean	± SD	Min	Max
<b>Season 2008/9</b>											
4	10-Dec	-	48	30.0	1.56	25.6	33.9	30.7	1.33	27.5	32.4
8	12-Dec	-	109	29.6	1.27	25.7	32.5	30.2	0.91	27.8	31.6
9	12-Dec	-	111	29.5	0.93	25.3	31.4	30.1	0.81	27.9	31.2
12*	21-Dec	70	88								
13	15-Dec	64	72	28.6	1.09	26.2	31.0	29.2	0.76	27.5	30.4
15	20-Dec	60	126	29.4	1.16	26.5	32.1	29.8	0.93	28.4	31.4
16	18-Dec	-	106	29.8	1.20	25.9	32.5	30.5	0.89	28.8	31.8
17	21-Dec	-	106	30.2	1.66	24.0	33.6	31.0	0.91	29.2	32.3
18	18-Dec	61	104	29.5	1.47	26.9	32.4	30.1	1.31	27.4	32.0
19	21-Dec	70	110	29.7	0.83	28.1	31.7	29.9	0.91	28.5	31.7
20	21-Dec	61	89	29.6	1.5	26.5	32.4	29.7	1.54	26.8	32.0
	<b>Average</b>	<b>64</b>	<b>97</b>	<b>29.6</b>	<b>1.27</b>	<b>26.1</b>	<b>32.4</b>	<b>30.1</b>	<b>1.03</b>	<b>28.0</b>	<b>31.7</b>

Nest #	Lay date	IP (days)	Clutch size (# eggs)	Total IP				TSP			
				Mean	± SD	Min	Max	Mean	± SD	Min	Max
<b>Season 2009/10</b>											
1	11-Dec	61	107	29.2	1.43	25.9	31.5	29.5	1.1	26.9	31.0
2	11-Dec	62	121	28.8	1.25	25.8	30.7	29.0	1.04	26.4	30.2
3	12-Dec	61	85	29.4	1.36	26.1	31.1	29.6	1.34	26.2	31.0
6	03-Jan	59	133	29.9	1.29	27.1	32.2	29.4	1.09	27.8	31.5
9	08-Jan	55	133	<b>31.4</b>	<b>1.98</b>	<b>27.6</b>	<b>34.9</b>	<b>31.1</b>	<b>2.01</b>	<b>27.6</b>	<b>34.4</b>
10*	08-Jan	60	111	-	-	-	-	-	-	-	-
11	09-Jan	56	91	31.1	1.58	28.2	33.8	30.8	1.75	28.2	33.7
12	09-Jan	58	78	30.1	1.32	28.2	32.2	30.8	1.43	28.4	32.2
13	09-Jan	57	88	29.8	1.16	27.0	31.8	29.5	1.59	27.0	31.8
15	09-Jan	60	86	29.9	1.05	27.4	32.2	29.8	1.33	27.4	31.4
17**	20-Dec	64	119	29.2	1.42	25.8	32.1	29.0	0.58	27.5	29.9
18**	24-Dec	58	71	30.0	1.73	26.2	33.7	29.5	0.68	27.4	30.4
20	25-Dec	58	101	30.3	1.23	27.2	32.6	30.3	0.43	29.2	31.0
21	26-Dec	59	84	30.2	1.30	26.9	32.7	29.6	0.65	28.2	30.6
24**	26-Dec	-	64	29.7	1.09	26.7	31.7	29.3	0.99	27.1	30.9
25**	05-Jan	-	110	29.4	1.18	26.2	31.2	28.9	1.12	27.1	30.7
<b>Average</b>		<b>59</b>	<b>99</b>	<b>29.9</b>	<b>1.36</b>	<b>26.8</b>	<b>32.3</b>	<b>29.7</b>	<b>1.14</b>	<b>27.5</b>	<b>31.4</b>
<b>All seasons (2008 - 10)</b>	<b>Average (±SD)</b>	<b>61</b>	<b>98</b>	<b>29.8</b>	<b>1.31</b>	<b>26.6</b>	<b>32.3</b>	<b>29.9</b>	<b>1.09</b>	<b>27.7</b>	<b>31.5</b>

beacon at which the highest sand temperature was recorded. Temperature profiles for successfully hatched nests are illustrated in Appendix B.

#### *Metabolic heating within nest*

There was an evident increase in nest temperature from the middle third of incubation. Seasonal changes in sand temperatures at nest depth were described by the control data loggers. Metabolic heating within the nest was calculated by subtracting the sand temperature from the incubation temperature from day 1 until day  $x$  of incubation (Godley *et al.*, 2001a). Mean sand temperature was  $29.0^{\circ}\text{C} \pm 1.11$  SD which suggest metabolic heating contributed *ca.*  $1.0^{\circ}\text{C}$  to the nest temperature. ANOVA and post-hoc Tukey HSD test analysis indicated significant differences for each stage and between stages respectively ( $F_{(2,35)} = 20.63$ ; mean first third =  $0.26^{\circ}\text{C} \pm 0.41$  SD; mean mid third =  $0.82^{\circ}\text{C} \pm 0.58$  SD; mean final third =  $1.61^{\circ}\text{C} \pm 0.76$  SD).

#### *Relationship between nest temperature & incubation period (IP)*

For the 18 complete nests where hatching success was higher than 20%, correlation analyses showed a weak negative correlation between the mean IP and mean nest temperature ( $r^2 = 0.39$ ) as well as with the mean IP and mean nest temperature during the TSP ( $r^2 = 0.31$ ). When the two seasons were analysed separately, in the 2008/9 season ( $n = 5$ ) the IP did not significantly correlate to mean nest temperatures. However, during the 2009/10 season ( $n = 13$ ) the IP showed a strong negative correlation ( $p < 0.05$ ;  $r^2 = 0.86$ ) to mean nest temperatures throughout the incubation period (Fig. 5.1).

Inter-seasonal variation showed a significant difference for the incubation period (ANOVA  $F_{(1,16)} = 5.61$ ,  $p = 0.03$ ), but mean nest temperatures showed no significant difference. The mean incubation period was significantly lower in the second season ( $59$  days  $\pm 2.63$  SD) than in the first season ( $63$  days  $\pm 4.09$  SD).

#### *Sex ratios*

##### *The effect of incubation temperature on sex ratios*

The mean overall sex ratio of specimens collected ( $n = 134$ ) over two seasons was 79% females. However, to establish the effect temperature has on sex ratios, the iButton nests were used. Histological results (from Chapter 4) of study nest specimens are tabulated in Table 5.2.

The mean percentage of females for iButton nests was 71% (n = 58). The percentage females was not related to the mean nest temperature throughout the IP as well as the incubation period (days). There was a (logistic) sigmoidal relationship with mean nest temperature during the TSP (Fig. 5.2) ( $p < 0.05$ ;  $r^2 = 0.88$ ). It is evident that mean TSP temperatures  $> 29^{\circ}\text{C}$  females are predominantly produced. Nests that displayed mean TSP temperatures higher than  $30^{\circ}\text{C}$  produced 100% females, with the exception of nest #17 and 18 (1 specimen).

#### *Estimation of sex ratios from sand- and incubation temperature*

Estimating sex ratios by using sand and incubation temperature has been implemented widely (Kaska *et al.*, 1998; Godley *et al.*, 2001a; Kaska *et al.*, 2006) rather than sacrificing hatchlings for histological purposes. In the previous study (Maxwell *et al.*, 1988), the relationship between incubation temperature (during TSP) and sex ratios was sigmoidal ( $y = 100 / (1.0 + 3.49\text{E}+70*\exp(-5.57x))$ ), where  $y$  was the percentage females and  $x$  represent temperature. To solve for  $y$ , we substituted  $x$  with the mean sand temperature throughout the incubation period ( $29.0^{\circ}\text{C}$ ), and with the mean nest temperature during the thermal sensitive period (TSP) ( $29.7^{\circ}\text{C}$ ). The percentage females predicted from the sand temperatures was 28% while the TSP temperature showed 95% females. Furthermore, the percentage of females per nest was calculated in the same way, by substituting the mean temperature during the TSP for each nest, and yielded a mean of 85% female  $\pm$  23.09 SD (Table 5.2). All the nests showed similar observed female percentage than the predicted female percentage, except for nest #18 (2008/9: 99% versus 0%) and nest #17 (2009/10: 28% versus 60%).

Table 5.1. Descriptive data of the nine study nests where specimens were collected for histological purposes, which include the sample size (n) per nest, incubation period (IP), mean nest temperature, mean nest temperature during the thermal sensitive period (TSP) and the predicted- and observed percentage (%) females.

Nest #	Sample size	IP (days)	Nest temp $\pm$ SD	TSP temp $\pm$ SD	Predicted %	Observed %
<b>Season 2008/9</b>						
8	1	-	29.6 $\pm$ 1.27	30.2 $\pm$ 0.91	100	100
17	3	-	30.2 $\pm$ 1.66	31.0 $\pm$ 0.91	100	67
18	1	61	29.5 $\pm$ 1.47	30.1 $\pm$ 1.31	99	0
<b>Season 2009/10</b>						
6	9	59	29.9 $\pm$ 1.29	29.4 $\pm$ 1.09	78	67
12	10	58	30.1 $\pm$ 1.32	30.8 $\pm$ 1.43	100	100
13	10	57	29.8 $\pm$ 1.16	29.5 $\pm$ 1.59	86	80
17**	5	64	29.2 $\pm$ 1.42	29.0 $\pm$ 0.58	28	60
18**	9	58	30.0 $\pm$ 1.73	29.5 $\pm$ 0.68	86	78
21	10	59	30.2 $\pm$ 1.30	29.6 $\pm$ 0.65	91	90
<b>Average</b>	<b>58</b>	<b>59</b>	<b>29.8 <math>\pm</math> 1.40</b>	<b>29.9 <math>\pm</math> 1.02</b>	<b>85 <math>\pm</math> 23.09</b>	<b>71 <math>\pm</math> 30.35</b>

\*\*Temperature data from moisture data loggers; The hatching date of nest #8 and 17 during the first season was missed; in this case, the IP was delineated as 60 days (average for loggerheads in this region) for temperature calculations.

