Comparison of the population growth potential of South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles

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Comparison of the population growth potential of South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles

by

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In accordance with Rule G4.6.3, I hereby declare that the above-mentioned thesis is my own work and that it has not previously been submitted for assessment to another University or for another qualification.

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LIST OF ABBREVIATIONS

AR	age at reproduction
AIC	Akaike Information Criterion
CC	*Caretta caretta* (loggerhead turtle)
CCL	curved carapace length
CMS	Convention on the Conservation of Migratory Species of Wild Animals
CTE	constant temperature equivalent
DC	*Dermochelys coriacea* (leatherback turtle)
ESD	environmental sex determination
Ezemvelo	Ezemvelo KwaZulu-Natal Wildlife
FM	female-male (pattern of sex determination)
FMF	female-male-female (pattern of sex determination)
GLM	general linear model
GLMM	mixed general linear model
GPS	global positioning system
GSD	genetic sex determination
IP	incubation period
iSimangaliso	iSimangaliso Wetland Park
KZN	KwaZulu-Natal (province in South Africa)
KZNW	KwaZulu-Natal Wildlife (equivalent to Ezemvelo)
MF	male-female (pattern of sex determination)
MPA	marine protected area
NMMU	Nelson Mandela Metropolitan University
PIT tag	passive integrated transponder tag
PT	pivotal temperature
PVA	population viability analyses
Ramsar	Convention on Wetlands of International Importance especially as Waterfowl Habitat
SAG	yolkless egg
SCL_{min}	straight carapace length (measured as minimum)
SCW	straight carapace width
SD	standard deviation
SE	standard error
SST	sea surface temperature
SWIO	South West Indian Ocean
TED	turtle excluder device
TRT	transitional range of temperature
TSD	Temperature-dependent sex determination
TSP	thermo-sensitive period

ABSTRACT

A beach conservation programme protecting nesting loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles in South Africa was started in 1963. As initial numbers of nesting females were low for both species (107 loggerheads and 24 leatherbacks) it was proposed that the protection of eggs, hatchlings and nesting females along the nesting beach would induce population growth and prohibit local extinction. Today, 50 years later, the loggerhead population exceeds 650 females per annum, whereas the leatherback population counts about 65 nesting females per year. The trend for leatherback turtles is that the population has been stable for about 30 years whereas loggerheads are increasing exponentially. Thus, this thesis investigated several life-history traits to explain the differing responses to the ongoing beach conservation programme.

Reproductive output and success were assessed for both species; it was hypothesised that environmental conditions are sub-optimal for leatherback turtles to reproduce successfully. It was ascertained that nesting loggerhead females deposit larger clutches than leatherbacks (112 ± SD 20 eggs and 100 ± SD 23 eggs, respectively), but that annual reproductive output per individual leatherback female exceeds that of loggerhead turtles (±700 eggs and ±448 eggs, respectively) because they exhibit a higher intra-seasonal nesting frequency (leatherbacks n = 7 and loggerheads n = 4 from Nel et al. 2013). Emergence success (i.e. the percentage of hatchlings produced) per nest was similar for both species (loggerhead 73.6 ± SD 27.68 % and leatherback turtles 73.8 ± SD 22.70 %), but as loggerhead turtles nest in greater numbers, i.e. producing more hatchlings per year, the absolute population growth potential favours the loggerhead turtle.

The second factor investigated was sex ratio because sea turtles display temperature-dependent sex determination (TSD) where extreme incubation temperatures can skew the sex ratio (i.e. feminising or masculinising a clutch). It was suspected that leatherback turtles are male-biased as this is the southern-most rookery (for both species). Further, leatherback nests are generally closer to the high tide mark, which might induce a cooling effect. Standard histological techniques were applied to sex hatchlings and a generalized linear model (GLM) was used to approximate annual sex ratio. Loggerhead sex ratio (2009 - 2011) was estimated at 86.9 ± SE 0.35 % female-biased; however, sufficient replication for the leatherback population was only obtained for season 2010, which indicated a 97.1 % (95 % CI 93.3 - 98.7) female bias. Both species are, thus, highly female-biased, and current sex ratio for leatherback turtles is not prohibiting population growth.

Current sex ratios, however, are not necessarily indicative of sex ratios in the past which would have induced present population growth. Thus, to account for present population growth profiles, sex ratios from the past needed to be ascertained. Annual sex ratios (1997 - 2011) were modelled from historical air and sea surface temperatures (SSTs) but no significant change over time was obtained for either loggerhead or leatherback turtles (linear regression; $p \geq 0.45$). The average sex ratio over this 15-year period for the South African loggerhead turtle was approximated at 77.1 ± SE 3.36 % female-biased, whereas leatherbacks exhibited a 99.5 ± SE 0.24 % female bias. Re-analysing data from the mid-80s by Maxwell et al. (1988) also indicated a 77.4 % female bias for the South African loggerhead population. It is, therefore, highly likely that sex ratios of the South African loggerhead and leatherback sea turtle populations have been stable for at least three decades and are not accountable for the differing population growth profiles as they are displayed today.

Another possibility that could explain the opposed population growth profiles is the time taken for animals to replace themselves, i.e. age at maturity. It was suspected that age at maturity for the South African loggerhead turtle is comparable with that for leatherbacks. Using data from a 30-year mutilation tagging experiment (i.e. notching), age at first reproduction for South African loggerhead females was estimated. Results ranged broadly but a mean of 36.2 ± SD 7.71 years was obtained using a Gaussian distribution. Age at reproduction of the South African leatherback turtle was not determined but the literature suggests a much younger age of 13.3 - 26.8 years (Zug & Parham 1996, Dutton et al. 2005, Avens et al. 2009, Jones et al. 2011). Therefore, population growth would favour leatherback turtles as they exhibit a much shorter generation time.

Finally, it was concluded that all life-history parameters investigated favour leatherback turtles, yet loggerheads are displaying population growth. However, as there were no obvious constraints to population growth on the nesting beach, it is suspected that population growth of the South African leatherback turtle is either unobserved (due to inadequate monitoring not capturing sufficient numbers of nesting events to establish a trend) or that population growth is prohibited by some offshore factor such as industrial fisheries (or some other driver not yet identified). Monitoring should, thus, be expanded and offshore mortality monitored as the leatherback population nesting in South Africa is still critically endangered with nesting numbers dangerously low.

Key words: Loggerhead, *Caretta caretta*, leatherback, *Dermochelys coriacea*, population growth potential, reproductive output, reproductive success, sex ratio, climate change, age at reproduction

Chapter 1: Introduction

1.1 Conservation of migratory species

Conservation of migratory species is particularly challenging as they are wide-ranging with distributions often spanning geopolitical borders (Duffy 2001, Walpole et al. 2001, Naidoo et al. 2014) and areas beyond national jurisdiction, i.e. high seas (Bolten et al. 1998, Da Silvaa et al. 2010, Silva et al. 2013). Consequently, international conventions (e.g. Ramsar[1] or CMS[2]) and networks of protected areas have been established to address trans-national protection (Griloa et al. 2012, Mackelworth 2012, CMS 2014). Recognising that areas outside the protected area networks are also important, several ecosystem-based management tools, such as marine zoning (in space or time) have been implemented to protect migratory species in particular from fisheries and by-catch issues (Murawski et al. 2000, Jaworski et al. 2006, Stokesa et al. 2014).

In cases where species are well-studied, it is possible to conduct detailed population viability analyses (PVAs) that provide an indication of the level of protection necessary or the rate of population growth required to maintain a healthy population (Coulson et al. 2001, Reed et al. 2002). This approach is successfully used for focal species in terrestrial habitats such as tigers or rhinos (Kenney et al. 1995, Goossens et al. 2013). However, it is necessary to have a reasonable estimation of the survivorship in each age-class, which is difficult in data-poor situations where neither the critical demographic parameters, nor the number or extent of threats are known (Holmes 2001). Such data-poor situations are frequently found in the marine environment and even more so for marine migratory species (Snover & Heppell 2009).

In the case of sea turtles only very few extinction risk analyses have been conducted. For example, on green turtles in the Indo-Pacific (Dethmers & Baxter 2011) modelling the levels of threats at different age- (or stage-) classes (Crowder et al. 1994). Results indicated that, even though nest protection and reduced harvest of adult nesting and foraging turtles can significantly affect population growth in the long-term, population stability is mostly dependent on the survival of large juveniles and adults in several turtle species (Crouse et al. 1987, Brooks et al. 1991, Congdon et al. 1993). Unfortunately, most conservation efforts are focused on the protection of eggs, hatchlings and females on the

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[1] *The Convention on Wetlands of International Importance especially as Waterfowl Habitat (Ramsar)* is an international agreement which provides the basic structure for national action and international cooperation for the conservation and wise use of wetlands and their resources (Ramsar 2014).

[2] *The Convention on the Conservation of Migratory Species of Wild Animals (CMS)* is an international agreement to conserve migratory species (terrestrial, marine and avian), as well as their habitats throughout their migratory range (CMS 2014).

nesting beach, as they are easier accessible than juveniles or adults in the open sea (Stewart et al. 2007, Thorson et al. 2012). The beach component, however, is only a small fraction in the life of a sea turtle and thus PVAs based on this type of data are linited.

1.1.1 Life cycle of sea turtles

A significant challenge to sea turtle conservation is their complex life cycle. Most sea turtles migrate (Fig. 1.1 - phase 1) thousands of kilometres from foraging grounds to their natal rookeries to reproduce (Carr 1975, Bowen et al. 1993). Thus, rookeries are often treated as genetically isolated through maternal philopatry (Bowen et al. 1989, Dutton et al. 1999), although the amount of male-mediated gene flow between rookieries has not been fully characterised. The onset of migration usually correlates with an increase in sea surface temperature (SST; Solow et al. 2002, Weishampel et al. 2004). Polyandrous and polygynous mating (Fig. 1.1 - phase 2) occurs off the nesting beach (Crim et al. 2002, Lee 2008) after which males return to their foraging grounds (Miller 1997). The females, which have the ability to store sperm (Hughes 1989, Pearse & Avise 2001), remain at the nesting beach for a few months to lay several clutches of eggs. Nesting (Fig. 1.1 - phase 3) usually takes place at night, characterised by females ascending the beach, excavating a body pit, digging an egg chamber, positioning the eggs, closing the egg chamber, covering up the body pit and returning to the sea[3]. After a female has deposited all her clutches (with no parental care or apparent knowledge of breeding success), she will leave the nesting area and migrate back to her foraging ground (Miller et al. 2003). As both migration to the nesting beach and egg-laying are energetically tasking activities, females generally skip one or two nesting seasons before returning (Hughes 1996, Miller et al. 2003), whereas males seem to mate every year or every second year (Miller 1997)[4].

The duration of a nesting season depends on the geographic location of the nesting beach. In the tropics sea turtles nest year-round, whereas subtropical nesting is strongly correlated with local summer months. Nesting in the northern hemisphere takes place typically from May to August and in the southern hemisphere from October to March (Bell et al. 2007, Nel et al. 2013). The incubation

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[3] Differences between species only occur in re-nesting intervals, clutch frequency, clutch size (i.e. number of eggs per clutch) and egg diameter (Miller 1997). Additionally, leatherback turtles also deposit yolkless eggs, so called ‘SAGs’ (Wallace et al. 2004), which occur towards the end of ovipositioning and which are smaller and irregular in size (Eckert et al. 2012). Those SAGs have no vitalline embryo (Miller 1985) and are assumed to be ‘production over run’ (Wallace et al. 2007). It must be acknowledged that SAGs (in low numbers) also occur in other species (Dodd 1988, pers. obs.) and that they are not included into the egg count but are recorded separately (Miller 1999).

[4] The absolute reproductive lifespan for sea turtles is not known but estimates for the South African loggerhead and leatherback population indicate a duration of 16 - 18 years (Nel et al. 2013).

period (Fig. 1.1 - phase 4) is about 60 days but varies according to species and incubation temperature.

Hatching seems to take place over a number of days; hatchlings within a nest crawl in a synchronised fashion towards the sand surface where they wait to emerge (Miller 1997), typically after sunset. Hatchlings emerge from their nest (Fig. 1.1 - phase 5) and orientate themselves towards the brightest point on the horizon, which is usually above the ocean (Hughes 1977, 1989). Once they reach the water, hatchlings enter the surf and swim away from the coast until they reach the main current, which will carry and ultimately disperse them for several years. These are known as the 'lost years' as little is known about the biology or ecology during this time (Hughes 1977, 1989).

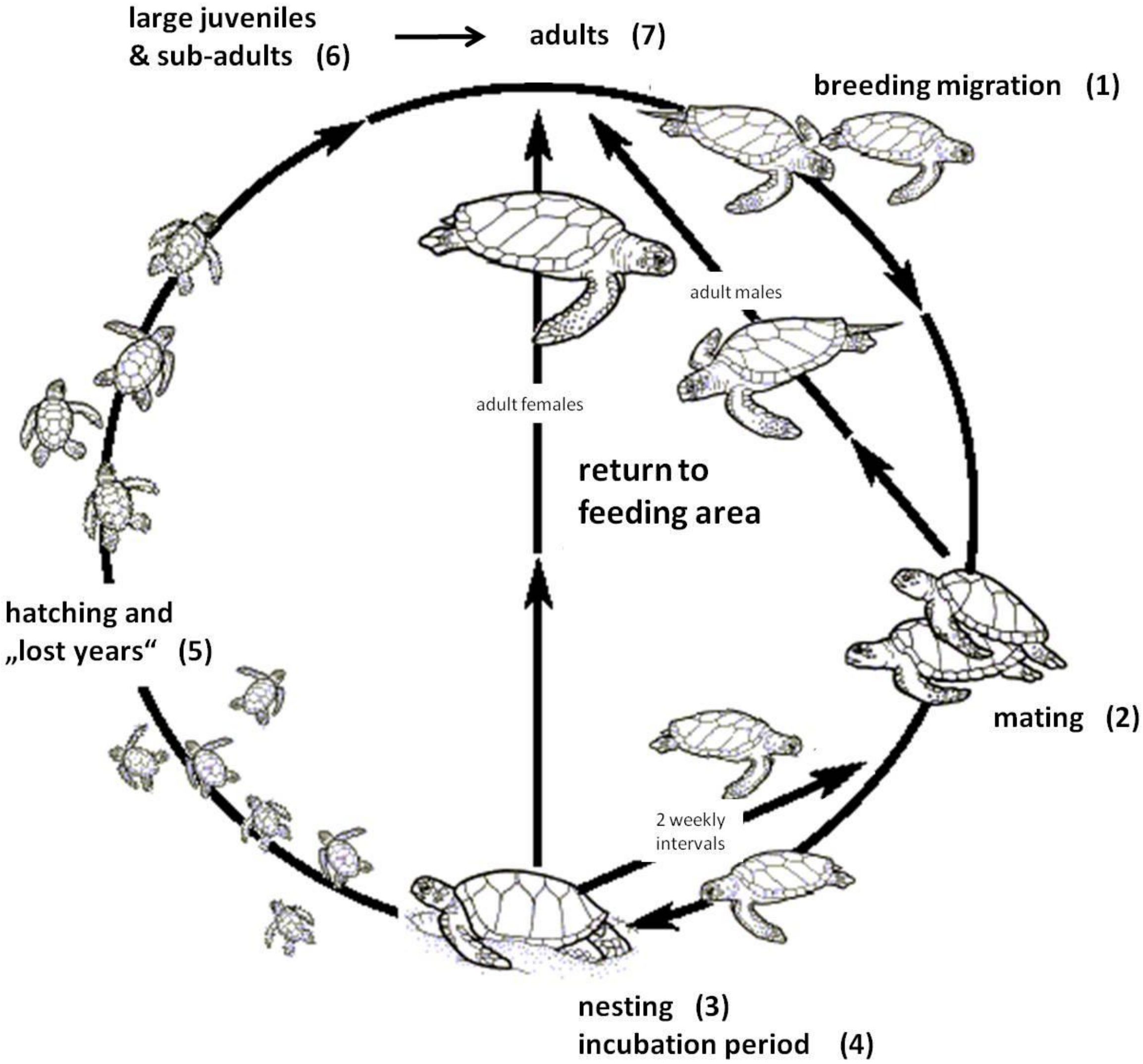

Figure 1.1: Illustration of the generalized life cycle of sea turtles (Lanyon et al. 1989; modified with permission) with reference numbers referring to the life history phases described in the text.

As large juveniles sea turtles migrate to shallow coastal regions (Fig. 1.1 - phase 6) where they change diet and feed on benthic organisms. Leatherback turtles are the exception as they maintain an exclusive pelagic lifestyle (Epperly 2003). The duration of the sub-adult stage is dependent on environmental conditions which can accelerate or reduce growth and maturation rates (Tucek et al. 2014; CHAPTER 6). Male and female turtles migrate to their natal rookeries to reproduce, once they have reached sexual maturity (Fig. 1.1 - phase 7 and 1).

1.1.2 Why conserve sea turtles?

Bjorndal and co-workers summarised the *ecological roles* of sea turtles in marine and coastal ecosystems as consumers, prey items to top predators, competitors, hosts to parasites and pathogens, substrate for epibionts (such as algae and barnacles), nutrient transporters and modifiers of the landscape (Bjorndal 2003, Bjorndal & Jackson 2003). Due to the range of habitats used and the range of diets consumed, sea turtles can also serve as indicators of the health status of marine ecosystems (Frazier 1999). There are some examples from the Caribbean where the decrease of hard coral cover over the first half of the twentieth century was linked to extensive exploitation of hawksbill turtles (Jackson 2001, Bjorndal & Jackson 2003), as they primarily feed on sponges and other invertebrates which are aggressive competitors for space on tropical reefs (León & Bjondal 2002). It was also speculated that the dramatic decline of green turtles in the Caribbean changed the structure and dynamics of the sea grass ecosystems. Green turtles primarily feed on sea grass, keeping the blades short, thus decreasing particle entrapment, which results in increased nutrient cycling (Moran & Bjorkland 2005). The loss of ecosystem resilience resulted in a mass dieback of sea grass beds in the 1980s (Jackson 1997, 2001). However, the relative contributions to each of these functions have not been quantified for most populations, hence it is difficult to spur interest in the protection of sea turtles based on the loss of ecosystem functions and services.

In contrast, the value of sea turtles to people is strongly recognised. The *cultural and spiritual value* of sea turtles dates back to the Middle Stone Age (Campbell 2003) when sea turtles provided nutritional and spiritual substance, or served as trade or barter commodity (Frazier 2003, Plug 2004). Currently, sea turtles are mostly valued alive for their *aesthetic, recreational and tourism value*. They also serve a particular scientific interest with their complex life history and *educational value* as charismatic species. Most importantly, sea turtles serve as flagship species for the conservation of marine habitats and raise public awareness of ocean vulnerability, particularly in the face of global change (Frazier 1999, Marcovaldi & Thome 1999, Hughes 2010). Finally, conservation success is often greatest where it is combined with sustainable activities. For example local communities that previously harvested sea turtles for meat, shell or other sea turtle products (Jacobson & Robels 1992, Marcovaldi & Marcovaldi 1999, Tisdell & Wilson 2000) now generate income either through toursim or conservation.

1.1.3 Conservation of sea turtles

Sea turtles are under extensive anthropogenic pressure, with many populations showing significant declines as a result of fisheries by-catch and illegal harvesting (Spotila et al. 1996, Chevalier et al. 1999, Tomillo et al. 2008). Fisheries by-catch, coastal development and climate change are identified as some of the main contemporary threats to sea turtles, while pollution and pathogens are considered as low-risk threats (Mast et al. 2005, Wallace et al. 2011). The combined global population trend per species is, however, stable and is even slightly increasing, which is likely due to the current efforts in conservation (Wallace et al. 2011). Sea turtle populations were found to respond well to protections afforded by marine protected areas (MPAs; Dutton et al. 2005, Lauret-Stepler et al. 2007, Nel et al. 2013) and by equipment adjustments in fisheries activities. For example, the use of turtle excluder devices (TEDs) and circle hooks were successful in saving significant numbers of sea turtles from drowning or capture (Epperly 2003, Read 2007). Climate change, however, poses a particular threat to sea turtle populations around the world as changes in temperature impose a direct effect on sex ratio.

1.1.4 Sex determination in sea turtles

One of the most characteristic but complicating factors in the life history of sea turtles is the effect of the environment, particularly temperature, on the sex ratio and hatching success of a nest. All sea turtles display temperature-dependent sex determination (TSD) where incubation temperatures less than 29 °C during the thermo-sensitive period (TSP) generally produce males and temperatures above 29 °C, females (Bull & Vogt 1979, Morreale et al. 1982, Standora & Spotila 1985, Janzen & Paukstis 1991, Godfrey & Mrosovsky 2006). The specific temperature range producing 100 % males to 100 % females is called the 'transitional range of temperature' (TRT; Valenzuela 2004, Hulin et al. 2009) and the temperature where a 1 : 1 sex ratio is produced the 'pivotal temperature' (PT; Mrosovsky & Pieau 1991, Merchant Larios 1999, Valenzuela 2004; Fig. 1.2).

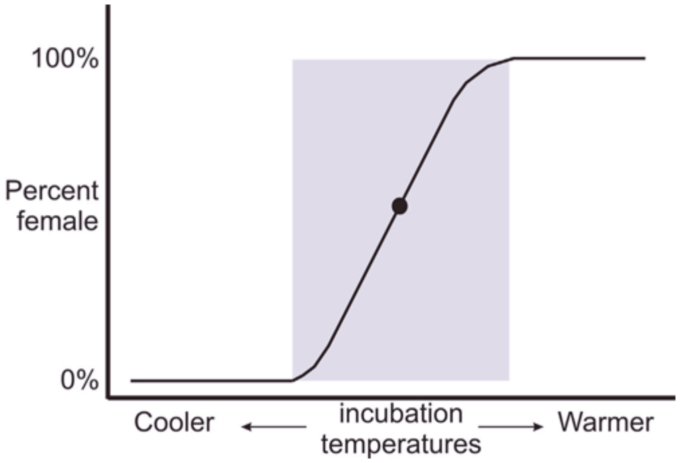

Figure 1.2: Pattern of temperature-dependent sex determination in sea turtles (Mrosovsky & Godfrey 2010). Low incubation temperatures (<29 °C) produce males and high incubation temperatures (>29 °C) females. The shaded area indicates the transitional range of temperature (TRT) and the solid dot the pivotal temperature (PT) which produces a 1 : 1 sex ratio.

Even though a PT of 29 °C to 30 °C is a generally accepted standard (Yntema & Mrosovsky 1980, Mrosovsky & Provancha 1989, Mrosovsky 1994, Merchant Larios 1999), it is assumed that dissimilarities in (estimated) PT between populations of the same species arise from genetic variations, inter-clutch variation, limited sample sizes or differences in methodology (Mrosovsky 1988, Janzen & Paukstis 1991, Binckley et al. 1998, Kaska et al. 1998, Wibbels et al. 1998, Godfrey et al. 1999, Merchant Larios 1999, Wibbels 2003). Additionally, a range of environmental factors influence incubation temperature and hence sex ratio. These include bright sunny or overcast conditions (Mrosovsky et al. 1984), rainfall (Standora & Spotila 1985, Godfrey et al. 1996, Houghton et al. 2007, Chu et al. 2008), beach orientation (Booth & Freeman 2006), vegetation shading (Janzen 1994, Mrosovsky et al. 1995), distance from the nest to the high tide mark (which also affects the possibility of inundation; Standora & Spotila 1985, Kaska et al. 1998, Godfrey & Mrosovsky 1999), egg position within a nest and nest depth (Mrosovsky et al. 1984, Standora & Spotila 1985, Hanson et al. 1998, Kaska et al. 1998). Nest substrate characteristics can have direct or indirect effects; sand colour and composition affect the albedo and, thus, temperature (Hays et al. 2001), whereas grain size regulates the respiratory gas exchange (Ackerman 1980, Janzen & Paukstis 1991). Metabolic heating can additionally alter the sex ratio if sand temperatures are just below pivotal temperature (Godfrey et al. 1997, Hanson et al. 1998, Broderick et al. 2001). Further, degradation of organic matter (for example egg decomposition) on beaches with extreme temperatures can push nest temperature to lethal incubation temperatures (Valverde et al. 2010).

Determining incubation temperature and resultant sex ratio provides a number of conservation options to manage threatened sea turtle populations. For example, nests laid too close to the water table ('doomed nests') or those in extremely exposed or sunny beaches can be relocated to a safe stretch of beach (or to a shaded hatchery) to enhance hatching and emergence success (Mrosovsky 2006, Pfaller et al. 2009). These nests might be saved, but the relocation of those nests might also have an impact on the long-term survival of the population if sex ratios are skewed in the process (Morreale et al. 1982, Dutton et al. 1985). However, these options have never been considered in South Africa.

1.2 Recovery potential of sea turtles in South Africa

South Africa has a long history of conservation in both terrestrial and marine environments, boasting some of the world's oldest terrestrial and marine reserves (Faasen 2006, Trinkel et al. 2008, Carruthers 2013). The country also had some significant conservation successes recovering species such as the white or black rhino (Emslie & Brooks 1999, Knight et al. 2011). However, South Africa also maintains one of the longest running conservation and monitoring programmes of sea turtles in the world (Hughes 2010), although sea turtle conservation in South Africa has had mixed success with one species recovering and the other one remaining at unchanged population levels (Nel et al. 2013).

Conserving sea turtles is particularly challenging because of their complex life cycle, slow growth rates, late sexual maturity and TSD. Therefore, population recovery might not be as simple as just proclaiming protected areas. Consideration of the biology of these animals in the context of the environment is required, to evaluate the potential for population growth. Thus, the main aim of this thesis is to evaluate possible causes of the mixed success of sea turtle conservation in South Africa.

1.2.1 Sea turtle conservation in South Africa

The nesting habitat of the South West Indian Ocean (SWIO) loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle populations is distributed along the east coast of South Africa, Mozambique and southern Madagascar (Baldwin et al. 2003). However, the majority of the current SWIO loggerhead nesting takes place in South Africa (annually 79 %; Mozambique hosts an additional 21 %; Madagascar ±20 nests) while leatherbacks nest almost exclusively in South Africa[5] and Mozambique (South Africa 75 - 80 %; Mozambique 20 - 25 %; Madagascar infrequently; Nel

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[5] As the majority of SWIO loggerhead and leatherback sea turtles nest in South Africa, they are further referred to as the South African population, which is equivalent to the Southwest Indian Ocean Regional Management Unit (Wallace et al. 2011).

2009, 2010; Fig. 1.3). This nesting emphasis in South Africa might be the result of historic preference maintained through natal-homing, intact nesting habitat or strong law-enforcement providing a safe nesting and hatching environment.

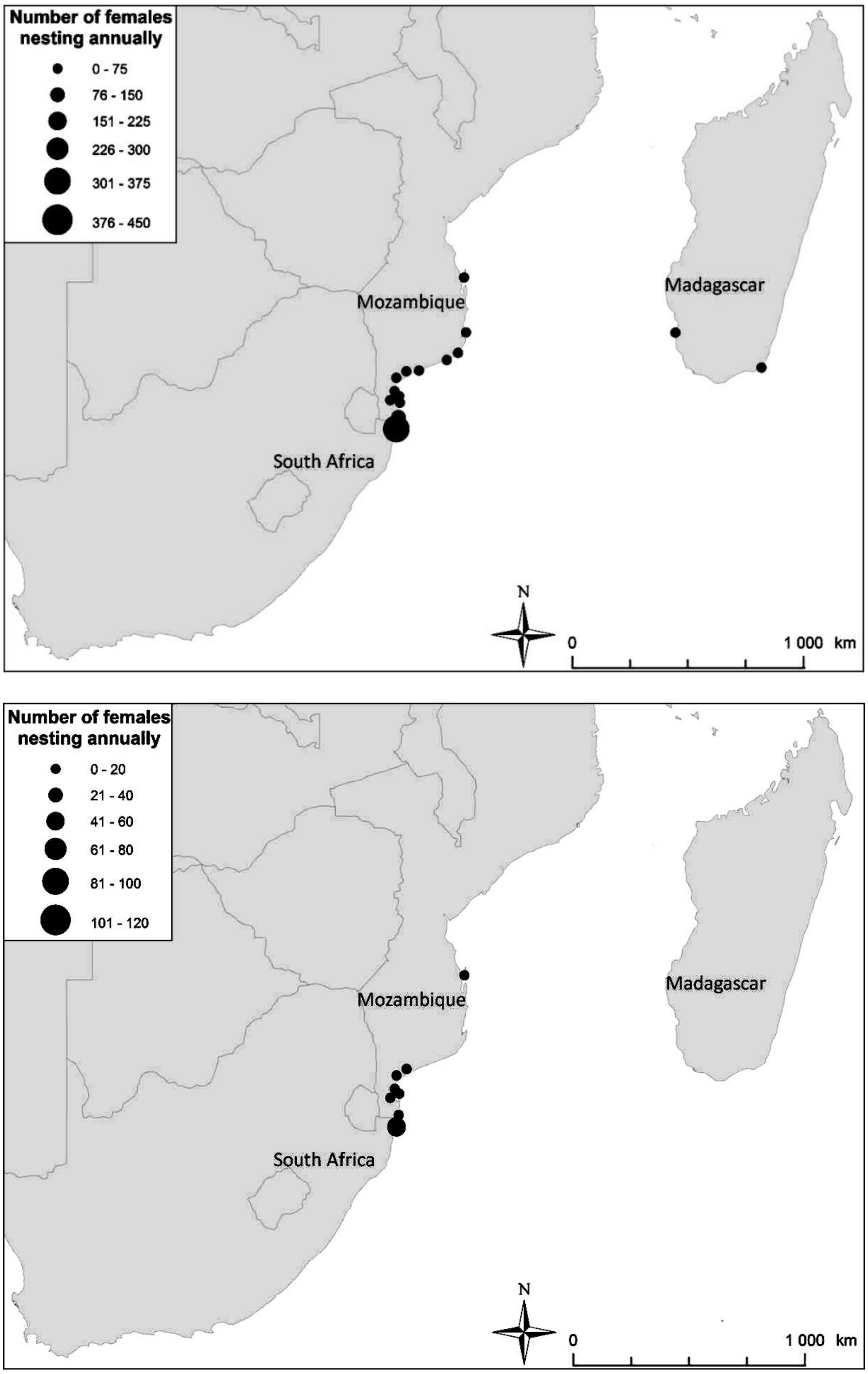

Figure 1.3: Distribution of annual nesting numbers of loggerhead (top) and leatherback (bottom) females in the South West Indian Ocean. (The data were obtained from Mast et al. 2006 and do not necessarily reflect recent numbers.)

In 1916 the first South African law for the protection of sea turtles was promulgated in the province of Natal (now known as KwaZulu-Natal and abbreviated here as KZN), but sea turtle poaching continued nevertheless (McAllister et al. 1965, Hughes 1989). Active sea turtle conservation only commenced in 1963 when the marine turtle monitoring programme was established by the Natal Parks Board (now known as Ezemvelo KZN Wildlife and hereafter referred to as Ezemvelo; Hughes 1989). In 1998, sea turtles in South Africa became further protected under the national legislation, the Marine Living Resource Act of 1998, and all activities around sea turtles became regulated.

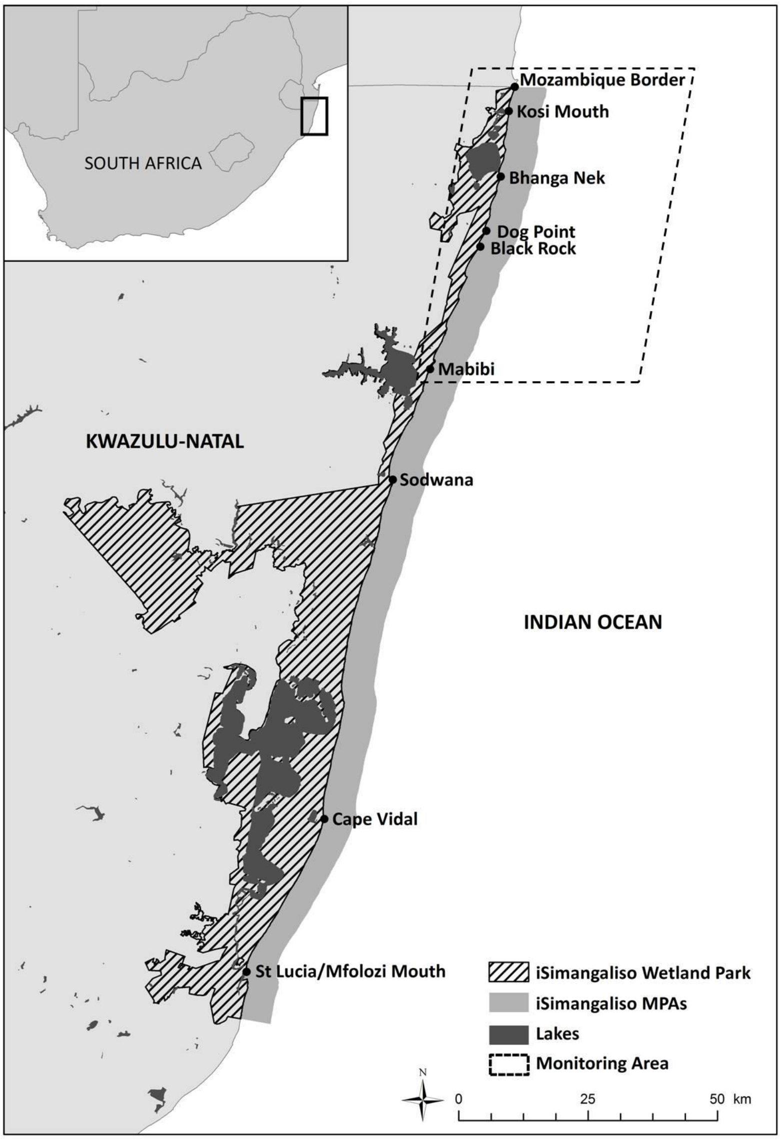

Figure 1.4: The South African sea turtle nesting beach is located in KwaZulu-Natal within the boundaries of iSimangaliso Wetland Park. The park includes an extent of the marine protected area with a 3-nautical mile seaward coverage. The turtle monitoring area stretches from the Mozambique border to Mabibi in the south. Sea turtle nesting occurs down to Sodwana and sporadically even to Cape Vidal and St Lucia.

In 1999 two marine protected areas (Maputaland and St Lucia) were joined and upgraded to the Greater St Lucia Wetland Park (recently re-named to iSimangaliso Wetland Park and further abbreviated as iSimangaliso; Fig. 1.4). Today this area is an UNESCO World Heritage and a Ramsar Site (Baldwin et al. 2003, Hughes 2010, Nel et al. 2013) which includes 150 km of the sea turtle nesting beach (Nel et al. 2013). In addition, the first and largest African Transfrontier Marine Conservation Area was established in 2009 to protect sea turtles across borders (Nel et al. 2013). Over time profitable tourism has developed around the turtles, providing employment for local communities (Hughes 1996, 2010, Nel et al. 2013). Many local people (amaThonga and Zulu) respect the turtles and believe that the protection of sea turtles and their nesting habitat will bring them economic benefit (Hughes 2010, pers. obs.).

1.2.2 Current threats to sea turtles in South Africa

Through law enforcement and the support of local communities, human-induced mortality along the nesting beach is incidental (<1 % including egg poaching; De Wet 2012). However, coastal development outside the borders of iSimangaliso is increasing dramatically, with a growing number of foreigners living in the rural communities (pers. obs.). It is proposed that the greater population density has led to an increased number of incidents of slaughtered females over the last two years (R. Nel pers. comm., NMMU).

There are no reported incidents of natural predation of adult females (for either species) on the nesting beach. However, natural predation of eggs, hatchlings and nests was estimated at 8.6 % for loggerheads and at 15.7 % for leatherbacks (De Wet 2012). Ghost crabs *(Ocypode ryderi)* were identified as the main predator for loggerhead hatchlings with 4 % predation per nest (Hughes 1974b, De Wet 2012). Hatchling survivorship to the water line was estimated at >70 % for both species (De Wet 2012), but kingfish, sharks, squids and rock cods depredate on hatchlings in the near shore zone (Hughes 1989).

Today, the greatest threats to South African sea turtles are commercial long-lining (specifically leatherbacks; Petersen et al. 2009), gill netting, and some trawling (Bourjea et al. 2008, Wallace et al. 2011, De Wet 2012). In addition, artisanal fisheries in neighbouring countries impact population growth, particularly that of loggerheads (Church & Palin 2003, Bourjea et al. 2008, De Wet 2012).

1.2.3 Population trends

Numbers of nesting females in 1963 were low due to the slaughter of females and egg poaching along the nesting beach (McAllister et al. 1965). Conservation management and the ensuing strong law enforcement put an end to the slaughter and poaching and led to the initiation of a conservation programme. In response, the leatherback population increased over the first 15 years of the programme and then stabilized (without any further growth). The loggerhead population, however, was slow to respond to conservation measures and despite an initial increase was reasonably stable for two decades before numbers of nesting females increased significantly, leading to current expanding growth (Nel et al. 2013; Fig. 1.5).

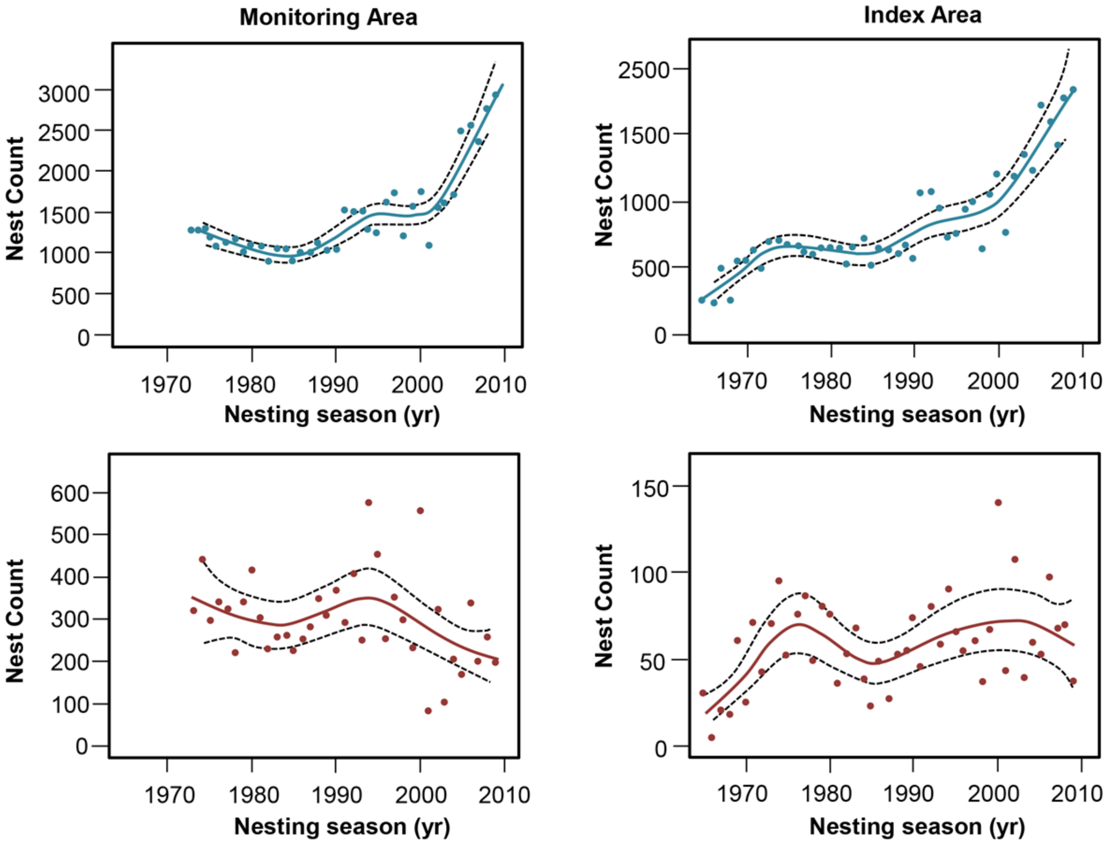

Figure 1.5: Population growth profiles of the South African loggerhead and leatherback population (Nel et al. 2013; modified with permission). Estimated loggerhead (upper section) and leatherback (lower section) nest counts and baseline model for the monitoring area (left-hand side) and the index area (right-hand side). (The index area, further explained in CHAPTER 2, represents a specific beach section which received continuous monitoring effort since the beginning of the programme.)

