The green sea turtle: adaptation and resilience to climate change

Submitted by

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Abstract

Sea turtles are a globally distributed migratory species that use a range of habitats during their life cycle, including both land and sea. As egg laying ectotherms they are particularly susceptible to variation in temperature, especially during clutch incubation. Climate change models predict increasing temperatures over the course of the century, along with sea level rise, and changes in weather patterns. Understanding how these factors impact the environment and such ectothermic species is key to their survival. The green sea turtle, *Chelonia mydas*, is one of the best studied sea turtle species, and like all seven species they are of conservation concern, thus understanding the impact of climate change on this group is of importance for their conservation. In this thesis I seek to investigate the impacts of temperature on offspring sex ratios and hatching success, two key parameters in the reproductive biology of sea turtles, using the Ascension Island rookery as model population. This will infer knowledge on adaptation and resilience to climate change.

I first carry out a literature review (Chapter 2) to evaluate the existing knowledge of current primary sex ratios, and find that despite nearly four decades of work on the topic, little progress has been made. Indeed, only four studies have been published on sex determining temperatures in laboratory conditions, and a range of varying methods have been used. I then carried out a laboratory-based study to establish the pivotal temperature, the temperature at which an equal proportion of male and females are produced, (29.3°C) for the Ascension Island green turtle rookery (Chapter 3) and carry out a translocation experiment to determine whether maternal philopatry confers any form of advantage to incubating clutches through localised adaptation (Chapter 4). Neither under laboratory conditions, nor *in-situ* do I find any evidence of localised thermal adaptation; hatching success drops with increasing temperatures, and clutches from different thermal backgrounds produce equivalent proportions of males and females. Finally, I use this information to evaluate island wide sex ratios and offspring output, based on different climate projection scenarios (Chapter 5). I find that the primary sex ratio will likely be extremely female biased (> 90%) by the end of the century, with hatching success starting to decrease in the most extreme scenarios, especially at the darker beaches where temperatures will exceed thresholds for successful incubation. The geographic isolation of Ascension Island means that there are limited opportunities for dispersal. A full assessment of the impacts of climate change on sea level rise, coastal erosion and changes in weather patterns may provide more information on the treats and opportunities that this population faces. In the meantime, a change in nesting seasonality or nesting distribution within the beaches of Ascension may provide critical to mitigate the impacts of increasing temperatures.

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AUTHOR’S DECLARATION

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All chapters within this thesis were written by D. Tilley under the supervision of Prof A.C. Broderick, Prof B.J. Godley and Dr S. Weber. All histology was carried out in the Ascension Island Government Fisheries and Conservation Department laboratory with guidance from E. Nolan. Fieldwork was carried out by D. Tilley with assistance from the Conservation Department Interns, A.C. Broderick, B.J. Godley, J. Ellick, S. Weber. Specific author contributions are detailed below:

Chapter 2: Current knowledge of pivotal temperature and sex ratios of green turtles

Authors: Dominic Tilley, Brendan J. Godley, Sam Weber, Annette C. Broderick

DT, BJG, SW and ACB planned the study design. DT carried out the review of the literature, assembled and analysed data, produced all figures and tables, and was the lead author on the manuscript. BJG, SW and ACB provided guidance on data analysis and writing and all co-authors provided useful comments on the manuscript.

Chapter 3: No evidence of fine scale thermal adaptation in green turtles

Authors: Dominic Tilley, Samantha Ball, Jacqui Ellick, Brendan J. Godley, Nicola Weber, Sam Weber, Annette C. Broderick

BJG, SW, NW and ACB were awarded funding for the project. DT, BJG, SW and ACB planned the study design and the field work.

DT, led the field work with help from, SB, JE, BG, SW. DT analysed the data, produced all figures and tables, and was the lead author on the manuscript. BJG, SW and ACB provided guidance on data analysis and writing. All co-authors provided useful comments and insight on the manuscript.

Chapter 4 Translocation of sea turtle clutches: effects on offspring phenotype and survival

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DT, led the field work with help from, SB, JE, BG, SW. DT analysed the data, produced all figures and tables, and was the lead author on the manuscript. BJG, SW and ACB provided guidance on data analysis and writing. All co-authors provided useful comments and insight on the manuscript.