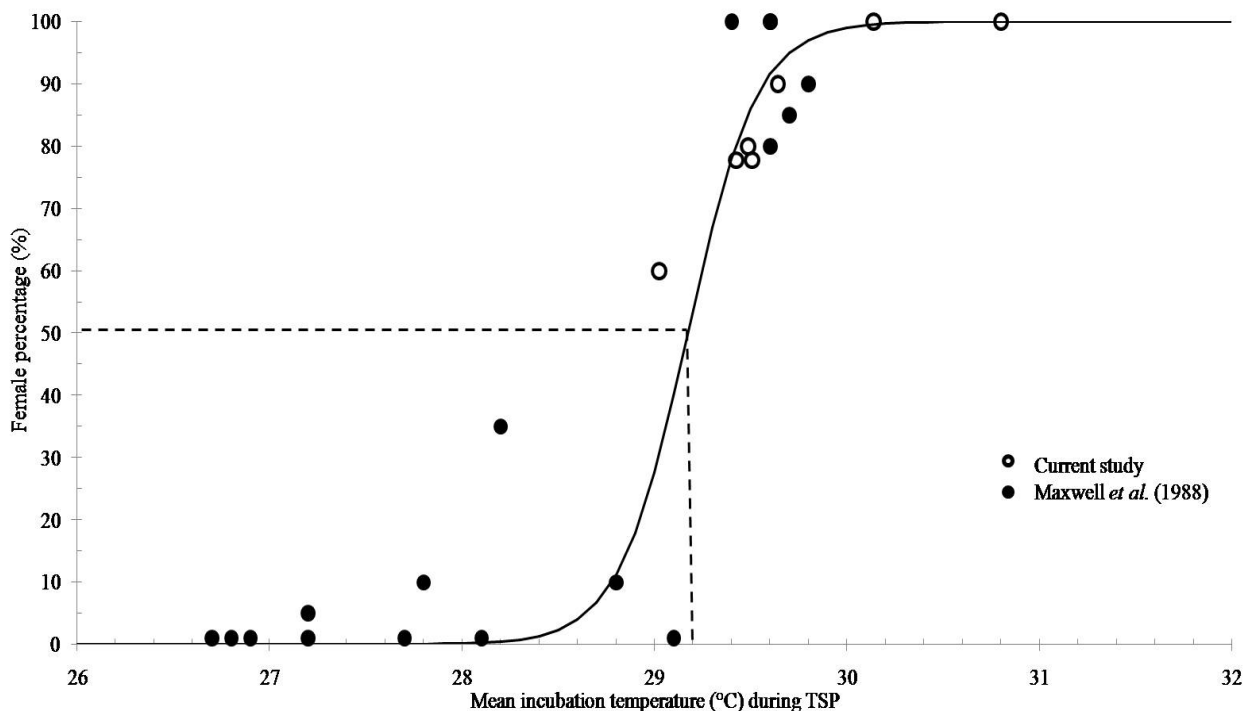


Figure 5.1. The sigmoidal relationship ( $y = 100 / (1.0 + 3.49E+70 * \exp(-5.57x))$ ;  $r^2 = 0.88$ ) with mean nest temperature during the TSP and the percentage of female hatchlings produced. Data from both the current study and Maxwell *et al.* (1988) were used. The broken line denotes equal sex ratio (y-axis) and pivotal temperature (x-axis).

### *Estimating pivotal temperature*

From the logistic equation of the relationship between the percentage female hatchlings produced and the mean nest temperature during the TSP, the pivotal temperature (sex ratio 1:1) was calculated. The sigmoidal equation was  $y = 100 / (1.0 + 3.49E+70*\exp(-5.57x))$ . The  $y$ -value was substituted with 50 (50% females) and the  $x$ -value was calculated (where  $x$  is the temperature). The pivotal temperature for loggerheads nesting in the study area was 29.2°C. This temperature was slightly higher than previously estimated for the same population (29.0°C; Maxwell *et al.*, 1988) and is higher than the pivotal temperature found for Australian loggerheads (28.3°C), which would be the most similar population with regards to latitude (see Table 5.3).

Table 5.2. Pivotal temperatures for other loggerhead turtle rookeries globally from north to south. The method(s) used to estimate the pivotal temperature is included such as the constant temperature equivalent (CTE) method.

<b>Region</b>	<b>Country</b>	<b>Pivotal temp</b>	<b>Latitude</b>	<b>Method</b>
<sup>1</sup> Kyparissia Bay	Greece	29.3°C	37°N	TSD Software & histology
<sup>2</sup> Patara beach	Turkey	28.8°C	36°N	CTE method
<sup>3</sup> Little Cumber Island, Georgia	USA	29.0°C	30°N	Laboratory & histology
<sup>4</sup> Little Cumber Island, Georgia	USA	30.0°C	30°N	Laboratory & histology
<sup>5</sup> Heron Island, Queensland	Australia	28.7°C	23°S	Field-based & histology
<sup>6</sup> Mon Repos, Queensland	Australia	27.7°C	24°S	Field-based & histology
<sup>7</sup> Mon Repos, Queensland	Australia	28.3°C	24°S	CTE method
<sup>8</sup> Maputaland, KwaZulu-Natal	South Africa	29.0°C	27°S	Field-based & histology

<sup>1</sup>Mrosovsky *et al.*(2002), <sup>2</sup>Öz *et al.*(2004), <sup>3</sup>Yntema and Mrosovsky (1980), <sup>4</sup>Yntema and Mrosovsky (1982), <sup>5,6</sup>Limpus *et al.*(1985), <sup>7</sup>Chu *et al.*(2008), <sup>8</sup>Maxwell *et al.*(1988)

### *Comparing results with Maxwell et al. (1988)*

In our study, we found that the incubation temperature during the TSP, and female sex ratios, increased significantly (ANOVA:  $F_{(2,21)} = 3.79$ ) from 22 years ago. From Maxwell *et al.* (1988), the mean incubation temperatures experienced during the TSP were *ca.* 28.8°C ± 1.15 SD (n = 17) while TSP temperatures, 22 years later, showed 29.8°C (n = 7). Due to ethical constraints during this study we were permitted to euthanize ten emergent hatchlings per nest (n = 5 nests), and the rest of the samples were specimens found dead in the nest. Maxwell *et al.* (1988) collected 20-25 embryos per nest, giving a better estimation of sex ratio per clutch.



The percentage females showed a sigmoidal relationship with mean TSP temperature. Temperature exceeding 30°C produced 100% females. Regarding sex ratios, the percentage of females also increased from 36% (one season) (Maxwell *et al.* 1988) to 79% (two seasons) in the current study (see Chapter 4). The increase in female percentage may be attributed to the increase in incubation temperatures during the TSP (Table 5.1).

## ***Discussion***

South Africa hosts the southernmost loggerhead (and leatherback) rookery in the world (27°S). The effect of temperature on population sex ratios for this region is lacking. Only one study on loggerhead hatchling sex ratios and temperature in Maputaland has been published to date (Maxwell *et al.*, 1988). The purpose of this study was to establish how current incubation temperatures effect turtle populations in South Africa. We wanted to establish whether incubation temperature can be used to predict sex ratios for loggerhead turtles (per nest and per the population). Further we estimated the pivotal temperature from the temperature data and corresponding sex ratios, and compared the findings with that of Maxwell *et al.* (1988) and other research.

Studies have found that sex ratios can vary in different parts of the nest (top, middle, bottom) as a result of thermal gradients in the sand (Kaska *et al.*, 1998). The upper eggs within a clutch are warmer, because sand temperatures are higher, closer to the surface when compared to deeper sands at the bottom of the clutch (Kaska *et al.*, 1998; Hanson *et al.*, 1998; Houghton and Hays, 2001). The centre of the clutch usually experiences the highest temperatures within the egg chamber due to metabolic heating (Booth and Astill, 2001). Mrosovsky and Yntema (1980) suggested that TSD is significantly affected by metabolic heating that is greater than 1°C. But, as found for green turtle nests in Australia, sex ratios are not always affected by the temperature spike, if the spike occurred after the sex determining period (Booth and Astill, 2001). In contrast, loggerhead nests in Florida experienced the hottest temperatures during the TSP (when compared to the top and bottom areas of the clutch) and Hanson *et al.* (1998) predicted highly female-biased sex ratios. However, during this study, the thermal variation within nests was not investigated, thus no clear statement can be made as to the depth at which the specimens were found, and hence sex determining temperature.

Sand- and nest temperatures were used to predict hatchling sex ratios. From the histology results (> 79% females), sand temperature gives a weak estimation of sex ratios (28% females) whereas incubation temperature overestimated (95% females) the histological results. This can be attributed to metabolic heat from the embryos produced in the nest (Booth and Freeman, 2006). Metabolic heat contributed *ca.* 1°C to overall mean nest temperature for this study. Metabolic heating was described for all study nests during the second season (2009/10).

Using the mean incubation temperature per nest to predict sex ratios is however closer to the observed sex ratio of samples from all the nests used in the histology study (85% versus 79%). Using the overall mean nest temperature may create sex ratio bias as some nests may experience very high or low incubation temperatures. Observed female percentage of hatchlings coincided with the predicted female percentage. As mentioned before, the small sample size may have limited the results and it is unknown whether the embryos were at the top, middle or bottom of the clutch. For future research, sex ratios may be predicted (per nest) by using the mean incubation temperature during TSP as opposed to sacrificing hatchlings for histological examinations of gonads. Even though incubation temperature predicts higher female ratios, more seasons of data (and greater sample size) may indicate a stronger relationship between sex ratio and incubation temperature.