1.3 Thesis outline

In sea turtles, as in many other R-selected species, a large amount of offspring is produced but only few individuals survive until adulthood. Therefore, population stability is mostly dependent on the survival of large juveniles and adults. Unfortunately, due to the complex life cycle of sea turtles those stages are difficult to address and thus most conservation effort is focussed on nesting and foraging areas.

In South Africa loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles reproduce on the same nesting ground but display different population growth profiles (see section 1.2.3). It is hypothesised that the leatherback population growth potential might be inhibited by environmental conditions (in the nesting and foraging areas) or by tremendous offshore mortality. This thesis, thus, investigates several life history characteristics (for both species), which may prohibit the population growth potential of the South African leatherback population.

CHAPTER 2 will review the existing information on the South African loggerhead and leatherback sea turtle populations. It will further describe the physical *environment of the nesting beach*, including ocean currents and climate conditions.

CHAPTER 3 will assess *reproductive output* (measured as clutch size) and *reproductive success* (measured as hatching and emergence success) per individual in each species and the effect of these parameters on population growth. The effect of female size (i.e. limited body capacity) on reproductive output will also be further investigated as it is hypothesized that larger individuals deposit larger clutches with larger eggs compared to smaller individuals.

CHAPTER 4 will determine *sex ratio* and *pivotal temperature* of the South African loggerhead and leatherback population to evaluate whether a skewed sex ratio (i.e. male-biased) inhibits leatherback population growth.

CHAPTER 5 will use historical air and sea surface temperatures (since 1997) in order to approximate the *effect of climate change on sex ratios* of the South African loggerhead and leatherback populations. Further, potential adaptations of sea turtles to climate change will be discussed with regards to sex ratio (based on a literature review).

CHAPTER 6 will investigate *age at reproduction* (for both species) as it is suggested that leatherback turtles exhibit an extended generation time, which will only induce population growth in the near future.

CHAPTER 7 will discuss key results (of the previous chapters) in an effort to explain differential population growth between the South African loggerhead and leatherback turtle populations.

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Chapter 2: Synopsis of South Africa's sea turtle nesting beaches and monitoring programme

The distribution of sea turtles and their nesting habitat is restricted by thermal boundaries, which are species specific. These boundaries are determined by ocean currents (i.e. water temperature; Coles & Musick 2000, Eckert 2002), which also affect climate (Rasmussen & Wallace 1983, Zhang et al. 1996), as well as the shape of coastal habitats, i.e. the morphodynamic state of the nesting beach (McLachlan et al. 1993). This section therefore provides information on the study site and reviews the dependence of sea turtles on the relevant environmental factors.

2.1 Ocean currents and their significance to sea turtles

South Africa is bounded by the warm Agulhas Current flowing southwards along the east and south coasts, and the cool Benguela Current flowing northwards up the west-coast (Fig. 2.1). The Agulhas Current is a major western boundary current in the southern hemisphere with an average volume flux of 73 x 10^6 m^3 s^{-1} making it one of the fastest flowing currents in the world (Beal & Bryden 1999). The flow comprises anti-cyclonic eddies, created within the narrow regions of the Mozambique Channel, the East Madagascar Current and the South Indian Ocean Gyre (Stapley 2009; Fig. 2.1). From the southern border of Mozambique (ca. S 27°) to S 34°, the Agulhas Current follows the South African coastline but then moves offshore as it runs along the continental shelf (Lutjeharms 1981b, Stapley 2009, Lutjeharms et al. 2010). Vortices and eddies form in the north and where the current diverges from the coast, such as at the Natal Bight (Duncan 1970, Harris 1978, Roberts et al. 2013; dotted in Fig. 2.1). The surface temperature at the centre of the Agulhas Current varies from 23 °C in winter to 28 °C (or more) during summer. Temperatures usually decrease about 3 °C from the centre of the current to the coast, as well as from north to south. When the current reaches the south-east coast (off the city of Port Elizabeth), the core current temperature has dropped by 4 °C (Schumann 1991, Roberts et al. 2010).

The Benguela System (S 34° to S 18°) in the west is one of the four major eastern boundary currents in the world. This current flows northwards up the southern African west coast (Nelson & Hutchings 1983, Shannon 1985; displayed by hatching in Fig. 2.1) to northern Angola. Like other boundary currents that are wide, slow-flowing and associated with strong upwelling, the Benguela Current is highly productive and is one of the most productive marine regions (>300 g C m^{-2} y^{-1}) in the world (Heileman & O'Toole 2009). The meeting of the Benguela and Agulhas Currents on the Agulhas Bank

(Fig. 2.1) creates a region of complicated dynamic interaction with retroflection and eddy formations (Shannon 1966, Lutjeharms 1981a, b).

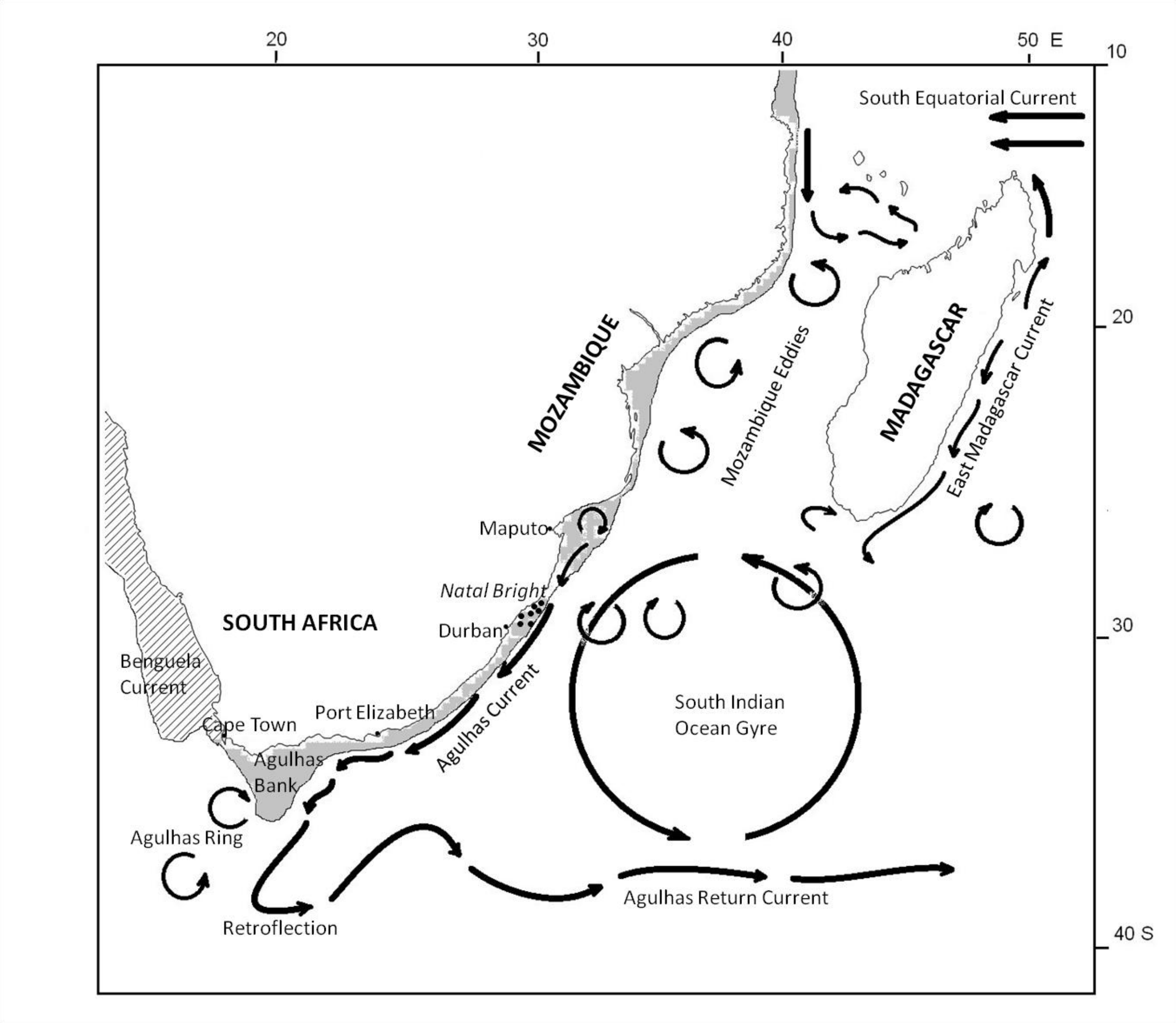

Figure 2.1: Schematic diagram of the Agulhas Current, showing the flow along the South African shoreline, the retroflection off Cape Agulhas and the Agulhas rings that leak into the South Atlantic (re-drawn following Lutjeharms 2006).

Further south, strong westerly winds direct the Agulhas Return Current (retroflection at about S 39°, E 13°) across the Agulhas Plateau (depths <3000 m) back into the Indian Ocean following the obstacles created by the ocean floor topography (Harris 1970, Gründlingh 1978, Lutjeharms 1981a, b, Beal et al. 2011). Only about 80 % of the original Agulhas volume flux proceeds with the Return Current. The other 20 % leaks either directly or as anti-cyclonic vortices into the South Atlantic (De Ruijter et al. 1999) affecting the Atlantic meridional overturning circulation (Biastoch et al. 2009).

This complicated set of oceanographic conditions creates a range of environments which affect the life history and habitat choices of ocean migrants such as sea turtles. Adult sea turtles follow main currents for a major part of their migrations (Luschi et al. 2003, Lambardi et al. 2008). Satellite tagging of South African sea turtles showed that loggerhead and leatherback turtles mostly remain in the warm Indian Ocean, but that leatherbacks also penetrate the cold water of the Benguela Current and travel north, as far as Angola (Luschi et al. 2006, Nel 2009). Ocean currents also play an important role in the distribution of sea turtle hatchlings and juveniles. It is suspected that the majority of South African sea turtles spend the first years of their life within the Agulhas Current, remaining in gyres and feeding on plankton or reef-associated species on the Agulhas Plateau (Baldwin et al. 2003; R. Nel from NMMU and A. Biastoch from IFM-GEOMAR pers. comm.). Environmental conditions like water temperature, food quality and availability of the ocean current or gyre in which the sea turtle travels thereby influence its fitness, growth rate, and age and size at reproduction (Bjorndal et al. 2003, Hatase et al. 2004, Tucek et al. 2014).Further, some hatchlings must inevitably be caught in the South Atlantic gyres or be carried away in the Agulhas Return Current as far as Western Australia (Hughes 1977, Stramma & Lutjeharms 1997, Steinke & Ward 2003, G. Hughes pers. comm.), although it is questionable if those individuals return to their natal beach to reproduce (Hughes 1989). These hypotheses of hatchling dispersal in the currents are supported by drift-card and drift-buoy experiments (Gründlingh 1978, Lutjeharms et al. 1981, Stramma & Lutjeharms 1997, Steinke & Ward 2003).

2.2 Climate and its impact on sea turtles

As heterotherms with temperature-dependent sex determination (TSD), the climate of the nesting site is especially important to sea turtles as it affects incubation temperature, which in turn determines sex ratio (Yntema & Mrosovsky 1982, Godfrey & Mrosovsky 2006), hatching success (Bull 1985) and individual fitness (Charnov & Bull 1977, Ewert & Nelson 1991). Green *(Chelonia mydas)*, hawksbill *(Eretmochelys imbricata)*, and olive ridley sea turtles *(Lepidochelys olivacea)* occur in the coastal waters off the east African shore (Hughes 1977, 1989), but only more sub-tropical nesters such as loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles reproduce in the southern extreme along the South African coast. The nesting distribution of strict tropical nesters (like green turtles) starts towards northern Mozambique or on the islands in the Mozambique Channel (Hughes 1974a, Lauret-Stepler et al. 2007).

The weather at the nesting beach in South Africa is strongly influenced by the Agulhas Current, resulting in a sub-tropical climate that is characterized by relatively high humidity (~77 % mean annual humidity) and a mean summer temperature of 23.3 °C (McAllister et al. 1965). Rainfall is seasonal (1000 - 1100 mm) with dry winter months and wet summer months (McAllister et al. 1965, Maud 1980). Precipitation and occasional cyclonic rainfall can decrease sand temperatures and thus affect incubation temperatures (Maud 1980, Godfrey et al. 1996). North-easterly and south-westerly breezes dominate along this coast but strong south-westerly winds precede the arrival of cold fronts and decrease ambient temperature (McAllister et al. 1965).

2.3 Coastal habitat and sea turtle nesting beach

The combination of a narrow continental margin with strong onshore swells, subtropical climate and strong wind has resulted in an almost uninterrupted dune forest along the entire east coast (Hughes 1974a, Van Wyk 1996). The primary dunes are colonised by pioneer plant species such as *Scaevola sericea, Scaevola plumeri, Ipomoea pes-caprae* and *Hydrophylax carnosa*. Behind these are secondary and tertiary dunes with stabilized climax plant communities including *Strelitzia nicolai*, *Brachylaena discolor* and *Diospyros rotundifolia*. Sea turtle nesting occurs along the entire coast within the boundaries of iSimangaliso (CHAPTER 1; Fig. 1.4) irrespective of the backshore vegetation. Most nesting takes place in open sand or on the primary dunes depending on the sea turtle species. Some individuals were also observed to nest on the primary dunes in patches of *Scaevola plumeri*, however, there is no vegetational shading along the entire nesting beach, which would affect nest site selection. In addition, there are very few constructions along the beach and, thus, nesting occurs undisturbed from anthropogenic stressors imposed by such structures (pers. obs.).

The shoreline utilized by sea turtles for nesting was classified as microtidal silica sandy beaches of intermediate and reflective morphodynamic types[6] (Harris et al. 2011). The stretch from Kosi Mouth to Dog Point (Fig. 2.2) is described as a medium-grained intermediate beach with a steep face and retrograded dunes. This area is also backed by a large freshwater lake system and seems to be preferred by loggerhead turtles for nesting because this is where the highest nest densities are observed (Hughes 1974b). The beach section to the south of Black Rock (a section of ±4 km) is extremely coarse and reflective and seems to be favoured by leatherback turtles (pers. obs). As one moves yet further south to Mabibi (Fig. 2.2) the sand becomes finer. It is speculated that a large number of leatherback turtles utilize this beach section, which is outside the monitoring area and thus not regularly observed (R. Nel pers. comm., NMMU).

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[6] An *intermediate beach* displays a microtidal range (<2 m) with medium grain size and a moderately steep beach face, with a moderate surf zone wasting wave energy in the surf. A *reflective beach* is typically microtidal with coarse sand and a steep beach face where wave energy dissipates on the beach (McLachlan & Brown 2006).

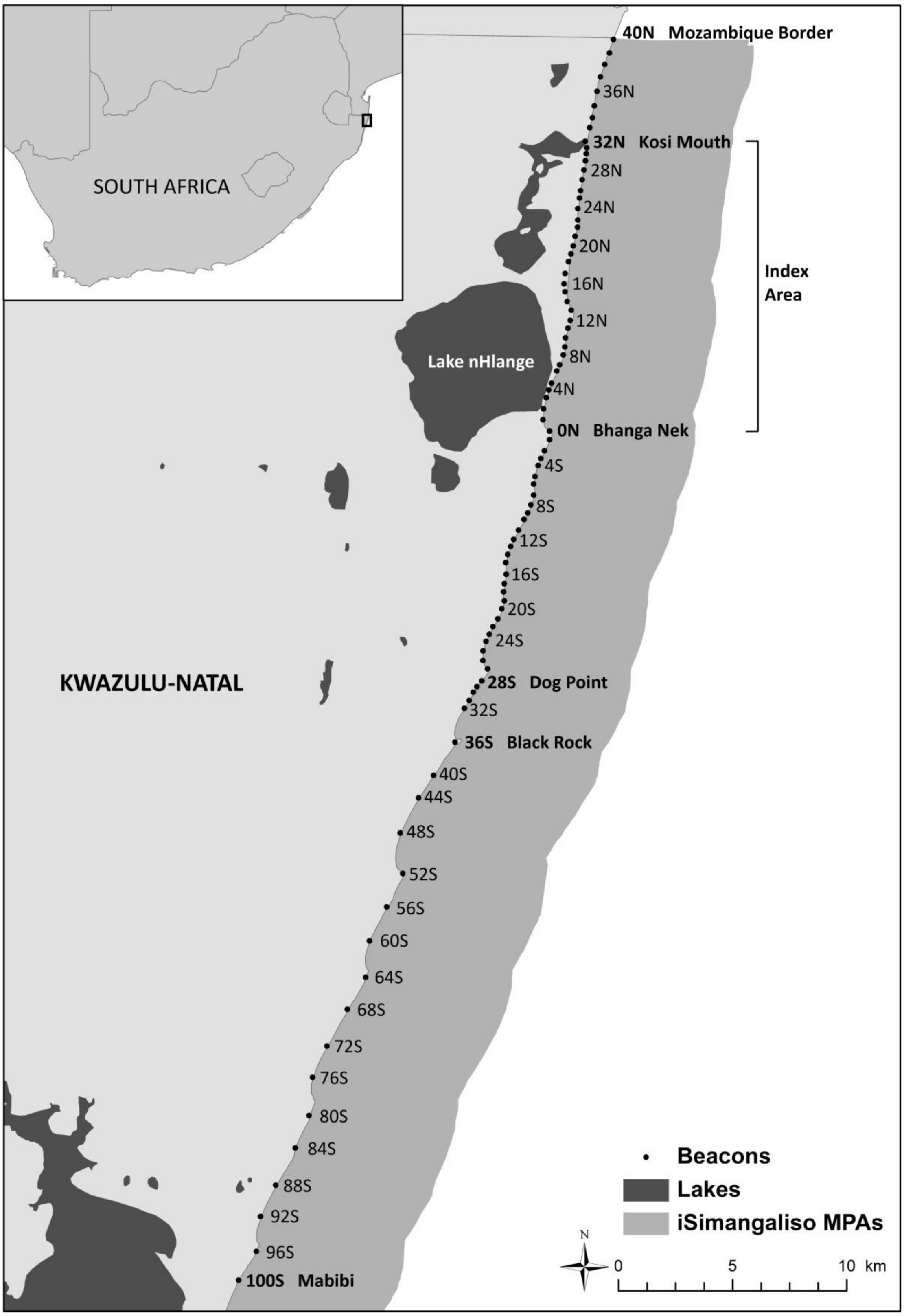

Figure 2.2: The monitoring area from the Mozambique border (40 N) to Mabibi (100 S) with the (13 km) index area between Kosi Mouth and Bhanga Nek.

2.4 Monitoring procedure

The nesting period in South Africa is restricted to the hot and wet austral summer months from October to March[7]. The monitoring of sea turtles in South Africa was originally conducted in the high-density nesting area only (1965 - 1972) but was later extended south to Mabibi (Hughes 1975, Nel et al. 2013) and in 2006 north to the Mozambique border (Fig. 2.2). Along this 56 km nesting beach, numbered poles (referred to here as beacons) are used as reference markers to record nesting activities. North of Bhanga Nek (ranger outpost at S 27.4770°, E 32.5980° and beacon 0 N) beacons are labelled north ‘N’ and south of Bhanga Nek beacons are marked south ‘S’. Beacons are positioned 400 m apart (Hughes 1996) but from Black Rock (36 S) southwards, nesting decreases, and only every fourth beacon is displayed (at one-mile intervals).

The section between Bhanga Nek and Kosi Mouth is referred to as the ‘index area’ (0 - 32 N; Fig. 2.2). Monitoring effort in this section of the beach has been constant since 1965 (Nel et al. 2013). This narrow strip of land between Lake nHlange and the sea (hence the name Bhanga *Nek*; Fig. 2.2) is also suspected to have a unique lake signature, with freshwater leaking through the coastal dunes into the sea. Hughes (1974b) hypothesised that this freshwater might be a cue for natal homing as this area has the highest loggerhead nesting density. However, the low-density widespread nesting of leatherbacks suggests that no such equivalent area exists for this species. The consistent monitoring of this section (0 - 32 N) thus serves as index area for between season comparisons for both species (CHAPTER 1; Fig. 1.5).

Monitoring methods have been standardised since 1973 (Hughes 1975, Nel et al. 2013), and nightly foot patrols take place between 19:00 to midnight. Tracks are recorded and scored with a large ‘S’ in the sand by all monitors to avoid duplication. Tracks discovered during the morning patrol are attributed to the previous nights’ nesting. Vehicle patrols are restricted to low tides, but are still conducted once each night. Data recorded are standard to most beach monitoring programmes: date, time observed, species, nesting activity (i.e. nesting or false crawl), flipper or PIT tags, tag scaring, size measurements (straight carapace length for loggerheads and curved for leatherbacks), position along the beach and notch codes[8] (Nel et al. 2013). Low tag return initiated double flipper tagging in 2008 to estimate tag loss (Nel 2009).

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[7] This period is further referred to as *season*, e.g. season 2009 equals the period mid-October 2009 to mid-March 2010.

[8] *Notching* (i.e. mutilation tagging) is further explained in CHAPTER 6.

2.5 Relevance to the thesis

This chapter summarised the most significant environmental facts for South African sea turtles, as it is important that one understands how environmental conditions affect sea turtle life history and thus population growth. Ocean currents (i.e. water temperature, food availability and quality) for example determine age and size at reproduction (CHAPTER 6). Size at reproduction is an important parameter as it affects reproductive output (i.e. clutch size and egg size; CHAPTER 3). Furthermore, reproductive success is highly dependent on environmental conditions such as the nesting habitat and the local climate. Both factors impact on incubation temperature, which affects reproductive success (CHAPTER 3) but also determines sex ratio (CHAPTER 4). In addition, the effect of climate change on the South African sea turtle sex ratio is investigated in CHAPTER 5. As several chapters of this thesis utilise data obtained in the beach monitoring programme, insight was given on the study site, nesting distribution and monitoring procedure.

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Chapter 3: The effect of reproductive output and success on population growth potential

Abstract

Populations are in a constant flux, increasing or decreasing in size depending on natality and mortality rates (such as predation and parasitism) or via anthropogenic means. When mortality exceeds natality populations decrease and may become imperilled. Loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles nesting in South Africa provide a good opportunity to investigate the relationship between natality and mortality with regards to reproductive output and population growth. Approximately 107 loggerhead and 24 leatherback females nested along the beaches of northern KwaZulu-Natal, South Africa, in 1965. But despite equal conservation efforts over the last 50 years, only the loggerhead population displayed (exponential) growth, whereas the leatherback population remained stable (after an initial increase). Reproductive output (i.e. clutch size as the mean number of eggs ± SD) over four consecutive seasons (2009 - 2012) was estimated for loggerhead and leatherback turtles at 112 ± 20 (n = 294) and 100 ± 23 eggs (n = 63), respectively. Additionally, emergence success (mean ± SD) for loggerheads was calculated at 73.6 ± 27.68 % (n = 1 089) and for leatherbacks at 73.8 ± 22.70 % (n = 138). Considering the higher intra-seasonal nesting frequency of leatherback turtles at 7 nests per season and loggerheads at only 4 nests per season, leatherbacks present a higher annual reproductive output per individual. In contrast, loggerhead turtles nest in greater numbers per annum and as a result have a higher absolute population growth potential. However, as neither reproductive output nor success seem to provide an explanation for the lack of 'population recovery', it is still uncertain why the leatherback population has not increased.

3.1 Introduction

Reproductive output per individual, i.e. the number of offspring produced per individual contributing to future generations, affects the population growth rate (Jenouvrier et al. 2005, Wallace et al. 2006a). Reproductive output per individual depends *(inter alia)* on the energy obtained and accumulated by the individual (Berner & Blanckenhorn 2007, Molles 2013). Therefore, food accessibility taking into account both environmental conditions and competitive interactions (Jenouvrier et al. 2005), as well as the rate at which an organism can process the food (West et al. 2004), regulate the metabolic investment of energy into reproduction or growth (Wallace et al. 2006a). The initial allocation of energy into growth rather than reproduction is a consequence of high adult survival rates, whereas low adult survival rates tend to induce greater energy allocation into reproduction and less into individual growth (Molles 2013). Further, energy resources and body capacity (i.e. size) lead to a trade-off between the number and size of the offspring[9] (Fleming & Gross 1990, Van Buskirk & Crowder 1994).

This trade-off is observed in sea turtles (Van Buskirk & Crowder 1994, Wallace et al. 2006a) in which the onset of reproduction requires a minimum (female) size (Hughes 1974b, Tucek et al. 2014), which depends on a number of intrinsic and extrinsic factors. The intrinsic factors that affect growth rate (and thus female size at reproduction) include species, genotype, health status and sex (Chaloupka & Limpus 1997, Van Dam 1999, Heppell et al. 2003). Extrinsic factors (environmental conditions) that regulate the growth rate include water temperature, food quality and food availability (Parker 1929, Bjorndal et al. 2003, Balazs & Chaloupka 2004). However, should a female grow up in unfavourable conditions and fail to reach this minimum nesting size she will eventually mature at a later age as a small individual with lower reproductive output (Gibbons 1981, Gibson & Hamilton 1984).

It therefore seems beneficial that female sea turtles allocate greater energy resources initially to growth to obtain the largest possible size at maturity to produce larger clutches with larger eggs (Heppell et al. 2003). The greater clutch size (i.e. number of yolked eggs) would increase the number of offspring produced, whereas larger eggs result in larger hatchlings (Hirth 1980, Van Buskirk & Crowder 1994) with increased fitness and possibly increased survival rates (Janzen 1993, Janzen et al. 2000). However, even if reproductive output is high, population growth potential could still be suppressed if reproductive success (i.e. successful fertilization or hatchling production) is low.

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[9] Due to limited body capacity females produce either fewer but larger or smaller and greater numbers of offspring to increase their individual fitness (Fleming & Gross 1990, Molles 2013).

Sea turtles do not maintain post-ovipositional care and thus reproductive success is highly dependent on environmental conditions during the incubation period. Climate change has been identified as one of the main threats to sea turtles, particularly during the incubation period. Climate effects can alter the physical environment in which turtle nests incubate (Van Houtan & Bass 2007). For example, climate change is linked to an increase in force and frequency of storms. Consequently, these storms lead to beach inundation, nest erosion and ultimately to temporary or permanent habitat loss, i.e. beach degradation (Fish et al. 2008). In the medium term, climate change and sea level rise can thus reduce hatching success (Fish et al. 2008). Beach inundation and erosion are especially likely to affect leatherback turtles as they place their nests closer to the high tide mark than any other sea turtle species (McAllister et al. 1965, Eckert 1987). Tidal inundation, storm surges and heavy rainfall can also induce a drop in nest temperature, which slows or stops embryonic development (below 26 °C; Ragotzkie 1959, Kraemer & Bell 1980, Yntema & Mrosovsky 1980, Whitmore & Dutton 1985, Mrosovsky 2006). Alternatively, if temperatures rise above ±34 °C (also due to climate change) increased nest mortality will occur (Yntema & Mrosovsky 1980, 1982, Matsuzawa et al. 2002). Other factors limiting reproductive success on the beach include depredation by natural predators, such as ants and ghost crabs (Hughes 1974b, De Wet 2012, pers. obs.), or predation and nest destruction by human-introduced species like pigs and dogs (Fowler 1979, pers. obs.). Direct take of eggs, frequently for human consumption, is probably one of the greatest conservation concerns as it has contributed to the near extinction of some populations, as demonstrated by the leatherback population in Costa Rica (Campbell 2003, Shillinger et al. 2008, Tomillo et al. 2008).

In South Africa, loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtles utilize the same nesting beach and, despite the initial low numbers of nesting females in both species (107 loggerheads and 24 leatherbacks), only the loggerhead population now displays exponential growth. In contrast, the leatherback population increased to about 80 - 100 nesting females per annum and then stabilized (Nel et al. 2013). It was thought that differences in reproductive output and reproductive success were the drivers for the opposed population growth rates but Hughes (1974b) investigated reproductive output and success during the first decade of turtle monitoring, and concluded that loggerhead and leatherback turtles then deposited similar clutch sizes (mean ± SD) at 105 ± 22 and 104 ± 21 eggs, with a similar emergence success at 77.8 % and 68.9 %, respectively. However, despite the apparent potential for population growth for both species (i.e. low population density and high reproductive output and success; Molles 2013) population growth profiles differ tremendously. Thus, it was proposed that environmental factors have changed since the 1970s, favouring conditions for loggerhead hatchling development while penalizing leatherback turtles.

Moreover, as the loggerhead population grew, the average nesting size of females decreased. This is explained by the growing number of neophyte nesters (Nel 2009), as size differences between neophyte nesters and re-migrants due to low but continuous growth after the commencement of reproduction exists (Carr & Goodman 1970). However, as female size affects reproductive output, i.e. clutch size and egg size (Van Buskirk & Crowder 1994) the effect of increasing numbers of neophyte nesters on the population growth potential needs to be clarified.

The aims of this chapter are i) to determine average reproductive output per female per species, ii) to approximate reproductive success and iii) to investigate female size and its effect on reproductive output. It is expected that a comparison with previous studies, as well as between species, will identify at least one factor responsible for the opposed population growth profiles of the South African loggerhead and leatherback population.

3.2 Material and Methods

Reproductive output and its likely effect on population growth were investigated by quantifying the clutch size and mean egg size (i.e. of 10 eggs per clutch) as a function of female size. The relationship between reproductive output and female size was established as it was hypothesised that female size affects the quantity and size (i.e. fitness) of the offspring. The same methods were applied to loggerhead and leatherback sea turtles, with possible variations described in the text.

3.2.1 Field sampling

Field sampling took place over four seasons: 2009 (early December until mid-March of the following year), 2010 (mid-November until mid-March 2011), 2011 (mid-October until late February 2012) and 2012 (mid-December until late February 2013). Most of the sampling effort took place using foot patrols in the high density loggerhead nesting area (0 - 12 N) with vehicle searches (using a two-seater Yamaha Rhino 660 quad-bike) between 32 N - 100 S for leatherbacks. Data collected on nesting females during oviposition included nesting date, species, flipper and passive integrated transponder (PIT) tag numbers, carapace size[10], clutch size and egg size (measuring egg diameter to

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[10] Carapace size of loggerhead turtles was measured as minimum straight carapace length (SCL_{min}) from the anterior point at midline (nuctual scute) to the posterior notch at midline between the supracaudals. Measurements of leatherback turtles were taken as curved carapace length (CCL) from the anterior end of the central carapace to the posterior tip of the carapace (Bolten 1999, Hughes et al. 1967), with the shortest direct line distance.

the nearest 0.1 mm) following Miller (1999). Nests were marked by flagging the position of the egg chamber. This was done by recording the GPS position (using a Garmin 60Cx with a ±5 m reported accuracy) and two weather resistant nest markers. The first marker remained in the nest while an attached string surfaced the sand and was tethered to the nearest vegetation or drift wood (Fig. 3.1). The second marker was made of a string connected to a plastic ping-pong ball at both ends, of which one end was buried among the eggs and the other one was allowed to roll on the sand surface (Fig. 3.1). Both ends of both markers contained nest information (i.e. date of oviposition, female tag numbers and a specific nest number) to relate nest information such as hatchling sizes to a specific female. All marked nests within the 5 km beach stretch north of the research station (0 - 12 N) were checked weekly, whereas nests away from public access points (with less potential human interference) were monitored at irregular intervals.

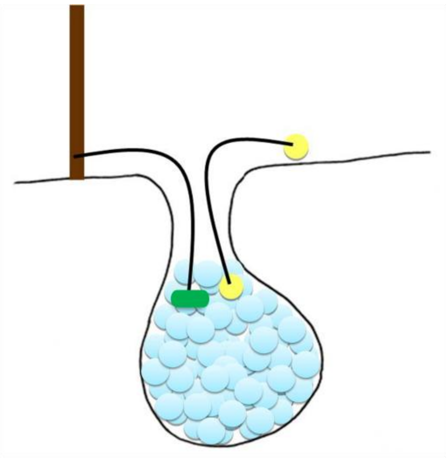

Figure 3.1: Schematic diagram of a sea turtle nest (not to scale) indicating nest markers; yellow specifies the nest marker with ping-pong balls and green the nest marker attached to vegetation or driftwood.

Figure 3.2: Example of a basket constructed on top of a nest to catch the emerging hatchlings. All baskets were marked 'Property of KZNW' to avoid theft as KwaZulu-Natal Wildlife (re-named Ezemvelo) is a well-known and respected governmental body within the study site.

Nylon mesh baskets were placed over the marked nests a few days prior to the expected hatching date to capture all emerging hatchlings (Fig. 3.2). These baskets were checked daily during the evening and sunrise patrols to ensure that emerged hatchlings were not trapped in the baskets for extended periods. Ten self-emerged hatchlings per nest (from both marked and unmarked nests) [11] were randomly selected and straight carapace length was measured (to the nearest 0.1 mm) using metal vernier calipers following Miller (1999). Four days after hatching nests were excavated and reproductive success was determined as per Miller (1999), whereby hatching success was defined as

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[11] Obtaining 10 self-emerged hatchlings from an unmarked nest was only possible if the emergence was observed by chance. In this case ten hatchlings were randomly captured.

the percentage of successfully developed individuals that emerged out of the eggs, and emergence success as the fraction of these hatchlings that reached the sand surface:

$$\text{hatching success (\%)} = (\#\ \text{shells}/(\#\ \text{shells} + \#\ \text{UD} + \#\ \text{UH} + \#\ \text{UHT} + \#\ \text{P})) \times 100$$

$$\text{emergence success (\%)} = ((\#\ \text{shells} - (\#\ \text{L} + \#\ \text{D}))/(\#\ \text{shells} + \#\ \text{UD} + \#\ \text{UH} + \#\ \text{UHT} + \#\ \text{P})) \times 100$$

Categories and definitions of nest content are adopted from Miller (1999): *shells* indicate the number of empty shells counted (only shells >50 % complete), *UD* the amount of undeveloped eggs with no obvious embryo, *UH* the number of unhatched eggs with obvious embryo (excluding *UHT*) and *UHT* the quantity of unhatched full-term embryos. Further, *P* describes the number of open, nearly complete shells containing egg residue (depredated), *L* indicates the amount of live hatchlings left in the nest (not those in the neck of the nest) and *D* the quantity of dead hatchlings that were outside their shells.

3.2.2 Analyses

Outliers in all data sets (excluding hatching and emergence success) were removed prior to analyses using the quartile method described in Zar (2010), i.e. 1.5 times above or below the Q_1 - Q_3 range. Additionally, a Shapiro-Wilk test was applied to test the data for normality. All statistics were conducted in R version 3.0.1 software (R Core Team 2013) with $\alpha = 0.05$.

Estimating reproductive output

Nest-specific clutch size, egg size and hatchling size were determined for turtles nesting during four consecutive seasons (2009 - 2012). Analysis of variance (ANOVA) with a post hoc Tukey-test (or a Kruskal-Wallis test with multiple comparisons) was applied to test homogeneity among seasons before data were pooled. A T-test with Welch’s approximate (in case of unequal variance or a Mann-Whitney test if data were non-normal) was used to compare clutch size between species.

Estimating reproductive success

Nest-specific hatching and emergence success were determined for clutches laid during the same four seasons (2009 - 2012). Reproductive failure of nests lost due to beach erosion and obvious depredation (i.e. dug up and scattered egg shells) were not assessed and are thus not included in the analyses. A Kruskal-Wallis test with multiple comparisons was applied to test homogeneity among seasons before the data were pooled. A Mann-Whitney test was used to investigate differences in hatching and emergence success between species. Finally, emergence success (*ES*) was multiplied with clutch size (*CS*), intra-seasonal nesting frequency (*NF*) and the estimated number of nesting females per season *(#F)* to determine the absolute annual population growth potential (as the estimated number of hatchlings produced):

$$\text{population growth potential}_{\text{(season)}} = ES \times CS \times NF \times \#F$$

Approximating mean nesting size

Mean size at reproduction was estimated by pooling all size measurements recorded in the Ezemvelo monitoring database (1965 - 2012), including repeated measurements of individuals. Further, annual mean size per season per species was obtained and plotted to identify temporal trends. Where information on carapace size was not captured in the database it was augmented with mean nesting size from annual season reports (Table 3.1). Only straight carapace length (SCL_{min}) measurements were used for loggerheads and only curved carapace length (CCL) for leatherbacks.

The annual numbers of neophytes (first-time nesters) and re-migrants (repeat nesters) were obtained to display the relative proportion of each category in the nesting population. It was assumed that the first recording of an individual (identified by tag numbers and the lack of any tag scars) resembles the first nesting season of a neophyte nester. Further, size measurements of neophytes and re-migrants were compared with a T-test and Welch's approximate (in case of unequal variance or a Mann-Whitney test if data were non-normal).

Determining the effect of female size on reproductive output

A general linear model (GLM) was applied to investigate the relationship between female size and its effect on clutch size and egg size (on data collected between 2009 and 2012). Only data from nests with measurements for all three metrics (female carapace length, clutch size and egg size) were used. The best model was determined by maximum likelihood (AIC values; Burnham & Anderson 2002). The final relationship between female carapace length, clutch size and egg size was verified by the coefficient of determination.

Estimating the effect of neophyte nesters on population growth

As reproductive output might be a function of female size, reproductive output was compared between neophyte and experienced nesters. Neophyte nesters and re-migrants for the period 2009 - 2012 were identified in the Ezemvelo database along with their respective clutch size, egg size and hatchling size, as well as hatching and emergence success for their clutches. A T-test with Welch's approximation (in the case of unequal variance or a Mann-Whitney test if data were non-normal) was applied to identify any differences between neophytes and re-migrants.

The effect of increasing numbers of neophyte nesters on reproductive output, as well as on hatchling fitness was tested by modelling clutch size, egg diameter and hatchling size over time (in years). Therefore, results obtained from previous studies as well as from the annual season reports by the Natal Parks Board were included (see Table 3.3). In order to determine any changes induced by the incubating environment hatching and emergence success were investigated in the same way.

3.3 Results

3.3.1 Reproductive output

The effect of reproductive output on population growth of the South African loggerhead and leatherback turtle was investigated (from season 2009 to season 2012). In this period, clutch size (mean ± SD) differed significantly between the two species with loggerheads having a higher output per female (T-Test: $t = 3.817$, $df = 82.718$, $p < 0.001$). Loggerhead turtle females deposited an average of 112 ± 20 normal yolked eggs (range 58 - 164) per clutch ($n = 294$) and leatherbacks oviposited 100 ± 23 eggs (range 56 - 158, $n = 63$). Leatherback clutch size was higher in 2009 and 2010 than in the following two seasons, but only differed significantly from the 2011 season (ANOVA; $p < 0.050$). In addition to the normal yolked eggs, leatherback turtles laid about 23 ± 10 SAGs[12] (range 4 - 50) on top of each clutch ($n = 60$)[13]. Annual reproductive output per individual female was approximated by multiplying clutch size with the intra-seasonal nesting frequency (loggerheads $n = 4$ and leatherbacks $n = 7$ from Nel et al. 2013). The estimated total number of eggs per female per season was lower for loggerheads ($n = \pm448$) than for leatherbacks ($n = \pm700$). In isolation, reproductive output per female (and per season) favours growth of the South African leatherback population[14].

As female body size limits reproductive capacity, the reproductive output per female is a trade-off between clutch size (quantity of eggs) and egg size. The mean size of eggs (measuring 10 eggs per clutch) for loggerhead turtles was 40.2 ± 1.27 mm (range 36.8 - 43.1, $n = 294$) and 51.0 ± 2.00 mm (range 46.0 - 56.0, $n = 38$) for leatherbacks. Loggerhead egg diameter differed slightly (ANOVA; $p < 0.050$) in season 2009. There was a strong positive relationship between egg size and hatchling size for loggerhead turtles ($r = 0.691$ with $p < 0.001$) but not for leatherbacks ($r = -0.396$, $p = 0.292$).

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[12] *SAGs* are yolkless eggs which were identified as 'production over-run' (Wallace et al. 2007) and are thus not included into egg counts but recorded separately (Miller 1999).

[13] The number of nests for clutch volume and amount of SAGs differs for leatherback turtles because it is unclear whether three nests were recorded without SAGs or if there were no SAGs to count.

[14] If completely predated and eroded clutches are incorporated (following De Wet 2012) the total number of eggs per female (and per season) is ±400 for loggerheads and ±546 for leatherbacks.

Mean loggerhead hatchling size was estimated at 44.0 ± 1.25 mm (range 40.8 - 46.6, n = 135) and leatherbacks at 58.6 ± 2.23 mm (range 54.1 - 63.9, n = 31).