Chapter 5: Nowhere to go – Modelling climate change impacts on a remote green turtle rookery

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LIST OF ABBREVIATIONS AND NOTATIONS

Abbreviations:

AIG: Ascension Island Government

CTE: Constant Temperature Equivalent

DPS: Distinct Population Segment

ESD: Environmental dependent Sex Determination

GAM: Generalised additive model

GHCNM: Global Historical Climatology Network monthly mean data set

GIS: Geographical Information System

GLM: Generalized linear model

GLMM: Generalized linear mixed model

IPCC: International Panel on Climate Change

IUCN: International Union of Conservation of Nature

KNMI: Royal Netherlands Meteorological Institute

LB: Long Beach

NEB: North East Bay

NOAA: National Oceanic and Atmospheric Administration

PAM: Pan-Am

PvT: Pivotal Temperature

RCP: Representative Concentration Pathway

RMU: Regional Management Unit

SLR: Sea Level Rise

TRT: Transitional Range of Temperatures

TSD: Temperature dependent Sex Determination

TSP: Thermosensitive Period

Notation:

ΔT: delta T

ca.: circa

e.g.: exempli gratia

CHAPTER 1: GENERAL INTRODUCTION

The climate is changing at unprecedented rates mainly due to human induced greenhouse gas emissions. Since the 1880’s, mean global temperatures have risen by 0.85°C, sea levels are rising and glaciers are melting (Stocker et al., 2013). All of this is having impacts on species and ecosystems alike (Parmesan, 2006; Parmesan and Yohe, 2003). Marine reptiles have been navigating the oceans for some 250 million years, with the ancestors of modern sea turtles *ca.* 120 million years old (Motani, 2009; Thorne et al., 2011). Over these time scales, the species have had to contend with vastly changing conditions, with changes in sea levels, large temperatures fluctuations and the formation/loss of habitats. As such, climate change is something turtles must have adapted to over the millennia.

Sea turtle ecology

From the ancient lineages, seven species emerged (Figure 1) and thrived to still be present today. They are split into two families, the Dermochelyidae that includes only the leatherback turtle (*Dermochelys coriacea*) and the Cheloniidae, comprising of the green turtle (*Chelonia mydas*), the flatback turtle (*Natator depressus*), the hawksbill turtle (*Eretmochelys imbricata*), the loggerhead turtle (*Caretta caretta*), the olive ridley (*Lepidochelys olivacea*) and the Kemp’s ridley (*Lepidochelys kempii*).

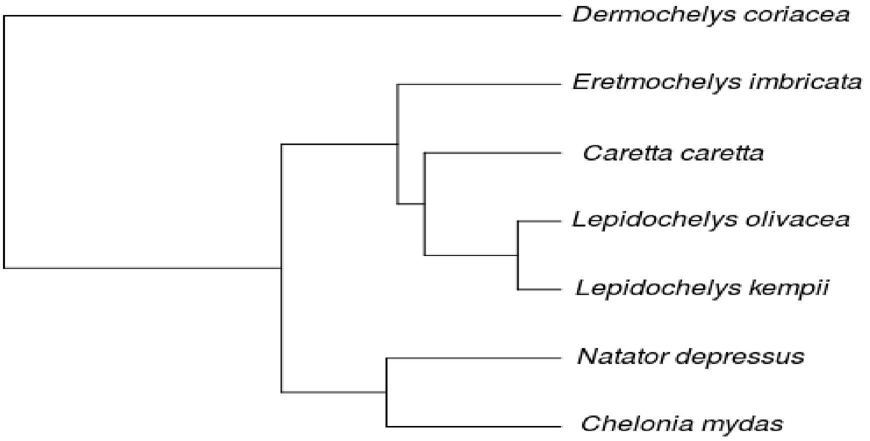

***Figure 1**: Phylogenetic relationship between the seven different species of sea turtles from two families, derived from* (Guillon et al., 2012).

The species have common characteristics, and each have specialisms. A main difference is that Cheloniidae are hard shelled turtles whereas the Dermochelyidae is soft shelled. Whilst most of the seven species are carnivorous, eating a range of jellyfish (*D. coriacea*), crustaceans (*L. olivacea, L. kempii, C. caretta),* soft bodied invertebrates *(N. depressus*) or sponges (*E. imbricata*), the adult green turtle stands out by being largely vegetarian, feeding on seagrass (Bjorndal, 1997).