Highly female-biased sex ratios for loggerheads have been reported globally in Australia (Chu *et al.*, 2008), eastern Mediterranean (Kaska *et al.*, 1998; Mrosovsky *et al.*, 2002), Turkey (Öz *et al.*, 2004; Kaska *et al.*, 2006), and the USA (Mrosovsky and Provanča, 1992; Hanson *et al.*, 1998). During this study, higher percentage of females were observed in the second season which may suggest higher incubation temperatures (this being confirmed) and/or shorter incubation periods as found in green turtles in the eastern Mediterranean (Broderick *et al.*, 2000). However, in this study sex ratios were not related to incubation duration, even though the incubation period was significantly higher in the second season (*ca.* 59 days) than in the first season (*ca.* 63 days). Unlike some studies where sex ratios (as the percentage of females) were correlated to incubation duration (Broderick *et al.*, 2000; Godley *et al.*, 2001b) and sex ratios were successfully estimated, for the population under study, incubation duration cannot be used to estimate sex ratios.

The pivotal temperature in this study (29.2°C) was slightly higher than that found in Maxwell *et al.* (1988) (29.0°C). Pivotal temperature for South African loggerheads is *ca.* 1°C higher than Australian loggerhead populations. We expected South African loggerheads to have similar pivotal temperatures as with Australian loggerheads (28.3°C; Chu *et al.*, 2008) since the latitudinal variation is similar (Australia being further north at 24°S than the South African rookery at 27°S). The estimate of pivotal temperature for South African loggerheads is close to the value of 29.0°C given by Yntema and Mrosovsky (1980) for loggerheads in Georgia, USA. There was also no tendency for pivotal temperatures to become progressively higher for populations as latitude decreased. Loggerhead populations in North Carolina, Georgia and Florida (east coast of USA) showed variations in pivotal temperatures (29.0°C, < 28.5°C and 29.2°C respectively) (Mrosovsky, 1988). It has been suggested that the differences in pivotal temperature may be natural selection working through variations in behaviour (Bull *et al.*, 1982).

Pivotal temperature was estimated by using the relationship equation of mean nest temperature during the TSP and the percentage females. In doing so, pivotal temperature might be biased due to the daily diel fluctuations of incubation temperature found in shallow loggerhead nests (Morreale *et al.*, 1982). If the nests experienced little temperature fluctuations, as with deeper nests such as leatherbacks in the same study area, pivotal temperature estimation may be closer to the true pivotal temperature.

Comparing the current data with the similar study conducted by Maxwell *et al.* (1988), indicated no significant change in thermal nest conditions, except nest temperature during the TSP which increased from *ca.* 28.8°C ± 1.15 SD (n = 17) to 29.8°C (n = 7) 22 years later. This data may suggest that there has been an increase in temperature over time, or that Maxwell *et al.* (1988) had a relatively cold season during which they monitored incubation temperature.

Global warming promotes feminizing sex ratios in sea turtles, and perhaps this may be advantageous for the population, since males mate with multiple females (Bowen, 2003). If more females are inseminated, more females are able to lay multiple clutches per season. Females are expected to store sperm from multiple males, alleviating the concern that males may become a limiting source (Bowen, 2003). The increase in female nesters over the years

in South Africa may be in part the result of positive conservation efforts. This may also be due to the female-biased hatchling sex ratios, but the primary sex ratios at other beaches in the South African nesting area may be male-biased. Highly female-biased sex ratios may ultimately aid in the recovery of sea turtle species, but a decrease in the population numbers may only be visible after many years since sexual maturity for loggerheads takes about 13 – 27 years (Bjorndal *et al.*, 2001).

### ***Conclusion***

Bhanga Nek is the peak nesting area for loggerheads in KZN, and the present study was the first attempt to examine the thermal gradient along the South African nesting beach. Maxwell *et al.* (1988) was the only other study conducted to date concerning the effect of nest temperatures on loggerhead hatchling sex ratios. Mean sand- and nest temperatures were generally high during the incubation period ( $> 28^{\circ}\text{C}$ ), but non-lethal ( $< 34^{\circ}\text{C}$ ). Shallow nests, such as the ones observed in this study, were expected to show diel temperature fluctuations than deeper nests of leatherbacks in the same area (J. Tucek, unpublished data). However, in this case, sand temperature cannot be used as a thermal baseline to determine effects of temperature on hatchling sex ratios due to the high metabolic heating in the nests. Incubation temperature per nest gave better sex ratio predictions and histological examinations of dead embryos/hatchlings confirmed these predictions (85% females predicted versus 79% females observed). Sex ratios may vary in different parts of the nests (i.e. top, middle and bottom), but for the present study this was not established. The estimated pivotal temperature for South African loggerheads was  $29.2^{\circ}\text{C}$ , slightly higher than previously found by Maxwell *et al.* (1988). Global warming may promote feminization of sex ratios, and perhaps this may be advantageous for the population in the short term, however the possibility of extinction may not be too far-fetched due to male-biased genetic constraints. The sample sizes were relatively small for both seasons (2008-10), nonetheless our results coincided with Maxwell *et al.* (1988). Future research in this field may need multiple seasonal data and larger sample size for histology. South African loggerheads are increasing in population size since nesting females are evidently increasing. This may result from positive conservation efforts and highly female-biased sex ratios.

## Chapter 6

### **Conclusions: The implications of incubation temperature on TSD in the light of future conservation strategies**

Sea turtles do not possess heteromorphic chromosomes, and incubation temperature determines the sex of individuals through a process called temperature-dependent sex determination (TSD). In sea turtles, higher temperatures ( $> 29^{\circ}\text{C}$ ) produce females and lower temperatures ( $< 29^{\circ}\text{C}$ ) produce males. Sex is determined during the middle trimester of incubation.

The critical low- and high temperature to ensure successful development of embryos was found to be  $25^{\circ}\text{C}$  and  $34^{\circ}\text{C}$  respectively (Mrosovsky and Yntema, 1980; Yntema and Mrosovsky, 1982). Thus, temperature not only affects sex of individuals, but also contributes to the successful development of embryos and post-hatchlings. Incubation temperature in turn is determined by environmental conditions and is hence influenced by factors including shading from vegetation, dunes or developments, seasonal temperature fluctuations, sand colour, rainfall, depth of eggs etc. Metabolic heating increases incubation temperature, especially towards the end of incubation. Only one South African study hitherto, conducted by Maxwell *et al.* (1988), examined the effect of temperature on hatchling sex ratios. The result from the present study indicated a mean incubation temperature (for 2008/9 season) of  $29.4^{\circ}\text{C}$  ( $n = 6$ ), and the overall nest success (i.e. nest that hatched) was high (94%,  $n = 110$ ). In the second season (2009/10) mean incubation temperature was  $30.0^{\circ}\text{C}$  ( $n = 14$ ) with a nest success of 88% ( $n = 223$ ). *In situ* temperature was recorded in the second season and was  $29.0^{\circ}\text{C}$  which suggests that metabolic heating contributed  $\pm 1.0^{\circ}\text{C}$ . There was a strong positive relationship with mean nest temperature and mean sand temperature ( $r^2 = 0.63$ ). From Maxwell *et al.* (1988), the mean incubation temperatures experienced during the TSP were *ca.*  $28.8^{\circ}\text{C} \pm 1.15$  SD ( $n = 17$ ) while the TSP temperature, 22 years later, was on average  $29.8^{\circ}\text{C}$  ( $n = 7$ ). Hatching success, in this study, was not affected by sand- and nest temperature, that suggests non-lethal ( $> 25^{\circ}\text{C}$  and  $< 34^{\circ}\text{C}$ ) sand temperatures in the region.

The sex of hatchlings cannot be morphologically distinguished and despite the search for alternatives, the most reliable method still for sexing hatchlings is through histological examinations of the gonads. To date, histological studies (Yntema and Mrosovsky, 1980; Yntema and Mrosovsky, 1982; Mrosovsky and Benabib, 1990; Ceriani and Wyneken, 2008) have demonstrated to give the most reliable results for sexual differentiation. The previous study conducted by Maxwell *et al.* (1988) indicated male-biased sex ratios (36% females; n = 373). The present study indicated 79% females (n = 134).

In this study, two methods were used to estimate population sex ratios, firstly direct measurement of hatchling sex ratios were determined by using histology on the gonads and secondly predicting sex ratios using sand- and nest temperatures. Results were then compared. There was a sigmoidal relationship with female percentage and the mean nest temperature during the thermal sensitive period, but no significant relationship with the incubation duration. Incubation temperature (per nest) predicted an overall sex ratio of 85% females, but histological examinations reported female-biased sex ratios of 79% (n = 139) for both seasons (2008-10). The results between the two seasons were similar, with one season producing 76% (n = 54) females and the 2009/10 season producing 83% females (n = 82). The higher incubation temperatures in 2009/10 therefore favoured female production. This study illustrated the importance of more seasonal sex ratio data and larger sample sizes per temperature monitored nest.

Female-biased production may be advantageous for the population in the short-term as this might assist in the recovery of the loggerhead population, in conjunction with a well-managed protection program. Future research in this field may be useful, but regardless of research being conducted on sex ratio data, human activities such as coastal development, pollution etc., may still compromise research and conservation efforts. Short term effects of increasing sand temperatures may occur when inexperienced first-time breeders choose less favourable nesting sites (Hawkes *et al.*, 2007).

In the long-term, increasing sand temperatures may create female-biased sex ratios (Hawkes *et al.*, 2007; Hawkes *et al.*, 2009), and with continued increases lead to decreasing hatching success (Hawkes *et al.*, 2007) and ultimately the extinction of the population. In this study, incubation temperatures were optimal for *C. caretta* eggs (Mrosovsky and Yntema, 1980; Yntema and Mrosovsky, 1982) and hatching success was high, thus feminization of embryos

would be the only threat, regarding the increase of temperature. To overcome this, managers may choose to protect male-producing beaches to ensure viable populations for the future (Booth and Astill, 2001; Hawkes *et al.*, 2007), but for that, we need sex ratio data conducted on the beaches beyond the high density area in KZN. However, these beaches are low density nesting areas, and little data has been gathered with regard to the degree of nest success in these regions. Hatcheries using a fence or wall with a roof over it to lower sand temperatures (Higginson and Vasquez, 1989) may be a cost effective and simple conservation strategy to overcome the problem of highly female skewed sex ratios in the high density nesting area.