3.3.2 Reproductive success

As not all deposited eggs result in healthy hatchlings, reproductive success (mean ± SD) and its effect on population growth of the South African loggerhead and leatherback sea turtles were approximated, with 'n' indicating the number of clutches investigated. Hatching success for loggerhead turtles was estimated at 74.9 ± 27.54 % (range 1 - 100, n = 1 094) with an emergence success of 73.6 ± 27.68 % (range 1 - 100, n = 1 089). Loggerhead hatching and emergence success were significantly higher in 2012 than in other seasons (Kruskal-Wallis test; $p < 0.050$). Hatching success for leatherback turtles was higher than for loggerheads at 76.3 ± 22.39 % (range 1 - 100, n = 140); leatherbacks had an emergence success at 73.8 ± 22.70 % (range 1 - 100, n = 138). A comparison of these metrics indicated a marginal difference in emergence success between species (Mann-Whitney test, W = 67 162, p = 0.042).

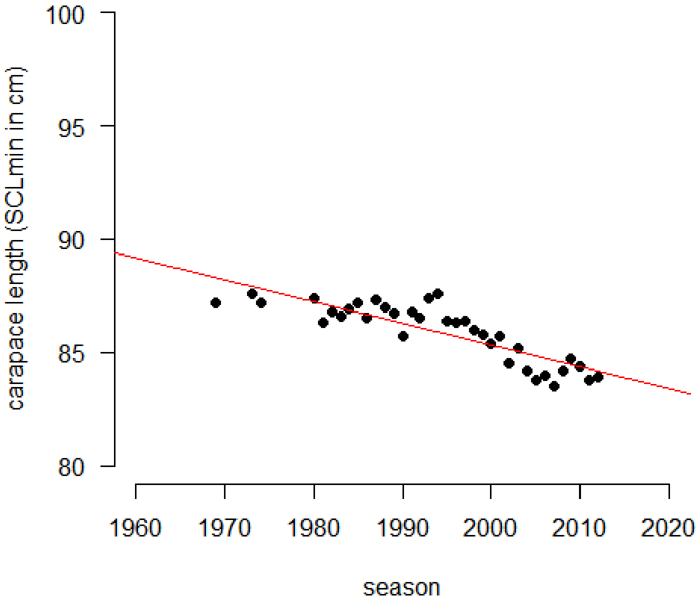
The effect of reproductive success was scaled to population level in order to determine the absolute population growth potential (as the estimated number of hatchlings produced). Individual annual contribution per female *(intra-seasonal nesting frequency x clutch size x emergence success)* was calculated at ±330 hatchlings for loggerhead and ±517 hatchlings for leatherback sea turtles. However, when multiplied by the number of individual females nesting each season (mean ± SD from 2008 - 2012; loggerheads n = 651 ± 103 and leatherbacks n = 65 ± 18) the absolute growth potential favours loggerheads with an annual hatchling production of 180 840 to 248 820 individuals, and 24 299 to 42 911 leatherback hatchlings respectively.

3.3.3 Average size of nesting females

Annual average size of nesting females, as well as its trend over time (1965 - 2012), was investigated as it was hypothesised that the decrease in average size of loggerhead females might affect current and future reproductive output (by affecting the number and fitness of hatchlings produced). The average size (mean ± SD) of loggerhead females was approximated at a straight carapace length (SCL_{min}) of 85.6 ± 4.31 cm (range 73.5 - 97.5, n = 15 733) and leatherbacks at a curved carapace length (CCL) of 160.9 ± 8.34 cm (range 136 - 185, n = 2 534). The annual average nesting size was estimated (for both species) from the Ezemvelo long-term monitoring database (see Table 3.1) and plotted over time (Fig. 3.3). Results confirmed a decrease in annual average size of nesting loggerhead females ($r^2 = 0.728$, $p << 0.001$) but not for the leatherback population ($r^2 = 0.039$, $p = 0.218$).

Table 3.1: Annual average size (cm) of nesting loggerhead and leatherback sea turtles in South Africa. Size measurements recorded in the Ezemvelo database were averaged (per season) and supplemented with data obtained from annual season reports by the Natal Parks Board* and Hughes (1974a)**, where omitted in the database. Loggerhead size is presented as minimum straight carapace length (SCL_{min}), measured from the anterior point at midline (nuctual scute) to the posterior notch at midline between the supracaudals. Measurements of leatherback turtles were taken as curved carapace length (CCL) from the anterior end of the central carapace to the posterior tip of the carapace (Bolten 1999, Hughes et al. 1967), with the shortest direct line distance. Further, 'n' provides the number of individuals measured and 'N' the number of individuals encountered during the season.

Season	Loggerhead (SCL_{min})	SD	n	N	Leatherback (CCL)	SD	n	N
1965	-	-	-	107	164*	-	26*	24
1966	-	-	-	211	165*	-	4*	4
1967	-	-	-	295	156.5*	-	17*	20
1968	-	-	-	218	-	-	-	20
1969	87.2*	-	30*	286	163.3*	-	8*	31
1970	-	-	-	245	160.3*	-	10*	18
1971	-	-	-	327	-	-	-	42
1972	-	-	-	262	160*	-	-	21
1973	87.6*	-	320*	337	160.8*	-	-	57
1974	87.2**	3.64**	29**	320	161.1**	7.0**	122**	70
1975	-	-	-	370	-	-	-	69
1976	-	-	-	329	-	-	-	61
1977	-	-	-	356	-	-	-	72
1978	-	-	-	415	-	-	-	68
1979	-	-	-	317	-	-	-	79
1980	87.4	4.10	370	351	158.5	9.59	85	86
1981	86.3	4.21	376	371	161.6	12.14	74	66
1982	86.8	3.92	309	308	161.9	6.61	70	65
1983	86.6	4.21	395	440	162.3	8.07	58	61
1984	86.9	3.87	328	378	157.6	11.71	84	96
1985	87.2	4.30	301	320	159.1	9.33	79	78
1986	86.5	3.68	374	385	155.3	15.27	103	101
1987	87.3	4.18	459	472	165.1	15.04	88	92
1988	87.0	3.84	361	363	161.3	12.92	87	87
1989	86.7	3.88	301	321	159.9	10.78	119	122
1990	85.7	4.16	452	437	160.9	10.95	109	103
1991	86.8	4.06	416	435	160.9	9.62	79	82
1992	86.5	3.95	468	476	158.6	12.38	100	117
1993	87.4	4.19	448	457	161.3	9.03	58	61
1994	87.6	4.24	406	413	158.8	10.03	124	137
1995	86.4	4.12	411	434	160.0	9.50	83	99
1996	86.3	3.79	427	437	159.3	8.72	60	66
1997	86.4	4.37	503	525	160.0	7.79	73	90
1998	86.0	3.86	468	507	159.2	11.79	96	103
1999	85.8	4.09	405	407	159.1	8.56	71	79
2000	85.4	3.89	473	469	161.5	10.45	183	159
2001	85.7	4.37	265	284	153.3	15.87	15	26
2002	84.5	4.52	153	161	164.3	11.13	21	32
2003	85.2	4.10	356	374	148.4	18.10	23	26
2004	84.2	4.09	269	237	154.9	14.37	22	34
2005	83.8	4.17	404	362	161.3	7.46	39	49
2006	84.0	3.83	514	403	159.7	7.51	79	66
2007	83.5	4.37	703	545	161.3	8.97	63	51
2008	84.2	4.17	656	514	159.8	7.34	58	44
2009	84.7	3.79	885	583	161.3	10.06	73	57
2010	84.4	3.68	950	662	161.6	14.04	91	65
2011	83.8	4.02	1053	742	160.8	8.84	145	94
2012	83.9	4.04	858	757	163.0	11.27	109	65



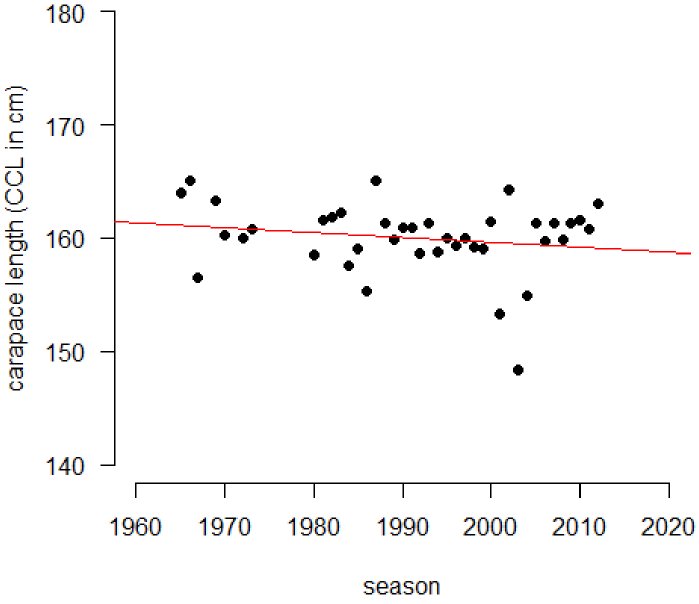


Figure 3.3: The trends in carapace length of the South African loggerhead (top) and leatherback (bottom) population over time. Straight carapace length (SCL_{min}) of the loggerhead population displayed a significant negative trend ($r^2 = 0.728$, $p << 0.001$, $SCL_{min} = -0.10*season + 277.58$) whereas leatherback curved carapace length (CCL) was stable ($r^2 = 0.039$, $p = 0.218$, $CCL = -0.04*season + 248.53$).

Assuming neophyte nesters are smaller in size than repeat nesters (due to continuous growth) an increase in the number of neophytes per annum could lower the average size of nesting females. For confirmation, annual numbers of nesting neophytes and re-migrants were plotted over time (Fig. 3.4). Results display that both nesting populations are dominated by neophyte nesters and indicate that most turtles in the South African loggerhead and leatherback populations nest one season only.

A size-comparison of neophytes and re-migrant nesters within each species further indicates a significant difference in loggerhead (T-test, $t = -17.5239$, $df = 8192.158$, $p << 0.001$) and leatherback turtles (T-test, $t = -8.1136$, $df = 777.644$, $p << 0.001$; Fig. 3.5). Size (mean ± SD) for neophyte loggerhead nesters was approximated at 85.2 ± 4.33 cm (range 73.5 - 97.5, n = 11 305) and re-migrants at 86.5 ± 4.10 cm (range 73.9 - 97.5, n = 4 302). The mean size for leatherback neophyte nesters was 160.2 ± 8.42 cm (range 136 - 185, n = 2 038) and for re-migrants 163.4 ± 7.47 cm (range 142 - 185, n = 474). As leatherback turtles tend to have a low flipper tag retention[15], the comparison was repeated for leatherbacks with PIT-tags. The repeat assessment, however, confirmed the previous results (Mann-Whitney test, $W = 4146.5$, $p < 0.001$).

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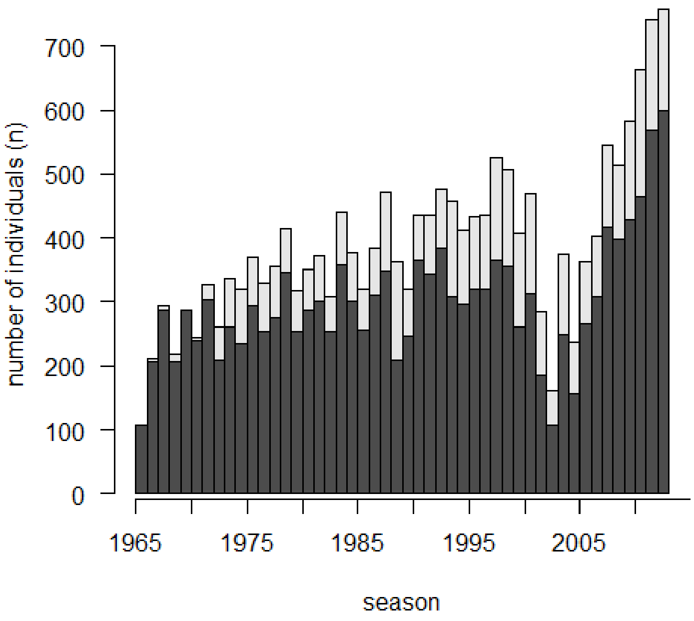
[15] Flipper tags on leatherback turtles are applied to the inner edge of the hind flippers (Nel & Papillon 2005) where the tissue is soft and tags easily pull out.



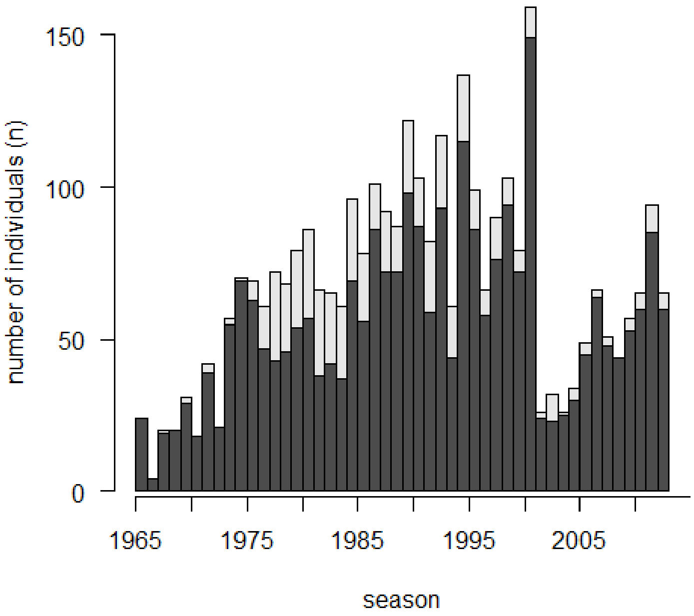
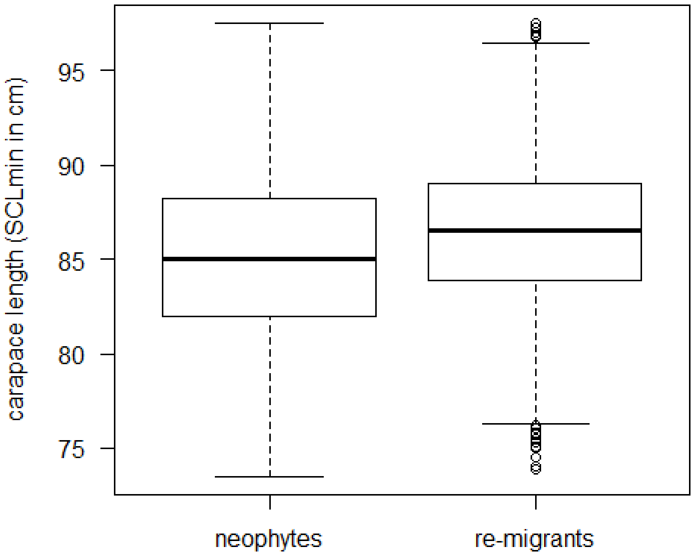


Figure 3.4: Population composition of the South African loggerhead (top) and leatherback (bottom) sea turtles displaying the total number of individuals handled per season with the proportion of neophyte nesters (black) and re-migrants (white). The decrease in the number of turtles handled in 2000 - 2005 is not an indication of population decrease (as the track numbers increased) but was caused by a change in monitoring method and effort (R. Nel pers. comm., NMMU).



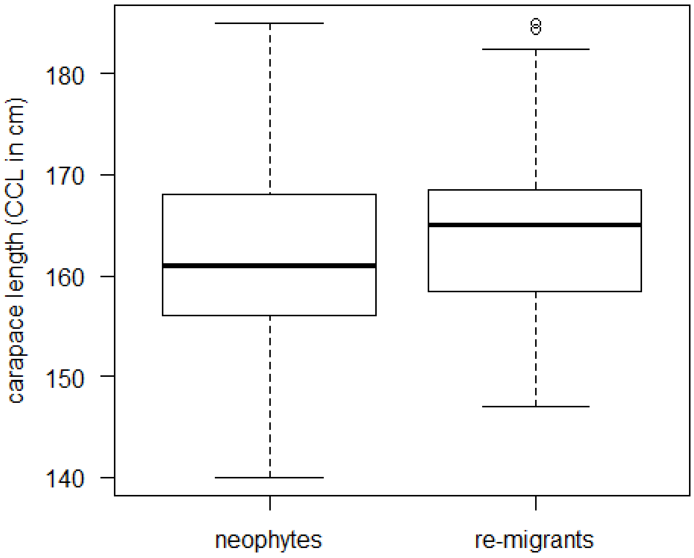


Figure 3.5: Size comparison between neophyte and re-migrant nesting sea turtles for the South African loggerhead (top) and leatherback (bottom) populations. Results indicate a significant difference in the carapace length between neophyte and re-migrant nesters for loggerhead (T-test, t = -17.5239, df = 8192.158, $p << 0.001$) and leatherback turtles (T-test, t = -8.1136, df = 777.644, $p << 0.001$).

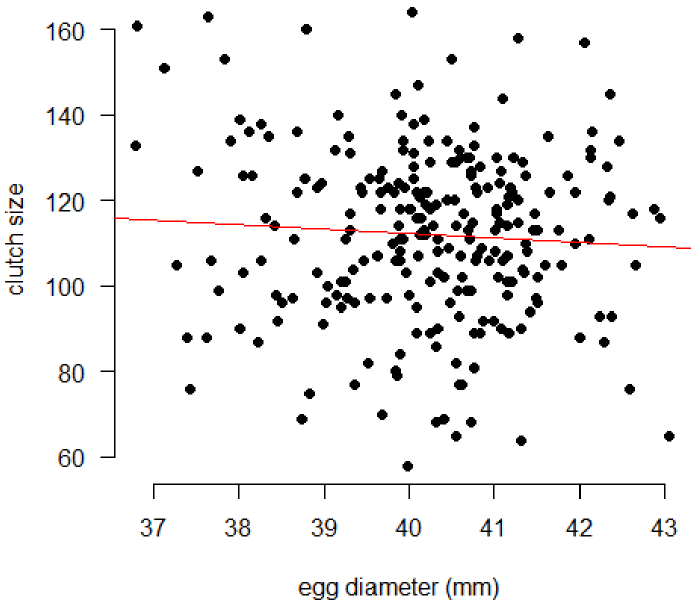
3.3.4 The effect of female size on reproductive output

The relationship between female size and reproductive output was analysed as it was hypothesised that larger females produce larger clutches with same-sized eggs or the same number but larger eggs. However, there was no correlation between the clutch size and egg diameter for loggerhead ($r = -0.064$, $p = 0.315$; Fig. 3.6) or leatherback turtles ($r = -0.1878$, $p = 0.441$, $n = 19$; Fig. 3.6) until female size was included into a GLM (Table 3.2). The GLM relationship indicates that large loggerhead females produce larger clutches irrespective of the size of eggs, whereas smaller loggerheads produce correspondingly smaller clutches also with a variety of eggs (Fig. 3.7). The size of the eggs are therefore unpredictable but smaller females produce fewer eggs per clutch and larger females more eggs per clutch.

Table 3.2: Statistical summary of the GLM for South African loggerhead and leatherback sea turtles explaining the effect of female size on reproductive output.

Model	r^2	p-value
Loggerheads		
clutch size = $0.24 \cdot SCL_{min}$ - (2.20 * egg diameter)	0.975	<<0.001
egg diameter = $0.05 \cdot SCL_{min}$ - (0.02 * clutch size)	0.998	<<0.001
neophytes only		
clutch size = $0.19 \cdot SCL_{min}$ - (1.21 * egg diameter)	0.971	<<0.001
egg diameter = $0.05 \cdot SCL_{min}$ - (0.01 * clutch size)	0.998	<<0.001
re-migrants only		
clutch size = $0.28 \cdot SCL_{min}$ - (3.08 * egg diameter)	0.977	<<0.001
egg diameter = $0.05 \cdot SCL_{min}$ - (0.03 * clutch size)	0.998	<<0.001
Leatherbacks		
clutch size = 0.14*CCL - (2.25 * egg diameter)	0.957	<< 0.001
egg diameter = 0.03*CCL - (0.06 * clutch size)	0.995	<< 0.001

Similar analyses were conducted for the South African leatherback population but because of the small sample size a significant correlation between egg size and hatchling size had to be assumed (Wallace et al. 2006b). Given this assumption larger leatherback females produce larger clutches irrespective of egg size, whereas smaller females produce correspondingly smaller clutches independent of egg size (Fig. 3.8). However, the relationship between female size, clutch size and egg diameter was highly variable.



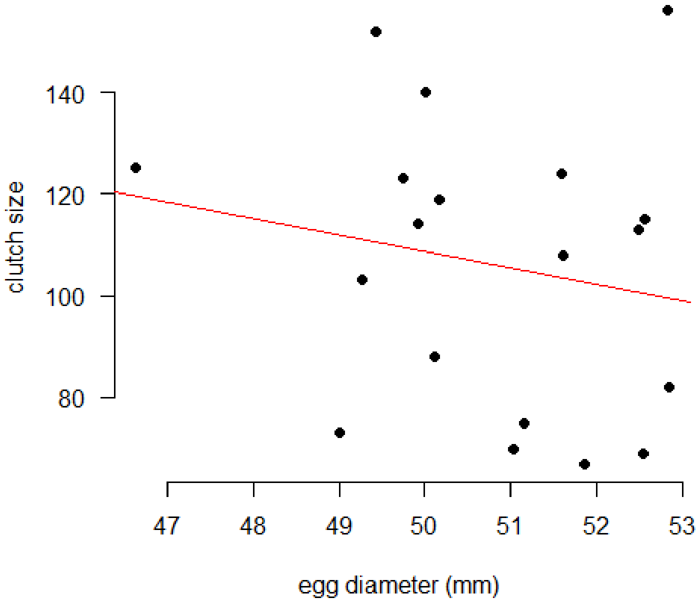
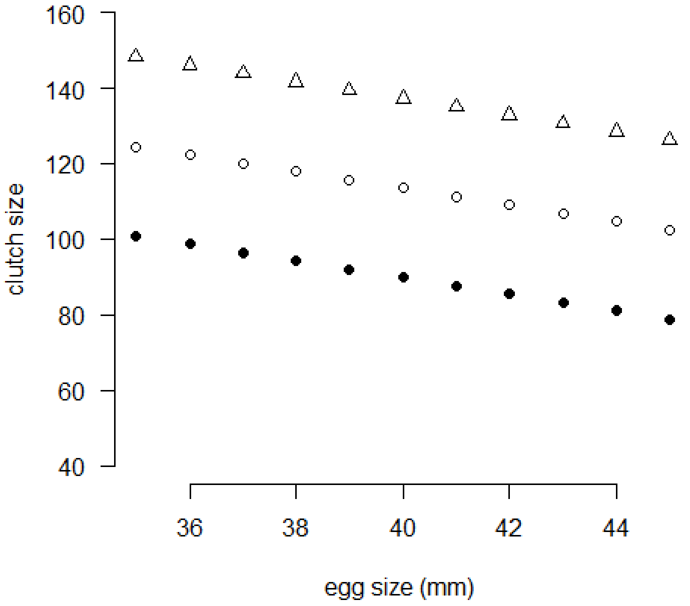


Figure 3.6: Relationship between clutch size and egg diameter of the South African loggerhead (at the top; r = -0.064, p = 0.315, n = 247) and leatherback population (at the bottom; r = -0.1878, p = 0.441, n = 19).



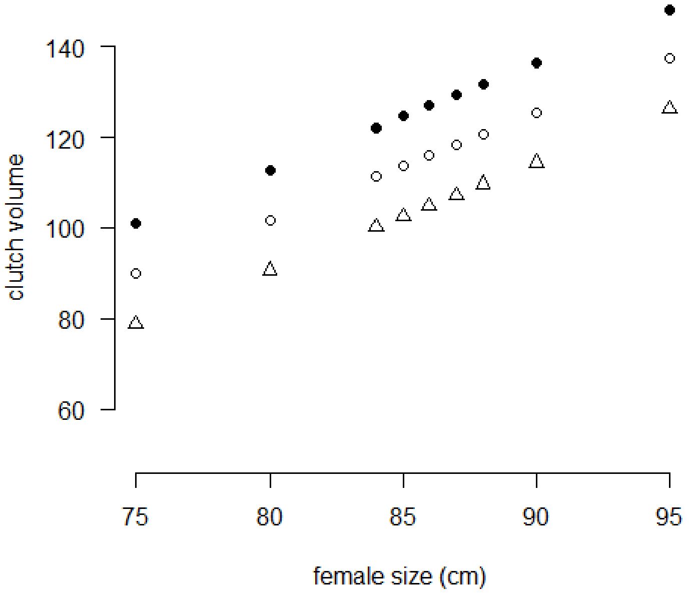


Figure 3.7: The relationship between clutch size and egg diameter correlated for the size of a female loggerhead turtle (following equations in Table 3.3). The figure at the top displays the relationship between clutch size and egg size (at constant female sizes: 75, 85 and 95 cm presented as dot, circle and triangle; r = -1; p << 0.001). The figure at the bottom demonstrates a correlation of clutch size and female size (at constant egg sizes: 35, 40 and 45 mm illustrated as dot, circle and triangle; r = 1; p << 0.001).

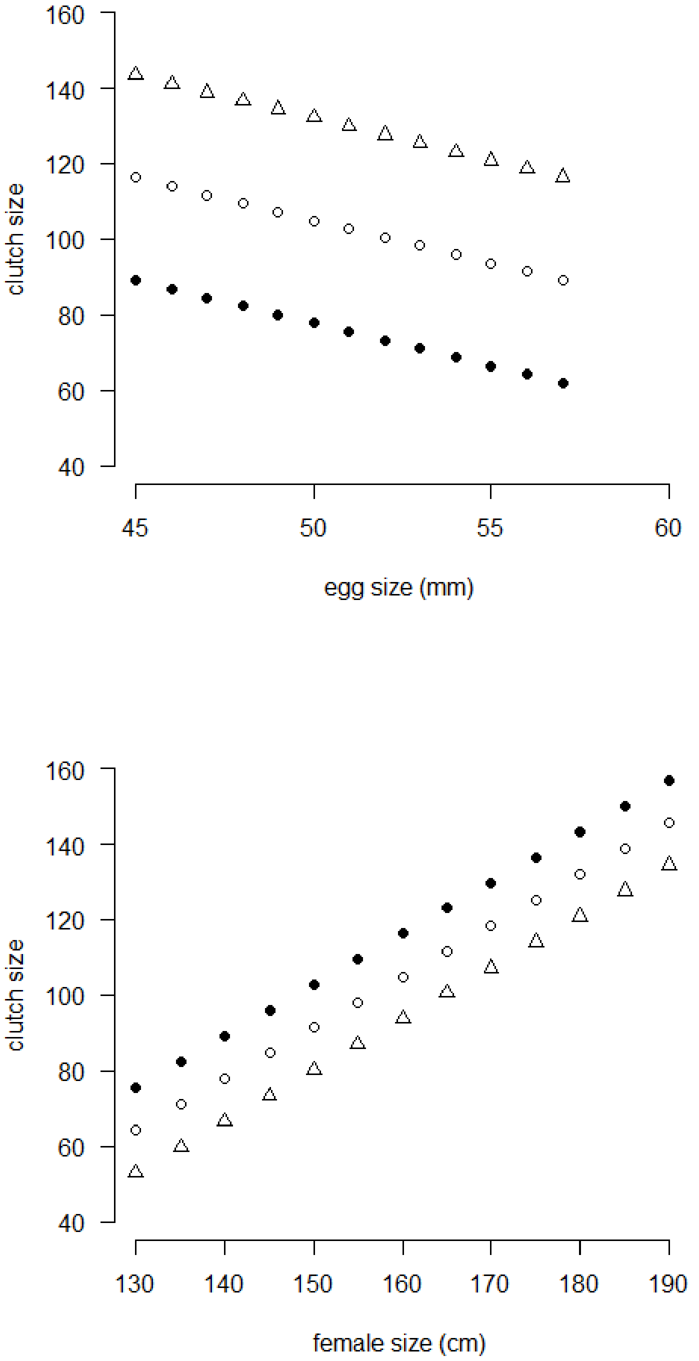


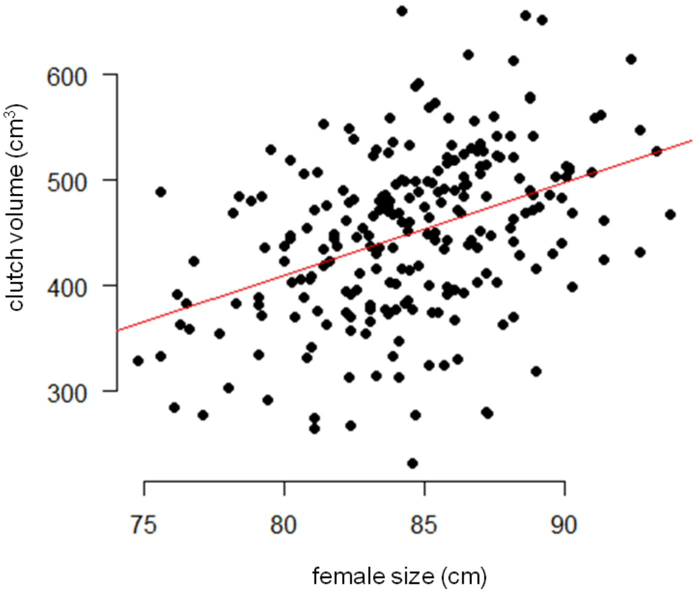
Figure 3.8: The relationship between clutch size and egg diameter correlated for the size of a female leatherback turtle (following equations in Table 3.3). The figure at the top displays the relationship between clutch size and egg size (at constant female sizes: 140, 160 and 180 cm presented as dot, circle and triangle; $r = -1$; $p << 0.001$). The figure at the bottom shows a correlation of clutch size and female size (at constant egg sizes: 45, 50 and 55 mm displayed as dot, circle and triangle; $r = 1$; $p << 0.001$).

A correlation between loggerhead egg and hatchling size ($r = 0.691$, $p < 0.001$) indicates that females which produce larger eggs also produce larger (i.e. fitter) hatchlings, whereas smaller females may produce smaller eggs and thus smaller (i.e. less fit) hatchlings. These relationships also held when neophyte nesters and re-migrants were investigated separately (Table 3.2). Because of the small sample size for leatherbacks neophyte nesters and re-migrants were not analysed separately.

3.3.5 The effect of neophyte nesters on population growth potential

The increase in numbers of neophyte nesters on reproductive output was investigated as neophytes are generally smaller in size (section 3.3.3). Clutch size (T-test; $p = 0.014$) and egg diameter (T-test; $p = 0.001$) were significantly different between neophytes and re-migrants of the South African loggerhead population, whereas hatchling size was not (T-test; $p = 0.977$). However, this outcome is not surprising as clutch volume (measured as clutch size *x* egg size) is also correlated with female size ($r = 0.406$, $p << 0.001$; Fig. 3.9). No differences in reproductive output between neophytes and re-migrants were found for the South African leatherback population (T-test; all $p \geq 0.051$), however, the correlation between clutch volume and female size was significant ($r = 0.535$, $p = 0.018$; Fig. 3.9). Furthermore, no differences in reproductive success between neophyte nesters and re-migrants were found for loggerheads (Mann-Whitney test; all $p > 0.251$) or leatherbacks (Mann-Whitney test; all $p > 0.686$).

To determine whether the increasing number of neophyte nesters affected reproductive output or success, a linear regression was applied to all data available (i.e. clutch size, egg size, hatchling size, as well as hatching and emergence success; Table 3.3). Results indicated that only loggerhead hatchling size has decreased marginally since the 1970s ($r^2 = 0.389$, $p = 0.040$).



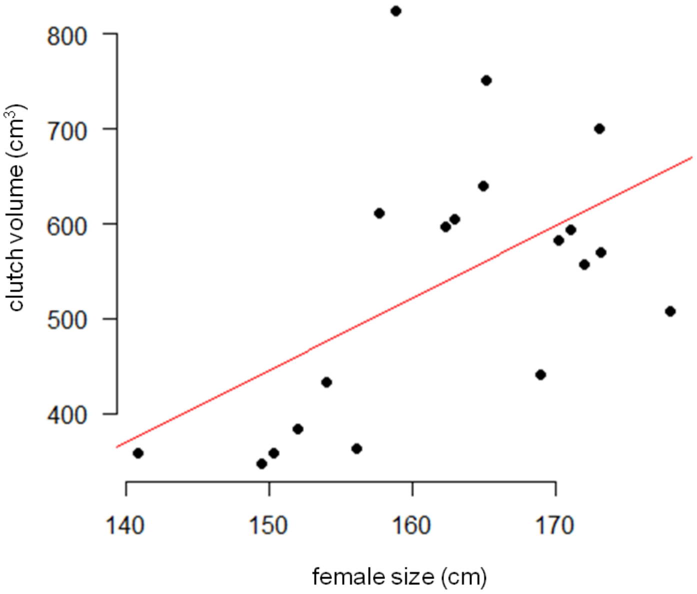


Figure 3.9: The relationship between clutch volume and female size for loggerhead (at the top; r = 0.406, p << 0.001, n = 235) and leatherback turtles (at the bottom; r = 0.535, p = 0.018, n = 19).

Table 3.3: Data summary on reproductive output and success of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle population. Loggerheads are displayed at the top and leatherbacks at the bottom. (Numbers in parentheses represent the number of nests investigated, whereas a * indicates number of measured individuals.)

Season	Clutch size	Egg size (mm)	Hatchling size (mm)	Hatching success (%)	Emergence success (%)	Source
1963	118	38.0	44.0	-	-	McAllister et al. (1965)
1965	112 (98)	49.9 (26)	44.7 (183*)	88.2 (26)	82.6 (26)	Hughes et al. (1967)
1966	118 (68)	-	45.0 (499*)	85.7 (91)	83.4 (91)	Hughes and Mentis (1967)
1967	118 (86)	-	44.4 (50*)	89.8 (81)	-	Hughes (1970)
1969	117 (19)	-	44.5 (30*)	75.3 (9)	-	Hughes (1971)
1970	104 (33)	-	45.2 (58*)	65.9 (25)	-	Hughes (1972)
1974	105 (272)	40.9 (26)	44.7 (121)	78.6 (72)	77.8 (72)	Hughes (1974a,b)
2008	97 (11)	-	-	78.3 (11)	77.9 (11)	Boonzaaier (2011)
2009	111 (80)	40.6 (80)	44.2 (30)	70.6 (267)	69.6 (262)	this study
2010	113 (107)	40.0 (121)	43.9 (64)	72.3 (452)	71.0 (452)	this study
2011	112 (85)	39.9 (67)	44.4 (30)	77.1 (160)	75.5 (160)	this study
2012	115 (22)	40.0 (20)	43.4 (11)	84.0 (215)	82.6 (215)	this study

Season	Clutch size	Egg size (mm)	Hatchling size (mm)	Hatching success (%)	Emergence success (%)	Source
1963	110	50.0	-	-	-	McAllister et al. (1965)
1965	106 (24)	53.1 (16)	59.8 (22*)	77.9 (4)	74.9 (4)	Hughes et al. (1967)
1966	97 (9)	-	60.8 (18*)	86.1 (6)	-	Hughes and Mentis (1967)
1967	104 (18)	-	59.0 (37*)	75.6 (11)	-	Hughes (1970)
1969	102 (4)	-	57.4 (19*)	-	-	Hughes (1971)
1972	104 (219)	-	-	-	68.9 (219)	Hughes (1975)
1974	103 (39)	53.1 (16)	58.7 (131*)	76.2 (39)	68.9 (39)	Hughes (1974a,b)
2009	127 (4)	51.1 (5)	60.4 (1)	77.4 (9)	76.0 (9)	this study
2010	125 (6)	50.9 (14)	59.2 (18)	76.2 (91)	73.0 (90)	this study
2011	94 (41)	51.4 (15)	57.7 (10)	70.9 (28)	70.2 (27)	this study
2012	101 (12)	49.7 (4)	56.3 (2)	89.4 (12)	86.0 (12)	this study

3.4 Discussion

Populations can increase in size, be stable or decrease in size depending on the availability and abundance of resources, natality and mortality (Chaloupka & Musick 1997). Thus, if population density is low and resources are abundant, population numbers may grow exponentially until environmental carrying capacity is reached (Molles 2013). Conversely, if mortality exceeds natality (from natural or anthropogenic sources) populations become imperilled (Dethmers & Baxter 2011, Simpkins et al. 2014). Responses of short-lived species to environmental perturbation (such as droughts or floods) are often straightforward (i.e. traceable) and maybe altered over few generations (Dunham 2012, Simpkins et al. 2014). Conversely, responses of long-lived, late maturing species are complex as consequences may only manifest after decades, which can lead to local extinction as the effects are difficult to reverse (Heppell et al. 2003, Reed et al. 2003).

Sea turtles are a long-lived endangered species which display high fecundity, low juvenile survival, and late maturity (Winemiller & Rose 1992, Van Buskirk & Crowder 1994). Thus, consequences from over-exploitation, habitat destruction (of nesting and foraging grounds) or disasters (such as the oil spill in the Mexican Gulf in 2010) will be reflected decades later (Heppell et al. 2003). Therefore, population growth (and its influencing factors) should be monitored in regular intervals in order to intervene if necessary. Such a revision is done annually on the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* populations. These populations started with low numbers of nesting females (107 loggerheads and 24 leatherbacks) in 1965. After 50 years of intensive beach conservation and strong law enforcement, the loggerhead population now displays exponential growth (Nel et al. 2013). The current number of nesting loggerhead females per season (average of the last 5 years) approximates ±650 individuals per annum whereas the leatherback population increased to about 90 individuals per annum (at its peak in the 90s; Table 3.1).

3.4.1 The effect of reproductive output and success on population growth potential

Reproductive output (measured as clutch size) was investigated for both species as it was hypothesized that loggerhead females deposit larger clutches and thus produce greater numbers of offspring per individual, which in turn would enhance population growth. Indeed, a comparison between loggerhead and leatherback clutch size (112 ± 20 eggs and 100 ± 23 eggs, respectively) displayed a significant difference (T-test; $p < 0.001$), supporting the hypothesis. Yet, a comparison with other populations demonstrated that loggerhead clutch size equals only standard values (Van Buskirk & Crowder 1994, Baptistotte et al. 2003, Antworth et al. 2006, Margaritoulis et al. 2011), whereas clutch size of the South African leatherback exceeds approximations of most other populations (in the Atlantic and Pacific; Eckert et al. 2012) except one from Sri Lanka with 100.5 eggs per clutch (Ekanayake et al. 2002). It is suggested that similarities between those populations might be due to analogous population demographics or shared environmental conditions in the Indian Ocean. However, South African leatherback turtles exhibit a higher intra-seasonal nesting frequency than loggerheads (Nel et al. 2013) and thus produce a larger number of eggs per female per season (i.e. ±700 eggs compared to ±448 eggs, respectively), facilitating population growth.

Sea turtles do not maintain post-ovipositional care and therefore it was suggested that environmental conditions during the incubation period might be sub-optimal for leatherback turtles, prohibiting population growth. Indeed, De Wet (2012) estimated that about 10.8 % of all deposited loggerhead nests and 22.0 % of all leatherback nests in South Africa are either completely predated or eroded each season, whereas anthropogenic sources of nest mortality (i.e. illegal egg harvest) were only incidental. Reproductive success (measured as emergence success) was calculated at 73.6 ± SD 27.68 % for South African loggerheads and at 73.8 ± SD 22.70 % for leatherbacks. On a global scale those estimates are amongst the highest reported as emergence success for loggerheads mostly ranges between 54 - 82.9 % (with an average at 70 %; Margaritoulis 2005, Lamont et al. 2012) and for leatherbacks between 11.6 - 71.1 % (with an average at 50 %; Eckert et al. 2012). This leads to the conclusion that the nesting environment (including anthropogenic impact) is not dampening population growth, especially as leatherback individuals produce a larger number of hatchlings per season than any loggerhead female (±517 and ±330, respectively).

As present reproductive output and success of the South African loggerhead and leatherback population are not accountable for current population growth, results were compared with previous studies, dating back to the 1960s. Nonetheless, outcomes indicated that neither clutch size nor emergence success have changed since the beginning of the programme (linear regression; all $p > 0.120$). Thus, it is proposed that an increasing number of neophyte nesters caused the current loggerhead population growth as a direct result of the initiated beach conservation programme in 1963. Indeed, many other sea turtle populations have benefitted in a similar way from beach conservation programmes such as the green turtles in Aldabra (Mortimer 1985), the hawksbill turtles on the Seychelles (Wood 1986) and the leatherbacks in the Caribbean (Dutton et al. 2005). Thus, it is unknown why the South African leatherback population has not responded similarly. However, if the number of annual nesting individuals (i.e. ±650 loggerheads and ±65 leatherbacks) is considered, the absolute growth potential favours the loggerhead population due to the greater number of females contributing to reproductive output (i.e. the amount of hatchlings produced).