Generally, sea turtles are described as long lived, migratory, and slow to mature (Musick and Limpus, 1997). Found in most of the temperate oceans of the world, they carry out seasonal breeding migrations on average every two to four years (Miller, 1997). Spending most of their life at sea, female sea turtles must come on land to deposit their clutches of eggs. They tend to be highly philopatric, returning to the nesting beaches from which they hatched to breed themselves (Bradshaw et al., 2018). After mating offshore from the nesting grounds, females emerge onto sandy shores to deposit their clutch of around 100 eggs. Females can lay up to 10 clutches per season, but more typically lay three to six clutches (Miller, 1997; Weber et al., 2013). Each clutch incubates for ≈ 50-60 days, when hatchlings emerge and find their way to the water (Miller, 1997). Combining swimming and drifting in the currents, the hatchlings reach pelagic waters where they spend several years feeding and growing (Briscoe et al., 2016a, 2016b; Mansfield et al., 2014). After an estimated 3 to 5 years, juveniles of most species recruit to foraging grounds and after reaching maturity at between 10 to 40 years of age (Avens and Snover, 2013; Scott et al., 2012) start their breeding cycle that may last for more than 30 years (Limpus, 2018).

In all species of sea turtle, offspring sex is determined by the incubation temperature, a form of environmental sex determination (ESD) known as temperature-dependent sex determination (TSD). In sea turtles, female offspring are produced at warmer temperatures and males at cooler temperatures, with the pivotal temperature at which a 1:1 sex ratio is produced typically around 29°C (Ackerman, 1997). Various hypotheses have been put forward for the evolutionary advantages of ESD in reptiles, for example, the Charnov-Bull hypothesis suggests that ESD enhances parental fitness by matching offspring sex to incubation conditions (Charnov and Bull, 1977) meaning that the embryo develops as the sex best-suited to those incubation conditions (Charnov and Bull, 1977; Warner and Shine, 2008, 2005).

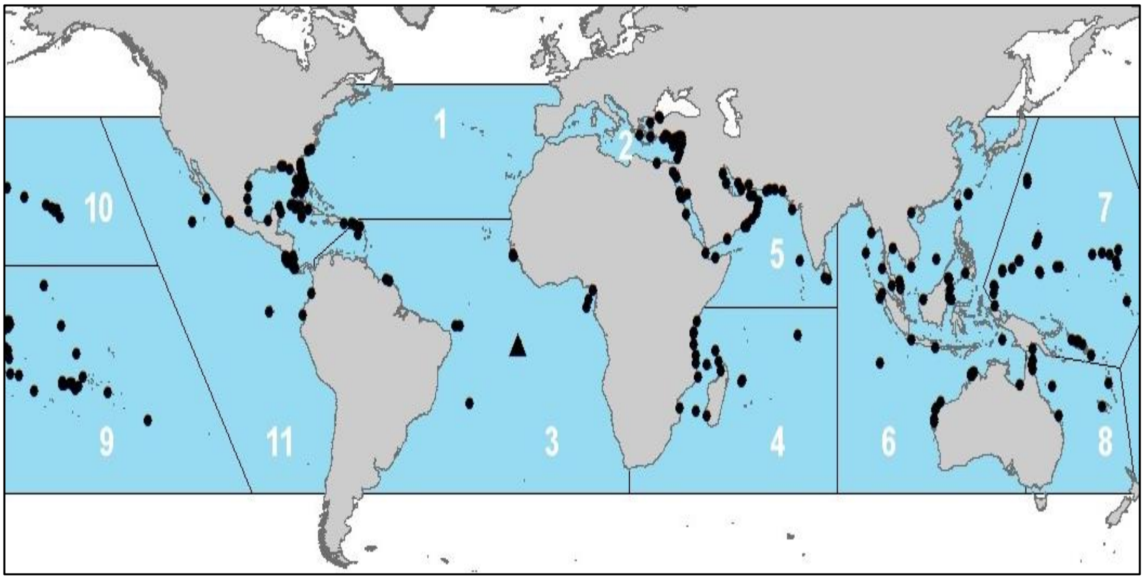

Figure 2: *Global distribution of green sea turtles, with filled circles representing known nesting sites, filled triangle marking Ascension Island. Data presented are from Seminoff et al 2015* (Seminoff et al., 2015)*, with each number corresponding to each Distinct Population Segment.*