## Reference List

- Ackerman,R.A., 1997. The nest environment and the embryonic development of sea turtles. In: Lutz,P.L., Musick,J.A. (Eds.), *The Biology of Sea Turtles*. CRC Press, Inc., Boca Raton, pp. 83-105.
- Alexander,G., Marais,J., 2007. Order: Testudines. *A guide to the reptiles of southern Africa*. Struik Publishers, Cape Town.
- Baldwin,R., Hughes,G.R., Prince,R.I.T., 2003. Loggerhead turtles in the Indian Ocean. In: Bolten,A.B., Witherington,B.E. (Eds.), *Loggerhead Sea Turtles*. Smithsonian Books, Washington, pp. 218-232.
- Bjorndal,K.A., Bolten,A.B., Koike,B., Schroeder,B.A., Shaver,D.J., Teas,W.G., Witzell,W.N., 2001. Somatic growth function for immature loggerhead sea turtles, *Caretta caretta*, in the southeastern U.S. waters. *Fishery Bulletin* 99, 240-246.
- Blamires,S.J., Guinea,M.L., 1998. Implications of nest site selection on egg predation at the sea turtle rookery at Fog Bay. In: Webb,K.A., Duff,G., Guinea,M., Hill,G. (Eds.), *Marine turtle conservation and management in Northern Australia*. Northern Territory University, Darwin, Australia, pp. 20-24.
- Booth,D.T., Astill,K., 2001. Temperature variation within and between nests of the green sea turtle, *Chelonia mydas* on Heron Island, Great Barrier Reef. *Australian Journal of Zoology* 49, 71-84.
- Booth,D.T., Freeman,C., 2006. Sand and nest temperatures and an estimate of hatchling sex ratio from the Heron Island green turtle (*Chelonia mydas*) rookery, Southern Great Barrier Reef. *Coral Reefs* 25, 629-633.
- Botha,M., 2010. Nest site fidelity and nest site selection of loggerhead, *Caretta caretta*, and leatherback, *Dermochelys coriacea*, turtles in KwaZulu-Natal, South Africa.
- Bowen,B.W., 2003. What is a loggerhead turtle? The genetic perspective. In: Bolten,A.B., Witherington,B.E. (Eds.), *Loggerhead Sea Turtles*. Smithsonian Institution, Washington, pp. 7-27.
- Bowen,B.W., Kamezaki,N., Limpus,C.J., Hughes,G.R., Meylan,A.B., Avise,J.C., 1994. Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. *Evolution* 48(6), 1820-1828.
- Bowen,B.W., Karl,S.A., 2007. Population genetics and phylogeography of sea turtles. *Molecular Ecology* 16, 4886-4907.
- Broderick,A.C., Godley,B.J., Hays,G.C., 2001. Metabolic heating and the prediction of sex ratios for green turtles (*Chelonia mydas*). *Physiological and Biochemical Zoology* 74(2), 161-170.
- Broderick,A.C., Godley,B.J., Reece,S., Downie,J.R., 2000. Incubation periods and sex ratios of green turtles: Highly female biased hatchling production in the eastern Mediterranean. *Marine Ecology Progress Series* 202, 273-281.



- Bull,J.J., Charnov,E.L., 1988. How fundamental are Fisherian sex ratios? *Oxford Surveys of Evolutionary Biology* 5, 96-135.
- Bull,J.J., Vogt,R.C., McCoy,C.J., 1982. Sex determining temperatures in turtles: a geographic comparison. *Evolution* 36, 333-341.
- Burgess,E.A., Booth,D.T., Lanyon,J.M., 2006. Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs* 25(341), 349.
- Carrasco,A.M., Marquez,M.R., Benitez,V.V., Diaz,F.J., Jimenez,J.Q., 2000. The effect of temperature change on the sex ratio of Kemp's ridley nests in the hatchery center at Rancho Nuevo, Tamaulipas, Mexico. In: Kalb,H.J., Wibbels,T. (Eds.), NOAA Technical Memorandum NMFS-SEFC-443, pp. 128-129.
- Carthy,R.R., Foley,A.M., Matsuzawa,Y., 2003. Incubation environment of loggerhead turtle nests. In: Bolten,A.B., Witherington,B.E. (Eds.), *Loggerhead Sea Turtles*. Smithsonian Books, Washington, pp. 144-153.
- Casale,P., Lazar,B., Pont,S., Tomás,J., Zizzo,N., Alegre,F., Badillo,J., Di Summa,A., Freggi,D., Lackovic,G., Raga,J.A., Rositani,L., Tvrtkovic,N., 2006. Sex ratios of juvenile loggerhead sea turtles *Caretta caretta* in the Mediterranean. *Marine Ecology Progress Series* 324, 281-285.
- Ceriani,S.A., Wyneken,J., 2008. Comparative morphology and sex identification of the reproductive system in formalin-preserved sea turtle specimens. *Zoology* 111(3), 179-187.
- Chan,E.H., Liew,H.C., 1995. Incubation temperatures and sex ratios of the Malaysian leatherback, *Dermochelys coriacea*. *Biological Conservation* 74, 169-174.
- Chu,C.T., Booth,D.T., Limpus,C.J., 2008. Estimating the sex ratio of loggerhead turtle hatchlings at Mon Repos rookery (Australia) from nest temperatures. *Australian Journal of Zoology* 56, 57-64.
- Davenport,J., 1997. Temperature and the life-history strategies of sea turtles. *Journal of Thermal Biology* 22(6), 479-488.
- Dodd,C.J., 1988. Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus, 1758). *Biological Report* 88(14), 1-110.
- Drake,D.L., Spotila,J.R., 2002. Thermal tolerances and the timing of sea turtle hatchling emergence. *Journal of Thermal Biology* 27(1), 71-81.
- Eckert,K.L., 1987. Environmental unpredictability and leatherback sea turtle (*Dermochelys coriacea*) nest loss. *Herpetologica* 43(3), 315-323.
- Foley,A.M., 1998. The nesting ecology of the loggerhead turtle (*Caretta caretta*) in the Ten Thousand Islands, Florida. University of South Florida, St. Petersburg.
- Foley,A.M., Peck,S.A., Harman,G.R., Richardson,L.W., 2000. Loggerhead turtle (*Caretta caretta*) nesting habitat on low-relief mangrove islands in southwest Florida and consequences to hatchling sex ratios. *Herpetologica* 56(4), 433-445.

- Fuentes, M.M.P.B., Hamann, M., Limpus, C.J., 2010. Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. *Journal of Experimental Marine Biology and Ecology* 383, 56-64.
- Garmestani, A.S., Percival, H.F., Portier, K.M., Rice, K.G., 2000. Nest-site selection by the loggerhead sea turtle in Florida's Ten Thousand Islands. *Journal of Herpetology* 34(4), 504-510.
- Georges, A., Limpus, C.J., Stoutesdijk, R., 1994. Hatchling sex ratio in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration exposure. *The Journal of Experimental Zoology* 270, 432-444.
- Godfrey, M.H., Barreto, R., Mrosovsky, N., 1997. Metabolically-generated heat of developing eggs and its potential effect on sex ratio of sea turtle hatchlings. *Journal of Herpetology* 31(4), 616-619.
- Godfrey, M.H., Barreto, R., 1995. Beach vegetation and seafinding orientation of turtle hatchlings. *Biological Conservation* 74(1), 29-32.
- Godfrey, M.H., Mrosovsky, N., 1997. Estimating the time between hatching of sea turtles and their emergence from the nest. *Chelonian Conservation Biology* 2, 581-585.
- Godley, B.J., Broderick, A.C., Downie, J.R., Glen, F., Houghton, J.D.R., Kirkwood, I., Reece, S., Hays, G.C., 2001a. 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.
- Godley, B.J., Broderick, A.C., Glen, F., Hays, G.C., 2002b. Temperature-dependent sex determination of Ascension Island green turtles. *Marine Ecology Progress Series* 226, 115-124.
- Godley, B.J., Broderick, A.C., Glen, F., Hays, G.C., 2002a. Temperature-dependent sex determination of Ascension Island green turtles. *Marine Ecology Progress Series* 226, 115-124.
- Godley, B.J., Broderick, A.C., Mrosovsky, N., 2001b. Estimating hatchling sex ratios of loggerhead turtles in Cyprus from incubation duration. *Marine Ecology Progress Series* 210, 195-201.
- Hamann, M., Limpus, J.C., Owens, W.D., 2003. Reproductive cycles of males and females. In: Lutz, P., Musick, J.A., Wyneken, J. (Eds.), *The biology of sea turtles*. CRC Press, Boca Raton, pp. 135-153.
- Hanson, J., Wibbels, T., Martin, R.E., 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from Florida nesting beach. *Canadian Journal of Zoology* 76, 1850-1861.
- Harlow, P.S., 2004. Temperature-dependent sex determination in lizards. In: Valenzuela, N., Lance, V.A. (Eds.), *Temperature-dependent sex determination in vertebrates*. Smithsonian Institution Press, Washington, DC, pp. 42-52.