Analysis of population composition further revealed that both species are dominated by neophyte nesters (in a ratio of 1 : 4 for loggerheads and 1 : 9 for leatherbacks). The dominance of neophyte nesters suggests that most individuals nest only one season (or an accumulation of repeat nesters should balance the number of neophyte nesters). A similar population structure was observed for olive ridley turtles in Orissa, India (Shanker et al. 2003). The dominance of neophyte nesters in the olive ridley population was explained by the high off-shore mortality of breeding adults. However, the dominance of neophyte nesters indicates that nesting females produce enough offspring to (at least) replace themselves in the breeding part of the population.

3.4.2 The effect of female size on population growth potential

The size of an individual is a widely accepted measure of fitness (Molles 2013). In sea turtles a minimum size should be reached before reproduction takes place (Hughes 1974b, Tucek et al. 2014; CHAPTER 6) as female size (i.e. body capacity) affects reproductive output (GLM; $p << 0.001$). In general, small individuals produce smaller clutches irrespective of egg size and larger individuals produce larger clutches independent of egg size (Wikelski & Romero 2003, Prado 2005, Cox et al. 2007, Wallace et al. 2007). It is suspected that larger eggs also produce larger hatchlings which might have increased survival rates (Janzen 1993, Janzen et al. 2000).

The average size of nesting loggerhead females (in South Africa) was approximated at SCL_{min} 85.6 ± 4.31 cm which is smaller than most other populations (Van Buskirk & Crowder 1994, Miller 1997, Tucek et al. 2014). However, it is proposed that the increasing numbers of neophyte nesters (section 3.4.1.) decreased average nesting size (linear regression; $p << 0.001$) as neophytes are smaller than re-migrants (T-test; $p << 0.001$). Those observations are confirmed by similar results in an olive ridley population in Orissa, India (Shanker et al. 2003). Average size of nesting leatherback turtles (in South Africa) was estimated at CCL 160.9 ± 8.34 cm and is only exceeded by the Western Pacific population (Eckert et al. 2012)[16]. Nonetheless, average size of nesting females has not decreased (linear regression; $p = 0.218$) since 1965 even though size differences between neophytes and re-migrants exist (T-test; $p <<0.001$). It is assumed that the consistent average size of nesting leatherback females is a consequence of a stable percentage of neophyte nesters to re-migrants.

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[16] This further suggests that the South African leatherback population is amongst the fittest on a worldwide scale and that population growth is not suppressed by intrinsic factors (i.e. genotype and health status) or extrinsic factors (i.e. food quality and availability).

As reproductive output is directly linked to female size (Table 3.2) it was suspected that size differences between neophytes and re-migrants might lead to a setback in population growth. However, differences in clutch size between neophytes and re-migrants was found for loggerhead turtles (T-test; p = 0.014) but not for leatherbacks (T-test; p = 0.051). As leatherback turtles do not maintain a hard shell it is proposed that the trade-off between clutch size and egg diameter is not as strict as in other sea turtle species and correlations may not be too obvious (within a small sample size). Further, a comparison with previous studies indicated no change in clutch size or egg diameter since the 1970s, neither for loggerheads (linear regression; p = 0.241 and p = 0.376, respectively) nor leatherbacks (linear regression; p = 0.297 and p = 0.318, respectively). Thus, it is concluded that the increase of neophyte nesters in the South African loggerhead and leatherback population has no negative effect on population growth.

In summary, clutch sizes of the South African loggerhead and leatherback population are large and environmental conditions at the nesting beach promote successful hatchling production. Successful reproduction is further confirmed by the dominance of neophyte nesters in both populations, which indicates that nesting females at least replace themselves. Thus, it is concluded that neither differences in clutch size nor emergence success (i.e. amount of successfully emerged hatchlings) per individual caused the different population growth profiles. At last, loggerhead population growth is enhanced by the greater number of females contributing to reproductive output.

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Chapter 4: Estimating sex ratio and pivotal temperature of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle populations

Abstract

In the light of global warming temperature-dependent sex determination (TSD) could lead to species extinction if only one sex is produced in a population. The sex ratios of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle populations were investigated. Standard histological techniques were used to sex hatchlings and a generalized linear model (GLM) combined with environmental data was used to model annual sex ratios for each population. Loggerhead sex ratio for this study (2009 - 2011) was estimated at 86.9 ± SE 0.35 % female-biased. Sufficient replication for the leatherback population was only obtained for season 2010, which indicated a 97.1 % (95 % CI 93.3 - 98.7) female bias. In order to approximate pivotal temperature (PT) *in situ* incubation temperatures were recorded (with iButtons) and the mean temperature of the thermo-sensitive period (TSP) was transferred to constant temperature equivalents (CTEs), using the length of the modelled TSP. Modelled embryonic growth (size) in dependence of incubation temperature was used to determine TSP *in situ*. As other methods before, this new approach confirmed that *in situ* obtained PT is not comparable with those from constant temperature incubation experiments (e.g. *in situ* PT for leatherbacks was estimated at 29.2 ± SE 0.35 °C with a CTE of 29.1 ± SE 0.17 °C). However, as gonad samples in this study were compromised it was assumed that sex ratio estimates were overestimated, which would also affect approximations of the PT. It was concluded that results from an earlier unpublished constant incubation experiment would describe PT of the South African loggerhead population more accurately at 29.7 ± SE 0.01 °C (Maxwell 1986). Unfortunately, no comparable study exists for the South African leatherback population.

4.1 Introduction

Sex-determining mechanisms can be categorized as *genotypic sex determination (GSD)*, which is typical for mammals and birds (Bull 1983), or *environmental sex determination (ESD)*, which is mandatory in reptile orders such as crocodilians (crocodiles and alligators) and sphenodontians (tuataras), and common among testudines (turtles) and squamates (snakes and lizards) (Janzen & Krenz 2004, Janzen & Phillips 2006). GSD is controlled by heteromorphic sex chromosomes, which define the gender of an individual at fertilization, whereby ESD depends on non-genetic cues (such as incubation temperature, nutrition, density, pH or exposure to the opposite sex) with sexual differentiation only after conception (Korpelainen 1990, Janzen & Paukstis 1991). The discovery of *temperature-dependent sex determination (TSD)* as a form of ESD in sea turtles (Bull & Vogt 1979, Mrosovsky 1980, Wyneken et al. 2007), fish (Devlin & Nagahama 2002), and other vertebrate and invertebrate taxa (Korpelainen 1990, Janzen & Paukstis 1991, Robert & Thompson 2001) suggests that sex determination by incubation temperature is more prevalent than previously thought (Valenzuela et al. 2003). Janzen and Phillips (2006) proposed that TSD is an ancient form of sex determination (≥300 million years) which has not changed in many taxa because it functions well. However, the evolution of TSD is still uncertain (Shine 1999, Janzen & Phillips 2006).

One of the greatest challenges to any population is to balance male and female sex ratios to maintain a prosperous population. Fischer (1930) suggested an even 1 : 1 sex ratio if parental investment for both sexes is equal. In GSD the genes of each of the parents are split through meiosis creating an equal chance to produce either male or female offspring and thus increasing the likelihood of a 1: 1 sex ratio in future generations (Janzen & Phillips 2006). ESD, in contrast, is potentially biased towards one sex; this is widely observed in plants, nematodes, amphipods, fish and amniote vertebrates (Charnov & Charnov 1982, Charnov & Bull 1989, Korpelainen 1990, Pieau et al. 1994). Spinach *(Spinacia oleracea* var. *Americana)*, for example, displays biased sex ratios based on precocious flowering of males, water abundance, photoperiod and seed source (Freeman & Vitale 1985), whereas sex ratios in some fish and most reptiles are skewed by high or low temperatures (Bull 1980, Conover 1984).

The understanding of ESD and optimal population sex ratios is also of practical interest as rapid climate change is expected to alter environmental conditions; i.e. global warming, sea level rise, droughts, wet periods and storms (Easterling 2000, IPCC 2013) can affect offspring sex ratios (Janzen 1994) and thereby the population growth potential of species. Knowing the sex ratio of a population can thus be applied in conservation modelling to predict impacts of (shifting) environmental conditions on sex ratios especially in species with TSD (Girondot et al. 1998, Hawkes et al. 2007, Fuentes & Porter 2013). It is also expected that populations which mostly produce one sex, due to changes in the environment, might not survive without anthropogenic assistance (Janzen 1994, Vogt 1994, Le Galliard et al. 2005, Mitchell et al. 2008).

Sea turtles for instance display TSD and recent literature suggests a female-biased sex ratio in all sea turtle species (ANNEX I). The degree of feminisation depends on the incubation temperature during the thermo-sensitive period (TSP) in which the sex of the offspring is determined (Janzen & Paukstis 1991, Mrosovsky & Pieau 1991). At constant incubation temperatures the TSP is the middle third of the incubation period (IP). If incubation temperatures during the TSP are mostly above the pivotal temperature (PT)[17], feminisation of the clutch is induced. The most commonly used method to estimate PT *in situ* seems to be histological analyses (determining the sex ratio) in combination with the mean temperature during the middle third of the incubation period (Kaska et al. 1998, Godley et al. 2002). When histological studies are not undertaken the most frequently applied method to estimate PT or sex ratio is from recorded nest temperatures with the approximated sex ratio from other experiments (Mrosovsky et al. 2009, Sieg et al. 2011). However, direct comparisons of PTs or sex ratios between populations, within or between species, can be misleading as there are a large number of factors that affect these estimates, including the number of nests or seasons monitored (i.e. replication), variation in histological interpretation to assign sex or the application of pivotal temperatures from other populations to approximate sex ratios (Wibbels 2003).

Marcovaldi et al. (1997) and Godfrey et al. (1999) further suggested that sex ratio could also be correlated with the duration of the incubation period (oviposition to emergence), indicating that female production displays a shorter IP than a male production. But in order to compare any *in situ* IPs to constant temperature experiments the time between piping and emergence needs to be known (Godfrey & Mrosovsky 1997). However, *in situ* obtained incubation temperatures are not comparable with constant temperature incubation experiments because of temperature fluctuations in the nest. The middle third of the *in situ* IP does not (necessarily) correspond to the TSP and should not be used to estimate PT (Georges 1989, Georges et al. 1994, Georges et al. 2004).

Girondot and Kaska (2014) developed a new method to determine the TSP *in situ*. They used embryo size at different stages of (stage-based) embryonic development from a constant temperature incubation experiment to determine the TSP and fitted a reaction norm model of daily embryonic

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[17] *Pivotal temperature* describes a threshold temperature which produces a 1 : 1 sex ratio (Mrosovsky & Pieau 1991).

growth with dependence on incubation temperature, the length of the IP and average hatchling size. Finally, stages for the TSP from the constant temperature incubation experiment were compared to modelled embryonic size and the *in situ* TSP was determined.

The objectives of this chapter are to i) establish sex ratio and ii) pivotal temperature for loggerhead *(Caretta caretta*) and leatherback *(Dermochelys coriacea)* sea turtles nesting in KwaZulu-Natal, South Africa, applying the method described by Girondot and Kaska (2014).

4.2 Material and Methods

In order to assess sex ratio of the South African loggerhead and leatherback sea turtle populations a histological survey of hatchling gonads was conducted. Research permits were obtained from both the iSimangaliso Park Authority to work in the World Heritage Site, as well as permits (RES 2009/08, RES 2010/55 and RES 2011/41) from the Department of Environmental Affairs: Directorate Oceans and Coasts (previously Marine and Coastal Management) to handle, collect and euthanize sea turtle hatchlings. In order to euthanize hatchlings animal ethics clearances were obtained from the Animal Ethics Committee at the Nelson Mandela Metropolitan University (A09-SCI-ZOO-005 and A11-SCI-ZOO-013). Together with histological examinations, nest temperatures were recorded in those nests where from hatchlings were sacrificed. As leatherback-nesting events are rarer and more dispersed, sample sizes were smaller. However, the same methods were applied to both loggerhead and leatherback sea turtles, unless otherwise stated.

4.2.1 Pre-treatment of temperature data

All temperatures were recorded using iButtons (from Fairbridge Technologies, DS1922L-F5#, ±0.5 °C accuracy) logging temperature every 30 minutes. To standardise the recordings, all iButtons were pre-tested before deployment, under controlled conditions (i.e. room temperature), and deviations to the median were assessed. To determine the accuracy of the readings, the 43 data loggers were calibrated against a mercury thermometer in a water bath at 30 °C (Gallenkamp, Cat. No. 1H350). Recordings from each iButton were normalised by adding the iButton specific deviation from the median, and setting the median deviation of the iButtons measured against the mercury thermometer.

To reduce the danger of corrosion *in situ*, iButtons were placed inside plastic shells (ping-pong balls) and sealed with duct tape. As the protective covering could affect the readings, an experiment was conducted to determine any effects. Recordings of data loggers with (n = 12) and without protection (n = 32) were compared under controlled conditions. A Shapiro-Wilk test was applied to test for normality and differences between the groups were assessed using a Mann-Whitney test, in R version 2.15.2 software (R Core Team 2012) with $\alpha = 0.05$.

4.2.2 Sex ratio

Field sampling

Field sampling was conducted over three consecutive seasons (2009 - 2011) as described in CHAPTER 3. In addition to these sampling procedures, temperature recordings of the nests were obtained by deploying temperature loggers (i.e. iButtons) sealed in ping-pong balls in the centre of the clutch during ovipositon (Table 4.1). To enhance leatherback sample size some nests were excavated within a few hours (<6 hours) if the actual nesting event was not observed to plant an iButton. Loggerhead nests with temperature loggers were situated between beacon 0 and 12 N (5 km) and leatherback nests between 0 - 12 N and 64 - 92 S (CHAPTER 2; Fig. 2.2) both representing areas with high nesting densities.

Table 4.1: Sample sizes of the number of nests (per season) in which temperature loggers (iButtons) were deployed.

Season	Loggerhead (n)	Leatherback (n)
2009	-	2
2010	18	16
2011	19	10

At hatching, ten self-emerged hatchlings were randomly selected for histology. The individuals were measured after Miller (1999) and then euthanized by injecting 2.5 ml sodium pentobarbital into the heart. Sample size was augmented with dead hatchlings and full-term (dead) embryos, which were excavated from the nests after hatching to retrieve the data loggers. All samples were stored for later examination by freezing[18] the specimens. Only the leatherback individuals (n = 11) from the 2009-season were preserved in 95 % ethanol.

As sampling was focussed on nests laid during the peak-nesting season (December), a dedicated experiment was carried out in 2011 to assess sex ratio development across the season. For this experiment, a temperature logger was deployed in four loggerhead nests (one per nest) at the beginning of the season (nesting started end of October) and every second week thereafter (until the end of December)[19]. Unfortunately, the permit (from Oceans and Coasts and iSimangaliso) limited

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[18] It was realized post-hoc that this method is sub-optimal as cell rupture complicated the sex assignment.

[19] In total 19 iButtons were positioned as only three data loggers were deployed end of November.

the number of hatchlings to be euthanized to only five self-emerged loggerhead hatchlings per nest[20].

Sex differentiation

Standard histology was applied to differentiate the sex of the sampled sea turtle hatchlings and embryos. Therefore, gonads were dehydrated, sliced and stained following Yntema and Mrosovsky (1980), Dutton et al. (1985), Wyneken et al. (2007) and Ceriani and Wyneken (2008). Processed glass slides carrying gonad cross-sections were examined under a light microscope (Leica, DM 750). The sex was distinguished using four characteristics (cortex, paramesonephric duct, medulla and gonad attachment), which are further described in the literature (Yntema & Mrosovsky 1980, Dutton et al. 1985, Rimblot et al. 1985, Maxwell 1986, Binckley et al. 1998, Miller & Limpus 2003, Wyneken et al. 2007, Ceriani & Wyneken 2008).

As a result of freezing the samples, sex identification was difficult as the integrity of the gonads were compromised. Consequently, sex assignment was conducted independently by two observers and then compared. All hatchlings displaying a perpendicularly arranged cortex (irrespective of the thickness) were considered as females. If the cortex was not clearly identifiable as female, the gonad was still considered an ovary if at least two other characteristics were feminine. If the cortex characteristics were deemed male, a minimum of two additional male characteristics were necessary in order to classify the gonad as a testis. All other configurations were considered as unclear and were removed from the data set (i.e. 219 loggerhead and 109 leatherback samples were eliminated). A detailed view of all described features is displayed in ANNEX II and ANNEX III. All photomicrographs presented in the annexes were obtained using an Olympus BX51 light microscope, an Olympus XC50 camera and analysis LS Research 3.4 software.

Estimating sex ratio

Sex ratio can differ within and between seasons as it is dependent on environmental temperature. A generalized linear model (GLM) with binomial distribution and a logit-link function was used to investigate differences in sex ratio over the course of a season and between different seasons[21]. To determine sex ratio per season, a backward selection model was set up with rank of time (quarter-

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[20] This limitation was only for loggerhead hatchlings, i.e. the sample size for leatherback turtles in 2011 remained the same (10 hatchlings per monitored nest).

[21] Data from Maxwell et al. (1988) for the 1984-season and Boonzaaier (2011) for the 2009-season were included for loggerhead turtles. It should be recognized that Maxwell's data were extracted from the publication whereas original gonad cross-sections from Boonzaaier were examined.

month periods)[22] over the course of a season (*model 1: y = f (a * quarter-month)*; where *y* equals sex ratio and *a* is a constant). To account for the possibility that more males than females were discarded during sex differentiation a second model with seasons as a category was set up. If one significant effect was observed within any year the effect was retained for all *(model 2: y = f (a + quarter-month))*. Additionally, nest distribution over the course of a season was integrated for both models after Girondot (2010)[23]. All statistical analyses were conducted in R version 2.15.2 software (R Core Team 2012) with $\alpha = 0.05$.

4.2.3 Pivotal temperature

As the middle third of the IP of *in situ* nests does not (necessarily) correspond to the TSP of constant incubation experiments, Girondot and Kaska (2014) developed a method to determine the TSP *in situ* (described in the introduction of this chapter). However, PT measured *in situ* is not comparable with those from constant temperature incubation experiments (Georges 1989, Georges et al. 1994, Georges et al. 2004) and needs to be adjusted. Mean temperatures of the TSPs (estimated following Girondot & Kaska 2014) were correlated with the duration (in days) of the TSPs, as well as the duration of the IPs[24]. Temperatures of the TSP were slightly better correlated with the duration of the TSP than with the IP (Table 4.2). The duration of the TSP was used to convert *in situ* incubation temperatures to constant temperature equivalents (CTEs).

Table 4.2: Correlations to transfer *in situ* incubation temperatures to constant temperature equivalents (CTEs) by the duration of the thermo-sensitive period (TSP) or incubation period (IP) for South African loggerhead and leatherback sea turtles.

	r	p-value
Loggerhead		
mean temperature of TSP & duration of TSP	0.801	<<0.001
mean temperature of TSP & duration of IP	-0.588	<0.001
Leatherback		
mean temperature of TSP & duration of TSP	-0.741	0.002
mean temperature of TSP & duration of IP	-0.738	0.003

In the process, incubation at constant temperatures were simulated from the established reaction norm (embryonic growth as a function of incubation temperature) and related durations of TSPs

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[22] For a detailed view on sex ratio the season was sub-divided into ‘quarter-month periods’ (equal to ±7 day periods) each allocated with an average sex ratio.

[23] Nest distribution was averaged (per quarter-month period) across each nesting season from 1965 - 2011. The data was extracted from the Ezemvelo database, which records annual nest counts since 1965.

[24] To increase the sample size for loggerhead turtles re-analysed data from Boonzaaier (2011) for season 2008 and 2009 were included.

were determined. Thereafter, a 3rd order polynomial was used to convert *in situ* TSP-durations to CTEs (Fig. 4.1), which act as an integrated measure of the effect of temperature on embryonic growth rate. Finally, PT was re-assessed by fitting CTEs and sex ratios following Girondot (1999). The fitting criterion was based on maximum likelihood with a binomial distribution.

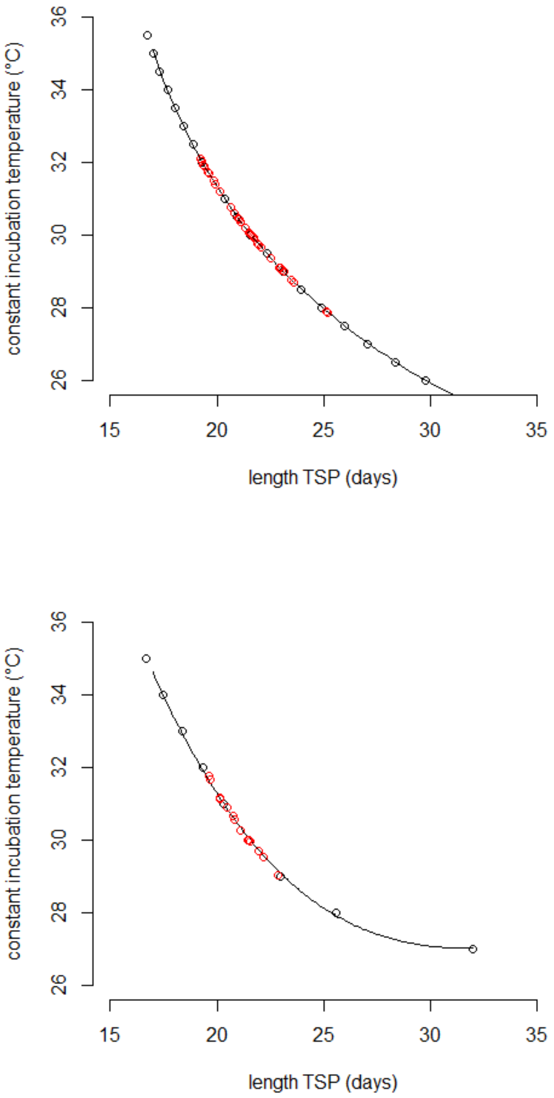


Figure 4.1: A polynomial model after simulated constant incubation temperatures (and thermo-sensitive periods) was applied to convert *in situ* incubation temperatures to constant temperature equivalents (CTEs) by the duration (length) of the thermo-sensitive period (TSP). At the top is the model for loggerhead turtles ($f(x) = -0.0024x^3 + 0.2107x^2 - 6.5573x + 97.2362$) and at the bottom for leatherbacks ($f(x) = -0.0014x^3 + 0.1457x^2 - 5.0837x + 85.7120$). The red circles indicate data from this study (obtained *in situ*).

To determine pivotal temperature (PT) for the South African loggerhead population more accurately, data from other sources (of the same population) were included. These include data from a constant incubation temperature experiment (Maxwell 1986) and data from Boonzaaier (2011). No previous study evaluating the pivotal temperature of the South African leatherback population exists and thus no adjustments were made. All statistical analyses were conducted in R version 2.15.2 software (R Core Team 2012) with $\alpha = 0.05$.

4.3 Results

4.3.1 Pre-treatment of temperature data

All temperature recordings from the 2010 and 2011 seasons were corrected by adding the iButton specific deviation from the median (range -0.8 to 0.8 °C, $n = 82$) to each set of values, as well as the correction factor of 0.23 ± SD 0.30 °C (range -0.43 to 1.07 °C, $n = 43$) which was measured against a mercury thermometer. In the 2008 and 2009 seasons, no iButton specific calibration tests were conducted and only the correction factor against the mercury thermometer was added to the measurements. However, temperature recordings of those iButtons were still included in analyses (as the potential error is very small) to increase sample size. Finally, the comparison of temperatures from data loggers sealed in ping-pong balls and those without protection displayed no difference (Mann-Whitney test, $W = 716\,537$, $p > 0.146$) and required no adjustments because of the plastic covering.

4.3.2 Sex ratio

Data on loggerhead sex ratio were collected over two consecutive seasons (2010 and 2011) with 23 successfully sampled nests (ANNEX IV). Sample size per nest ranged 3 - 26 individuals even though the objective was 10. Reasons for the deviation were that monitored nests were depredated, hatchlings escaped, hatching success was too low (however dead hatchlings or full-term embryos were available to enhance the numbers) or because of permit restrictions (as in the case of loggerhead hatchlings in season 2011). A comparison of sex ratios across the season (including all available data) displayed a significant increase in female percentage over time (GLM, $z = -7.710$, $p < 0.000$; Fig. 4.2), producing >80 % females after the first week of December (Fig 4.2). To test the consistency of this trend, the number of seasons was compared and showed that female percentages produced in the more recent seasons were significantly higher than in 1984 (GLM, 2010: $z = -3.427$, $p < 0.000$ and 2011: $z = -2.819$, $p < 0.005$; Fig. 4.3).

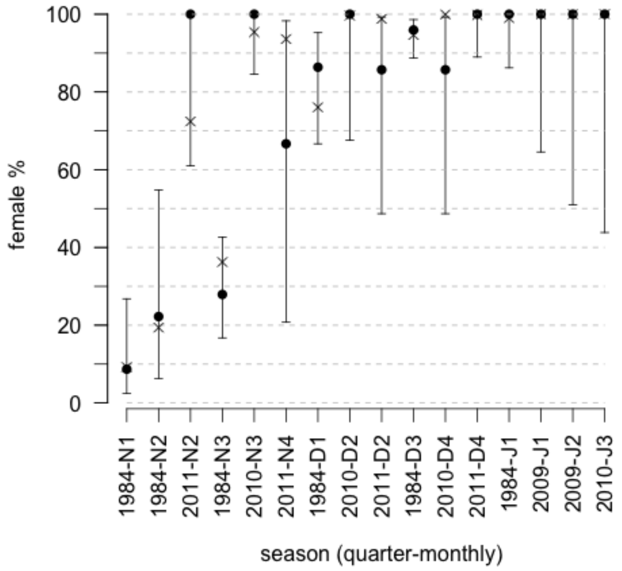


Figure 4.2: Loggerhead sex ratio (black dot) plotted in quarter-month intervals over the course of the season. The season indicates the date of oviposition (N-November, D-December, J-January; numbers indicate the quarter of the month). Additionally, an 'X' (with 95 % CI) indicates sex ratio estimate from the backward selection model *(model 1).*

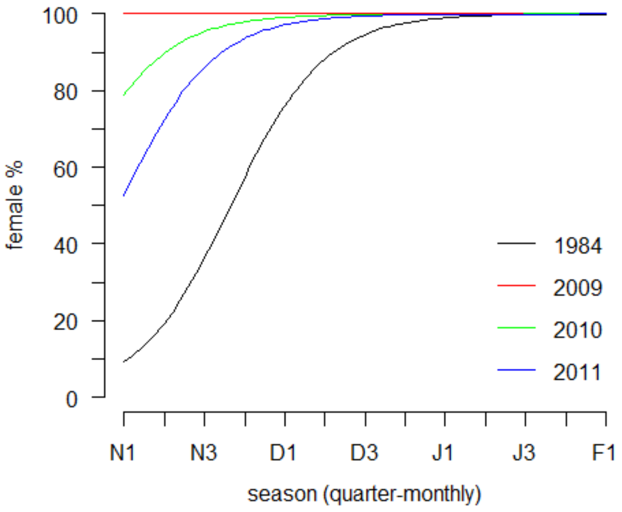


Figure 4.3: Annual estimated sex ratio of loggerhead turtles (*model 1*) displayed in quarter-month intervals over the course of each season whereby the season indicates date of oviposition (N-November, D-December, J-January; numbers indicate the quarter of the month).

Using the backward selection model with rank of time over the course of a season *(model 1)*, the current sex ratio for loggerhead turtles was estimated at 98.1 ± SE 1.16 % females (Table 4.3). This is about 20 % higher than (re-)estimations for the data obtained from Maxwell et al. (1988). As sex ratio could be overestimated in this study (as gonads were compromised), a backward selection model with years as category *(model 2)* was applied to each season. This model suggested a more rational sex ratio of 86.9 ± SE 0.35 % females for the 2009 - 2011 data (Table 4.3).

Table 4.3: Comparison of sex ratio (% female) per season of loggerhead turtles as estimated by two different GLMs (SE in parentheses). (In the models *y* equals sex ratio and *a* is a constant.)

	1984	**2009**	**2010**	**2011**	**Combined Data**
Model 1	77.4	100.0	98.2	96.0	98.1
y = f (a*quarter-month)	(2.79)	(0.13)	(1.59)	(2.50)	(1.16)
Model 2	83.2	86.8	86.4	87.6	86.9
y = f (a+quarter-month)	(2.32)	(1.96)	(2.11)	(1.96)	(0.35)

The sex ratio of leatherback nests was only investigated in season 2010 (n = 15) with one additional nest from 2009 and one from 2011. The sample size per nest was 1 - 23 individuals for the same reasons as for loggerheads (ANNEX V). The results from the across-season analysis suggest no difference in the sex ratio over the season or between seasons (GLM; all $p > 0.300$; Fig. 4.4). However, these results are likely to be an artefact of sample size and gonad deformations and should be interpreted with caution. Applying the backward selection model (i.e. *model 1*) with rank of time over the 2010 season data, the sex ratio for leatherback turtles was estimated to be 97.1 % (95 % CI 93.3 - 98.7) female-biased.

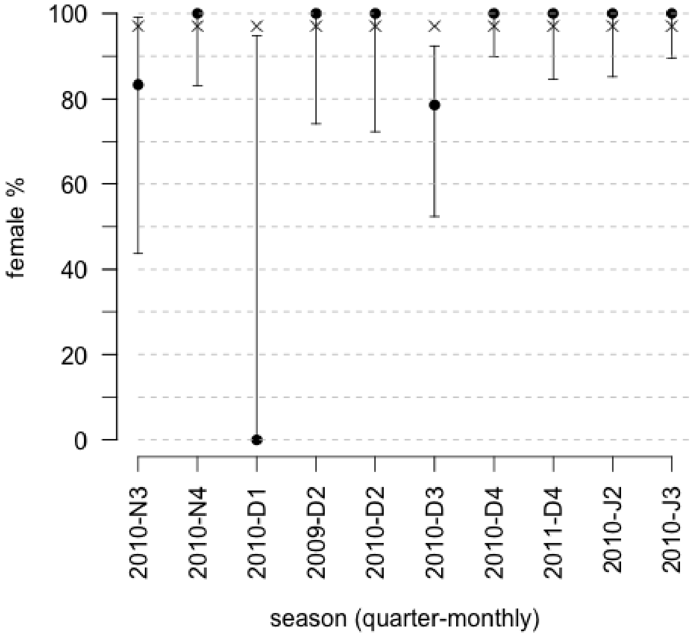


Figure 4.4: Leatherback sex ratio (black dot) plotted in quarter-month intervals over the course of the season. The season indicates date of oviposition (N-November, D-December, J-January; numbers indicate the quarter of the month). The 'X' (with 95 % CI) indicates the sex ratio estimates from the backward selection model *(model 1).*

4.3.3 Pivotal temperature

A total of 39 iButtons were successfully retrieved from loggerhead nests (ANNEX IV) and TSPs were estimated following Girondot and Kaska (2014). However, sex ratio was only determined for 12 nests (two nests in season 2009, four nests in 2010 and six in 2011; ANNEX IV). Consequently data (incubation temperature with sex ratio) of 12 nests were applied to assess pivotal temperature (PT) after Girondot (1999) using two different approaches. The first attempt used mean temperatures during the TSP and estimated sex ratios, i.e. 'CC (*in situ)*', and the second approach 'CC (CTE)' modelled sex ratio with CTEs. It was found that males are underrepresented in this study as estimated PTs for 'CC *(in situ)*' and 'CC (CTE)' were below minimum temperatures for successful embryonic development (Yntema & Mrosovsky 1980, 1982). Also, standard errors are very large (Table 4.4) which indicates that the data is insufficient for modelling PT. Thus, data obtained from a constant temperature incubation experiment were re-analysed 'CC Maxwell (1986)' and finally combined with 'CC (CTE)'. The combined, re-estimated pivotal temperature for the South African loggerhead population is 29.3 ± SE 0.01 °C (Table 4.4; Fig. 4.5).

Table 4.4: Summary of estimated pivotal temperatures (°C) for South African loggerhead and leatherback sea turtles (estimated with different data sets) and SE in parentheses. The re-analysed data from Maxwell thereby reflects original results after Maxwell (1986). Abbreviations are as followed: pivotal temperature - PT, transitional range of temperature - TRT and constant temperature equivalent - CTE.

PT	TRT	Data set
22.8 (100.98)	13.87 (59.48)	CC *(in situ)*
21.9 (159.25)	15.98 (93.44)	CC (CTE)
29.7 (0.01)	0.93 (0.01)	CC (Maxwell 1986)
29.3 (0.01)	2.50 (0.03)	CC (combination)
29.2 (0.35)	1.18 (0.25)	DC *(in situ)*
29.1 (0.17)	1.25 (0.08)	DC (CTE)

In total, 14 data loggers were successfully retrieved from leatherback nests (ANNEX V) and TSPs were approximated following Girondot and Kaska (2014)**.** Average incubation temperature (during TSP) and sex ratio of leatherback turtles were established for seven nests (one from 2009 and six from season 2010; ANNEX V). *In situ* PT for leatherbacks was estimated to be 29.2 ± SE 0.35 °C, whereas PT calculated with CTEs was assessed to be 29.1 ± SE 0.17 °C (Table 4.4; Fig. 4.6).

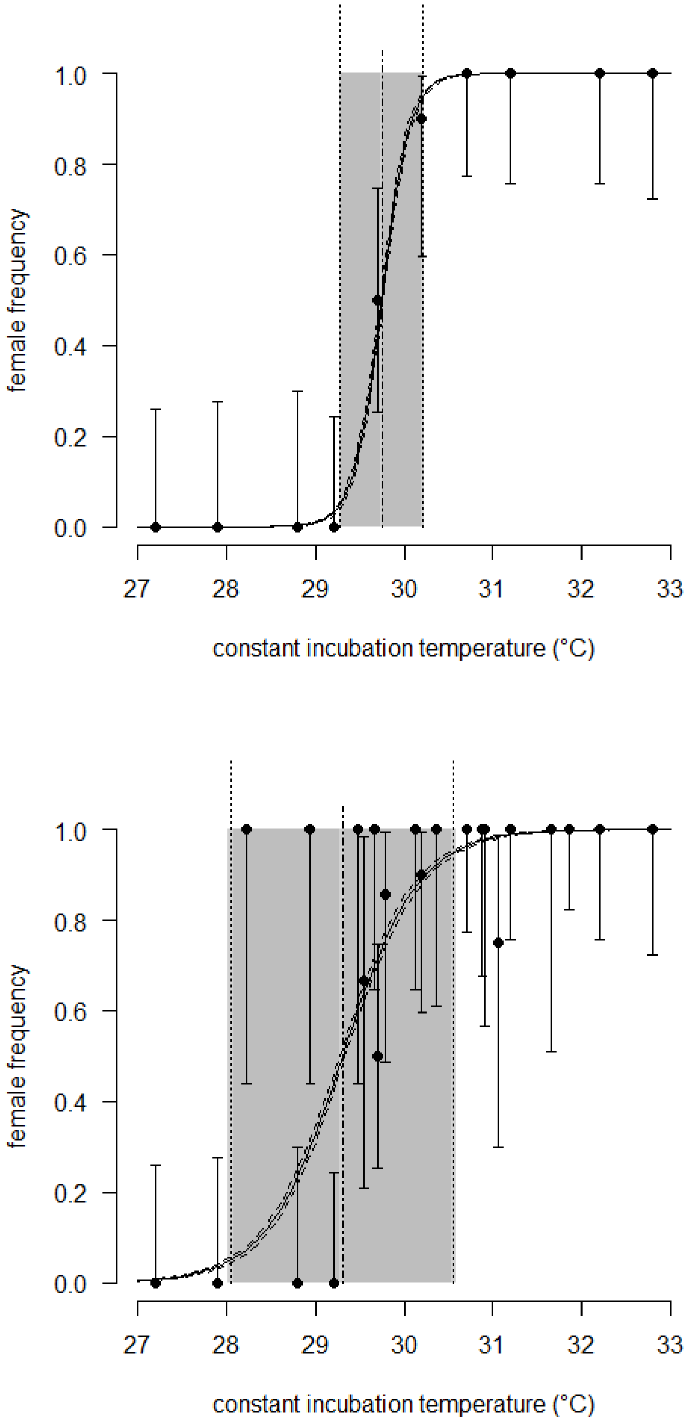
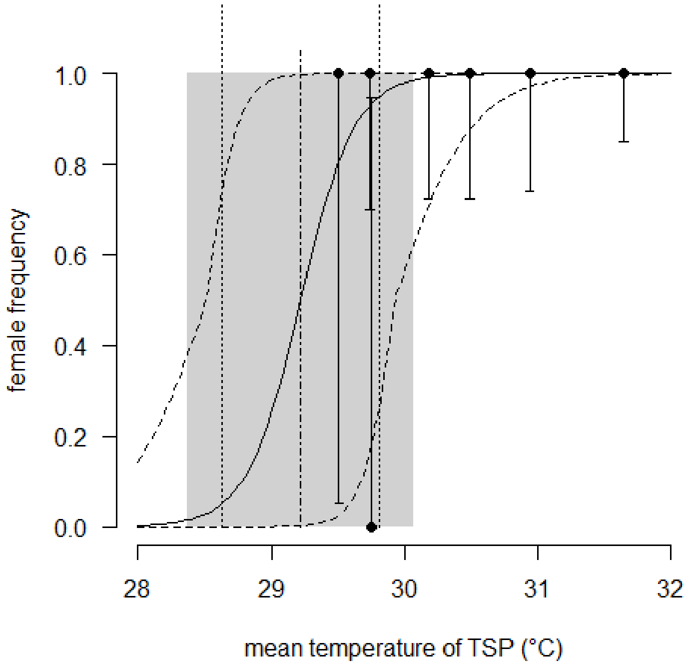


Figure 4.5: Pivotal temperature of the South African loggerhead turtle using data from Maxwell (1986) is displayed at the top. Results from the constant temperature experiment (Maxwell 1986) determined pivotal temperature (PT) at 29.7 °C. PT estimated from Maxwell (1986) and the present study (CTEs; bottom) is = 29.3 °C. The outer vertical dashed lines indicate the transitional range of temperature (TRT).



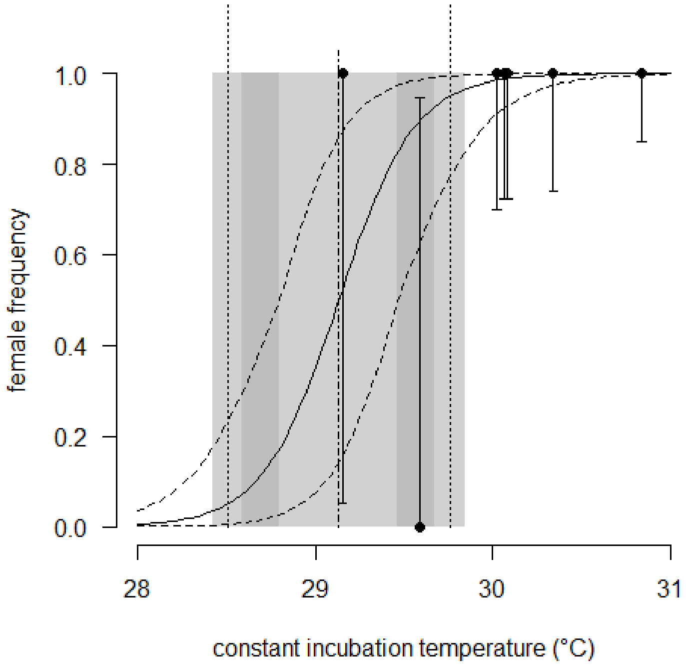


Figure 4.6: Using *in situ* nest temperatures pivotal temperature (PT) of the South African leatherback turtle was estimated at 29.2 °C (at the top). Applying constant temperature equivalents (CTEs) instead the estimated PT was 29.1 °C (displayed at the right). The outer vertical dashed lines indicate the transitional range of temperature (TRT) whereas 95 % CIs for PT and TRT overlap (in the picture at the top), but are displayed in light grey in the bottom figure.

4.4 Discussion

A variety of methods are applied to approximate pivotal temperature and sex ratio of sea turtles, but none of these estimates are straightforward. The most common technique to assign sex to individuals (hatchlings or late staged embryos) are gross-morphology (Wibbels et al. 1998, Wyneken et al. 2007, Ceriani & Wyneken 2008) or histology of the gonads (Yntema & Mrosovsky 1980, Whitmore et al. 1985, Mrosovsky & Benabib 1990). However, interpretation of the sex depends on the observer's skill and the method applied (Whitmore et al. 1985, Mrosovsky & Benabib 1990).

Other difficulties are found with estimating pivotal temperature (PT) obtained from constant incubation temperature experiments or *in situ* experiments (Marcovaldi et al. 1997, Kaska et al. 1998, Godley et al. 2002, Godfrey & Mrosovsky 2006), although those results are not comparable (Georges 1989, Georges et al. 1994, Georges et al. 2004). Conditions in constant temperature experiments can differ according to methodology and equipment; the accuracy of the incubator, sand texture, moisture, number of incubated eggs or a correction factor considering the cooling effect by evaporation can lead to different sex ratios at similar incubation temperatures (Limpus et al. 1985, Steyermark 1999, Godfrey & Mrosovsky 2006).

The applied statistics and different models to determine PT (e.g. simple method, maximum likelihood analysis or a sigmoidal curve) can also result in different PTs for the same dataset (Mrosovsky & Pieau 1991, Godfrey & Mrosovsky 2006). Thus, it is not surprising that PT in sea turtles, even of the same species, vary widely around the globe (ANNEX I). Godfrey and Mrosovsky (2006) also implied that *in situ* obtained PTs differ between populations not only because of methodology, but also in terms of impact of substrate, water content (i.e. hydric conditions) and non-random hatchling collection (for sex differentiation). Georges et al. (1994) also indicated that feminisation is enhanced with repeated large temperature fluctuations (around the mean) during the TSP. This variation may also explain the wide range of PTs (in general).

The most reliable results seem to be from constant incubation temperature experiments in combination with histology of the gonads (Godfrey & Mrosovsky 2006) and the sigmoidal curve method to approximate PT (Marcovaldi et al. 1997, Mrosovsky et al. 2002). Godfrey and Mrosovsky (2006) further suggested that estimated PTs for sea turtles aggregate around 29 °C when exactly the same methodologies are applied.

This study, however, applied a novel technique developed by Girondot and Kaska (2014) by which similar results can be obtained *in situ* (described in the introduction). The thermo-sensitive period

(TSP) is thereby determined from the developmental stage of the embryo (instead of the middle third incubation period) following a constant temperature experiment from Miller (1985). The only disadvantage of the model seems to be that it depends on temperature recordings only in the centre of the nest, as temperature decreases at the periphery (Maxwell et al. 1988, Kaska et al. 1998). Therefore, it has been assumed that fewer females are produced at the edge of the nest, as long as sand temperatures are not close to or above PT (Godfrey et al. 1997, Broderick et al. 2001). In addition to the technique developed by Girondot and Kaska (2014), the duration of the modelled TSP (instead of the mean incubation temperature or the incubation period) was used to convert *in situ* temperatures to constant temperature equivalents (CTEs). A comparison between *in situ* PTs and CTEs for the South African loggerhead (*in situ* 22.8 °C and CTE 21.9 °C) and leatherback (*in situ* 29.2 °C and CTE 29.1 °C) population confirmed that PTs obtained from diverse methodologies differ.

As sampled gonads were compromised it was assumed that approximated female sex ratios were overestimated in this study. Unfortunately, this complication affects estimates of the PT. It was attempted to correct the complication by being very conservative in sex assignment, by repeating the assignments with different observers and by including a previous study on South African loggerhead turtles (Maxwell et al. 1988) into the modelling. The final estimate suggests a loggerhead sex ratio (in 2009 - 2011) of 86.9 % female-biased. This study, however, was the first assessment of sex ratios on South African leatherback turtles, so no additional data were available. The sex ratio estimate for leatherback turtles was calculated at 97.1 % female-biased. Although it is supposed that those values are overestimates, they are still within the range of other populations (ANNEX I).

Further, corrections were done to estimate PT for the loggerhead population, whereby *in situ* obtained sex ratios and CTEs were combined with data from a constant incubation experiment by Maxwell (1986). The approximated PT (29.3 ± SE 0.01°C) is 0.4 °C lower than the previous estimate by Maxwell (1986) but consistent with those from other populations (ANNEX I). However, as Maxwell (1986) conducted a constant incubation temperature experiment with sex ratio differentiated by histology (with uncompromised gonads) it is concluded that the PT for the South African loggerhead population is closer to 29.7 ± SE 0.01 °C (Maxwell 1986; Table 4.4). PT of the South African leatherback population was estimated to be 29.2 ± SE 0.35 °C (CTE 29.1 ± SE 0.17 °C), which is slightly lower than the PT of other leatherback populations (ANNEX I); the Costa Rican and French Guiana populations, for example, have an estimated PT of 29.4 and 29.5 °C, respectively (Lescure et al. 1985, Rimblot-Baly et al. 1987, Binckley et al. 1998). It is suspected that the value of the PT for the South African leatherback population falls in that range (29.1 - 29.5 °C).

In conclusion, a novel approach to assess PT *in situ* was developed and tested. Results confirmed that *in situ* obtained PT is not comparable with those from constant incubation experiments. However, it is possible to transfer *in situ* recorded temperatures to CTEs, for example by the length of the estimated TSP. Further, it is reasoned that PTs will remain as estimates (around the mean), as it is not possible to account for all factors that affect incubation temperature (such as substrate, hydric conditions, temperature fluctuations) and their resultant effects on sex ratios.

The hatchling production for the South African loggerhead and leatherback sea turtle populations thus both seem severely female-biased. However, even though a female-biased population is assumed to facilitate (short to medium term) population increase, it is clearly no guarantee that a population will grow, as demonstrated by the South African leatherback population, which has maintained a more or less stable size for the last 40 years.

4.5 References

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Chapter 5: The effect of climate change on species with temperature-dependent sex determination (TSD)

Abstract

Climate change affects the phenology, survival and distribution of many species and poses a particular threat to species with temperature-dependent sex determination (TSD) like sea turtles. Increased incubation temperatures induce feminisation, decrease hatching success and even manipulate the phenotype of hatchlings. As the effects of climate change are not uniform across species or habitats the consequences on individual sea turtle populations should be examined. Sex ratios of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle population (from the most southern rookeries in the world) were investigated to determine the level of feminisation and to establish a baseline to which future sex ratios can be compared. Annual sex ratios were approximated from historical air and sea surface temperatures (1997 - 2011) but no significant trend in the sex ratio of the South African loggerhead (linear regression; $p = 0.45$) or leatherback (linear regression; $p = 0.47$) population during this period was found, which suggests that feminisation is not a recent development. The baseline average over 15 years was estimated for loggerheads at a 77.1 ± SE 3.36 % female bias and for leatherback sea turtles at 99.5 ± SE 0.24 % female-biased. Additionally, it is concluded that sea turtles use nest site selection to increase their own fitness (i.e. contribution of offspring to future generations) by increasing hatching success, whereby sex ratio is indirectly balanced. Thus, it is suggested that new nesting beaches will be established if successful development (including an appropriate sex ratio) can no longer be guaranteed, i.e. increased sand temperatures or unfavourable hydric conditions.

5.1 Introduction

Climate change describes a lasting change in modal weather conditions, which is usually measured by changes in patterns of season, temperature, precipitation, humidity and wind. Environmental responses to the current climate change regime have already been observed, with *inter alia* a pole-ward shift of isotherms and a concomitant change in habitats and species distribution (Hughes 2000, Walther et al. 2002, Root et al. 2003, Davies et al. 2006, McMahon & Hays 2006). Such a pole-ward shift was shown for 35 non-migratory European butterfly species which moved the range of their distribution 35 - 240 km northwards (Parmesan et al. 1999). Climate change has also induced shifts in the phenology of species (Parmesan & Yohe 2003, Pike et al. 2006, Hawkes et al. 2007). Zhu et al. (2012) investigated 73 plant types at 802 sites across North America and suggested that the growing season has extended due to a protracted autumn. Roy and Sparks (2000) found that a temperature increase of 1 °C advanced the arrival of butterflies in Great Britain by 2 - 10 days and Both et al. (2004) demonstrated that *Ficedula* flycatchers across Europe reproduce earlier as spring temperatures have increased. However, not all species will be able to adapt to climate change.

The current challenge of climate change is the accelerated rate brought on by human activities and the concomitant habitat destruction (i.e. global change). For example, the burning of fossil fuels along with deforestation and development (destroying natural vegetation) lead to an increase in atmospheric CO_2 and other greenhouse gases (Vitousek 1994, Pierrehumbert 2006, Forster et al. 2007). The progress of current climate change is observed in global temperature increase, CO_2 increase, ocean acidification, sea-level rise, and the amplification of acute weather events such as extreme temperatures and increased storminess, plus changes in precipitation inducing drought and wet periods (Easterling 2000, IPCC 2013). Because of the rate of climate change it is expected that many species are not or will not be able to adjust, which may lead to mass extinctions in the anthropogenic era (Thomas et al. 2004, Parmesan 2006, Willis et al. 2008). Polar bears are a classic example as they depend heavily on Arctic sea ice for their survival. However, with rising temperatures the ice breaks up, platforms drift further away and their natural habitat is lost (Hsiung & Sunstein 2007). Another example is the British ring ouzel, a thrush. Their population decreased by 42 % between 1988 and 1999. The proposed cause is extreme temperatures and rainfall regimes which led to a decrease in food availability (Beale et al. 2006).

Species with temperature-dependent sex determination (TSD), like sea turtles, are particularly vulnerable to climate change as incubation temperatures and sex ratio are dependent on ambient temperature (Janzen & Paukstis 1991, Janzen 1994a, Miller et al. 2004). As different patterns of TSD exist (Fig. 5.1) some species would endure feminisation and others masculinisation. It is also implied that sex ratios would be skewed at different rates due to the width of the transitional range of temperature (TRT)[25]. Hulin et al. (2009) suggested that species with a wide TRT are more likely to adjust to new thermal conditions than those with a very narrow transitional range. Furthermore, species with a short life cycle and short generation span are more likely to go extinct as populations are likely to reach a severely skewed sex ratio quicker than those which are long-lived and late-maturing (O'Grady et al. 2008, Mitchell & Janzen 2010). The latter group might be able to re-balance the sex ratio at a later stage. Further, it is assumed that species, which exhibit a female bias, would have an advantage with a likely increase in reproductive output and the possibility to recover (or normalise) over time (Miller et al. 2004, Mitchell & Janzen 2010).

Increased incubation temperatures, however, decrease reproductive success (Janzen 1994b, Matsuzawa et al. 2002, Fuentes et al. 2011) by affecting the offspring phenotype. In the case of sea turtles, increased numbers of female hatchlings are produced at higher incubation temperatures but high incubation temperatures may also lead to an increased number of deformities, as well as lower swimming performance, post-hatchling survival and post-hatchling growth (Du & Ji 2003, Booth et al. 2004). In green turtles high incubation temperatures may also result in smaller offspring, with poor crawling abilities but with good swimming performances (Burgess et al. 2006, Ischer et al. 2009).

Sea turtles are long-lived, late-maturing reptiles with TSD (Bull & Vogt 1979, Mrosovsky 1980, Wyneken et al. 2007) and female-biased hatchling production around the globe (ANNEX I). However, a biased sex ratio might be the ‘default state’ that corresponds to environmental sex determination (ESD; Charnov & Charnov 1982, Charnov & Bull 1989, Korpelainen 1990). Nonetheless, increasing temperatures resulting from climate change could induce absolute feminisation. It is already predicted that absolute feminisation might be as rapid as the next 50 - 100 years for some sea turtle populations (Hawkes et al. 2007, Fuentes et al. 2010, Fuentes & Porter 2013). A robust ‘baseline’ would be useful to indicate the present situation and future changes in sex ratio (Hays et al. 2003a, Pinnegar & Engelhard 2007). However, current baselines are usually measured against previous reference points, which themselves may not represent the original state, resulting in a gradual shift of the baseline perception (Pinnegar & Engelhard 2007).

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[25] The transitional range of temperature (TRT) specifies the temperature range which produces 100 % females to 100 % males *(vice versa;* Valenzuela 2004, Hulin et al. 2009).

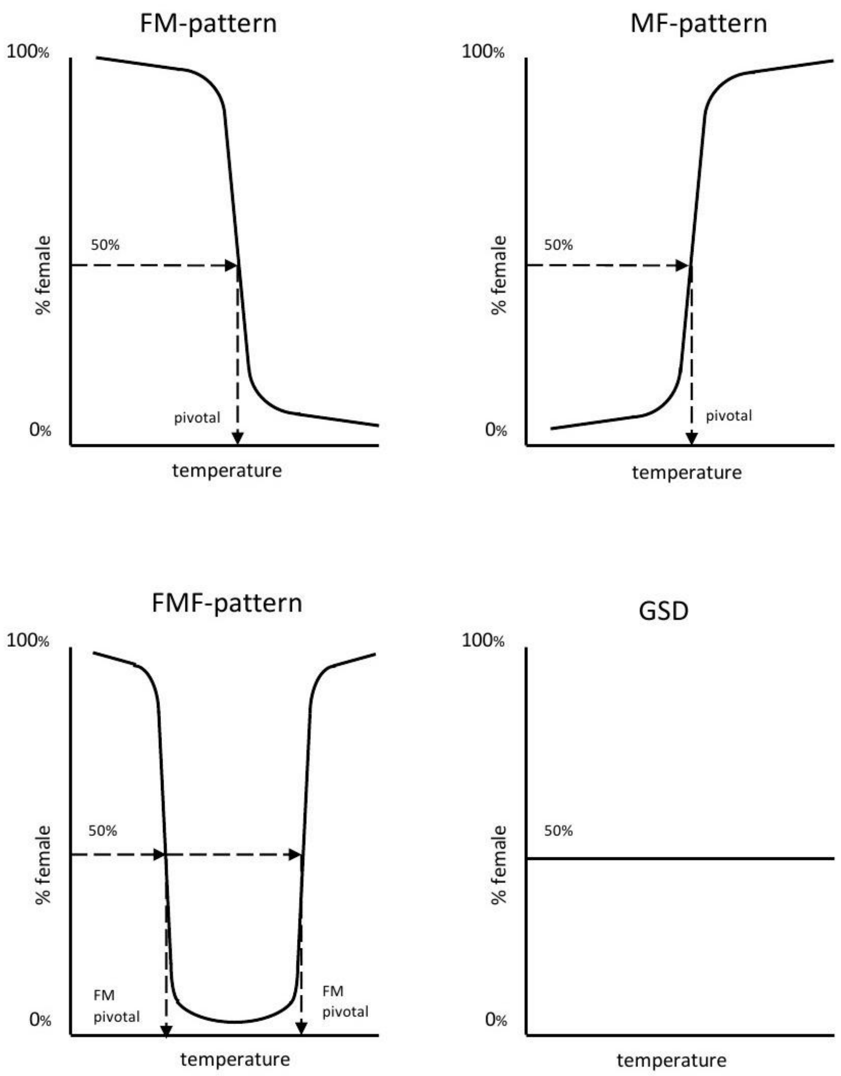

Figure 5.1: Different patterns of sex determination in reptiles (re-drawn following Bull 1980). Sex ratio is expressed as female percentage in dependence of incubation temperature (°C). Contradictory to the temperature-dependent profiles (FM, MF, FMF) displayed here the sex ratio in species with genotypic sex determination (GSD) remains unchanged with changes in temperature. (Abbreviations are as followed: FM = female-male, MF = male-female and FMF = female-male-female.)

A few studies have attempted to estimate past baselines using a variety of different approaches. Godfrey et al. (1996) used daily rainfall (which affects ambient temperatures) from 1981 to 1993 to approximate sex ratios for a green turtle population nesting in Suriname. Annual hatchling sex ratio estimates spanned a 20 - 90 % female bias. Hawkes et al. (2007) estimated annual sex ratio of a loggerhead population in North Carolina from daily average air temperature (1980 to 2005). There was no change in sex ratio over time with a reasonably consistent annual female production of 58 %. Fuentes et al. (2010) approximated sex ratios of green turtles on the Great Barrier Reef from 1990 onwards using daily average air and sea surface temperatures (SSTs). Results indicated a current female bias without a significant change in incubation temperature over the past 18 years. Hays et al. (2003a) used a similar approach, e.g. daily average air temperature, to model nest temperatures since 1855 for green turtles on Ascension Island. Estimated nest temperatures displayed a general warming trend (≥0.36 °C) over the last 100 years.

Rouault et al. (2010) detected an increase of 0.25 °C per decade (1982 - 2009) of summer sea surface temperature (SST) in the Agulhas Current, bounding the South African sea turtle nesting beach. As sea turtle nest temperature is a function of air temperature and SST (Fuentes et al. 2010, Girondot & Kaska 2014b) this temperature change in the Agulhas Current could induce further feminisation of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* population. The aims of this chapter are i) to define a 'baseline' to which future sex ratios can be compared and ii) to discuss possible adaptations of sea turtles to climate change (based on a literature review).

5.2 Material and Methods

Annual hatchling sex ratios over the past 15 years (1997 - 2011) were estimated in order to establish if there is a change over time and to ascertain a 'baseline' for future comparisons. Records on daily average air and sea surface temperature (together with recorded nest temperatures in 2008 - 2011) were applied to approximate daily nest temperatures (from 1997 - 2011), as well as the nest specific TSP and sex ratio. As the methodology is complicated, the approach was broken down into a flow diagram (with numbered paragraphs and displayed step by step in Fig. 5.2). All analyses were conducted in R version 3.0.1 software (R Core Team 2013) with $\alpha = 0.05$ (when necessary). The same methods were applied for both loggerhead and leatherback sea turtles. Any differences between species are described in the text.

1-2: Estimating daily mean air temperature from a daily minimum and maximum

Air temperature was recorded for three nesting seasons (2009 - 2011)[26] using iButtons (from Fairbridge Technologies, DS1922L-F5#, ±0.5 °C accuracy; frequency 30 minutes)[27] at the Bhanga Nek Research Station (S 27.4770, E 32.5980). However, a longer continuous data set in proximity of the nesting beach was available from the South African Weather Service for the Mbazwana Airfield

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[26] Unfortunately, the iButton in season 2010 corroded and temperature data are thus not available. In total, air temperature at Bhanga Nek was recorded for 235 days.

[27] See CHAPTER 4 for further information on the iButton and adjustments to the recordings.

(S 27.0123, E 32.8662) which recorded daily minimum and maximum temperature from 1997 onwards. In order to estimate a daily mean from daily min-max temperature data, the usefulness of the approach had to be verified first. A generalized linear model (GLM) with a Gaussian distribution and an identity link function was applied to model daily mean air temperature (daily_mean_AIRT) recorded at Bhanga Nek from daily minimum (daily_min_AIRT) and maximum values (daily_max_AIRT):

$$\text{daily\_mean\_AIRT} \sim \text{daily\_min\_AIRT} + \text{daily\_max\_AIRT}$$

3: Estimating daily mean air temperatures from 1997 to 2012 at Mbazwana Airfield

The daily mean air temperature at the Mbazwana Airfield (1997 - 2012) was approximated from daily minimum and maximum values (using the model described in section 1 - 2).

4-5: Model for daily nest temperature (2008 - 2011) based on air and sea surface temperatures

The daily mean temperature recorded in each nest (dNEST)[28] was fitted into a GLM following Girondot and Kaska (2014b), which assimilates daily air and sea surface temperatures:

$$\text{dNEST} \sim \text{dAIRT}_{\text{j\_lagged}} + \text{dSST}_{\text{k\_lagged}} + \text{dAIRT}_{\text{lagged}} \text{ x } \text{dSST}_{\text{lagged}} + \text{dMH}$$

In this model *dAIRT* stands for daily mean air temperature, *dSST* represents daily mean sea surface temperature[29] and *dMH* corresponds to daily mean metabolic heat. Metabolic heating is added during incubation as a linear increase of temperature from +0 °C (beginning of incubation) to +dMH °C (end of incubation). It was further considered that the effect of *SST* or *AIRT* on *NEST* could be lagged by *j* and *k* days respectively (e.g. nest temperature at day *d* is linked to *SST j* days before day *d*, as well as to *AIRT k* days before day *j*). A temporal autocorrelation was introduced into the model for *dNEST* and the identity of each nest was used as a random factor. A general linear mixed model (GLMM) was fitted using a Gaussian distribution with an identity link and pseudo-likelihood (Wolfinger & O'Connell 1993). The value of the autocorrelation was adjusted during the fitting process. The GLMM ran with *j* and *k* (lagged daily effect for air temperature and SST, respectively) varying from 0 to 10. The final combination of *j* and *k* retained is the first one with a significant signal for all co-variables.

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[28] In total iButtons from 39 loggerhead loggerhead nests and 14 leatherback nests (season 2008 - 2011) were successfully retrieved and used for further analyses. The procedures of iButton placement and adjustments to the recordings are described in CHAPTER 4.

[29] Daily sea surface temperatures were obtained from *NOAA/OAR/ESRL PSD, Boulder, Colorado, USA (www.esrl.noaa.gov/psd).*

6: Estimating daily mean sand and nest temperatures from 1997 - 2012

Time series of sand (with MH = 0) and nest temperatures at the end of incubation (using fitted MH values) were generated, for the period of 1997 to 2012, using the relationship between sand temperature, lagged SST and air temperature (section 4 - 5).

7: Reaction norm of embryonic growth rate as a function of incubation temperature

Based on the method described in Girondot and Kaska (2014a) results from 39 *in situ* obtained time series of loggerhead nest temperatures (and 14 time series for leatherbacks) were used to obtain a reaction norm model of embryonic growth[30] as a function of incubation temperature, incubation period and hatchling size. The dependency of embryonic growth rate on temperature *r(T)* is thereby expressed (following Johnson & Lewin 1946, Schoolfield et al. 1981) using a 4- and a 6-parameter equation (only the 6-parameter equation is displayed):

$$r(T) = \frac{\rho_{(24.85°C)}\ \dfrac{T}{298}\, exp\left(\dfrac{\Delta H_A^{\neq}}{R}\left(\dfrac{1}{298} - \dfrac{1}{T}\right)\right)}{1 + exp\left[\dfrac{\Delta H_H}{R}\left(\dfrac{1}{T_{1/2_H}} - \dfrac{1}{T}\right)\right]}$$

In this equation, *r(T)* equals the mean development rate at temperature $T(time^{-1})$, $p_{(24.85\,°C)}$ is the growth rate at 24.85 °C (assuming no enzyme inactivation), *T* represents the temperature in *K* (298 K = 24.85 °C), $\Delta H^{\neq}_A$ corresponds to the enthalpy of activation of the reaction catalyzed by the enzymes (J mol^{-1}), *R* equals the universal gas constant (JK^{-1} mol^{-1}), ΔH_L is the change in enthalpy associated with the low-temperature inactivation of the enzymes (J mol^{-1}), $T_{1/2_L}$ represents the temperature in *K* at which the enzymes are ½ active and ½ low-temperature inactive, ΔH_H corresponds to the change in enthalpy associated with the high-temperature inactivation of the enzymes (J mol^{-1}) and $T_{1/2_H}$ equals to the temperature in K at which the enzymes are ½ active and ½ high-temperature inactive.

To approximate changes in embryo size over time the equation by Schoolfield et al. (1981) was incorporated into a modified Gompertz model:

$$X(t) = K \exp\left(ln\left(\frac{X(0)}{K}\right)\exp(-r(T)t)\right)$$

In this model (by Laird 1964) *X(0)* represents hatchling size at oviposition (time = 0) which cannot be fitted from observation data. Thus, the diameter of the gastrula disk (1.7 mm; Kaska & Downie 1999)

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[30] It should be recognized that embryonic growth, as well as hatchling size, in this chapter refer to length measurements.

was used instead. Further, *r(T)* equals the growth rate dependent on the temperature and *K*, a factor that slows growth rate down towards hatching. Thus, *K* = *rK* [hatchling SCL], with *rK* = 2.0933, was applied following Girondot and Kaska (2014a). The dynamic of *X(t)* is thereby regulated by the Gompertz differential equation:

$$X'(t) = r(T)ln\left(\frac{K}{X(t)}\right)X(t)$$

In total 39 *in situ* obtained time series sets of loggerhead nest temperatures and 14 sets for leatherback turtles were used to establish a thermal reaction norm. The Runge-Kutta method of the fourth order was applied to approximate solutions of ordinary differential equations. Parameters of the parametric thermal reaction norm equation were fitted to adjust modelled hatchling size as close as possible to the actual observed value. Maximum likelihood, with an identity link and a Gaussian distribution of SCL (μ = 4.3 cm, σ = 0.19 cm and μ = 5.9 cm, σ = 0.23 cm, respectively), was used to estimate those parameters. The Akaike Information Criterion (AIC; Akaike 1974) was applied to evaluate the fit, whereby the goodness of fit is rewarded (-2 ln L: likelihood component of AIC formula) while additional parameters are penalised (2 p: number of parameters of AIC formula). Similarly, the 4- and 6-parameter models for the thermal reaction norm were chosen (based on AIC). Subsequently, parameters which describe the thermal reaction norm were fitted again using the Bayesian Monte-Carlo Method, with a Markov Chain (MCMC) to estimate their standard errors. Finally, the standard errors of the parameters were corrected for being calculated from time series (Plummer et al. 2006).

8: Simulation of incubation periods for 1997 - 2012

Through the estimated sand and nest temperatures (section 6) and the reaction norm model of embryonic growth as a function of incubation temperature (section 7), the incubation period for 20 eggs per day was simulated over a 16 years period. In doing so, parameters which describe embryo growth for each egg replicate were randomly obtained from a Gaussian distribution with the mean and standard error already estimated in section 7. The simulated size of each embryo during embryogenesis was used to define the egg specific thermo-sensitive period (TSP), i.e. stage 21 to 26 after Miller (1985).

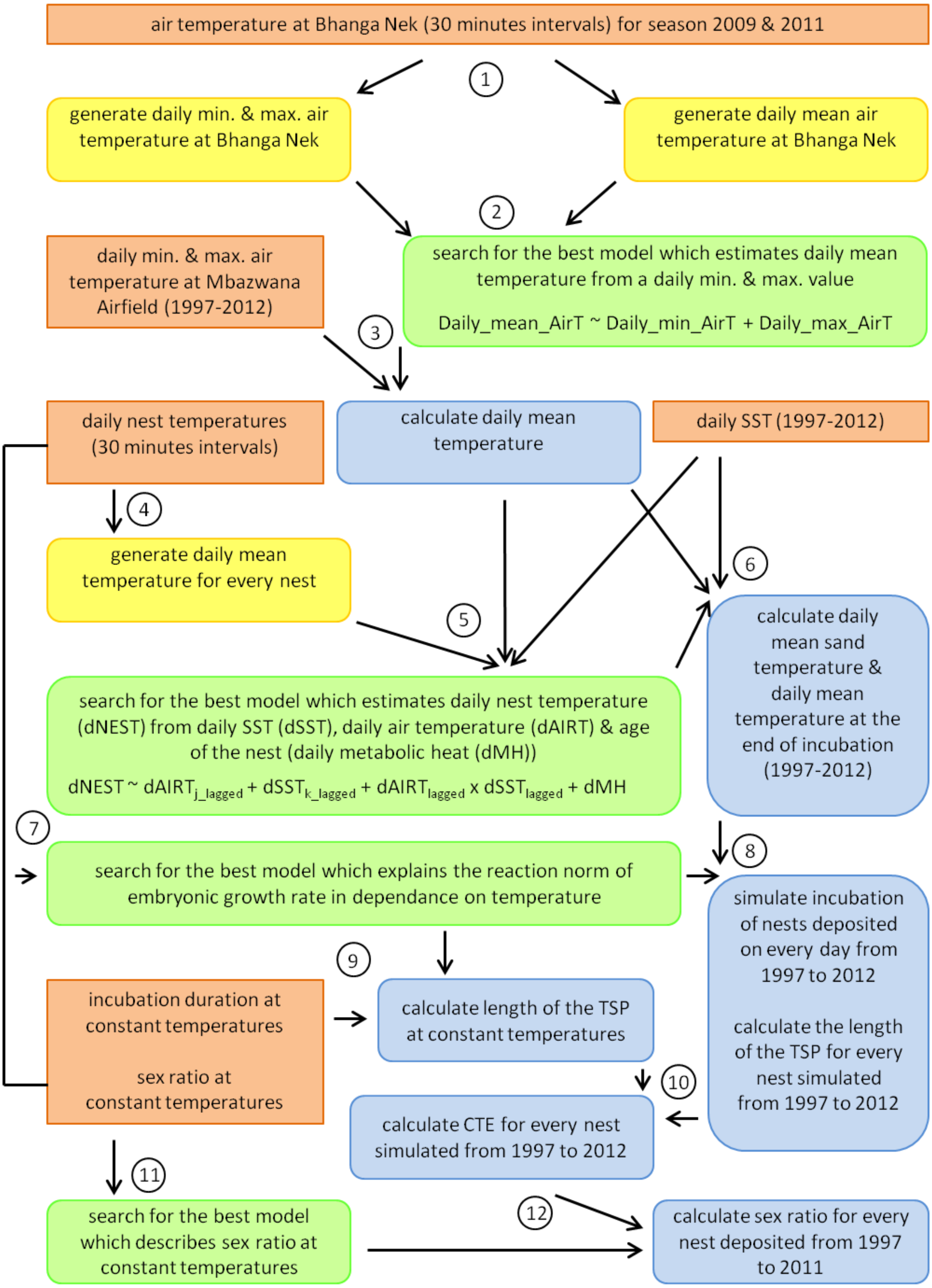

Figure 5.2: Graphical presentation on the process of estimating seasonal sex ratio in dependence on daily air and sea surface temperatures (SSTs) from 1997 - 2011. Squared orange boxes specify available data and rounded boxes generated estimates. Thereby yellow indicates the aggregation of data, green the process of model fitting and blue the analysis of previously obtained data. Abbreviations are as followed: thermo-sensitive period (TSP) and constant temperature equivalent (CTE).

9: TSPs at constant incubation temperatures

The growth pattern of embryos incubated at constant temperatures was modelled after the reaction norm in section 7. During the simulation process of embryonic growth the TSP was determined as described in section 8. A polynomial model to the 3rd order was applied to fit constant incubation temperature to the duration of the TSP.

10: Constant temperature equivalents (CTEs) for in situ simulated nest temperatures

The duration of the TSP of each nest which was simulated in section 8 was converted to a constant temperature equivalent (CTE) based on the relationship described in section 9. Thereby, the CTE acts as an integrated measure of the effect of temperature on embryonic growth.

11: Reaction norm of sex ratio according to constant temperature experiments

Constant incubation temperatures and sex ratios of loggerhead turtles were obtained from Maxwell (1986). Similar data for leatherback turtles were gathered from Rimblot et al. (1985), Rimblot-Baly et al. (1987) and Binckley et al. (1998)[31]. The data were fitted following Girondot (1999) where sex ratios were modelled against constant incubation temperatures using a logistic equation. The fitting criterion was based on maximum likelihood (of the observed number of males and females against the fitted ones) using a binomial distribution.

12: Sex ratio of the simulated nests from 1997 - 2011

The CTE (section 10), which mimics the pattern of embryonic growth, was estimated for every simulated egg (section 8). Those CTEs were then converted to sex ratios based on the relationship obtained in section 11. Sex ratio of every simulated nest deposited during any day between 1997 - 2011 was approximated. Furthermore, the number of nests (per day and per season) within the monitoring area is documented in the Ezemvelo database.

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[31] Leatherback data were either obtained from the publications or directly from the authors. Only data which were gathered under similar incubation conditions (i.e. incubated in sand) were included in the analysis. The data are however not from the South African population but from leatherbacks nesting in Suriname, French Guinea and Costa Rica.

The number of nests on every day during the nesting season was fitted using an equation which models seasonal occurrence at a migratory site (following Girondot 2010b). The system of equations used is best described as:

$$\begin{cases} t < B \rightarrow MinB \\ t \in [B, P - F/2] \rightarrow \left(\left(1 + cos\left(\pi\left(P - \frac{F}{2} - t\right)\left(P - \frac{F}{2} - B\right)\right)\right)/2\right)(Max - MinB) + MinB \\ t \in \left[P - \frac{F}{2}, P + f/2\right] \rightarrow Max \\ t \in [P + F/2, E] \rightarrow \left(\left(1 + cos\left(\pi(t - P + F/2)(E - P + F/2)\right)\right)/2\right)(Max - MinE) + MinE \\ t > E \rightarrow MinE \end{cases}$$

The model requires seven parameters (at most) and every parameter features a direct biological interpretation: *MinB* equals the mean nightly nest number before the beginning of the nesting season, *MinE* is the mean nightly nest number after the end of the nesting season, *Max* represents the mean number of nests at the peak of the nesting season, *P* is the day of the year on which the the nesting season peaks, *F* equals the number of days around day *P* on which the curve of the graph flattens, *B* corresponds to the day of the year on which the nesting season begins and *E* is the day of the year on which the nesting season ends.

Whereas a nesting season is usually described by segments, this model assembles all segments in continuity and thus defines a nesting season as the interval [*B*, *E*]. Various constraints can be set up to simplify this model: *MinB* = *MinE* for the same number of nests outside of the nesting season, *MinB* and/or *MinE* = 0 when no nests are observed outside of the nesting season, *L* = *P* - *B* = *E* - *P* when the nesting season is symmetric around *P* with *L* being half the length of the nesting season or *F* = 0 if there is no flat portion.

The parameters of this equation were fitted using maximum likelihood and a negative binomial distribution for the amount of daily deposited nests. As zero nest counts were never reported conditional probabilities were used for the likelihood to avoid a bias (Girondot 2010a)[32]. With the knowledge of the number of nests deposited on each day from 1997 - 2011 and its predicted sex ratio it was possible to estimate annual sex ratios since 1997, which is the daily sex ratio weight by the relative number of nests for this day among all the nests of the season.

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[32] This was done under the assumption that sporadic sea turtle nesting also occurs outside the monitored time period, i.e. before mid-October and after mid-March.

5.3 Results

The relationship between the observed daily mean air temperature at Bhanga Nek and the calculated daily mean air temperature, using only the minimum and maximum values, was very strong ($r^2 = 0.937$; Fig. 5.3). A correlation between daily mean air temperatures calculated for the Mbazwana Airfield and Bhanga Nek was also strong ($r = 0.597$, $p << 0.001$; Fig. 5.4). Given that the Mbazwana Airfield is only about 3 km away from the nesting beach, and the time series available (1979 onwards) is longer for the Mbazwana Airfield, the daily mean air temperatures estimated for the Mbazwana Airfield were used in the analyses as an approximation of air temperature at the nesting beach.

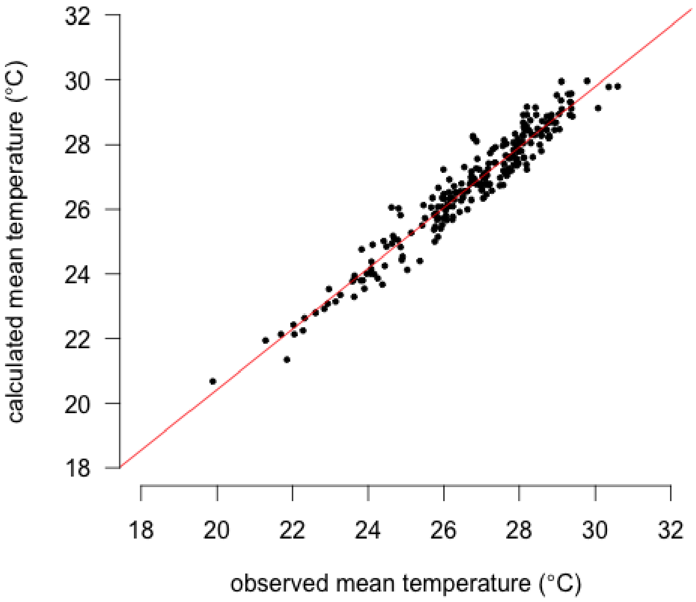

Figure 5.3: Regression of daily mean temperature (°C) observed at Bhanga Nek (during season 2009 and 2011) with a daily mean temperature (°C) calculated from daily minimum and maximum values ($r^2 = 0.937$, $y = 0.937x + 1.675$).

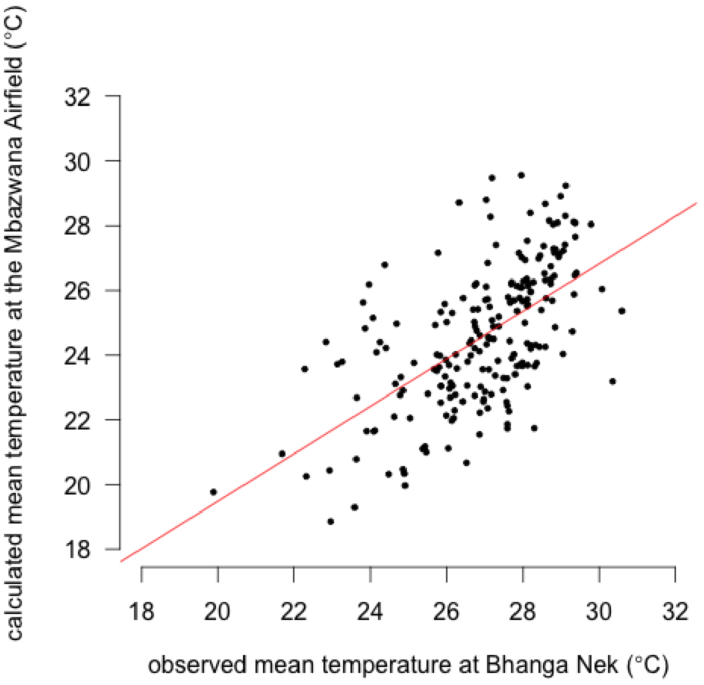

Figure 5.4: Correlation of daily mean temperature (°C) observed at Bhanga Nek (during season 2009 and 2011) with calculated daily mean temperature (°C) at the Mbazwana Airfield ($r = 0.597$, $y = 0.734\,x + 4.800$, $p << 0.001$).

Daily mean air temperatures (Mbazwana Airfield), daily mean SSTs and the age of the nests[33] were fitted to recorded daily mean nest temperatures from 2008 - 2011. There was a strong positive relationship between the observed nest temperatures and estimated nest temperature values for the same time period (loggerhead $r^2 = 0.501$ and leatherback $r^2 = 0.704$; Fig. 5.5).

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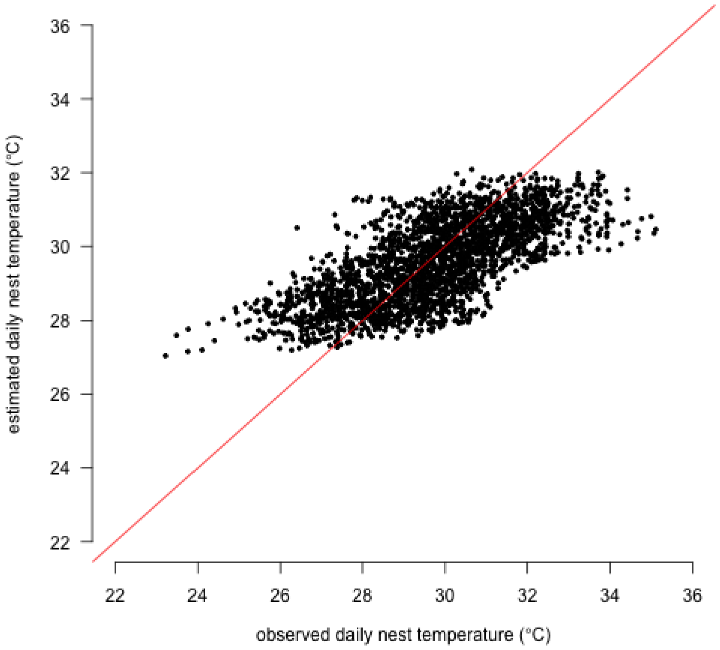
[33] During the incubation period metabolic heat builds up inside the egg chamber (before it drops just before hatching). As metabolic heat was recorded for the entire incubation period it was used to age the nests.