- Harris,L.R., 2008. The ecological implications of sea-level rise and storms for sandy beaches in KwaZulu-Natal. University of KwaZulu-Natal, Westville, Durban.
- Hawkes,L.A., Broderick,A.C., Godfrey,M.H., Godley,B.J., 2007. Investigating the potential impacts of climate change on a marine turtle population. *Global Change Biology* 13, 1-10.
- Hawkes,L.A., Broderick,A.C., Godfrey,M.H., Godley,B.J., 2009. Climate change and marine turtles. *Endangered Species Research* 7, 137-154.
- Hays,G.C., Ashworth,J.S., Barnsley,M.J., Broderick,A.C., Emery,D.R., Godley,B.J., Henwood,A., Jones,E.L., 2001. The importance of sand albedo for the thermal conditions on sea turtle nesting beaches. *Oikos* 93(1), 87-94.
- Hays,G.C., Broderick,A.C., Glen,F., Godley,B.J., 2003. Climate change and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Change Biology* 9, 642-646.
- Hays,G.C., Godley,B.J., Broderick,A.C., 1999. Long-term thermal conditions on the nesting beaches of green turtles on Ascension Island. *Marine Ecology Progress Series* 185, 297-299.
- Hays,G.C., Mackay,A., Adams,C.R., Mortimer,J.A., Speakman,J.R., Boerema,M., 1995. Nest site selection by sea turtles. *Journal of the Marine Biological Association of the United Kingdom* 75(3), 667-674.
- Hays,G.C., Speakman,J.R., 1993. Nest placement by loggerhead turtles, *Caretta caretta*. *Animal Behaviour* 45(1), 47-53.
- Hays,G.C., Speakman,J.R., Hayes,J.P., 1992. The pattern of emergence by loggerhead turtle (*Caretta caretta*) hatchlings on Cephalonia, Greece. *Herpetologica* 48(4), 396-401.
- Hewavisenthi,D., Parmenter,C.J., 2000. Hydric environment and sex determination in the flatback turtle (*Natator depressus* Garman) (Chelonia: Cheloniidae). *Australian Journal of Zoology* 48, 653-659.
- Hewavisenthi,S., Parmenter,C.J., 2002. Incubation environment and nest success of the flatback turtle (*Natator depressus*) from a natural nesting beach. *Copeia*(2), 302-312.
- Higginson,J., Vasquez,F., 1989. Hatchery design and the production of female hatchlings. pp. 7-12.
- Houghton,J.D.R., Hays,G.C., 2001. Asynchronous emergence by loggerhead turtle (*Caretta caretta*) hatchlings. *Naturwissenschaften* 88, 133-136.
- Houghton,J.D.R., Myers,A.E., Lloyd,C., King,R.S., Isaacs,C., Hays,G.C., 2007. Protracted rainfall decreases temperature within leatherback turtle (*Dermochelys coriacea*) clutches in Grenada, West Indies: Ecological implications for a species displaying temperature dependent sex determination. *Journal of Experimental Marine Biology and Ecology* 345, 71-77.
- Hughes,G.R., 1974a. The sea turtles of south east Africa. PhD thesis. University of Natal.

- Hughes,G.R., 1974b. The sea turtles of South-east Africa I. Status, morphology and distributions. Rep 35, 1-144, Oceanographic Research Institute.
- Hughes,G.R., 1989. Sea turtles. In: Pelletier,D., Crawford,R.J.M. (Eds.), Oceans of Life off Southern Africa. Vlaeberg, Cape Town, pp. 230-243.
- Hughes,G.R., 1996. The status of sea turtle conservation in South Africa. In: Humphrey,S.L., Salm,R.V. (Eds.), Status of sea turtle conservation in the Western Indian Ocean. IUCN / UNEP, Nairobi, Kenya, pp. 95-101.
- Hughes,G.R., Bass,A.J., Mentis,M.T., 1967. Further studies on the marine turtles in Tongaland, I. The Lammergeyer 7, 5-54.
- Hulin,V., Delmas,V., Girondot,M., Godfrey,M.H., Guillon,J.-M., 2009. Temperature-dependent sex determination and global change: are some species at risk? *Oecologia* 160, 493-506.
- Janzen,F.J., 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the Natural Academy of Science* 91, 7487-7490.
- Kamel,S.J., Mrosovsky,N., 2004. Nest site selection in leatherbacks, *Dermochelys coriacea*: individual patterns and their consequences. *Animal Behaviour* 68, 357-366.
- Kamel,S.J., Mrosovsky,N., 2005. Repeatability of nesting preferences in the hawksbill sea turtle, *Eretmochelys imbricata*, and their fitness consequences. *Animal Behaviour* 70, 819-828.
- Kamezaki,N., 2003. What is a loggerhead turtle? The morphological perspective. In: Bolten,A.B., Witherington,B.E. (Eds.), *Loggerhead Sea Turtles*. Smithsonian Books, Washington, pp. 28-43.
- Kaska,Y., Downie,R., Tippett,R., Furness,R.W., 1998. Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. *Canadian Journal of Zoology* 76, 723-729.
- Kaska,Y., Ilgaz,Ç., Özdemir,A., Baskale,E., Türkozan,O., Baran,Y., Stachowitsch,M., 2006. Sex ratio estimations of loggerhead sea turtle hatchlings by histological examination and nest temperatures at Fethiye beach, Turkey. *Naturwissenschaften* 93, 338-343.
- Kikukawa,A., Kamezaki,N., Ota,H., 1999. Factors affecting nesting beach selection by loggerhead turtles (*Caretta caretta*): a multiple regression approach. *Journal of Zoology* 249(4), 447-454.
- Kolbe,J.J., Janzen,F.J., 2002. Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology* 83(1), 269-281.
- Limpus,C.J., 1985. Study of the loggerhead sea turtle, *Caretta caretta*, in eastern Australia. University of Queensland, Brisbane.
- Limpus,C.J., Reed,P.C., Miller,J.D., 1985. Temperature dependent sex determination in Queensland sea turtles: Intraspecific variation in *Caretta caretta*. In: Grigg,G.,

- Shine,R., Ehmann,H. (Eds.), *Biology of Australian Frogs and Reptiles*. Royal Zoological Society, New South Wales, pp. 343-351.
- López-Castro,M.C., Carmona,R., Nichols,W.J., 2004. Nesting characteristics of the olive ridley turtle (*Lepidochelys olivacea*) in Cabo Pulmo, southern Baja California. *Marine Biology* 145, 811-820.
- Luschi,P., Lutjeharms,J.R.E., Lambardi,P., Mencacci,R., Hughes,G.R., Hays,G.C., 2006. A review of migratory behaviour of sea turtles off southeastern Africa. *South African Journal of Science* 102, 51-58.
- Lutjeharms,J.R.E., 2001. Agulhas current. In: Steele,J.H., Turekian,K.K., Thorpe,S.A. (Eds.), *Encyclopedia of ocean sciences*. Academic Press, London, pp. 104-113.
- Lutjeharms,J.R.E., Ansorge,I.J., 2001. The Agulhas return current. *Journal of Marine Systems* 30, 115-138.
- Maloney,J.E., Darian-Smith,C., Takahashi,Y., Limpus,C.J., 1990. The environment for development of the embryonic loggerhead turtle (*Caretta caretta*) in Queensland. *Copeia* 1990(2), 378-387.
- Marcovaldi,M.A., Godfrey,M.H., Mrosovsky,N., 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. *Canadian Journal of Zoology* 75, 755-770.
- Marine Living Resources Act, 1998. Government gazette. Marine and Coastal Management, Cape Town.
- Matsuzawa,Y., Sato,K., Sakamoto,W., Bjorndal,K.A., 2002. Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of loggerhead sea turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. *Marine Biology* 140, 639-646.
- Maxwell,J.A., Motara,M.A., Frank,G.H., 1988. A micro-environmental study of the effect of temperature on the sex ratios of the loggerhead turtle, *Caretta caretta*, from Tongaland, Natal. *South African Journal of Zoology* 23(4), 342-350.
- Mazaris,A.D., Matsinos,Y.G., Margaritoulis,D., 2006. Nest site selection of loggerhead sea turtles: The case of the island of Zakynthos, W Greece. *Journal of Experimental Marine Biology and Ecology* 336, 157-162.
- McAllister,H.J., Bass,A.J., van Schoor,H.J., 1965. Marine turtles on the coast of Tongaland, Natal. In: Bigalke,R.C., Crass,R.S. (Eds.), pp. 12-40.
- McGehee,M.A., 1990. Effects of moisture on eggs and hatchlings of loggerhead sea turtles (*Caretta caretta*). *Herpetologica* 46(3), 251-258.
- McGinley,M.A., 1989. The influence of a positive correlation between clutch size and offspring fitness on the optimal offspring size. *Evolutionary Ecology* 3(2), 150-156.
- Miller,J.D., 1997. Reproduction In Sea Turtles. In: Lutz,P.L., Musick,J.A. (Eds.), *The Biology of Sea Turtles*. CRC Press, Inc., Florida, pp. 51-81.

- Miller, J.D., 1999. Determining clutch size and hatching success. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group, Washington, DC, pp. 124-129.
- Miller, J.D., Limpus, C.J., Godfrey, M.H., 2003. Nest site selection, oviposition, eggs, development, hatching, and emergence of loggerhead turtles. In: Bolten, A.B., Witherington, B.E. (Eds.), *Loggerhead Sea Turtles*. Smithsonian Books, Washington, pp. 125-143.
- Miller, S.A., Harley, J.P., 1996. Reptiles: The First Amniotes. *Zoology*. Wm.C. Brown Publishers, pp. 467-481.
- Moran, K.L., Bjorndal, K.A., Bolten, A.B., 1999. Effects of the thermal environment on the temporal pattern of emergence of hatchling loggerhead turtles *Caretta caretta*. *Marine Ecology Progress Series* 189, 251-261.
- Morreale, S.J., Ruiz, G.J., Spotila, J.R., Standora, E.A., 1982. Temperature dependent sex determination: current practices threaten conservation of sea turtles. *Science* 216, 1245-1247.
- Mortimer, J.A., 1990. Influence of beach sand characteristics on nesting behavior and clutch survival in green turtles. In: Richardson, T.H., Richardson, J.I., Donnelly, M. (Eds.), NOAA Technical Memorandum NMFS-SEFC-278.
- Mortimer, J.A., 1995. Factors influencing beach selection by nesting sea turtles. In: Bjorndal, K.A. (Ed.), *Biology and conservation of sea turtles*. Smithsonian Institution Press, Washington D.C..
- Mrosovsky, N., 1980. Thermal biology of sea turtles. *American Zoologist* 20(3), 531-547.
- Mrosovsky, N., 1983. Ecology and nest-site selection of leatherback turtles *Dermochelys coriacea*. *Biological Conservation* 26, 47-56.
- Mrosovsky, N., 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. *Canadian Journal of Zoology* 66, 661-669.
- Mrosovsky, N., Bass, A., Corliss, L.A., Richardson, J.I., Richardson, T.H., 1992. Pivotal and beach temperatures for the hawksbill turtles nesting in Antigua. *Canadian Journal of Zoology* 70, 1920-1925.
- Mrosovsky, N., Benabib, M., 1990. An assessment of two methods of sexing hatchling sea turtles. *Copeia* 1990(2), 589-591.
- Mrosovsky, N., Camel, S., Rees, A.F., Margaritoulis, D., 2002. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. *Canadian Journal of Zoology* 80, 2118-2124.
- Mrosovsky, N., Dutton, P.H., Whitmore, C.P., 1984. Sex ratios of two species of sea turtle nesting in Suriname. *Canadian Journal of Zoology* 62(11), 2227-2239.