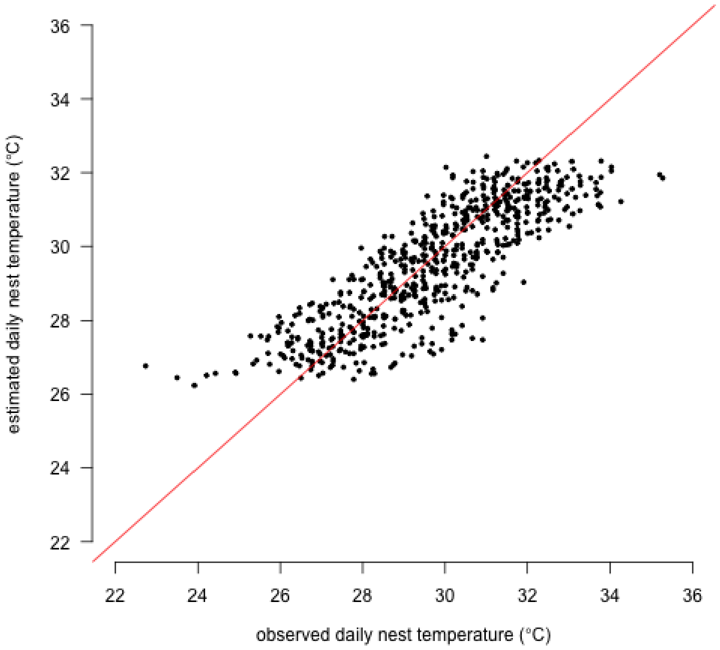


Figure 5.5: Illustration of the relationship between daily observed mean nest temperatures (°C) and estimated daily mean nest temperatures (°C) from air and sea surface temperatures. The regression at the top displays the fit for loggerheads (season 2008 - 2011, $r^2 = 0.501$, y = x, n = 39) and the figure at the bottom for leatherbacks (season 2009 - 2011, $r^2 = 0.704$, y = x, n = 14).

Daily mean sand (i.e. beach) and nest temperature profiles were established for the period 1997 - 2012 based on the relationship between sand temperature, lagged SST and air temperature. The established reaction norms were further used to model embryo growth patterns at constant incubation temperatures. In addition, a polynomial model to the 3rd order (CHAPTER 4; Fig. 4.1) was applied to convert the durations of the TSPs of the simulated nests (1997 - 2011) to constant temperature equivalents (CTEs). The relationship between constant incubation temperature and sex ratio (for loggerhead and leatherback turtles) was modelled from constant incubation temperature experiments (Fig. 5.6). Consequently, the CTE of every simulated nest (1997 - 2011) was assigned to a sex ratio which was multiplied with the approximate number of deposited nests (for each day from 1997 - 2011) to establish annual sex ratios (Table 5.1 and Figure 5.7). No significant change in sex ratio over time was found for the South African loggerhead or leatherback population (loggerhead ANOVA: F (1, 13) = 0.62, p = 0.45 and leatherback ANOVA: F (1, 13) = 0.55, p = 0.47). The average sex ratios for this study (1997 - 2011) were estimated at 77.1 ± SE 1.86 % female-biased for loggerhead and 99.5 ± SE 0.24 % female-biased for leatherback turtles.

Table 5.1: Estimated annual sex ratio (female percentage) of the South African loggerhead and leatherback sea turtle population dating back to 1997.

Season	Loggerhead sex ratio (SD)	Leatherback sex ratio (SD)
1997	79.9 (1.91)	98.5 (0.39)
1998	86.2 (1.81)	100.0 (0.18)
1999	87.0 (1.99)	100.0 (0.24)
2000	63.0 (1.99)	99.9 (0.45)
2001	71.3 (1.99)	99.9 (0.51)
2002	90.5 (1.50)	99.7 (0.29)
2003	80.3 (1.85)	99.9 (0.47)
2004	90.5 (1.71)	100.0 (0.13)
2005	76.0 (1.97)	99.8 (0.34)
2006	79.9 (1.50)	99.3 (0.28)
2007	52.6 (1.91)	98.6 (0.69)
2008	76.7 (2.29)	99.9 (0.28)
2009	49.5 (2.36)	96.6 (0.65)
2010	90.9 (1.49)	100.0 (0.19)
2011	81.7 (1.66)	100.0 (0.24)

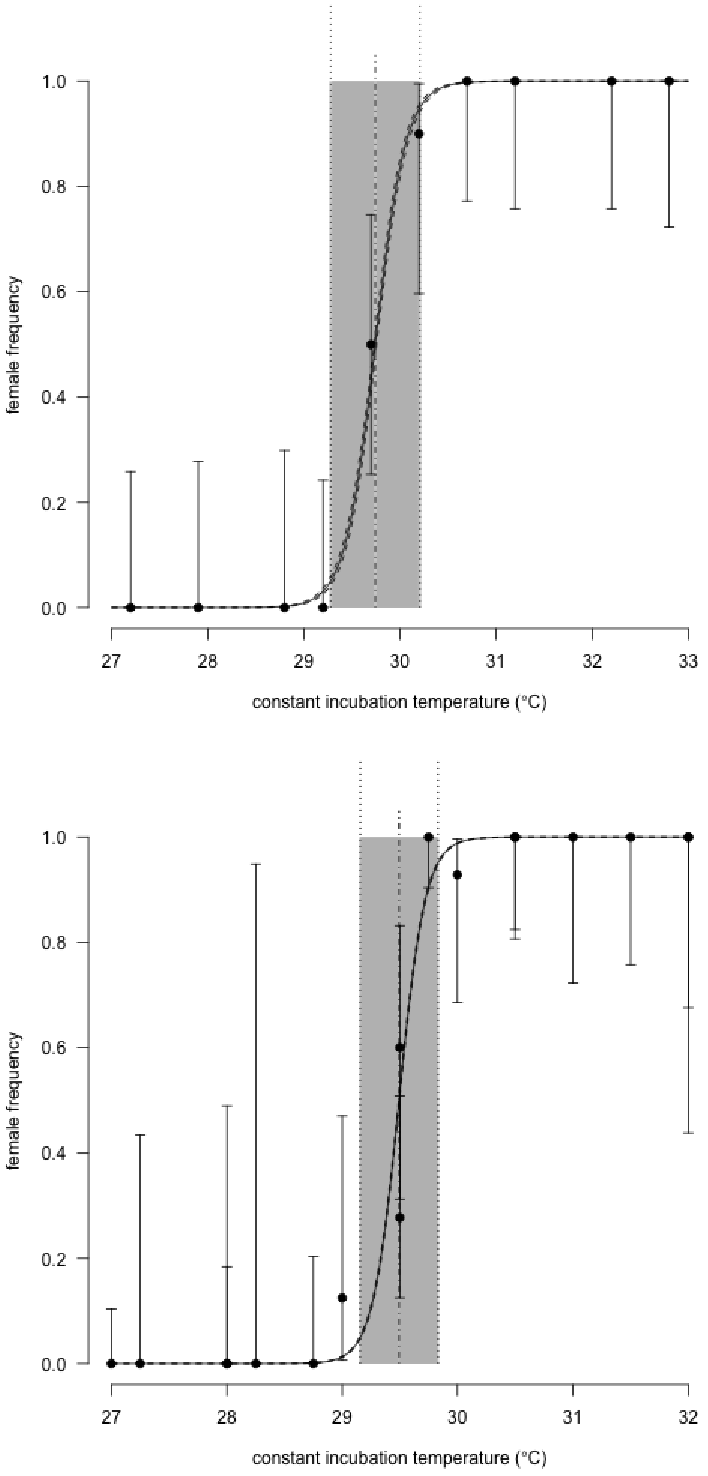
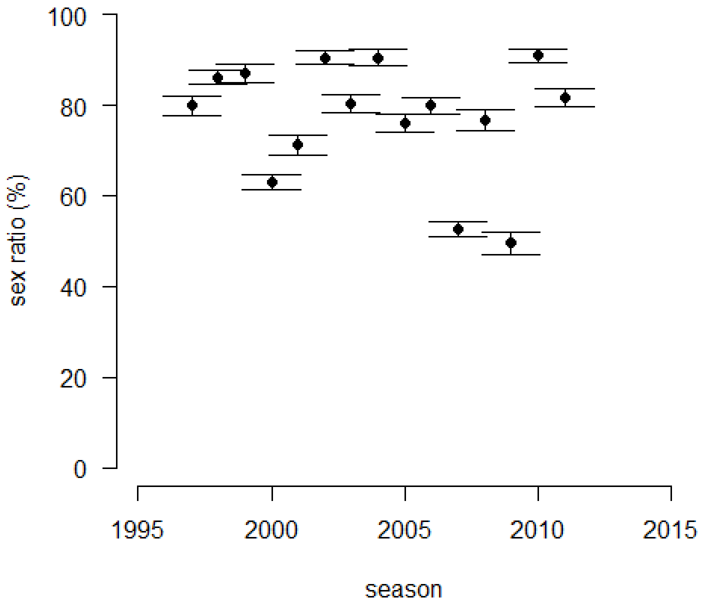


Figure 5.6: The relationship between incubation temperature (°C) and sex ratio (female %) of the South African loggerhead population (after Maxwell 1986) at the top and the same for a South American leatherback population (after Rimblot et al. 1985, Rimblot-Baly et al. 1987, Binckley et al. 1998) at the bottom. Thereby a 1 : 1 sex ratio is obtained for loggerheads at a pivotal temperature of 29.7 ± SE 0.01 °C and for leatherbacks at 29.5 ± SE 0.00 °C. The transitional range of temperature (range of 0 - 100 % female frequency) is displayed in grey.



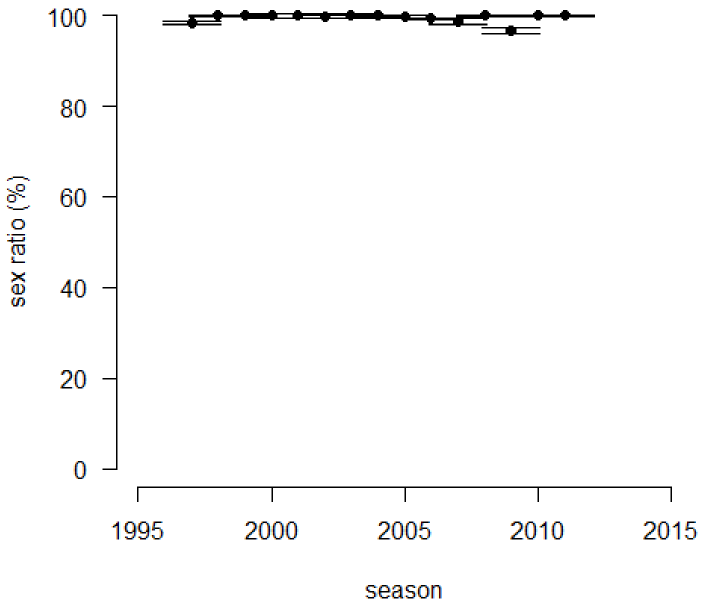


Figure 5.7: Approximated annual sex ratio (±SD; 1997 - 2011) for the South African loggerhead population at the top and for leatherbacks at the bottom.

5.4 Discussion

One of the main problems in conservation is the 'shifting baseline syndrome' (Pauly 1995), which accepts that the current state is also representative of the past (Pinnegar & Engelhard 2007). But in many cases the pre-human state has been severely altered and the converse is true. Any change or disruption in what appears to be the norm is frequently interpreted as human interference or the effect of climate change. For example, species from different taxa, including plants, nematodes, fish and amniotes with environmental sex determination (ESD) produce naturally biased sex ratios (Charnov & Charnov 1982, Charnov & Bull 1989, Korpelainen 1990). However, nowadays, a skewed sex ratio is often incorrectly interpreted as an effect of climate change. Climate change affects the environment in many different ways; for example, global warming, sea level rise, droughts, wet periods and storms (Easterling 2000, IPCC 2013). Species with ESD are more vulnerable and probably at a greater risk of extinction via climate change than those with genetic sex determination (in the context of a rapid changing environment).

The objective of this chapter was to evaluate if the sex ratios (obtained in CHAPTER 4) for loggerhead and leatherback turtles nesting in South Africa are a recent condition (i.e. an effect of climate change) or if it existed for at least the last 15 years. If sex ratios have been constant, the mean could serve as a baseline value against which future climate change impact studies can be measured. The main results of this chapter indicated that the current sex ratios have been similar for the last three decades (see next paragraph), with the average sex ratios estimated at 77.1 ± SE 1.86 % female-biased for loggerhead and 99.5 ± SE 0.24 % female-biased for leatherback turtles and with no trend over time (Fig. 5.7).

Many sea turtle populations around the globe display a female bias (ANNEX I). However, it is difficult to make direct comparisons between sex ratios from different populations, within or between species as there are many factors that affect sex ratio. These include the number of nests or seasons monitored (i.e. pseudo replication), variation in histological interpretations to identify sex, and the use of theoretical or estimated pivotal temperatures (PTs) from other populations (Wibbels 2003). This also applies to the South African loggerhead population. Maxwell et al. (1988) estimated sex ratio in season 1984 at a 1 : 1 ratio (i.e. 50 % females). The previous chapter (CHAPTER 4), however, estimated sex ratio from histology and phenology at 86.9 % female-biased. In the current study (CHAPTER 5) sex ratio approximate 77.1 % female bias based on simulations from data derived from a constant temperature experiment (Maxwell 1986). However, re-analyses of the field data obtained from Maxwell et al. (1988), i.e. *model 1* in CHAPTER 4, also estimated the sex ratio at 77.4 % female bias and a female bias of ~77 % seems realistic and has existed since 1984 (the year in which Maxwell et al. (1988) executed their field experiments and 30 years before the present study). It is concluded that there is no obvious effect of climate change on the sex ratio of loggerhead turtles in the South African population for at least three decades (Table 5.1 and Fig. 5.7).

Similar results were obtained by Hawkes et al. (2007) who recorded an increase in SST but no change in sex ratio for loggerhead turtles nesting in North Carolina (1980 - 2005). It must be mentioned though that Hawkes et al. (2007) estimated sex ratio from decreasing air temperatures while SSTs increased. Fuentes et al. (2010) reported no significant increase in beach temperatures at a green turtle nesting beach in Australia (1990 - 2008). Hays et al. (2003a) approximated an increase in nest temperatures of 0.36 - 0.49 °C for a green turtle population on Ascension Island over the last 100 years. Thus, even though sea surface temperatures increased at various locations, nest temperatures seem to have remained quite stable (i.e. sand temperatures at nest depth). It appears that feminisation by climate change is not yet apparent in sea turtle populations or that the observed periods (in contrast to Hays et al. (2003a)) are too short to draw any conclusions.

Sex ratio in a given turtle population is also a function of location. Sea turtle populations closer to the equator are expected to be more female-biased due to consistent, elevated temperatures, but as one moves to more temperate latitudes, population sex ratios should become more balanced (Mrosovsky 1988, Marcovaldi et al. 1997). South Africa represents the most southern rookery for loggerhead and leatherback turtles in the world (Wallace et al. 2010) and is assumed to be buffered against the temperature effect of climate change. Developing a ‘baseline’ against which potential future climate-induced changes in sex ratios could be measured seems appropriate and potentially advantageous. It is proposed that the mean annual modelled sex ratios (±SD; 1997 - 2012) from this study present a suitable baseline (loggerhead sex ratio was estimated at 77.1 ± 1.86 % and that of leatherbacks at 99.5 ± SE 0.24 % female-biased) against which future data can be compared.

Operational sex ratios are not directly related to the demographic sex ratio. Sea turtles practice polygyny (Crim et al. 2002) and males possibly re-migrate on an annual basis (Miller 1997) and female re-migrations only every second or third year or more. Thus, female-biased sex ratios, help to maintain a healthy population. However, considering the high annual mortality of sea turtles (Hays et al. 2003b, Koch et al. 2006), no population can maintain a 99.5 % female bias in the long term (Chan & Liew 1995, Chu et al. 2008). The reproductive success, and by implication fertilization, in the South African leatherback population is high (emergence success 73.6 %; CHAPTER 3), which implies a presence of male turtles in the nesting area. Brazier et al. (2012) further estimated a 2 : 1 adult male : female ratio caught in the shark nets off Durban. Another possibility to explain the absolute feminisation but high fertility might be that females are fertilized away from the nesting ground from males belonging to another population, such as Gabon in the Atlantic (as females regularily migrate as far as Angola; Nel 2009).

Although corrections were applied to this study it is recognised that the leatherback hatchling sex ratio is an overestimate because no population can sustain with a continuous 99.5 % female-bias. It is also recognised that this overestimation might be caused by the assumption that the South African and South American leatherback populations display the same PT (i.e. PT 29.5 °C). Another possible reason for the extreme bias could be the localised nature of the study (i.e. nests obtained from 56 km; beacons 32 N - 100 S) whereas nesting takes place over 300 km (Nel et al. 2013). Extending the study area southward may obtain results from cooler nests (i.e. more males; Mrosovsky 1988, Marcovaldi et al. 1997). A definitive answer on leatherback sex ratio is not available at this time and further research (e.g. an extended beach temperature profile) would be beneficial to determine *inter alia* the PT and the sex ratio bias for the South African leatherback population.

Assuming that feminisation of sex ratios will increase as a result of climate change (Mrosovsky 1984, Hawkes et al. 2007, Fuentes et al. 2010, Fuentes & Porter 2013) it is speculated that sea turtles might benefit from a shift to genetic sex determination (GSD; Janzen & Paukstis 1991, Pen et al. 2010). Pen et al. (2010) already demonstrated how two populations of the snow skink *(Niveoscincus ocellatus)* adjusted their sex-determining mechanism in accordance with the environment, with GSD operating in the highlands and TSD in lowlands. The genetic mechanism of TSD in sea turtles is still not well understood (Wibbels 2003) but several studies suggest that GSD might be expressed if nests incubate in an environment close to PT, while TSD is dominant at temperatures below or above pivotal temperature (Bull et al. 1982, Girondot et al. 1994). However, even if facultative GSD is expressed close to PT, it will be 'overwritten' by TSD because of rising environmental temperatures at nesting beaches. It may be advantageous for sea turtles to 'adjust' their PT in order to keep a more balanced sex ratio. However, it is suspected that the current rate of global warming is progressing too fast for sea turtles to adapt (Janzen 1994a, Doody et al. 2006, Hawkes et al. 2007).

Another potential mechanism through which sea turtles can respond to climate change is by colonizing new nesting habitats and so extending their geographic range (Morjan 2003, Ewert et al. 2005, Doody et al. 2006, Mrosovsky & Godfrey 2010). Colonization and the establishment of new founding populations is common in sea turtles and has occurred prior to the (current) climate change (Poloczanska et al. 2009). This is well-documented in the genetic relationships between populations

(Karl et al. 1992, Bowen et al. 1994, Shamblin et al. 2014), e.g. the South African loggerhead population descends from central Atlantic populations. Although natal homing does complicate the establishment of new nesting grounds (Hawkes et al. 2007), not all turtle nesting events are at the home beaches and beaches also do disappear. Recent shifts, possibly result from climate change, have already been observed (Hamann et al. 2007, Mrosovsky & Godfrey 2010).

Temporal alterations in the onset of the nesting season provide a mechanism by which sea turtles could acclimate to climate change. A changed phenology has already been observed in some populations which was speculated to be a response to changed environmental temperatures (Weishampel et al. 2004, Doody et al. 2006, Hawkes et al. 2007). Warmer sea water temperatures off the nesting beaches accelerated maturation of the eggs (Hays et al. 2002) which enabled earlier nesting.

The real questions, however, concern weather sea turtles choose their nesting habitat deliberately and whether they 'know' that the habitat is un-/favourable for successful development (Stoneburner & Richardson 1981, Schwarzkopf & Brooks 1987, Doody et al. 2006, Hamann et al. 2007, McGaugh et al. 2010). Because successful development depends on temperature, moisture and gas exchange (Miller 1997), amongst other factors. Are nesting females aware of marginal conditions that affect the fitness of their offspring when there is no parental care? The theory of maternal nest site selection is partly confirmed in other species with TSD (Ewert et al. 2005, Doody et al. 2006), for example in painted turtles. Painted turtles seem to regulate nest temperatures by choosing the amount of shade likely to be on the nest, as well as the distance to standing water. There are a number of factors that have been identified to affect nest site placement in sea turtles (which differ among species), but it is not known how deliberate these selections are. For example, leatherbacks tend to nest on open sand, whereas green turtles prefer vegetation (Whitmore & Dutton 1985) and loggerheads the edge of vegetation (Botha 2010).

Hughes (1974) noted that sea turtle nesting activities in South Africa accumulate at locations backed by coastal lakes. He also speculated that loggerheads 'sniff the sand' on the way up the nesting beach. It is suspected that sea turtles select nesting sites for specific sand temperature and moisture content (or some other factors not yet recognised). Further, it is assumed that nest site selection is used to increase offspring fitness (Stoneburner & Richardson 1981, Gutzke & Paukstis 1983, Ackerman et al. 1985, Gutzke et al. 1987, Mortimer 1990) and also indirectly affects sex ratio (but it is difficult to demonstrate this). Ultimately though, human-induced climate change is occurring at an accelerated rate (Brohan et al. 2006, Hawkes et al. 2009, IPCC 2013) and the response by marine turtles to changing environmental conditions will lead to changes in the sex ratios and probably their distribution. The question remains: Can sea turtle populations adapt quickly enough to climate change to survive?

5.5 References

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Chapter 6: The effect of age at reproduction on generation time

NOTE: The results of this chapter were published in a peer-reviewed journal. The authors were J. Tucek (student and first author) under supervision of Dr. R. Nel with statistical input by Prof. M. Girondot. The project was initiated and maintained over 30 years by Dr. G. Hughes who also provided input to the manuscript. However, the project was limited to loggerhead turtles. Thus for the purpose of this thesis the publication is augmented with information on leatherback turtles (based on a literature review). The additional information is displayed in *italic* and the original publication is attached as ANNEX VI.

Age-size relationship at reproduction of South African female loggerhead turtles *(Caretta caretta)*

Abstract

Average age and size at first reproduction are important demographic parameters used in the management and conservation of populations. For sea turtles absolute values for these parameters are still ambiguous as most species are slow-growing, late-maturing migrants, which are rarely encountered during the first 1 - 2 decades of their lives. Additionally, growth is significantly influenced by a variety of intrinsic and extrinsic factors which make it difficult to describe age-size relationships. Extensive notching (mutilation-tagging) of South African loggerhead turtles was used to determine age and size at reproduction (putative first nesting season), and thus to identify the trigger for sexual maturation. To date 137 clearly identified notched adult females have been encountered at the nesting beach. A Gaussian and log-normal distribution were fitted to the age distribution data but only the Gaussian distribution can be used to safely estimate age at first observation, as the right part ('older ages') of the distribution is still unknown. The estimated age at first observation was corrected for the possibility that a female was not encountered during its first nesting season. Results indicate an average age of 36.2 ± SD 7.71 years (95 % CI 28.2 - 44.3 years) with a straight carapace length (SCL_{min}) of 83.7 ± SD 4.15 cm (95 % CI 83.0 - 84.4 cm). Ultimately, it was concluded that size is a more important threshold for the initiation of the maturation process than age, and that the onset of sexual maturity is dependent on intrinsic and extrinsic factors. *Further, it is concluded that generation times for leatherback turtles are shorter than for other sea turtle species and that population growth of the South African leatherback population is suppressed by high offshore mortality of mature individuals (as the population is dominated by neophyte nesters). Ultimately, it is proposed that age at reproduction in long-lived, slow-growing, late-maturing species is generally under-estimated.*

6.1 Introduction

Age and size at first reproduction, reproductive lifespan, and the number and size of offspring produced per individual are fundamental life history characteristics required to model population dynamics or productivity. They are frequently used in conservation to model the likelihood of a population going extinct or in wild stocks to estimate the off-take (Scott et al. 2012), e.g. setting fishing quotas. Life history characteristics are easy to track in captive animals but it is much more difficult in wild populations (Scott et al. 2012), especially those of wide-ranging or migratory species. Due to the range of factors affecting individuals/populations it is also difficult to predict/model these parameters.

The innate life history characteristics of a species/population is highly modified by energy availability (Berner & Blanckenhorn 2007). Therefore, food accessibility and the rate at which an organism can process food (West et al. 2004, Molles 2010) regulate the metabolic investment of energy into reproduction or growth. Thus, the onset of sexual maturity (as indicated by a reduction in energy investment into somatic growth and a greater energy allocation into the growth and maturation of reproductive organs (Hatase et al. 2004, Berner & Blanckenhorn 2007, Snover et al. 2007, Bjorndal et al. 2012)) is dependent on both population characteristics (intrinsic) and the environment (extrinsic).

For sea turtles, growth rates, and thereby age and size at sexual maturity, are influenced by intrinsic and extrinsic factors. Intrinsic factors that affect the growth rate in sea turtles include species, genotype, health status and sex (Chaloupka & Limpus 1997, Van Dam 1999, Heppell et al. 2003). Extrinsic factors that regulate growth rate are, for example, water temperature, food quality and food availability (Parker 1929, Anderson & Cummins 1979, Temming 1994, Bjorndal et al. 2003, Balazs & Chaloupka 2004, West et al. 2004, Koch et al. 2007). These factors are easy to regulate in captive studies but are often highly variable and difficult to measure under natural conditions (Mendonca 1981) as sea turtles migrate over thousands of kilometres and experience large habitat variations that additionally affect their growth rate (Musick & Limpus 1997, Casale et al. 2007, Koch et al. 2007). The variability in growth rate has already been documented in different species and several populations. Green turtles from the Hawaiian Archipelago (Balazs & Chaloupka 2004) or the southern Great Barrier Reef (Chaloupka et al. 2004) grow at different rates due to differences in food quality and availability, as well as the different sea surface temperatures of their specific foraging grounds. Furthermore, Bjorndal et al. (2003) and Hughes (1974) found that compensatory growth (accelerated growth) appears in juvenile sea turtles when they move from a suboptimal environment to better conditions. Similarly, different sexes can follow different growth patterns, as shown for green (Chaloupka et al. 2004) and hawksbill turtles on the Great Barrier Reef (Chaloupka & Limpus 1997). Here immature female green turtles grow significantly faster than males after a juvenile growth spurt (>60 cm curved carapace length; CCL), resulting in sexual dimorphism in adult size (Limpus & Chaloupka 1997). Immature hawksbill females grow faster than males at all recorded sizes (Chaloupka & Limpus 1997). Although variability in growth rate in natural populations has been documented, the link between age and size at sexual maturity remains unidentified. Size *per se* is thus not a reliable indicator of age in turtles, but a minimum size should be reached before reproduction can take place (Hughes 1974).

Current methods used to estimate the growth rate of individuals in sea turtle populations require substantial investment in in-water studies (e.g. on the feeding ground). The most common field methods include capture-mark-recapture techniques (Frazer & Ehrhart 1985, Limpus & Chaloupka 1997, Bresette & Gorham 2001, Seminoff et al. 2002, Casale et al. 2009b) and length-frequency analysis (Bjorndal et al. 2001, Casale et al. 2009a). However, none of these provides a definitive measure of age at maturity, but rather an estimate of growth. Estimating age and size at first reproduction in wild sea turtle populations would thus require a permanent mark, which can be applied to the hatchling and which grows with the turtle throughout its life. Mutilation tagging, i.e. living tags (Bell & Parsons 2002), notching of hatchlings with a year-code (Hughes & Brent 1972) or DNA sampling (Dutton et al. 2005) allow the determination of concrete information of age and size at first reproduction. Guastella and Hughes (unpubl. data) later confirmed the validity of notch codes, as they established that notching has no harmful effect on a turtle’s health (as indicated through growth and mortality rates). However, mutilation tagging is a labour intensive (Bjorndal et al. 2001) and enduring method as it requires notching large numbers of individuals to compensate for high annual mortality (Table 6.1).

In addition, surviving individuals are expected to return only decades later. To date, results are only known from two mutilation tagging studies: the Ezemvelo KZN Wildlife (previously known as Natal Parks Board) notching programme in South Africa and the Queensland’s Environmental Protection Agency study in Australia. The notching programme in South Africa started 40 years ago and Baldwin et al. (2003) estimated an arithmetic mean age at first nesting of 19.5 years (range 10 - 29 years), based on a limited sample size and not taking into account that the sample was time-censored. The notching programme in Australia, which was conducted from 1976 to 1983 with 129 921 loggerhead hatchlings notched (Limpus et al. 1994), on the other hand, had very few returns, suggesting a minimum age at first nesting around 29 years (Limpus 2008). This paper aims to re-assess the average age and size during the putative first nesting season of loggerhead females nesting in South Africa through the evaluation of notch codes (mutilation tagging), as well as to identify size versus age as driver for sexual maturity.

Table 6.1: Summary of the South African notching data. Displayed are the amounts of hatchlings notched per season and the number of observed notched females per year of notching. The numbers of notched hatchlings were extracted from annual season reports by the Natal Parks Board (1972 - 1998) and Ezemvelo KZN Wildlife (1999 - 2002).

Season	Hatchlings notched	Notched females
1972	5000	6
1973	10250	7
1974	12133	22
1975	11267	13
1976	14720	18
1977	13463	24
1978	15130	-
1979	15981	1
1980	8489	-
1981	7507	2
1982	5634	-
1983	7383	-
1984	7585	3
1985	9712	-
1986	3784	1
1987	18038	3
1988	11380	5
1989	9071	2
1990	5189	16
1991	9406	3
1992	12521	-
1993	18046	6
1994	12068	1
1995	12856	2
1996	12203	1
1997	10038	1
1998	14981	-
1999	9028	-
2000	12424	-
2001	9928	-
2002	7596	-

6.2 Material and Methods

The South African nesting beach is situated within the iSimangaliso Wetland Park, at the southern end of the tropics (-27 °S) in the north-east coastal province of KwaZulu-Natal (KZN; Fig. 6.1). Between the 1972 and 2002 hatching seasons (which last from January to March with a peak in February) about 320 000 loggerhead hatchlings were notched by clipping out marginal scales with a leather punch, applying a unique seasonal code (Hughes 2002, Baldwin et al. 2003). Single notch codes were used during the first years, but this was replaced with a double-notch procedure eight years into the programme. This amendment was due to the possibility of a turtle coming ashore with an injury representing a single notch. The nesting data obtained from returning females in the beach monitoring programme are standard to most nest monitoring programmes: i.e. recording species, flipper/PIT tags, carapace size (SCL_{min} and SCW after Hughes et al. (1967) and Bolten (1999)), numbers of nesting females, nest position along the beach, as well as the notching code. The age of a notched female on its first encounter on the nesting beach is further referred to as 'age at first observation' as it was assumed that not all females are encountered during their first nesting season (Thorson et al. 2012, Nel et al. 2013). Additionally, individuals with unclear or contradictory notch codes were removed from the sample size. Further, the age of a notched female was calculated under the assumption that the turtle had hatched on the 1st February (peak hatching) of its cohort. If an adult female was encountered more than once during its observed first nesting season its mean size for that season was used to reduce measuring error.

A Shapiro-Wilk test was used to confirm normality for the size distribution of notched females at first observation. GLM was applied to identify significant factors influencing size at first observation. The factors tested were age, year of notching and the number of notches (single or double notched). A Gaussian identity link based on the result of the Shapiro-Wilk normality test was employed. The significance of co-factors was assessed by an analysis of deviance using chi-square statistics. All interactions were investigated and backward selection was used to simplify the model.

In contrast to the size of notched individuals, the distribution of age at first observation is biased; the distribution is naturally truncated because the highest (or oldest) age classes have not yet been observed (Fig. 6.2). To take into account the truncated nature of the age distribution, the parameters providing the best explanation for the age distribution were identified using the maximum likelihood method (Gupta 1973). As the full distribution of 'age at first observation' is not known, both Gaussian and log-normal distributions were tested. Let $D(\mu, \sigma)$ be the age distribution at first observation (*D* being Gaussian or log-normal) and y the time period since an individual was notched. The likelihood L of observing a female during its first nesting season at an age $x = A$:

$$L\ (y, A, D\ (\mu, \sigma))\ \#\ \Pr\ (x = A \mid D\ (\mu, \sigma))\ /\ \Pr\ (x < y \mid D\ (\mu, \sigma))$$

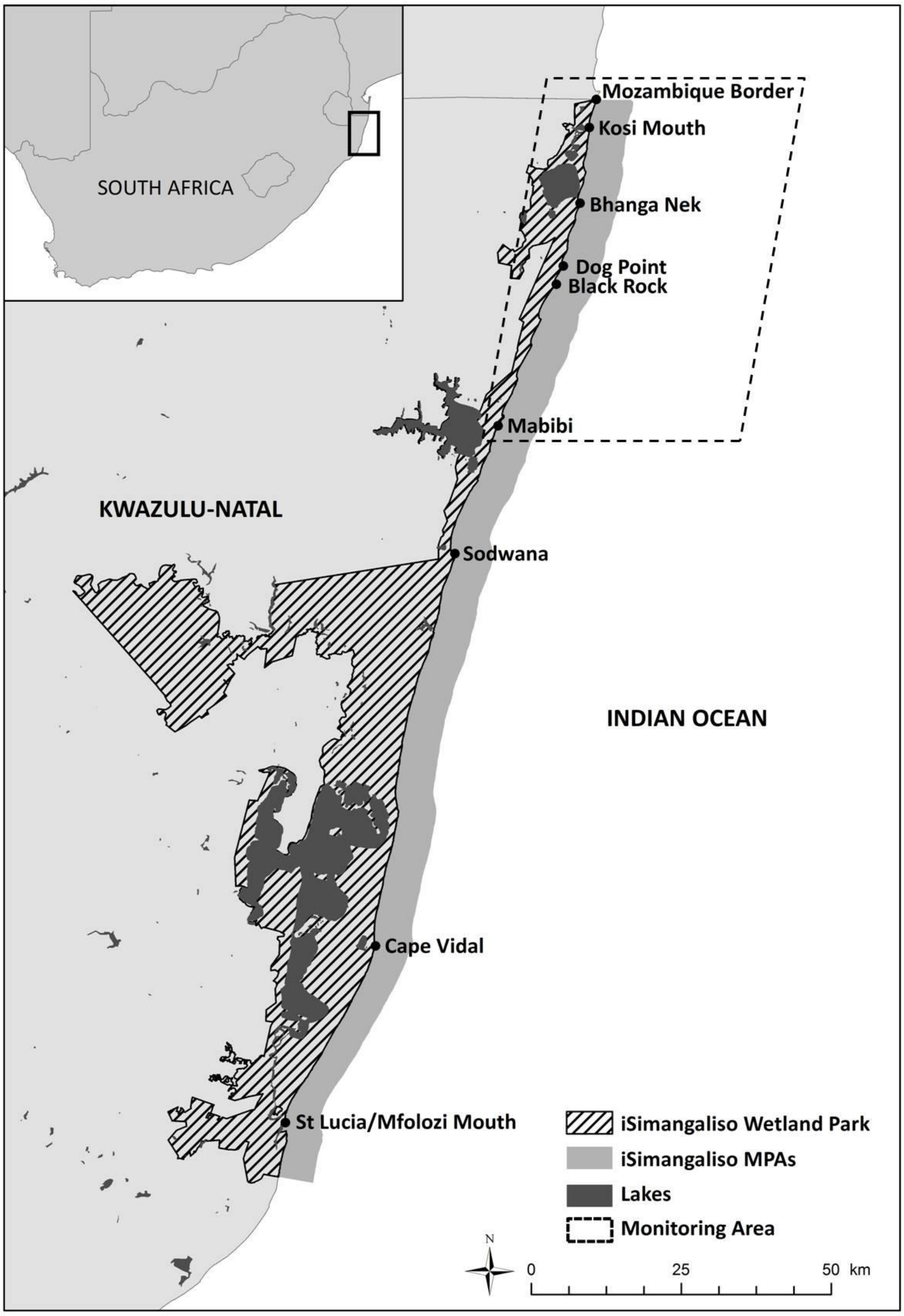

Figure 6.1: The iSimangaliso Wetland Park, including the extent of the Marine Protected Areas (3 nautical miles) and the monitoring area south of the Mozambique border.

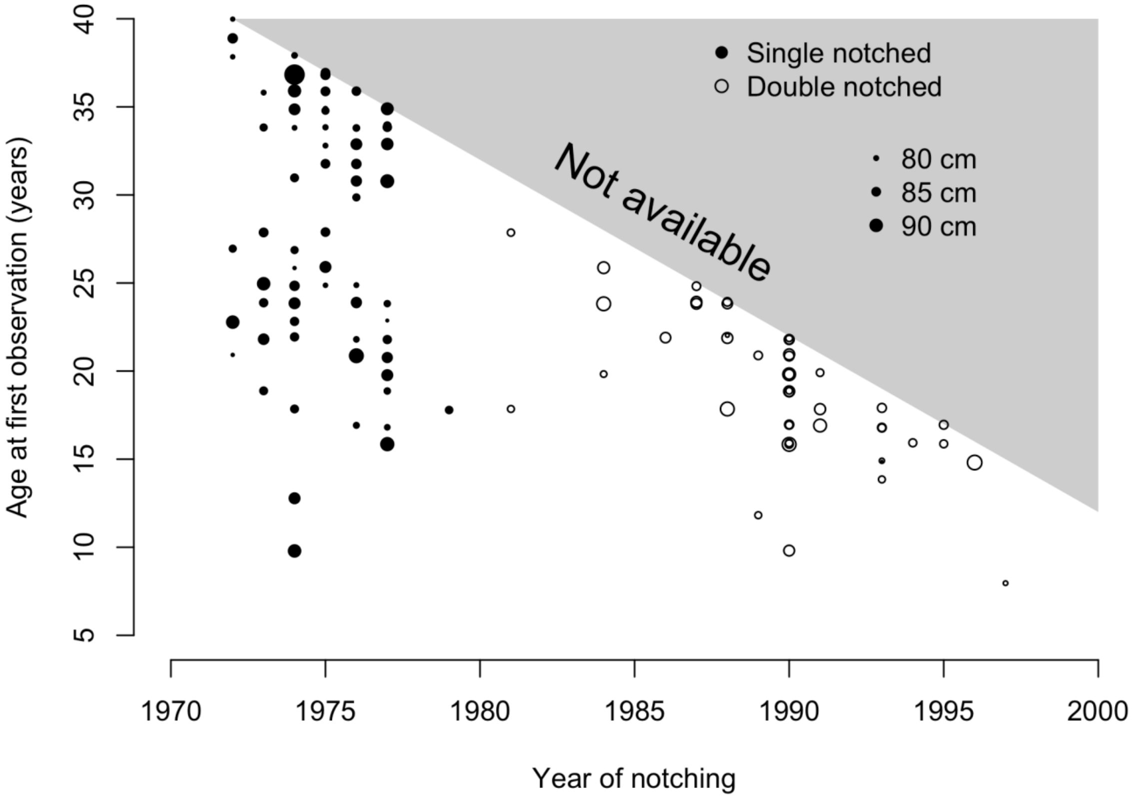

Figure 6.2: Age and size distribution of notched loggerhead females (n = 137) during their putative first nesting season in South Africa, displayed after year of notching (see Table 6.1). (Size, e.g. dot diameter, is expressed as a quantitative measure.)

Both Gaussian and log-normal distributions were fitted and their respective likelihoods were compared through Akaike Information Criteria (AIC) and Akaike weight (Burnham & Anderson 2002). The standard error of the parameters was estimated using the square root of the inverse of the Fisher information matrix at the point of maximum likelihood. R version 2.15.2 software (R Core Team 2012) was used for all of the statistical analysis. To correct for the possibility of missing the first nesting season of a female, the total number of nests per season (within the monitoring area) was divided by 3.7 (average nesting frequency for loggerheads after Nel et al. (2013)) to estimate the number of nesting females. The probability of encountering a nesting female at least once in a season is thus the number of recorded nesting individuals (identified through flipper or PIT tags) divided by the estimated number of females on the nesting beach. As this probability is constant over time, the average over four years (2008 - 2011), referred to as p_{ind}, was used. When a female nested in year Y, it was seen with a probability of p_{ind} and not seen with a probability of $1 - p_{ind}$. If not seen this year, it could have been seen each subsequent nesting season always with a probability of p_{ind}

and not *1 -* p_{ind}. Thus the probability that a female which first nested in year *Y* was first observed in year $Y + a$ is $p_a = (1 - p_{ind})^a * p_{ind}$. The mean observed age at first observation A_{obs} has thus been corrected using these p_a to obtain an estimate of age at first nesting: $A_{nest} = A_{obs} \cdot p_0 + (A_{obs} - 1) * p_1 + \ldots + (A_{obs} - n) * p_n$.

6.3 Results

The size-frequency distribution of females at first observation was normal (Shapiro-Wilk normality test, W = 0.99, p = 0.381) or log-normally distributed (W = 0.99, p = 0.560). Female size at first nesting (n = 137) was investigated with regards to age, year of notching and single or double notch code. No significant effect of any of these parameters was found (all p > 0.1; Fig. 6.3). Average size (SCL_{min}) at first observation of loggerhead females nesting in South Africa was 83.7 ± SD 4.15 cm (95% CI 83.0 - 84.4 cm, range 72.0 - 99.2 cm, median 83.5 cm).