- Mrosovsky,N., Provancha,J., 1992. Sex ratio of hatchling loggerhead sea turtles: Data and estimates from a 5-year study. *Canadian Journal of Zoology* 70, 530-538.
- Mrosovsky,N., Yntema,C.L., 1980. Temperature dependence of sexual differentiation in sea turtles: Implications for conservation practices. *Biological Conservation* 18, 271-280.
- Musick,J.A., 1999. Ecology and conservation of long-lived marine animals. *American Fisheries Society Symposium* 23, 1-10.
- National Research Council, 1990. *The decline of the sea turtles: Causes and prevention*. National Academy of Science Press, Washington, D.C., pp. -259.
- Nel,R., 2008. Sea turtles of KwaZulu-Natal: Data report for 2007/8 season. Report for Ezemvelo KwaZulu-Natal Wildlife. 1-31.
- Nel,R., 2010. Sea turtles of KwaZulu-Natal: Data report for the 2009/10 season. Report for Ezemvelo KZN Wildlife. 1-32.
- Nel,R., Lawrence,C., 2007. Provincial species monitoring plan: Leatherback (*Dermochelys coriacea*) and loggerhead (*Caretta caretta*) sea turtles. Report for EKZNW. 1-25, EKZNW.
- Nel,R., Papillon,J., 2005. Turtle monitoring programme: Season report for 2004-2005. Report for Ezemvelo KwaZulu-Natal Wildlife. 1-22.
- Owens,D.W., 1999. Reproductive cycles and endocrinology. In: Eckert,K.L., Bjorndal,K.A., Abreu-Grobois,F.A., Donnelly,M. (Eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group, Blanchard, Pennsylvania, USA, pp. 119-123.
- Owens,D.W., Hendrickson,J.R., Lance,V., Callard,I.P., 1978. A technique for determining sex of immature *Chelonia mydas* using radioimmunoassay. *Herpetologica* 34, 270-273.
- Öz,M., Erdogan,A., Kaska,Y., Düsen,S., Aslan,A., Sert,H., Yavuz,M., Tunç,M.R., 2004. Nest temperatures and sex-ratio estimates of loggerhead turtles at Patara beach on the southwestern coast of Turkey. *Canadian Journal of Zoology* 82, 94-101.
- Pearce,A.F., 2001. Contrasting population structure of the loggerhead turtle (*Caretta caretta*) using mitochondrial and nuclear DNA markers. Master's Thesis. University of Florida, Gainesville.
- Rees,A.F., Margaritoulis,D., 2004. Beach temperatures, incubation durations and estimating hatchling sex ratio for loggerhead sea turtle nests in southern Kyparissia Bay, Greece. *Testudo* 6(1), 23-36.
- Ross,J.P., Barwani,M.A., 1995. Review of sea turtles in the Arabian area. In: Bjorndal,K.A. (Ed.), *Biology and conservation of sea turtles*. Smithsonian Institution Press, Washington D.C., pp. 373-383.
- Schumann,E.H., Orren,M., 1980. The physio-chemical characteristics of the South-West Indian Ocean in relation to Maputaland. In: Bruton,M.N., Cooper,K.H. (Eds.), *Studies on the ecology of Maputaland*. Wildlife Society of South Africa, Durban, pp. 8-11.

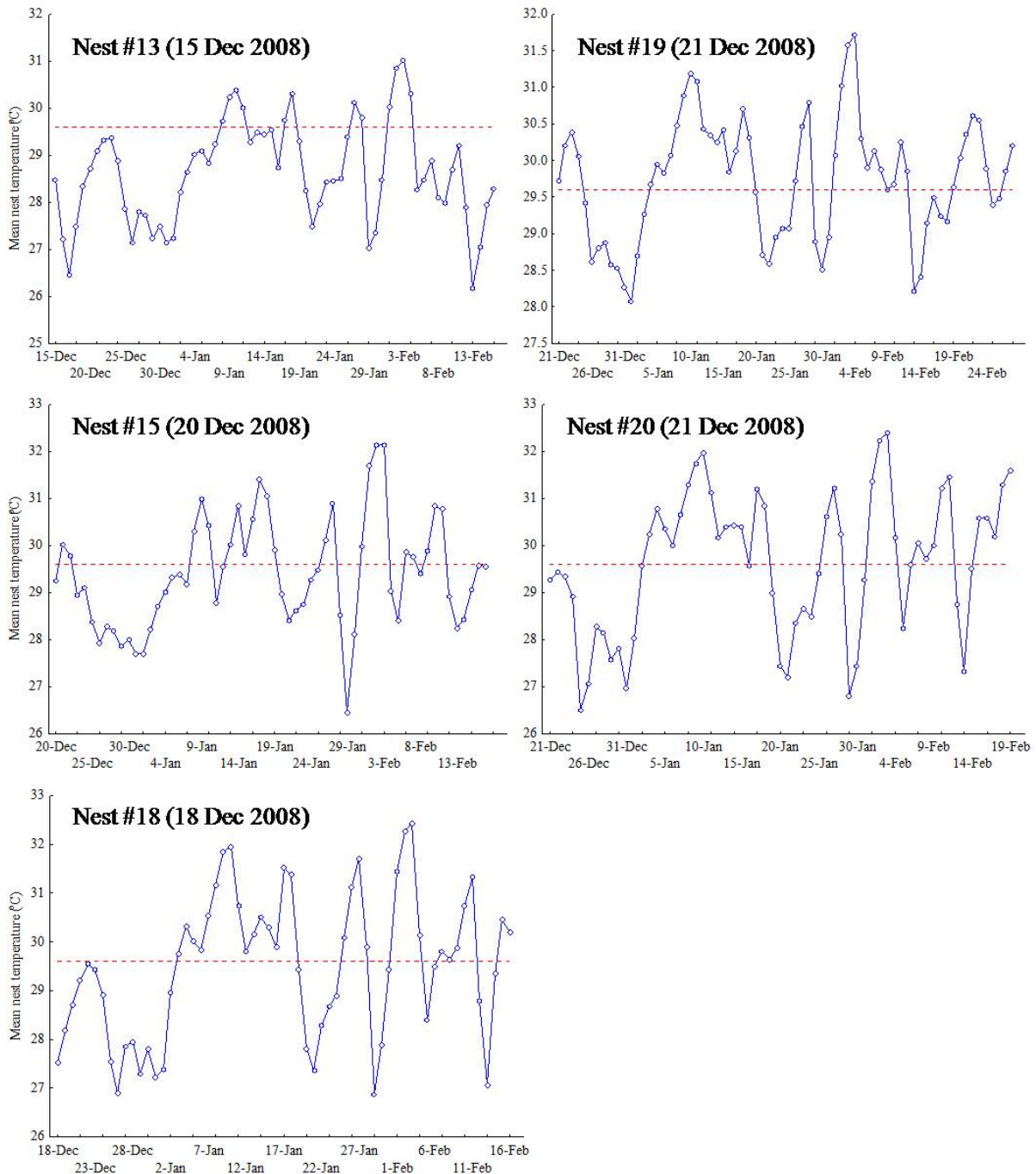
- Serafini, T.Z., Lopez, G.G., da Rocha, P.L.B., 2009. Nest site selection and hatching success of hawksbill and loggerhead sea turtles (Testudines, Cheloniidae) at Arembepe Beach, northern Brazil. *Phyllomedusa* 8(1), 3-17.
- Speakman, J.R., Hays, G.C., Lindblad, E., 1998. Thermal conductivity of sand and its effect on the temperature of loggerhead sea turtle (*Caretta caretta*) nests. *Journal of the Marine Biological Association of the United Kingdom* 78(4), 1337-1352.
- Spotila, J.R., 2004. *Sea Turtles: a complete guide to their biology, behavior and conservation*. John Hopkins University Press, Maryland, USA.
- Standora, E.A., Spotila, J.R., 1985. Temperature dependent sex determination in sea turtles. *Copeia* 1985, 711-722.
- Storfer, A., 1996. Quantative genetics: a promising approach for the assessment of genetic variation in endangered species. *Trends in Ecology & Evolution* 11(8), 343-348.
- Valenzuela, N., 2004a. Evolution and maintenance of temperature-dependent sex determination. In: Valenzuela, N., Lance, V.A. (Eds.), *Temperature-dependent sex determination in vertebrates*. Smithsonian Books, Washington, DC, pp. 131-147.
- Valenzuela, N., 2004b. Temperature-dependent sex determination. In: Deeming, D.C. (Ed.), *Reptilian incubation: Environment and behaviour*. Nottingham University Press, Nottingham, UK, pp. 211-227.
- Valverde, R.A., Wingard, S., Gómez, F., Tordoir, M.T., Orrego, C.M., 2010. Field lethal incubation temperature of olive ridley sea turtle *Lepidochelys olivacea* embryos at a mass nesting rookery. *Endangered Species Research* 12, 77-86.
- van Buskirk, J., Crowder, L.B., 1994. Life-history variation in marine turtles. *Copeia* 1994(1), 66-81.
- Vogt, R.C., Bull, J.J., 1984. Ecology of hatchling sex ratio in map turtles. *Ecology* 65, 582-587.
- Wang, J.W., Andrews, H.V., 1994. Temperature-dependent sex determination in crocodylians. *Journal of Experimental Zoology* 270(1), 28-44.
- Whitmore, C.P., Dutton, P.H., 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. *Biological Conservation* 34(3), 251-272.
- Wibbels, T., Rostal, D.C., Byles, R., 1998. High pivotal temperature in the sex determination of the olive ridley turtle *Lepidochelys olivacea* from Playa Nancite, Costa Rica. *Copeia* 1998, 1086-1088.
- Wilson, A., Humphrey, S.L., 2004. Marine turtle update: Recent news from the WWF Africa and Madagascar marine turtle programme. 1-16, WWF.
- Wood, D.W., Bjorndal, K.A., 2000a. Relation of temperature, moisture, salinity, and slope to nest site selection in Loggerhead Sea Turtles. *Copeia* 2000(1), 119-128.

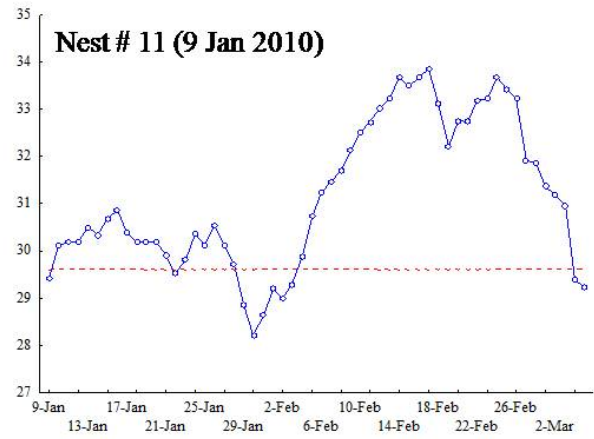
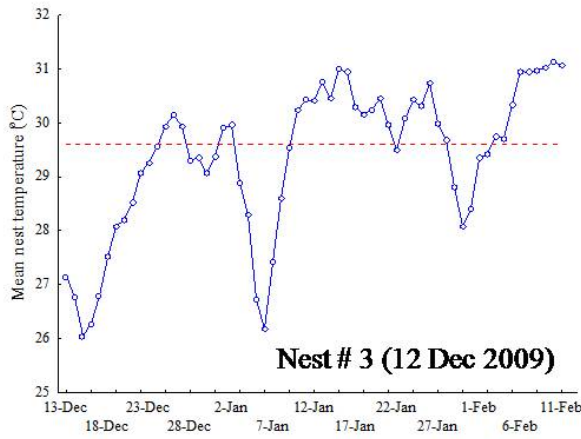
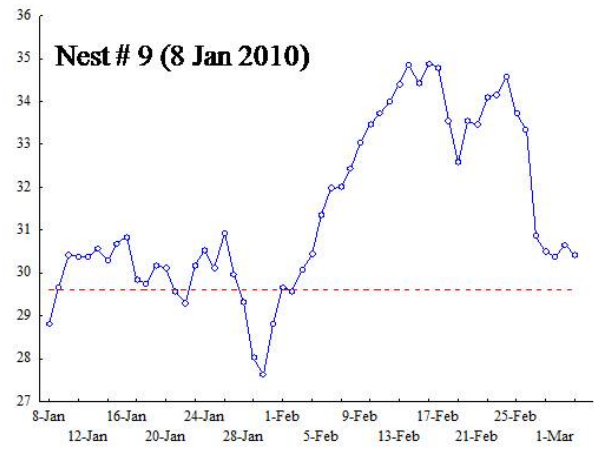
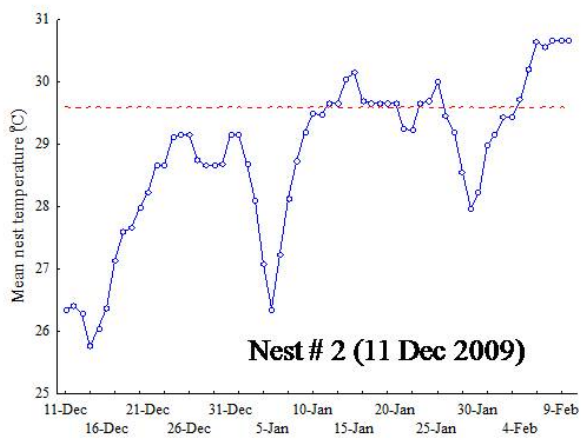
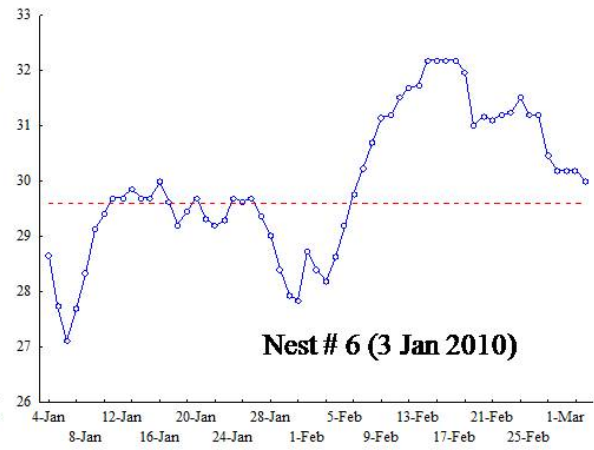
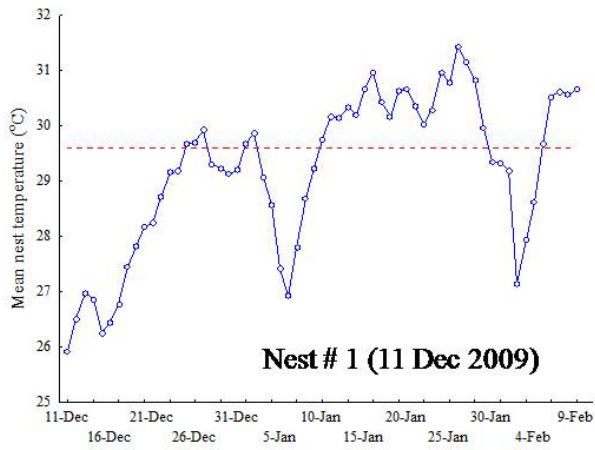


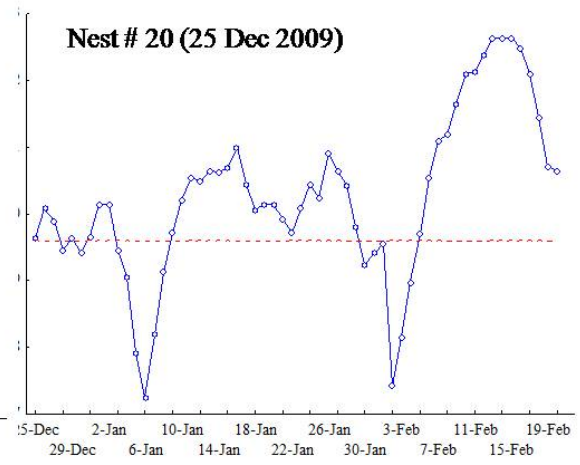
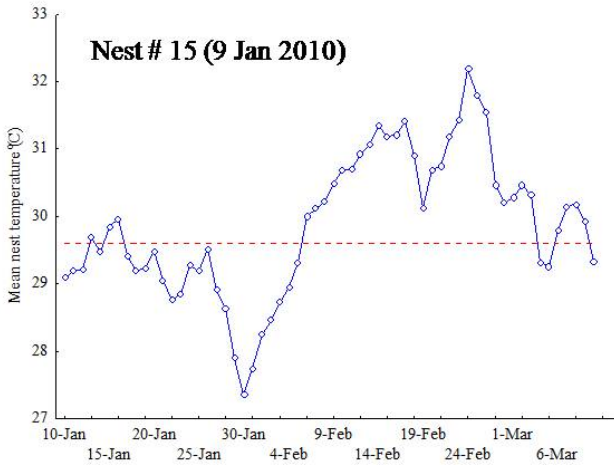
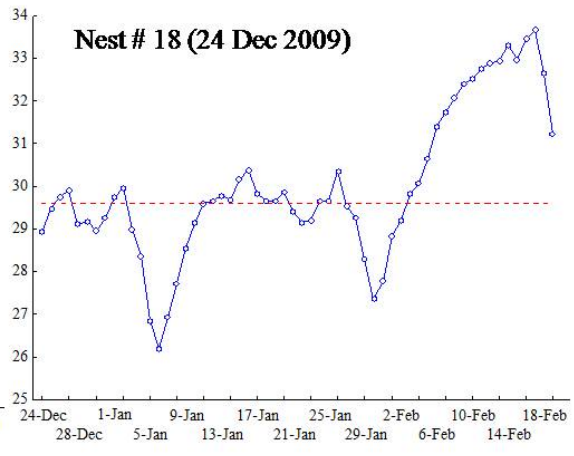
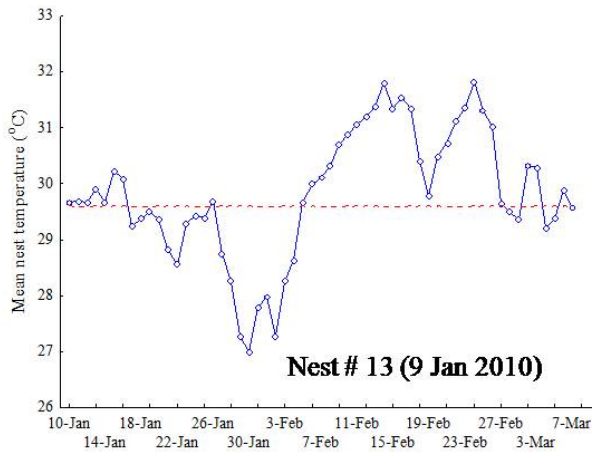
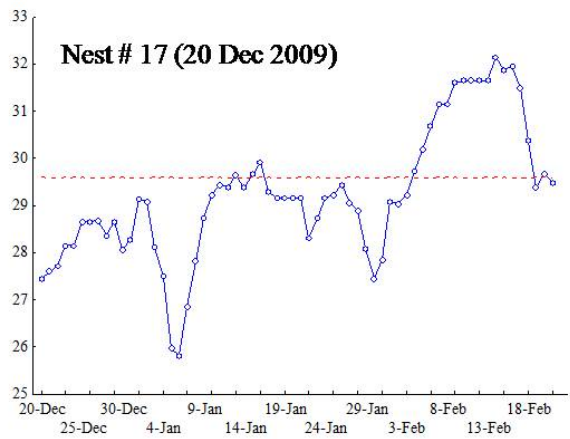
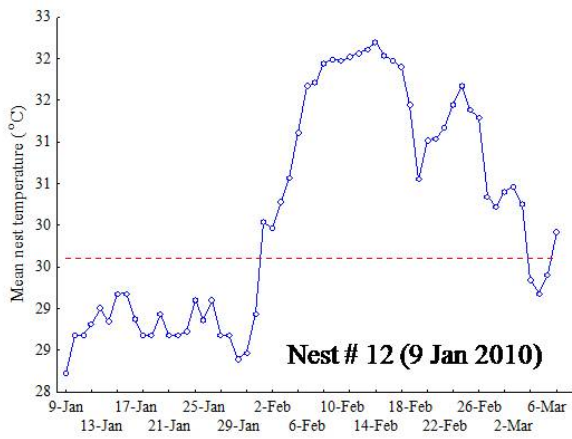
- Wood,D.W., Bjorndal,K.A., 2000b. Relation of temperature, moisture, salinity, and slope to nest site selection in loggerhead sea turtles. *Copeia* 2000(1), 119-128.
- Wyneken,J., Epperley,S.P., Crowder,L.B., Vaughan,J., Esper,K.B., 2007. Determining sex in posthatchling loggerhead sea turtles using multiple gonadal and accessory duct characteristics. *Herpetologica* 63(1), 19-30.
- Yntema,C.L., Mrosovsky,N., 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) incubated at different controlled temperatures. *Herpetologica* 36, 33-36.
- Yntema,C.L., Mrosovsky,N., 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. *Canadian Journal of Zoology* 60, 1012-1016.
- Zbinden,J.A., Margaritoulis,D., Arlettaz,R., 2006. Metabolic heating in Mediterranean loggerhead sea turtle clutches. *Journal of Experimental Marine Biology and Ecology* 334, 151-157.