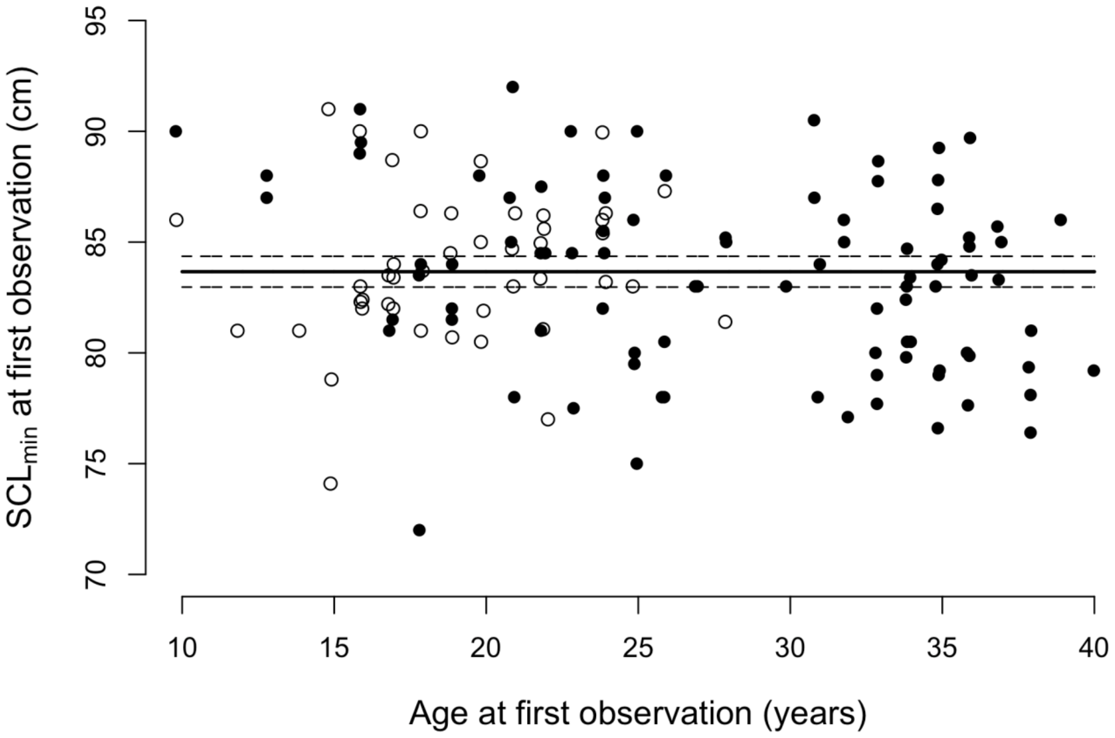

Figure 6.3: Age and size distribution of notched loggerhead females (n = 137) at first observation at the nesting beach in South Africa. Single notched individuals are displayed as dots and double notches with circles. The regression line indicates that no age-size relationship exists.

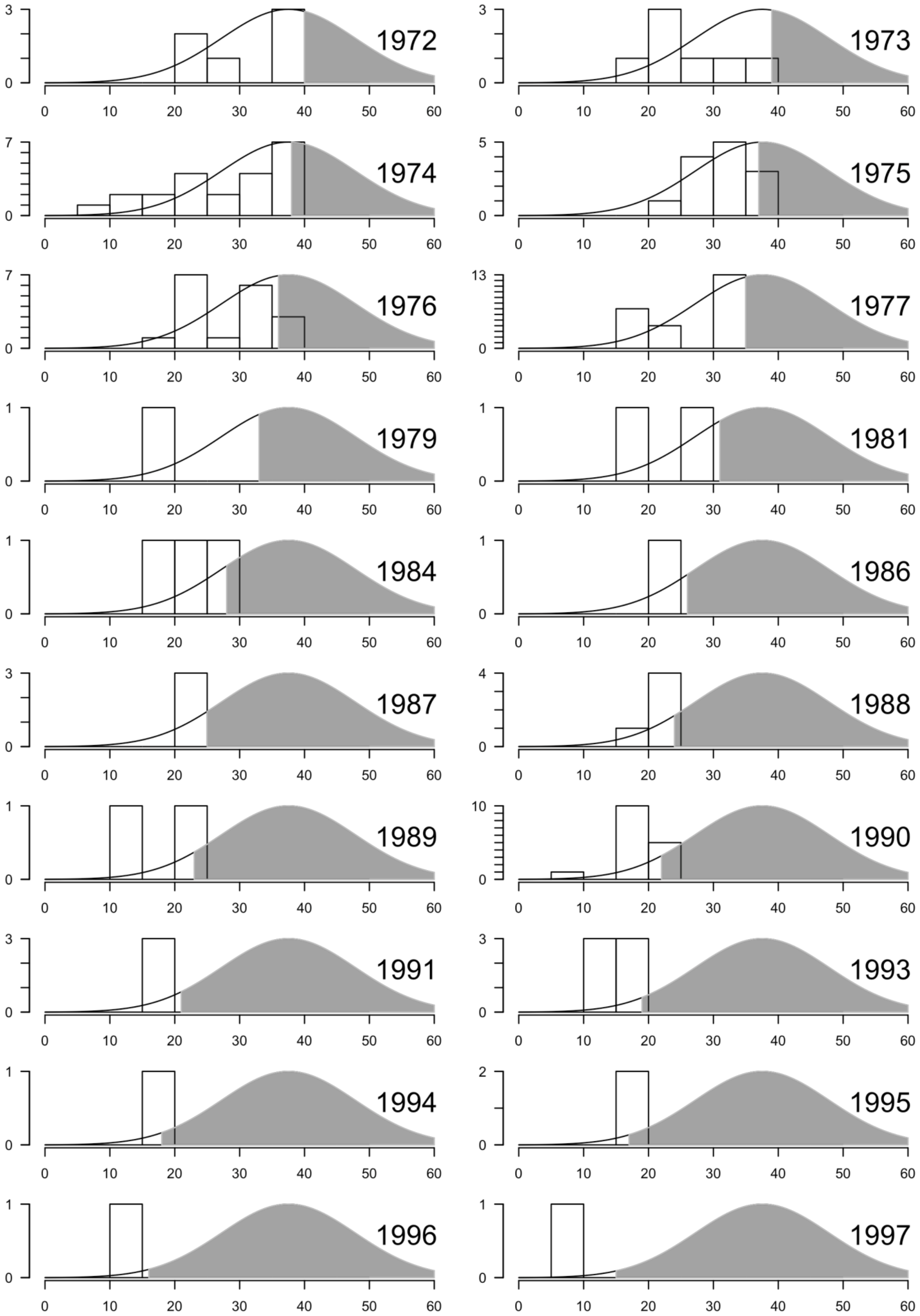

Figure 6.4: Histogram of age at first observation for each year of notching. The curve shows the density of the fitted truncated Gaussian model. The shaded area indicates the lack of data for each distribution. Age in years is displayed on the x-axis and number of notched individuals on the y-axis.

The age distribution of notched females that returned per year is displayed in Figure 6.4. This also includes the truncated Gaussian distribution. The fit of the truncated Gaussian distribution estimated an average age of 37.5 ± SD 10.31 years for loggerhead turtles at their first observation (age 95 % CI = 29.3 - 45.8 years; SD 95 % CI = 7.45 - 13.17; -ln L = 391.37; AIC 786.7397). The truncated log-normal distribution approximated an average age of 134.7 ± SD 2.04 years at first observation (age 95 % CI = 11.8 - 1541.8 years; SD 95 % CI = 1.29 - 3.22; -ln L = 387.12; AIC 778.2328). The log-normal distribution was selected against the Gaussian distribution as describing age at first observation best ($p > 0.9859$). However, when both distributions are superimposed on the truncated area (Fig. 6.5), it is obvious that data are only available for the left side of the distribution. The right side of the distribution is only known for the Gaussian distribution, based on the symmetry of the model.

Figure 6.5: Gaussian and log-normal distributions of age at first observation for loggerhead turtles nesting in South Africa. The shaded area indicates the lack of data.

The log-normal distribution is much less constrained than the Gaussian distribution but the right side of the distribution is unknown. Thus only the Gaussian distribution can be used to estimate age at first observation. If we assume that some notched females were not encountered during their first nesting season but had nested previously, then the absolute age at first reproduction for South African loggerhead turtles can be estimated as 36.2 ± SD 7.71 years (95 % CI 28.2-44.3 years, range 8.0 - 40.0 years; Table 6.2).

Table 6.2: Estimated age (in years) of notched loggerhead females at first observation on the South African nesting ground (n = 137). The second row corrects for the possibility that a notched female was not observed during its first nesting season and hence indicates the real age at first reproduction (years).

	Observed values			**Mean age from Gaussian model**		
	Min.	**Arithmetic average**	**Max.**	**Min. 95 % CI**	**Average**	**Max. 95 % CI**
Observed data	8.0	25.1	40.0	29.3	37.5	45.8
Corrected data	7.3	24.1	38.7	28.2	36.2	44.3

6.4 Discussion

The age and size of putative neophyte nesters were determined through mutilation-tagging and the results used to identify whether size or age drives sexual maturity. Over a 30 years period about 320 000 hatchlings were marked of which only 137 notched females were encountered at the natal nesting beach. This low recovery rate (Table 6.1) could be due to variable numbers of notched hatchlings, high natural or induced mortality (extrinsic factors), unclear notch codes that were discarded from the analysis, (male-) biased sex ratios of notched hatchlings and/or the fact that a large number of turtles have not yet matured and will only return over the next 2 - 3 decades. Thus, simply calculating the arithmetic mean age would present a false outcome, as all samples are time-censored, i.e. ‘oldest age classes’ are not observed yet.

When including the possibility that a female loggerhead was not encountered during her first nesting season, the average age at reproduction (putative first nesting season) for the South African population was estimated at 36.2 ± SD 7.71 years. This is much higher than reported for most other loggerhead populations (Table 6.3), but it is in agreement with the recovery profile of the South African population. The South African conservation and monitoring programme was initiated in 1963 and about 40 years later the loggerhead population displays almost exponential population growth (Nel et al. 2013). This indicates that the elimination of poaching along the nesting beach has resulted in an increased reproductive success leading to population growth.

Table 6.3: Summary of growth studies on age and size at first reproduction of loggerhead turtles. Method refers to the type of used data as capture-mark-recapture (CMR), skeletochronology (SC), length-frequency-analysis (LFA), notching or size-specific growth rates (SSGR). Size measurements are given as published and described in Bolten (1999): $SCL_{n\text{-}t\text{ and}}$ $CCL_{n\text{-}t}$ are measured from notch to tip; SCL_{min} and CCL_{min} are measured from the anterior point at midline (nuctual scute) to the posterior notch at midline between the supracaudals.

Method	Age (yrs.)	Size (cm)	Location	Source
CMR	30+	-	Australia	Limpus (1979)
CMR	10-15	75.0 $SCL_{n\text{-}t}$	Florida	Mendonca (1981)
CMR	30	92.22 SCL	Florida	Frazer and Ehrhart (1985)
CMR	16-28	66.5-84.7 $CCL_{n\text{-}t}$	Mediterranean	Casale et al. (2009b)
SC	22	92.5 SCL	Chesapeake Bay	Klinger and Musick (1995)
SC	20-24	92.4 SCL	Georgia	Parham and Zug (1997)
SC	14.9-28.5	66.5-84.7 $CCL_{n\text{-}t}$	Mediterranean	Casale et al. (2011a)
SC	24	69	Mediterranean	Piovano et al. (2011)
LFA	26.5	87 $CCL_{n\text{-}t}$	Southeast USA	Bjorndal et al. (2001)
LFA	23.5-29.3	80 $CCL_{n\text{-}t}$	Mediterranean	Casale et al. (2011b)
notching	19.5	72.8-98.5 SCL_{min}	South Africa	Baldwin et al. (2003)
notching	29	95.7-95.76 CCL_{min}[a]	Australia	Limpus (2008)
notching	36.2[b]	83.7 SCL_{min}[c]	South Africa	this study
SSGR	45.0	98.2 CCL	Pacific & Atlantic	Scott et al. (2012)

[a] Mean size of nesting loggerhead females (n = 2587) in eastern Australia; [b] 95 % CI 28.2 to 44.3 yr; [c] 95 % CI 83.0 to 84.4 cm.

Average size (SCL_{min}) at first observation was 83.7 ± SD 4.15 cm. This was not corrected for the possibility that the female could have nested in a previous season as growth after first reproduction is negligible (Limpus & Chaloupka 1997). South African loggerheads are similar in size to those from the south-eastern USA, but larger than those from the Mediterranean and smaller than the Australian populations (Table 6.3). Discrepancies in age and size at reproduction within and between populations (of the same species) could be based on intrinsic and/or extrinsic factors, or different methodologies (see Casale et al. 2009b, Casale et al. 2011a, Casale et al. 2011b, Piovano et al. 2011 in Table 6.3). In the former case, South Africa’s unique position between the warm Indian Ocean and the cold South Atlantic Ocean could have increased age and size ranges at first observation as hatchlings may grow up in a variety of environments.

The available age-size data at first observation of 137 loggerhead females indicated that neither age, year of notching nor notch code (single or double) affect female size at reproduction and thus no age-size relationship exists (Fig. 6.3). It is concluded that the maturation process of the reproductive organs, as well as the onset of reproduction itself, are correlated with a minimum size of the female (Hughes 1974), which may be reached at any given age dependent on intrinsic and extrinsic factors

(Fig. 6.3). Similar results were found by Bjorndal et al. (2012) who raised 47 female green turtles in captivity until after first reproduction. This hypothesis is also supported by Hatase et al. (2002) who conducted an isotope and telemetry study and found that immature turtles that migrate to coastal waters at a small size grow larger by feeding on nutrient-rich prey, such as benthos until they reach sexual maturity. In contrast, immature turtles that stay in the pelagic zone for a longer period are smaller because of the scarcity of nutrient-rich prey in this habitat. Additionally, Bjorndal et al. (2003) determined that compensatory growth occurs in juvenile turtles. This decreases as the turtles gain control over their movements. As this study identified size as the driver for sexual maturity, Hatase et al. (2002) and Bjorndal et al. (2003) could have provided the link between minor differences in size and the wide age distribution of notched females during their putative first nesting season.

Even though mutilation-tagging (or DNA sampling) is a labour intensive and long-enduring method, we suggest that it is the only way to ultimately determine the turnover rate of a population. Additionally, this older age at first reproduction (putative first nesting season) might question the sustainability of sea turtle populations in future; as they are less resilient to anthropogenic stress than previously presumed (Scott et al. 2012).

For leatherback turtles few data are availible on growth rates, survival probabilities and age at maturity. The general lack of knowledge is (in part) caused by the inaccaccebility of younger age-/size-classes in their pelagic distribution (Eckert 2002) and because they are difficult to rear in captivity (Jones et al. 2011). However, current estimations of age at reproduction include growth rate studies for some year-classes (Birkenmeier 1971, Jones et al. 2011), skeletochronology (Rhodin 1985, Zug & Parham 1996, Avens et al. 2009), population trend analysis and DNA fingerprinting (Dutton et al. 2005). Published data suggest that leatherback turtles mature at a younger age but a larger size than all other sea turtle species (Zug & Parham 1996, Jones et al. 2011). While previous research suggested an age of 2 - 6 years at reproduction (Birkenmeier 1971, Rhodin 1985), more recent studies estimated that leatherback turtles reproduce at an average age of 13.3 - 26.8 years (Zug & Parham 1996, Dutton et al. 2005, Avens et al. 2009, Jones et al. 2011). The age discrepancy of earlier studies was caused by extrapolating hatchling growth rates and not considering the flexibility of growth rate (i.e. effects of intrinsic and extrinsic factors) nor that growth rate slows down with age or increased size (Avens et al. 2009, Jones et al. 2011). However, age estimates do not exist for South African leatherbacks but population trend analysis indicated that the age at reproduction might be ±16 years (Nel et al. 2013), which overlaps with the initial increase in population size.

In addition, results from CHAPTER 3 indicate that young individuals (i.e. neophytes) dominate the South African loggerhead and leatherback sea turtle populations. This demonstrates that the banishment of egg poaching (since 1963) has resulted in a greater reproductive output and success. However, population growth profiles for loggerhead and leatherback turtles processed differently. From an initial ratio of 0.27 : 1 leatherbacks to loggerheads, the leatherback population had increased over the first 15 years of the conservation programme before it declined below the initial ratio by 1990. The loggerhead population, on the other hand, increased continuously over time with tremendous growth in the last decade (Nel et al. 2013). Differences in population growth profiles between the South African loggerhead and leatherback populations could, however, not be induced by the unequal generation times, i.e. for loggerheads 36.2 ± SD 7.71 years and for leatherbacks at 13.3 - 26.8 years, as it would predict the opposite trend.

In conclusion, the elimination of poaching along the South African nesting beach initiated an increase in the leatherback population but it was not enough to overcome offshore mortalities of large juveniles and adults. Thus, more effort should be invested to determine absolute generation times in order to adjust conservation methods. Additionally, not only population growth should be monitored to assess conservation success but also age and size distribution, as they affect the future population growth potential and may display potential threats.

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Chapter 7: Discussion and conclusion

7.1 Discussion

This thesis investigated the potential for population growth in long-lived, slow-growing, late-maturing, migratory species based on the example of the South African loggerhead *(Caretta caretta)* and leatherback *(Dermochelys coriacea)* sea turtle populations. The number of sea turtles in South Africa was low at the start of the conservation programme with 107 loggerheads and 24 leatherback females recorded to nest in 1965. However, despite equal conservation efforts (i.e. a beach conservation programme) over the last 50 years only the loggerhead population currently displays increasing growth (Nel et al. 2013). Nonetheless, as many leatherback populations are declining or are at the brink of extinction, because of poaching (Tomillo et al. 2008), by-catch (i.e. long-lining; Petersen et al. 2009) and climate change (Fish et al. 2008), a stable leatherback population, as displayed in South Africa, is already a conservation success (Spotila et al. 2000, Nel et al. 2013).

Populations tend to be stable if carrying capacity is reached (Molles 2013). However, in the case of the South African leatherback population, it is unlikely that environmental carrying capacity has been reached at the nesting site or at the foraging areas. This hypothesis is supported by the small population size, with annually less than 100 nesting females, which utilizes >300 km nesting beach, as well as the worldwide proliferating food abundance (Lynam et al. 2006). The (spatial) nesting density of the South African leatherback population ranges between 3 - 19 nests per km (Nel et al. 2013) with foraging areas as far as St Brandon, Mauritius, to the east or (off) Angola to the west (Nel 2009). The lack of population growth is not to the result of population density being near carrying capacity on the beach as other much larger populations, for example the Gabonese leatherback population, display nest densities at 83 - 292 nests per km (Witt et al. 2009, Nel et al. 2013). Further, environmental conditions, such as water temperature, food quality and food availability, determine growth rates and body size (Parker 1929, Bjorndal et al. 2003, Balazs & Chaloupka 2004). The South African leatherback turtles with an average size (CCL) of 160.9 cm are among the largest individuals in the world (Eckert et al. 2012), which suggests that individuals in the population are healthy. Therefore, it is assumed that the South African leatherback population has not reached its carrying capacity and that population growth is suppressed for some other reason.

Since 1963, when the South African sea turtle conservation and monitoring programme was set in place by the Natal Parks Board, anthropogenic stressors along the nesting beach have been reduced substantially and human-induced mortality now occurs only incidental (<1 % including egg poaching;

De Wet 2012, pers. obs.). However, increasing populations with greater demands for coastal property outside the boarders of iSimangaliso, in combination with high (and increasing) unemployment put more and more pressure on the natural environment. Further, recent road construction in the park has facilitated access to the beach, which has increased human presence and as a result there is a growing number of incidents of egg poaching and slaughtered females (R. Nel pers. comm., NMMU). Nevertheless, because the increase in human population density and coastal development are very recent events, they did not affect the population growth potential of the South African loggerhead and leatherback sea turtle.

Reproductive output was investigated as possible cause for the difference between population growth profiles of loggerhead and leatherback populations, as the number of offspring can significantly affect the population growth potential. However, even though leatherbacks displayed a higher annual reproductive output per female (±700 eggs per season compared to about ±448 loggerhead eggs), loggerhead turtles nest in greater numbers (650 loggerhead females compared to 65 leatherback individuals) and exhibit a higher absolute population growth potential (i.e. the number of hatchlings produced per season). Nonetheless, it is unclear why the leatherback population has not responded more favourably to the conservation efforts applied to the beach portion of their life cycle. Further, even if reproductive output is high population growth potential would still be reduced if reproductive success (i.e. successful incubation) is low. However, reproductive success is equally high for loggerhead and leatherback turtles, with an emergence success of 73.6 and 73.8 %, respectively, and has not changed since the 1970s (linear regression; $p \geq 0.121$). It seems that reproductive success driven by environmental conditions does not favour population growth of either of the species.

De Wet (2012) approximated that about 10.8 % of deposited loggerhead nests and 22.0 % of leatherback nests are either completely depredated or eroded each season. As the effects of climate change (i.e. sea-level rise, storms, inundation and beach degradation) intensify (Fish et al. 2008, IPCC 2013) it should be recognized that leatherback turtles will be particularly affected because their nests are placed closer to the high tide mark than those of any other sea turtle species (McAllister et al. 1965, Eckert 1987). Thus, one should consider re-locating doomed leatherback nests to safer grounds as it is standard in many beach conservation programmes (Dutton & Whitmore 1983, Mrosovsky 2006, Pfaller et al. 2009). Further, 55 % of all depredated leatherback nests are destructed by honey badgers (De Wet 2012). This displays a localized threat in one of the moderate density nesting areas for leatherbacks and a possible measure to protect leatherback nests should be introduced; such a protecting the nests *in situ* with cages. Nonetheless, even though the absolute population growth potential for leatherback turtles could be enhanced this way it does not explain why the South African leatherback population has not expanded.

Sex ratios of the South African loggerhead and leatherback turtle were approximated because disparate sex ratios between the two populations seemed like a reasonable explanation for the opposed population growth profiles. Sea turtles exhibit temperature-dependent sex determination (TSD), as a form of environmental sex determination (ESD), in which sex is only determined after fertilization by the incubation temperature during the thermo-sensitive period (TSP; Bull & Vogt 1979, Mrosovsky 1980, Janzen & Paukstis 1991, Mrosovsky & Pieau 1991, Wyneken et al. 2007). It was proposed that the South African leatherback population is male-biased, which prohibits population growth, whereas loggerhead population growth is enhanced by a female bias. However, results indicated that both species are severely female-biased (loggerheads 86.9 % and leatherbacks 97.1 %), which is quite common for sea turtle populations around the world (ANNEX I). Even if it is assumed that approximated sex ratios in this study are overestimated, because samples in this study were compromised (i.e. frozen for storage), current sex ratios of the South African loggerhead and leatherback populations are both female-biased. The extent of this bias is uncertain, although it seems substantial (>70 %). Unfortunately, it is also unknown whether historical differences in sex ratio account for the differences in the population growth profiles.

One of the main reasons why different historical sex ratios are considered is the progressive effect of climate change. Climate change is manifested in acute weather events such as extreme temperatures and changes in precipitation (including droughts and wet periods; Easterling 2000, IPCC 2013). In sea turtles, increasing temperatures during the TSP, for example due to climate change, lead to feminisation of the clutch (Hawkes et al. 2007, Fuentes et al. 2010, Fuentes & Porter 2013), a decrease in reproductive success (Janzen 1994, Matsuzawa et al. 2002, Fuentes et al. 2011) and even to manipulation of the phenotype (Du & Ji 2003, Booth et al. 2004). In order to determine whether current sex ratios are a recent development or not, air and SSTs were used to model sex ratios in the past. Because of the partial destruction of the gonads in this study, sex ratios of constant incubation experiments were applied using data from Maxwell (1986) for loggerheads. Because no constant incubation temperature experiment has been conducted for the South African leatherback population sex ratio data from Rimblot et al. (1985), Rimblot-Baly et al. (1987) and Binckley et al. (1998) for South American leatherback populations were used. Air temperatures were only recorded for the entire duration of this study but historical data for the area date back to 1997. However, there was no significant change in annual sex ratio for loggerheads (linear regression; p = 0.45) or leatherbacks (linear regression; p = 0.47) for these 15 seasons (1997 to 2011). Further, re-analysed loggerhead data from Maxwell et al. (1988) indicated that a 77.4 % female bias existed in 1984. It is proposed that sex ratios have not been affected by global warming over the last three decades. It is also suggested that the 15-year average (loggerheads 77.1 % and leatherbacks 99.5 %) should be used as a robust baseline for future sex ratio comparisons.

It is possible that the female-biased sex ratio for the South African leatherback population is an overestimate based on the assumption that the PT (i.e. equal sex ratio at fixed incubation temperatures) of this population is the same as in South American leatherback populations. However, the estimate may be somewhat realistic and the sex ratio bias not a mistake in estimation but rather results from factors *in situ*. For example, leatherback nesting is not restricted to the monitoring area even though nest monitoring was conducted in the central and northern nesting areas. Leatherback nesting accumulates to the south also outside the monitored area (McAllister et al. 1965, Nel et al. 2013), which might result in cooler nests and, therefore, more males (Mrosovsky 1988, Marcovaldi et al. 1997). Factors driving long-shore leatherback nest site selection are unclear and it may be an artefact of beach accessibility, i.e. sub-tidal rock formations (McAllister et al. 1965, Botha 2010), rather than the attempt to manipulate reproductive success (or sex ratio). Further research is required to establish a spatial beach temperature profile and to determine the PT for the South African leatherback population.

As leatherback nesting continues beyond the monitoring area (McAllister et al. 1965, Nel et al. 2013) it was suggested that the South African leatherback population might be larger than expected, as some individuals are not recorded (Nel 2009, Nel et al. 2013). However, South African leatherback turtles have a very low nest site fidelity (9.7 ± SE 0.29 km, n = 953; Botha 2010) nesting over large distances and Thorson et al. (2012) estimated that a leatherback female has only a 0.4 probability (or 40 % likelihood) of being recorded in this programme. It is therefore accepted that the South African leatherback population size might be underreported but stable.

The last possibility to explain the disparate population trends is age at reproduction. It was proposed that leatherback turtles may have an equal or longer generation time than loggerheads. However, age at reproduction for South African loggerhead turtles was determined using mutilation tagging over a 30 year period. The estimated age at first reproduction was at 36.2 years. Considering that the conservation programme started in 1963 loggerhead age at reproduction fits the population growth profile perfectly, displaying a long retraction period and a steep population growth around year 2000

(CHAPTER 1; Fig. 1.5). Thus, it is concluded that the current (exponential) growth of the South African loggerhead population is a direct result of the beach conservation programme. Unfortunately, age at reproduction for leatherback turtles was not determined in this study, but a literature review suggested that leatherbacks mature at a much younger age (13.3 - 26.8 years) than all other sea turtle species (Zug & Parham 1996, Dutton et al. 2005, Avens et al. 2009, Jones et al. 2011). Therefore, population growth of the South African leatherback population must have commenced much earlier, even if it mostly remained unnoticed.

As the age structure of a population can be used to draw conclusions about success of the population reproductive success and survival rates (Molles 2013), the loggerhead and leatherback populations were analysed. Results indicated that both species are dominated by neophyte nesters (CHAPTER 3; Fig. 3.4), which suggests successful reproduction and survival in which each individual replaces itself (maybe even at a surplus). Also the predominance of neophyte nesters indicates that most individuals reproduce only once in their lifetime, which implies significant adult mortality. Nonetheless, as neophyte nesters dominate the South African loggerhead and leatherback population it is proposed that different mortality rates occur for immature and mature[34] individuals off the nesting beach.

7.2 Conclusion

It is concluded that the South African sea turtle conservation programme is successful because neophyte nesters dominate both populations, which suggests population growth. A comparison of clutch size between loggerhead and leatherback turtles indicated that leatherbacks produce more eggs per individual per nesting season, but loggerhead turtles nest in greater numbers, therefore exhibiting a higher absolute population growth potential. In addition, emergence success is high for both species (>70 %) and has persisted since 1965, facilitating population growth. Furthermore, a substantially female-biased (>70 %) sex ratio in loggerhead and leatherback hatchlings presumably has endured for at least 30 years, enhancing population growth (in the short term). Finally, age at maturity (i.e. age at reproduction) was approximated for loggerhead turtles at 36.2 years, which appears to be about twice the generation time of leatherback turtles. Thus, it is reasoned that all biological metrics support the population growth of the South African loggerhead and leatherback population, whereas leatherbacks are favoured in all aspects (i.e. reproductive output, sex ratio and age to maturity). However, as no constraints to population growth are evident on the nesting beach it is proposed that population growth of the South African leatherback turtle is either not adequately

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[34] In this contxt ‘mature’ describes those individuals which reproduced at least once.

reported or prohibited by industrial fisheries (or some other driver for offshore mortality). Thus, further research particularity with dedicated cooperation from fisheries is required.

7.3 References

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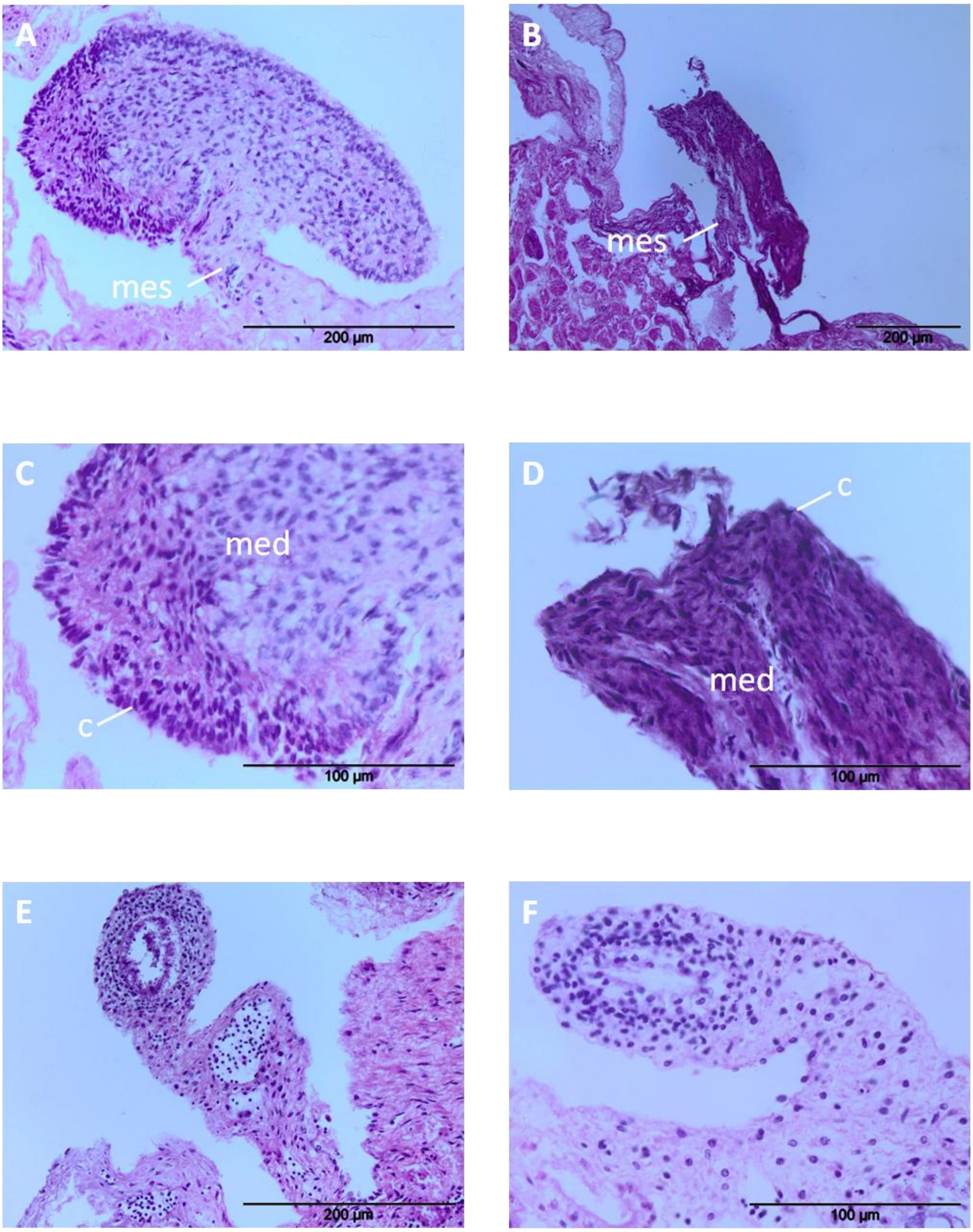
ANNEX I: Summary of pivotal temperature and sex ratio of sea turtle populations - sorted after species (CC - *Caretta caretta*, CM - *Chelonia mydas*, DC - *Dermochelys coriacea*, EI - *Eretmochelys imbricata*, LK – *Lepidochelys kempii*, LO – *Lepidochelys olivacea,* CTE – constant temperature equivalent and CIT – constant incubation temperature).

location	study period	species	PT (°C)	TRT (°C)	PT (method)	female%	source
Australia	-	CC	28.6±0.5	-	CIT	-	Limpus et al. (1985)
Australia	1990-1992	CC	±29.4	28.1-30.1	CIT; CTE	-	Georges et al. (1994)
Australia	2005&2006	CC	28.3±SE0.1	-	*in situ*; CTE	70-100	Chu et al. (2008)
Brazil	1995	CC	29.2	28.0-30.6	CIT	82.5	Marcovaldi et al. (1997)
Greece	2001	CC	29.3	28.3-30.5	CIT	-	Mrosovsky et al. (2002)
Cyprus & Turkey	1995&1996	CC & CM	29	-	*in situ*	81	Kaska et al. (1998)
Japan	1989	CC	29.4	27.7-30.7	CIT	-	Tokunaga et al. (1999)
South Africa	1984&1985	CC	29.7	29.2-30.7	CIT	±50	Maxwell (1986), Maxwell et al. (1988)
Turkey	2000&2001	CC	-	-	-	67-74	Öz et al. (2004)
Turkey	2000-2002	CC	-	-	-	60-65	Kaska et al. (2006)
USA	1978	CC	30	-	CIT	-	Yntema and Mrosovsky (1980, 1982)
USA	1980-2005	CC	-	-	-	58	Hawkes et al. (2007)
USA	1984-1986	CC	±29.2	<27.5-≥30.4	CIT	-	Mrosovsky (1988)
USA	1986-1988	CC	-	-	-	87.0-100	Mrosovsky and Provancha (1992)
Ascension Island	1999	CM	28.8	-	*in situ*	75	Godley et al. (2002)
Australia	2002	CM	-	-	-	94	Booth and Freeman (2006)
Costa Rica	1977	CM	-	<28.5->30.3	-	67	Spotila et al. (1987)
Suriname	1983	CM	28.75	-	CIT	53.9	Mrosovsky et al. (1984)
Suriname	1993	CM	-	-	-	63.8	Godfrey et al. (1996)
Suriname	1995	CM	29.2-29.5	-	CIT	-	Godfrey and Mrosovsky (2006)
Costa Rica	1998-2007	DC	-	-	-	83	Sieg et al. (2011)
Costa Rica	1993-1995	DC	29.4	29.0-30.0	CIT	74.3-100	Binckley et al. (1998)
French Guiana	-	DC	29.5		CIT	-	Rimblot-Baly et al. (1987)
Malaysia	1986	DC	-	29.21-30.4	-	100	Chan and Liew (1995)
Mexico	1983-1984	DC	-	-	-	52	Banabib (1984)

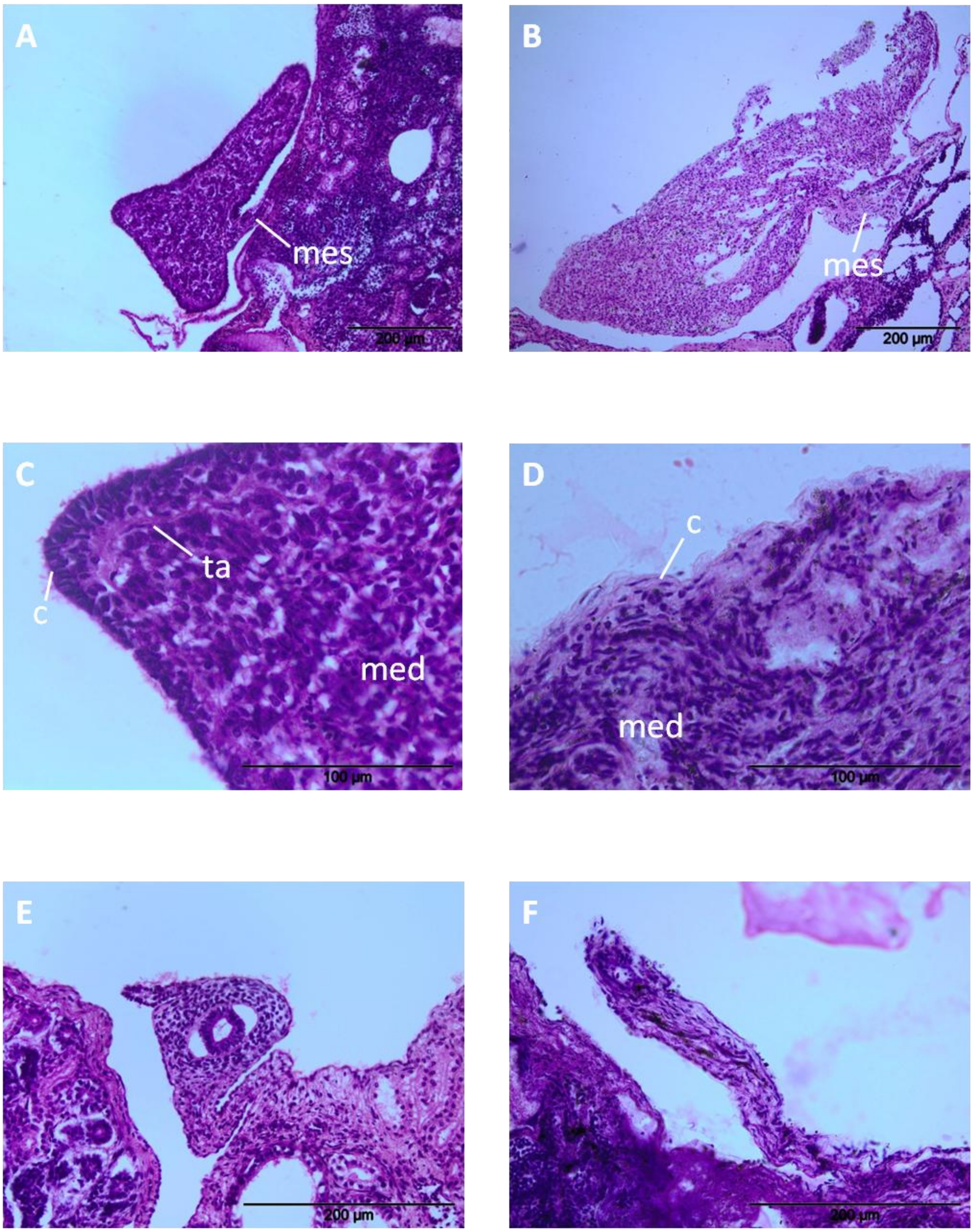
ANNEX I: Continued summary of pivotal temperature and sex ratio of sea turtle populations worldwide.

location	study period	species	PT (°C)	TRT (°C)	PT (method)	female %	source
P. New Guinea	2007	DC	-	-	-	7.7	Steckenreuter et al. (2010)
Suriname	1980&1982	DC	-	28.1-30.5	-	-	Dutton et al. (1985)
Suriname	1982	DC	-	-	-	49.0	Mrosovsky et al. (1984)
French Guiana	1983-1984	DC	29.5	29.25-29.75	CIT	-	Lescure et al. (1985)
Suriname & French Guiana	-	DC	-	28.75-29.75	CIT	-	Rimblot et al. (1985)
Suriname	1993	DC	-	-	-	69.4	Godfrey et al. (1996)
Brazil	1991-1997	EI	29.65	28.4-30.4	CIT	>90	Godfrey et al. (1999)
Puerto Rico	-	EI	29.6	1.3 *	CIT	-	Mrosovsky et al. (2009)
Costa Rica	1981	LO	±30	25.0-32.0	CIT	-	McCoy et al. (1983)
Costa Rica	-	LO	30.5	27.0-32.0	CIT	-	Wibbels et al. (1998)

*Calculated after Girondot (1999).