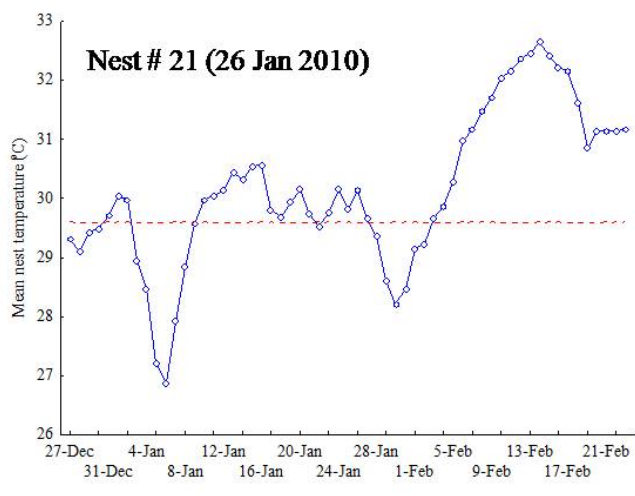
# Appendices

**Appendix A.** Temperature profiles of 18 successfully hatched nests (hatching success > 20%) over two seasons (2008/9 and 2009/10) for loggerhead turtles nesting in KZN as measured with iButton temperature loggers. Broken line denotes the temperature at which 100% females were produced at 29.6°C (from Maxwell *et al.* 1988).









**Appendix B.** Information on all the study nests found and excavated over two nesting seasons. The data include lay date, incubation period (IP) in days (if applicable), date of emergence (DOE), clutch size, whether the nest was predated or not, hatching success (HS), emergence success (ES) and mean temperature data during the whole IP and the TSP.

Nest #	Lay date	IP (days)	DOE	Clutch size (# eggs)	Predated	HS (%)	ES (%)	Total IP				TSP			
								Mean	± SD	Min	Max	Mean	± SD	Min	Max
<b>Season 2008/9</b>															
1	09-Dec	Not hatched	-	107	Yes	-	-	-	-	-	-	-	-	-	-
4	10-Dec	-	No	48	No	68.8	68.8	30.0	1.56	25.6	33.9	30.7	1.33	27.5	32.4
5	12-Dec	Not hatched	-	97	Yes	-	-	-	-	-	-	-	-	-	-
7	18-Dec	Not hatched	-	84	Yes	-	-	-	-	-	-	-	-	-	-
8	12-Dec	-	No	109	No	75.2	75.2	29.6	1.27	25.7	32.5	30.2	0.91	27.8	31.6
9	12-Dec	-	No	111	No	97.3	97.3	29.5	0.93	25.3	31.4	30.1	0.81	27.9	31.2
12*	21-Dec	70	Yes	88	No	9.1	9.1	-	-	-	-	-	-	-	-
13	15-Dec	64	Yes	72	No	98.6	95.8	28.6	1.09	26.2	31.0	29.2	0.76	27.5	30.4
15	20-Dec	60	Yes	126	No	94.4	94.4	29.4	1.16	26.5	32.1	29.8	0.93	28.4	31.4
16	18-Dec	-	No	106	No	96.2	94.3	29.8	1.20	25.9	32.5	30.5	0.89	28.8	31.8
17	21-Dec	-	No	106	No	87.7	87.7	30.2	1.66	24.0	33.6	31.0	0.91	29.2	32.3
18	18-Dec	61	Yes	104	No	71.2	71.1	29.5	1.47	26.9	32.4	30.1	1.31	27.4	32.0
19	21-Dec	70	Yes	110	No	75.2	75.2	29.7	0.83	28.1	31.7	29.9	0.91	28.5	31.7
20	21-Dec	61	Yes	89	No	87.6	87.6	29.6	1.5	26.5	32.4	29.7	1.54	26.8	32.0
21	21-Dec	Not hatched	-	110	Yes	-	-	-	-	-	-	-	-	-	-
<b>Average</b>		<b>64</b>		<b>96</b>		<b>78.3</b>	<b>77.9</b>	<b>29.6</b>	<b>1.27</b>	<b>26.1</b>	<b>32.4</b>	<b>30.1</b>	<b>1.03</b>	<b>28.0</b>	<b>31.7</b>

Nest #	Lay date	IP (days)	DOE	Clutch size (# eggs)	Predated	HS (%)	ES (%)	Total IP				TSP			
								Mean	± SD	Min	Max	Mean	± SD	Min	Max
<b>Season 2009/10</b>															
1	11-Dec	62	Yes	121	No	88.4	88.4	28.8	1.25	25.8	31.5	29.5	1.1	26.9	31.0
2	12-Dec	61	Yes	85	No	90.6	90.6	29.4	1.36	26.1	30.7	29.0	1.04	26.4	30.2
3	12-Dec	Not hatched	-	128	No	-	-	-	-	-	31.1	29.6	1.34	26.2	31.0
4	15-Dec	Not hatched	-	106	Yes	-	-	-	-	-	-	-	-	-	-
5	03-Jan	59	Yes	133	No	87.2	85.0	29.9	1.29	27.1	-	-	-	-	-
6	03-Jan	Not hatched	-	111	Yes	-	-	-	-	-	32.2	29.4	1.09	27.8	31.5
7	06-Jan	Not hatched	-	82	Yes	-	-	-	-	-	-	-	-	-	-
8	08-Jan	55	Yes	133	No	92.5	88.0	<b>31.4</b>	<b>1.98</b>	<b>27.6</b>	-	-	-	-	-
9	08-Jan	60	Yes	111	Yes	18.0	18.0	-	-	-	<b>34.9</b>	<b>31.1</b>	<b>2.01</b>	<b>27.6</b>	<b>34.4</b>
10*	09-Jan	56	Yes	91	No	92.3	92.3	31.1	1.58	28.2	-	-	-	-	-
11	09-Jan	58	Yes	78	No	69.2	69.2	30.1	1.32	28.2	33.8	30.8	1.75	28.2	33.7
12	09-Jan	57	Yes	88	No	96.6	96.6	29.8	1.16	27.0	32.2	30.8	1.43	28.4	32.2
13	09-Jan	Not hatched	-	43	Yes	-	-	-	-	-	31.8	29.5	1.59	27.0	31.8
14	09-Jan	60	Yes	86	No	88.4	88.4	29.9	1.05	27.4	-	-	-	-	-
15	09-Jan	Not hatched	-	109	Yes	-	-	-	-	-	32.2	29.8	1.33	27.4	31.4
16	20-Dec	64	Yes	119	No	89.1	89.1	29.2	1.42	25.8	-	-	-	-	-
17**	24-Dec	58	Yes	71	No	53.5	43.7	30.0	1.73	26.2	32.1	29.0	0.58	27.5	29.9
18**	24-Dec	Not hatched	-	135	Yes	-	-	-	-	-	33.7	29.5	0.68	27.4	30.4
19*	25-Dec	58	Yes	101	No	97.0	96.0	30.3	1.23	27.2	-	-	-	-	-
20	26-Dec	59	Yes	84	No	94.1	94.1	30.2	1.30	26.9	32.6	30.3	0.43	29.2	31.0
21	26-Dec	Not hatched	-	113	Yes	-	-	-	-	-	32.7	29.6	0.65	28.2	30.6
22*	28-Dec	Not hatched	-	116	Yes	-	-	-	-	-	-	-	-	-	-
23*	26-Dec	-	No	64	No	56.3	54.7	29.7	1.09	26.7	-	-	-	-	-
24**	05-Jan	-	No	110	No	49.1	48.2	29.4	1.18	26.2	31.7	29.3	0.99	27.1	30.9
25**	<b>Average</b>	<b>59</b>		<b>101</b>		<b>78.1</b>	<b>76.8</b>	<b>29.9</b>	<b>1.36</b>	<b>26.8</b>	31.2	28.9	1.12	27.1	30.7
	<b>Average (±SD)</b>	<b>61</b>		<b>99</b>		<b>78.2</b>	<b>77.2</b>	<b>29.8</b>	<b>1.31</b>	<b>26.6</b>	<b>32.3</b>	<b>29.7</b>	<b>1.14</b>	<b>27.5</b>	<b>31.4</b>

**All seasons (2008 - 10)**

\* Hatching success lower than 20% in proportion to the clutch size; \*\* Temperature data from iButtons also recording relative humidity.