ANNEX II: Microscopic cross sections of loggerhead gonads and the paramesonephric duct. A) ovary with mesorchium (mes), B) testis with mesorchium (mes), C) close-up of an ovary with perpendicular arranged cortex (c) and medulla (med), D) close-up of a testis with poorly developed cortex (c) and medulla (med), E) female paramesonephric duct with a complete lumen and high mobility and F) male paramesonepgric duct with a reduced lumen and low mobility.



ANNEX III: Microscopic cross sections of leatherback gonads and the paramesonephric duct. A) ovary with mesorchium (mes), B) testis with mesorchium (mes), C) close-up of an ovary with pseudostratisfied cortex (c), tunica albuginea (ta) and medulla (med), D) close-up of a testis with poorly developed cortex (c) and a dense medulla (med), E) female paramesonephric duct with a complete lumen and high mobility and F) male paramesonepgric duct with a reduced lumen.

ANNEX IV: Descriptive statistics of sex ratio and nest parameters of loggerhead turtles in South Africa. Non self-emerged hatchlings and full-term (dead) embryos are included in the total count (n) and additionally indicated in parentheses. ‘Hatchling size’ (mm) indicates average size of self-emerged hatchlings per nest, ‘IP’ the total incubation period in days (oviposition to emergence), ‘TSP’ the thermo-sensitive period in days and ‘TSP (begin)’ the onset of the TSP in days of total IP. ‘TSP temperature (°C)’ specifies *in situ* mean nest temperature during TSP and ‘CTE (°C)’ the constant temperature equivalent thereof. (*In situ* data from 1984 were obtained from a graph in Maxwell et al. (1988) whereby TSP temperature equals mean temperature of the middle third of the total IP. Laboratory data from 1984 were obtained from Maxwell (1986) where IP is the time period from oviposition to hatching, TSP exactly equals the middle third of IP and CTE indicate constant incubation temperatures. Available raw data from 2008 and 2009 from Boonzaaier (2011) were re-analysed.)

season	oviposition	nest #	n	males	females	female %	hatchling size	IP	TSP	TSP (begin)	TSP temperature (°C)	CTE (°C)
1984	11/01/1984	1	23	-	-	8	-	-	-	-	28.5	-
1984	11/15/1984	2	9	-	-	20	-	-	-	-	28.9	-
1984	11/16/1984	3	21	-	-	40	-	72	-	-	28.8	-
1984	11/22/1984	4	22	-	-	20	-	-	-	-	28.7	-
1984	12/01/1984	5	22	-	-	85	-	60	-	-	29.3	-
1984	12/17/1984	6	21	-	-	97	-	-	-	-	29.4	-
1984	12/18/1984	7	9	-	-	100	-	-	-	-	29.6	-
1984	12/19/1984	8	21	-	-	92	-	-	-	-	29.7	-
1984	12/19/1984	9	23	-	-	100	-	-	-	-	29.9	-
1984	01/01/1985	10	24	-	-	100	-	-	-	-	30.2	-
2008	12/15/2008	11	-	-	-	-	-	63	22	21	29.43	29.95
2008	12/18/2008	12	-	-	-	-	-	60	21	21	30.17	30.59
2008	12/20/2008	13	-	-	-	-	-	59	21	20	29.73	30.36
2008	12/21/2008	14	-	-	-	-	-	69	24	23	30.22	28.76
2008	12/21/2008	15	-	-	-	-	-	60	22	20	29.64	29.96
2009	01/03/2010	16	7	0	7	100	-	59	21	20	30.13	30.20
2009	01/09/2010	17	4	0	4	100	-	57	19	20	31.65	32.12
2009	01/30/2010	18	-	-	-	-	-	60	21	21	30.00	30.43
2009	01/30/2010	19	-	-	-	-	-	62	22	22	29.36	29.77
2009	01/31/2010	20	-	-	-	-	-	61	22	21	29.95	30.07
2009	02/07/2010	21	-	-	-	-	-	64	23	23	29.17	29.11
2009	02/11/2010	22	-	-	-	-	-	58	22	20	29.51	30.04
2009	02/12/2010	23	-	-	-	-	-	58	21	20	30.05	30.46

ANNEX IV: Continued descriptive statistics of sex ratio and nest parameters of loggerhead turtles in South Africa.

season	oviposition	nest #	n	males	females	female %	hatchling size	IP	TSP	TSP (begin)	TSP temperature (°C)	CTE (°C)
2009	02/14/2010	24	-	-	-	-	-	59	22	20	29.86	30.01
2009	02/27/2010	25	-	-	-	-	-	54	19	19	32.16	32.03
2009	02/28/2010	26	-	-	-	-	-	55	20	19	31.80	31.73
2009	02/28/2010	27	-	-	-	-	-	57	21	19	30.33	30.78
2010	11/18/2010	28	5	0	5	100	-	-	-	-	-	-
2010	11/21/2010	29	6	0	6	100	43.2	66	22	25	30.36	29.92
2010	11/21/2010	30	7	0	7	100	41.3	66	22	25	29.66	29.65
2010	11/21/2010	31	-	-	-	-	-	69	23	26	29.72	29.08
2010	11/22/2010	32	3	0	3	100	42.8	69	23	26	29.48	29.03
2010	11/25/2010	33	-	-	-	-	-	61	20	23	31.24	31.18
2010	11/27/2010	34	-	-	-	-	-	65	22	25	30.60	30.03
2010	11/29/2010	35	-	-	-	-	-	64	22	24	30.01	29.92
2010	12/11/2010	36	8	0	8	100	-	-	-	-	-	-
2010	12/13/2010	37	-	-	-	-	-	60	22	21	29.75	29.98
2010	12/20/2010	38	-	-	-	-	41.7	62	23	21	30.06	29.37
2010	12/25/2010	39	7	1	6	86	39.9	61	22	20	29.79	29.73
2010	01/16/2011	40	(3)	0	(3)	100	-	-	-	-	-	-
2011	11/03/2011	41	-	-	-	-	44.4	71	25	27	28.69	27.85
2011	11/02/2011	42	-	-	-	-	40.8	72	25	27	29.47	27.89
2011	11/14/2011	43	3 (2)	0	3 (2)	100	44.6	64	24	24	28.22	28.69
2011	11/15/2011	44	3	0	3	100	-	58	22	22	28.94	30.07
2011	11/15/2011	45	-	-	-	-	44.0	69	23	28	29.59	28.98
2011	11/27/2011	46	3	1	2	67	45.7	64	22	24	29.55	29.95
2011	11/27/2011	47	-	-	-	-	40.8	64	22	24	30.47	29.98
2011	12/10/2011	48	4 (1)	(1)	3	75	45.2	59	20	22	31.06	31.41
2011	12/10/2011	49	-	-	-	-	-	58	20	21	31.47	31.48

ANNEX IV: Continued descriptive statistics of sex ratio and nest parameters of loggerhead turtles in South Africa.

season	oviposition	nest #	n	males	females	female %	hatchling size	IP	TSP	TSP (begin)	TSP temperature (°C)	CTE (°C)
2011	12/12/2011	50	(3)	0	(3)	100	-	-	-	-	-	-
2011	12/25/2011	51	8 (3)	0	8 (3)	100	42.6	54	20	18	30.88	31.70
2011	12/25/2011	52	18	0	18	100	44.0	54	19	19	31.86	31.94
2011	12/25/2011	53	5	0	5	100	44.2	54	19	19	30.91	31.90
2008	-	54	(4)	0	(4)	100	-	-	-	-	-	-
2010	-	55	(5)	0	(5)	100	-	-	-	-	-	-
2010	-	56	5	0	5	100	-	-	-	-	-	-
2010	-	57	(4)	0	(4)	100	-	-	-	-	-	-
2010	-	58	(7)	0	(7)	100	-	-	-	-	-	-
2010	-	59	(6)	0	(6)	100	-	-	-	-	-	-
2010	-	60	(5)	0	(5)	100	-	-	-	-	-	-
2010	-	61	26	0	26	100	-	-	-	-	-	-
2011	-	62	(14)	(1)	(13)	93	-	-	-	-	-	-
1984	laboratory	63	11	11	0	0	-	69-71	-	-	-	27.2
1984	laboratory	64	10	10	0	0	-	65	22	23	-	27.9
1984	laboratory	65	9	9	0	0	-	60-61	-	-	-	28.8
1984	laboratory	66	12	12	0	0	-	58	19	20	-	29.2
1984	laboratory	67	12	6	6	50	-	55	18	19	-	29.7
1984	laboratory	68	10	1	9	90	-	54	18	19	-	30.2
1984	laboratory	69	13	0	13	100	-	52	17	18	-	30.7
1984	laboratory	70	12	0	12	100	-	51	17	18	-	31.2
1984	laboratory	71	12	0	12	100	-	49	16	17	-	32.2
1984	laboratory	72	10	0	10	100	-	47	16	17	-	32.8

ANNEX V: Descriptive statistics on sex ratio and nest parameters of leatherback turtles in South Africa. Non self-emerged hatchlings and full-term (dead) embryos are included in the total count (n) and additionally indicated in parenthesis. 'Hatchling size' (mm) indicates average size of self-emerged hatchlings per nest, 'IP' the total incubation period in days (oviposition to emergence), 'TSP' the thermo-sensitive period in days and 'TSP (begin)' the onset of TSP in days of total IP. 'TSP temperature (°C)' specifies *in situ* mean nest temperature during TSP and 'CTE (°C)' the constant temperature equivalent thereof.

season	oviposition	nest #	n	males	females	female %	hatchling size	IP	TSP	TSP (begin)	TSP temperature (°C)	CTE (°C)
2009	12/15/2009	A	11 (1)	0	11 (1)	100	60.5	64	21	25	30.94	30.34
2010	11/18/2010	B	(5)	(1)	(4)	80	-	-	-	-	-	-
2010	11/21/2010	C	1	0	1	100	58.8	77	23	35	29.51	29.15
2010	11/25/2010	D	10	0	10	100	-	67	21	29	30.56	30.63
2010	11/25/2010	E	9	0	9	100	60.6	71	22	31	29.74	30.02
2010	12/06/2010	F	1	1	0	0	58.4	69	22	29	29.75	29.59
2010	12/08/2010	G	-	-	-	-	57.0	65	21	28	30.32	30.57
2010	12/12/2010	H	10	0	10	100	58.7	63	21	25	30.18	30.08
2010	12/19/2010	I	9	3	6	67	-	-	-	-	-	-
2010	12/21/2010	J	(5)	0	(5)	100	-	-	-	-	-	-
2010	12/24/2010	K	(3)	0	(3)	100	-	-	-	-	-	-
2010	12/27/2010	L	10	0	10	100	61.5	65	21	26	30.49	30.06
2010	12/29/2010	M	12	0	12	100	-	-	-	-	-	-
2010	12/31/2010	N	(9)	0	(9)	100	-	59	21	23	30.81	31.13
2010	01/08/2011	O	22 (6)	0	22 (6)	100	57.0	64	21	25	31.65	30.84
2010	01/17/2011	P	10	0	10	100	-	-	-	-	-	-
2010	01/22/2011	Q	(23)	0	(23)	100	-	-	-	-	-	-
2011	11/10/2011	R	-	-	-	-	62.5	73	22	35	29.31	29.73
2011	11/25/2011	S	-	-	-	-	-	67	20	32	30.43	31.56
2011	12/01/2011	T	-	-	-	-	57.7	63	20	27	31.22	31.63
2011	12/18/2011	U	-	-	-	-	59.8	60	20	24	30.52	31.17
2011	12/27/2011	V	21	0	21	100	-	-	-	-	-	-

ANNEX VI: Publication 'Age-size relationship at reproduction of South African female loggerhead turtles *Caretta caretta'*

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ENDANGERED SPECIES RESEARCH
Endang Species Res

Age–size relationship at reproduction of South African female loggerhead turtles *Caretta caretta*

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ABSTRACT: Average age and size at first reproduction are important demographic parameters used in the management and conservation of populations. For sea turtles, absolute values for these parameters are still ambiguous, as most species are slow-growing, late-maturing migrants which are rarely encountered during the first 1 to 2 decades of their lives. Additionally, growth is significantly influenced by a variety of intrinsic and extrinsic factors which make it difficult to describe age–size relationships. Extensive notching (mutilation-tagging) of South African loggerhead turtles was used to determine age and size at reproduction (putative first nesting season), and thus to identify the trigger for sexual maturation. To date, 137 clearly identified notched adult females have been encountered at the nesting beach. A Gaussian and log-normal distribution were fitted to the age distribution data but only the Gaussian distribution can be used to safely estimate age at first observation, as the right part ('older ages') of the distribution is still unknown. The estimated age at first observation was corrected for the possibility that a female was not encountered during her first nesting season. Results indicate an average age of 36.2 ± SD 7.71 yr (95% CI 28.2 to 44.3 yr) with a straight carapace length (SCL_{min}) of 83.7 ± 4.15 cm (95% CI 83.0 to 84.4 cm). It was concluded that size is a more important threshold for the initiation of the maturation process than age and that the onset of sexual maturity is dependent on intrinsic and extrinsic factors.

KEY WORDS: Age and size at reproduction · Loggerhead turtle · Mutilation tagging

INTRODUCTION

Age and size at first reproduction, reproductive lifespan, and the number and size of offspring produced per individual are fundamental life history characteristics required to model population dynamics or productivity. They are frequently used in conservation to model the likelihood of a population going extinct or in wild stocks to estimate the off-take (Scott et al. 2012), e.g. for setting fishing quotas. Life history characteristics are easy to track in captive animals but this is much more difficult in wild populations (Scott et al. 2012), especially those of wide-ranging or migratory species. Due to the range of factors affecting individuals/populations it is also difficult to predict/model these parameters.

The innate life history characteristics of a species/population are highly modified by energy availability (Berner & Blanckenhorn 2007). Therefore, food accessibility and the rate at which an organism can process food (West et al. 2004, Molles 2010) regulate the metabolic investment of energy into reproduction or growth. Thus, the onset of sexual maturity — as indicated by a reduction in energy investment into somatic growth and a greater energy allocation into the growth and maturation of reproductive organs (Hatase et al. 2004, Berner & Blanckenhorn 2007, Snover et al. 2007, Bjorndal et al. 2012) — is dependent on both population characteristics (intrinsic) and the environment (extrinsic).

For sea turtles, growth rates, and thereby age and size at sexual maturity, are influenced by intrinsic and

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extrinsic factors. Intrinsic factors that affect the growth rate in sea turtles include species, genotype, health status and sex (Chaloupka & Limpus 1997, Van Dam 1999, Heppell et al. 2003). Extrinsic factors that regulate growth rate are, for example, water temperature, food quality and food availability (Parker 1929, Bjorndal et al. 2003, Balazs & Chaloupka 2004). These factors are easy to regulate in captive studies but are often highly variable and difficult to measure under natural conditions (Mendonca 1981) as sea turtles migrate over thousands of kilometres and experience large habitat variations that additionally affect their growth rate (Musick & Limpus 1997, Casale et al. 2007, Koch et al. 2007). The variability in growth rate has already been documented in different species and several populations. Green turtles from the Hawaiian archipelago (Balazs & Chaloupka 2004) and the southern Great Barrier Reef (Chaloupka et al. 2004) grow at different rates due to differences in food quality and availability, as well as in sea surface temperatures of their foraging grounds. Furthermore, Hughes (1974) and Bjorndal et al. (2003) found that compensatory growth (accelerated growth) appears in juvenile sea turtles when they move from a suboptimal environment to better conditions. Similarly, different sexes can follow different growth patterns, as shown for green (Chaloupka et al. 2004) and hawksbill (Chaloupka & Limpus 1997) turtles on the Great Barrier Reef. Here immature female green turtles grow significantly faster than males after a juvenile growth spurt, resulting in sexual dimorphism in adult size (Limpus & Chaloupka 1997). Immature hawksbill females grow faster than males at all recorded sizes (Chaloupka & Limpus 1997). Although variability in growth rate in natural populations has been documented, the link between age and size at sexual maturity remains unidentified. Size per se is thus not a reliable indicator of age in turtles, but a minimum size has to be reached before reproduction can take place (Hughes 1974).

Current methods used to estimate the growth rate of individuals in sea turtle populations require substantial investment in in-water studies (e.g. on the feeding ground). The most common field methods include capture-mark-recapture techniques (Frazer & Ehrhart 1985, Limpus & Chaloupka 1997, Bresette & Gorham 2001, Seminoff et al. 2002, Casale et al. 2009b) and length-frequency analysis (Bjorndal et al. 2001, Casale et al. 2009a). However, these methods do not provide a definitive measure of age at maturity, but rather an estimate of growth. Estimating age and size at first reproduction in wild sea turtle populations requires a permanent mark, which can be applied to the hatchling and which grows with the turtle throughout its life. Mutilation tagging, i.e. living tags (Bell & Parsons 2002), notching of hatchlings with a year-code (Hughes & Brent 1972) or DNA sampling (Dutton et al. 2005) allow the definitive determination of age and size at first reproduction. L. Guastella and G. Hughes (unpubl. data) later confirmed the validity of notch codes, as they established that notching has no harmful effect on a turtle's health (as indicated through growth and mortality rates). However, mutilation tagging is labour intensive (Bjorndal et al. 2001) as it requires notching large numbers of individuals to compensate for high annual mortality (Table 1). Moreover, it takes many years to produce results as surviving individuals are expected to return only decades later. To date, results

Table 1. Summary of the South African notching data, showing numbers of loggerhead turtle hatchlings notched per season and the number of notched females subsequently observed from each year of notching. The numbers of notched hatchlings were extracted from annual season reports by the Natal Parks Board (1972–1998) and Ezemvelo KZN Wildlife (1999–2002)

Season	Hatchlings notched	Notched females observed
1972	5000	6
1973	10 250	7
1974	12 133	22
1975	11 267	13
1976	14 720	18
1977	13 463	24
1978	15 130	–
1979	15 981	1
1980	8489	–
1981	7507	2
1982	5634	–
1983	7383	–
1984	7585	3
1985	9712	–
1986	3784	1
1987	18 038	3
1988	11 380	5
1989	9071	2
1990	5189	16
1991	9406	3
1992	12 521	–
1993	18 046	6
1994	12 068	1
1995	12 856	2
1996	12 203	1
1997	10 038	1
1998	14 981	–
1999	9028	–
2000	12 424	–
2001	9928	–
2002	7596	–

are only known from 2 mutilation tagging studies: the Ezemvelo KZN Wildlife (previously known as Natal Parks Board) notching programme in South Africa and the study carried out by Queensland's Environmental Protection Agency in Australia. The notching programme in South Africa started 40 yr ago and Baldwin et al. (2003) estimated an arithmetic mean age at first nesting of 19.5 yr (range 10 to 29 yr), based on a limited sample size and not taking into account that the sample was time-censored. The notching programme in Australia, which was conducted from 1976 to 1983 with 129 921 loggerhead hatchlings notched (Limpus et al. 1994), on the other hand, had very few returns; these suggested a minimum age at first nesting of around 29 yr (Limpus 2008). The present study aims to re-assess the average age and size during the putative first nesting season of loggerhead females nesting in South Africa through the evaluation of notch codes (mutilation tagging), as well as to determine whether size or age is the principal driver of sexual maturity.

MATERIALS AND METHODS

The South African nesting beach is situated within the iSimangaliso Wetland Park, at the southern end of the tropics (27°S) in the north-eastern coastal province of KwaZulu-Natal (KZN) (Fig. 1). Between the 1972 and 2002 hatching seasons (which last from January to March with a peak in February) about 320 000 loggerhead hatchlings were notched by clipping out marginal scales with a leather punch, applying a unique seasonal code (Hughes 2002, Baldwin et al. 2003). Single notch codes were used during the first years, but were replaced with a double-notch procedure 8 yr into the programme, to avoid possible confusion caused by a turtle coming ashore with an injury representing a single notch. The nesting data obtained from returning females in the beach monitoring programme are standard to most nest monitoring programmes and included: species, flipper and passive integrated transponder (PIT) tag numbers, carapace size and nest position along the beach, as well as the notching code. Thereby, carapace length was measured from the anterior point at midline to the posterior notch at midline (SCL_{min}) and width (SCW) at the widest point (after Hughes et al. 1967 and Bolten 1999). The age of a notched female on its first encounter on the nesting beach is hereafter referred to as 'age at first observation' as it was assumed that not all females are encountered during their first nesting season (Thorson et al. 2012, Nel et al. 2013). Additionally, individuals with unclear or contradictory notch codes were removed from the sample. Further, the age of a notched female was calculated under the assumption that the turtle had hatched on 1 February (peak hatching) of its cohort. If an adult female was encountered more than once during its observed first nesting season its mean size for that season was used to reduce measuring error.

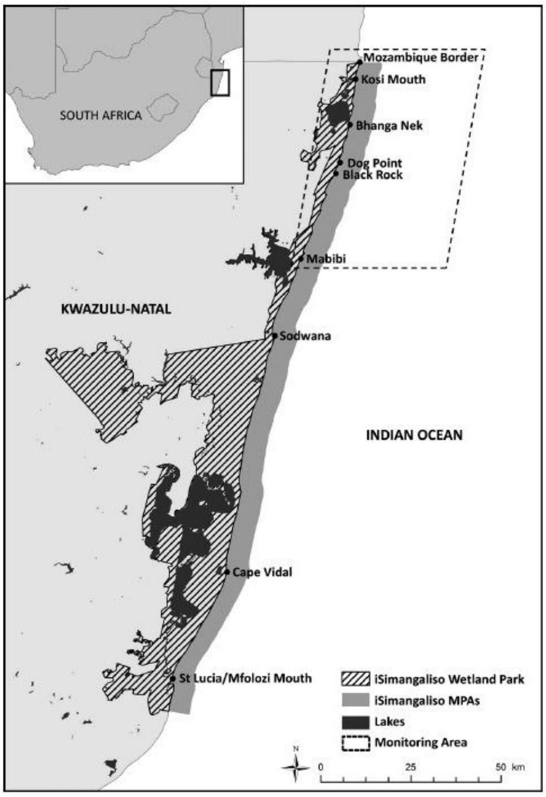

Fig. 1. The iSimangaliso Wetland Park, South Africa, including the extent of the Marine Protected Area (MPA), extending to 3 nautical miles from the coast, and the monitoring area south of the Mozambique border

A Shapiro-Wilk test was used to confirm normality for the size distribution of notched females at first observation. A Generalised Linear Model (GLM) was applied to identify significant factors influencing size at first observation. The factors tested were age, year of notching and the number of notches (single or double notched). A Gaussian identity link based on the result of the Shapiro-Wilk normality test was employed. The significance of co-factors was assessed by an analysis of deviance using chi-square statistics. All interactions were investigated, and backward selection was used to simplify the model.

In contrast to the size of notched individuals, the distribution of age at first observation is biased; the distribution is naturally truncated because the highest (or oldest) age classes have not yet been observed (Fig. 2). To take into account the truncated nature of the age distribution, the parameters providing the best explanation for the age distribution were identified using the maximum likelihood method (Gupta 1973). As the full distribution of 'age at first observation' is not known, both Gaussian and log-normal distributions were tested. Let D (μ, σ) be the age distribution at first observation (D being Gaussian or log-normal) and y the time period since an individual was notched. The likelihood L of observing a female during its first nesting season at an age $x = A$:

$$L\,[y, A, D\,(\mu, \sigma)] \;\#\; \Pr\,[x = A \mid D\,(\mu, \sigma)]\;/\;\Pr\,[x < y \mid D\,(\mu, \sigma)] \qquad (1)$$

Both Gaussian and log-normal distributions were fitted and their respective likelihoods were compared through Akaike Information Criteria (AIC) and Akaike weight (Burnham & Anderson 2002). The standard error of each parameter was estimated using the square root of the inverse of the Fisher information matrix at the point of maximum likelihood. R version 2.15.2 software (R Core Team 2012) was used for all of the statistical analysis. To correct for the possibility of missing the first nesting season of a female, the total number of nests per season (within the monitoring area) was divided by 3.7 (average nesting frequency for loggerheads after Nel et al. 2013) to estimate the number of nesting females. The probability of encountering a nesting female at least once in a season is thus the number of recorded nesting individuals (identified through flipper or PIT tags) divided by the estimated number of females on the nesting beach. As this probability is constant over time, the average over 4 yr (2008–2011), referred to as p_{ind}, was used. When a female nested in year Y, it was seen with a probability of p_{ind} and not seen with a probability of $1 - p_{ind}$. If not seen this year, it could be seen each subsequent nesting season, always with a probability of p_{ind}, and not seen with a probability of $1 - p_{ind}$. Thus the probability that a female which first nested in year Y was first observed in year $Y + a$ is:

$$p_a = (1 - p_{ind})^a \times p_{ind} \qquad (2)$$

The mean observed age at first observation A_{obs} has thus been corrected using these p_a to obtain an estimate of age at first nesting:

$$A_{nest} = A_{obs\cdot} \times p_0 + (A_{obs} - 1) \times p_1 + \ldots + (A_{obs} - n) \times p_n \qquad (3)$$

RESULTS

The size-frequency distribution of females at first observation was normal (Shapiro-Wilk normality test, $W = 0.99$, $p = 0.381$) or log-normally distributed ($W = 0.99$, $p = 0.560$). Female size at first nesting (n = 137) was investigated with regards to age, year of notching and single or double notch code. No significant effect of any of these parameters was found (all $p > 0.1$; Fig. 3). The mean ± SD size (SCL_{min}) at first observation of loggerhead females nesting in South Africa was 83.7 ± 4.15 cm (95% CI 83.0 to 84.4 cm, range 72.0 to 99.2 cm, median 83.5 cm).

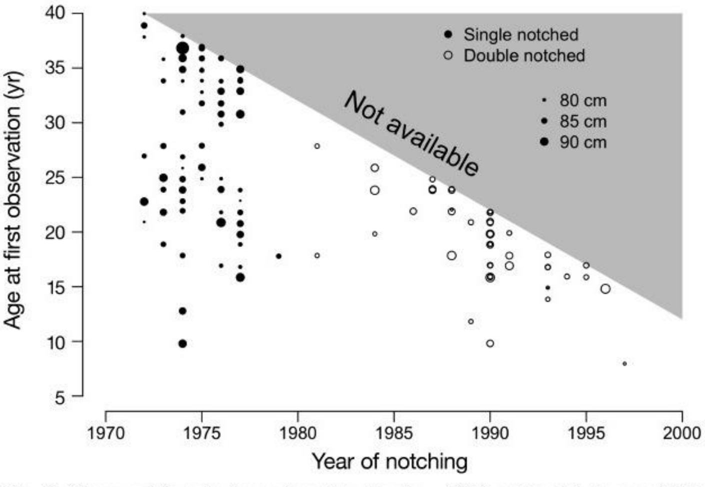

Fig. 2. Observed female loggerhead turtles (n = 137) notched between 1972 and 1997, showing age and size during their putative first nesting season in South Africa (see Table 1). Dot diameter indicates size, i.e. straight carapace length (SCL_{min})

The age distribution of notched females that returned each year is displayed in Fig. 4. This also includes the truncated Gaussian distribution. The fit of the truncated Gaussian distribution estimated a mean ± SD age of 37.5 ± 10.31 yr for loggerhead turtles at their first observation (age 95% CI = 29.3 to 45.8 yr; SD 95% CI = 7.45 to 13.17; $-\ln L = 391.37$; AIC 786.7397). The truncated log-normal distribution estimated a mean ± SD age of 134.7 ± 2.04 yr at first observation (age 95% CI = 11.8 to 1541.8 yr; SD 95% CI = 1.29 to 3.22; $-\ln L = 387.12$; AIC 778.2328). The log-normal distribution was selected against the Gaussian distribution as describing age at first observation better ($p > 0.9859$). However, when both distributions are superimposed on the truncated area (Fig. 5), it is obvious that data are only available for the left side of the distribution. The right side of the distribution is only known for the Gaussian distribution, based on the symmetry of the model. The log-normal distribution is much less constrained than the Gaussian distribution but the right side of the distribution is unknown. Thus, only the Gaussian distribution can be used to estimate age at first observation. If we assume that some notched females were not encountered during their first nesting season but had nested previously, then the 'real' age at first reproduction for South African loggerhead turtles can be estimated as 36.2 ± 7.71 yr (95 % CI 28.2 to 44.3 yr, range 8.0 to 40.0 yr; Table 2).

DISCUSSION

The age and size of putative neophyte nesters were determined through mutilation tagging and the results used to identify whether size or age drives sexual maturity. Over a 30 yr period about 320 000 hatchlings were marked, of which only 137 notched females were encountered at the natal nesting beach. This low recovery rate (Table 1) could be due to variable numbers of notched hatchlings, high natural or induced mortality (extrinsic factors), unclear notch codes that were discarded from the analysis, (male-)biased sex ratios of notched hatchlings and/or the fact that a large number of turtles have not yet matured and will only return over the next 2 to 3 decades. Thus, simply calculating the mean age would present a false outcome, as all samples are time-censored, i.e. 'oldest age classes' are not observed yet.

When including the possibility that a female loggerhead was not encountered during her first nesting season, the mean age at reproduction (putative first nesting season) for the South African population was estimated at 36.2 ± SD 7.71 yr. This is much higher than reported for most other loggerhead populations (Table 3), but it is in agreement with the recovery profile of the South African population. The South African conservation and monitoring programme was initiated in 1963 and about 40 yr later the loggerhead population displays almost exponential population growth (Nel et al. 2013). This indicates that the elimination of poaching along the nesting beach has resulted in an increased reproductive success leading to population growth.

Mean size (SCL_{min}) at first observation was 83.7 ± SD 4.15 cm. This was not corrected for the possibility that the female could have nested in a previous season, as growth after first reproduction is negligible (Limpus & Chaloupka 1997). South African loggerheads are similar in size to those from the south-eastern USA, but larger than those from the Mediterranean and smaller than the Australian populations (Table 3). Discrepancies in age and size at reproduction within and between populations (of the same species) could be based on intrinsic and/or extrinsic factors, or different methodologies (see Casale et al. 2009b, 2011a,b, Piovano et al. 2011 in Table 3). In the former case, South Africa's unique position between the warm Indian Ocean and the cold South Atlantic Ocean could have increased age and size ranges at first observation as hatchlings may grow up in a variety of environments.

The available age-size data at first observation of 137 loggerhead females indicated that neither age, year of notching nor notch code (single or double) affect female size at reproduction and thus no age–size relationship exists (Fig. 3). It is concluded that the maturation process of the reproductive organs, as well as the onset of reproduction itself, are correlated with a minimum size of the female (Hughes 1974), which may be reached at any given age dependent on intrinsic and extrinsic factors (Fig. 3). Similar results were found by Bjorndal et al. (2012)

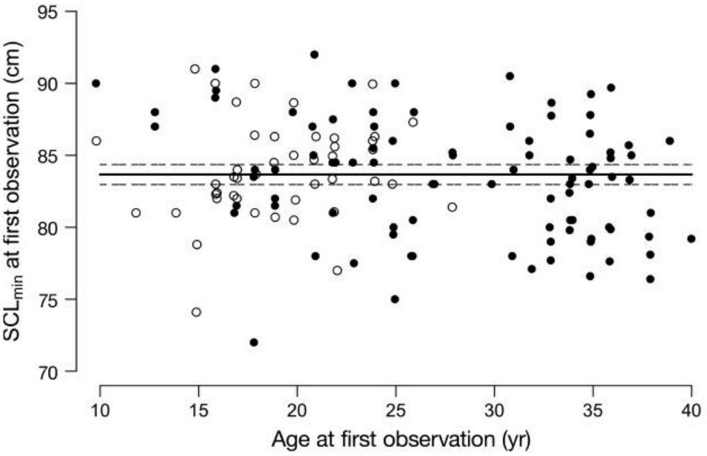

Fig. 3. Age and size distribution of single-notched (●) and double-notched (○) loggerhead females (n = 137) at first observation at the nesting beach in South Africa. SCL_{min}: straight carapace length. The regression line indicates that no age–size relationship exists; dashed lines indicate 95 % CI

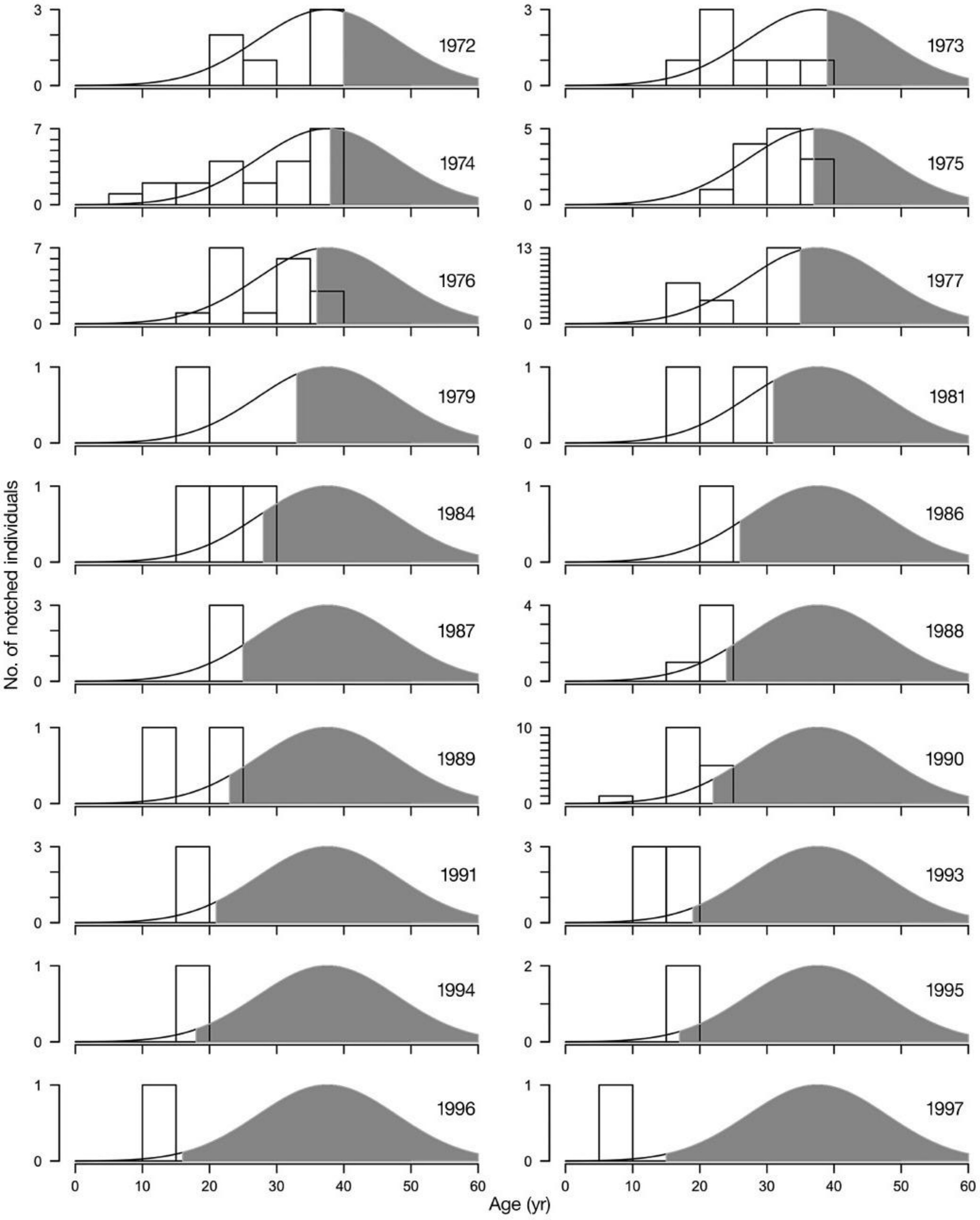

Fig. 4. Histograms of age at first observation of notched female loggerhead turtles, for each cohort. The curve shows the density of the fitted truncated Gaussian model. The shaded area indicates the lack of data for each distribution

who raised 47 female green turtles in captivity until after first reproduction. This hypothesis is also supported by Hatase et al. (2002) who conducted an isotope and telemetry study and found that immature turtles that migrate to coastal waters at a small size grow larger by feeding on nutrient-rich prey, such as benthos, until they reach sexual maturity. In contrast, immature turtles that stay in the pelagic zone for a longer period are smaller because of the scarcity of nutrient-rich prey in this habitat. Additionally, Bjorndal et al. (2003) determined that compensatory growth occurs in juvenile turtles but decreases as the turtles gain control over their movements.

Even though mutilation tagging is a labour-intensive method that takes a long time to produce results, we suggest that it (or DNA sampling) is the only way to ultimately determine the turnover rate of a population. In conclusion, the older age at first reproduction highlights the vulnerability of sea turtles as it has a negative effect on population replacement. Thus, with growing anthropogenic stresses the sustainability of turtle populations will become increasingly difficult (Scott et al. 2012).

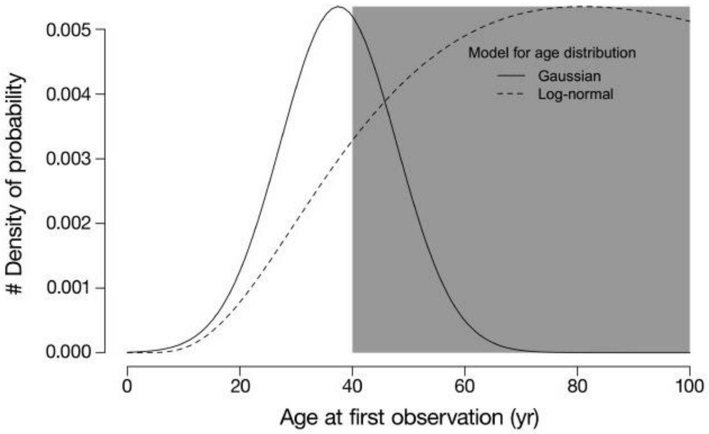

Fig. 5. Gaussian and log-normal distributions of age at first observation of female loggerhead turtles nesting in South Africa. The shaded area indicates the lack of data

Table 2. Estimated age (yr) of notched loggerhead females at first observation on the South African nesting ground (n = 137). The second row corrects for the possibility that a notched female was not observed during its first nesting season and hence indicates the real age at first reproduction

	Observed values			Age from Gaussian model		
	Min.	Mean	Max.	Min. 95% CI	Mean	Max. 95% CI
Observed data	8.0	25.1	40.0	29.3	37.5	45.8
Corrected data	7.3	24.1	38.7	28.2	36.2	44.3

Table 3. Summary of growth studies on age and size at first reproduction of loggerhead turtles. CMR: capture-mark-recapture; SC: skeletochronology; LFA: length-frequency-analysis; MT: mutilation tagging, i. e. notching; SSGR: notching or size-specific growth rates; SCL: straight carapace length: CCL: curved carapace length. SCL_{n-t} and CCL_{n-t} are measured from notch to tip; SCL_{min} and CCL_{min} are measured from the anterior point at midline (nuctual scute) to the posterior notch at midline between the supracaudals (Bolten 1999)

Method	Age (yr)	Size (cm)	Location	Source
CMR	30+	–	Australia	Limpus (1979)
CMR	10–15	75.0 SCL_{n-t}	Florida	Mendonca (1981)
CMR	30	92.22 SCL	Florida	Frazer & Ehrhart (1985)
CMR	16–28	66.5–84.7 CCL_{n-t}	Mediterranean	Casale et al. (2009b)
SC	22	92.5 SCL	Chesapeake Bay	Klinger & Musick (1995)
SC	20–24	92.4 SCL	Georgia	Parham and Zug (1997)
SC	14.9–28.5	66.5–84.7 CCL_{n-t}	Mediterranean	Casale et al. (2011a)
SC	24	69	Mediterranean	Piovano et al. (2011)
LFA	26.5	87 CCL_{n-t}	Southeast USA	Bjorndal et al. (2001)
LFA	23.5–29.3	80 CCL_{n-t}	Mediterranean	Casale et al. (2011b)
MT	19.5	72.8–98.5 SCL_{min}	South Africa	Baldwin et al. (2003)
MT	29	95.7–95.76 CCL_{min}^{a}	Australia	Limpus (2008)
MT	36.2^{b}	83.7 SCL_{min}^{c}	South Africa	This study
SSGR	45.0	98.2 CCL	Pacific and Atlantic	Scott et al. (2012)

[a] Mean size of nesting loggerhead females (n = 2587) in eastern Australia; [b] 95% CI 28.2 to 44.3 yr; [c] 95% CI 83.0 to 84.4 cm

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