Conservation concern

All sea turtle species are globally of conservation concern, due to historic exploitation, habitat degradation, pollution, and fisheries interaction among others; As such they are all on the IUCN Redlist of threatened species and protected under various global conservation conventions such as CITES (Convention on international trade in endangered species of wild fauna and flora) and the Convention on Migratory Species. For globally distributed species however, sub-population or regional assessments are often needed in order to prioritise populations that require conservation action, thus more recently marine turtle populations have been divided into regional management units (RMUs; (Wallace et al., 2010)) or distinct populations segments (DPS; (Seminoff et al., 2015)) for regional assessments. For instance, the leatherback turtle is listed as globally vulnerable, but of least concern in the Northwest Atlantic Ocean, critically endangered in the East Pacific Ocean and data deficient in the Northeast Indian Ocean (Wallace et al., 2013), highlighting the importance of local or regional assessments.

Impacts of climate change

Climate change was identified as a potential threat to sea turtles some three decades ago (Davenport, 1989; Mrosovsky, 1984) and impacts predicted at all life stages by a multitude of climatic processes. At the nesting beaches however, impacts are likely to be multi-faceted; rising temperatures will affect incubation conditions, impacting offspring survival (Hays et al., 2017), sex and phenotype (Horne et al., 2014; Micheli-Campbell et al., 2012), with sea level rise resulting in habitat loss and impacting egg development (Fish et al., 2005; Fuentes et al., 2012; Katselidis et al., 2014; Varela et al., 2018). Some nesting beaches in Australia for example are forecast to reach up to 40°C (Butt et al., 2016), temperatures at which embryonic development would be drastically impacted (Hays et al., 2017). The often distant foraging and breeding grounds may not be experiencing the same climatic influences, as such responses to environmental cues may not happen fast enough to cope with these forecast changes (Hamann et al., 2007). Whilst no clear pattern emerges, neither within species nor among regions, phenological shifts are a common response to increasing temperatures. For instance loggerhead turtles in Florida seemed to respond to increasing temperatures by nesting earlier (Weishampel et al., 2010, 2004), whilst there appeared to be no shift in the seasonality of nesting in North Carolina, possibly due to the lack of change in temperatures (Hawkes et al., 2007). Leatherback turtles nesting on the Pacific coast of Costa Rica were found to be nesting later in response to changing temperatures, however those in the Atlantic seem to be nesting earlier (Neeman et al., 2015; Robinson et al., 2014).

Changes in climatic conditions may however expand the habitat range (Witt et al., 2010) opening new nesting grounds leading to colonisation (Carreras et al., 2018), site specific characteristics (e.g. lack of warming in North Carolina (Hawkes et al., 2007)), or shaded area (e.g. coastal forest in Guinea Bissau (Patrício et al., 2017)) may lead to these areas producing enough male offspring to mitigate the impacts of high female producing beaches in other areas. Furthermore, whilst beaches in Cape Verde are currently producing 70% to 90% female offspring (Laloë et al., 2014), those in Chagos (Esteban et al., 2016) and Guinea Bissau (Patrício et al., 2017) are reported to be relatively balanced. Whether this variation will be enough to counter the extremes is difficult to tell, but with new nesting areas, for instance in Spain (Carreras et al., 2018) becoming available, there may be new opportunities for turtles to explore and exploit new regions. Changes in weather patterns may increase storminess and rainfall; whilst this may cause destruction of incubating nests, rainfall has been shown to decrease incubation temperature (Houghton et al., 2007) and therefore may also counter some of the negative impacts of increasing temperatures.

Nevertheless, much still needs to be done to assess site specific issues, with more detailed studies needed of the thermal regimes of nesting beaches worldwide, in addition to better understanding of sea level rise impacts and how ocean currents and predicted increased storminess will affect coastal erosion. Further studies are needed to understand how individual turtles may adapt or be adapted to the thermal environment they use. I explore this possibility in Chapter 3.

The green turtle

The green turtle, the study species of this thesis, has a circumglobal distribution within a general subtropical and Mediterranean range of temperatures (Figure 2) (Seminoff, 2004). Long harvested as a source of food, many populations are now recovering worldwide (Balazs and Chaloupka, 2004; Piacenza et al., 2016; Velez-Zuazo et al., 2014; Weber et al., 2014). Ascension Island, a small (≈ 90 km^2), remote (closest significant land masses: Brazil: 2250 km; Liberia: 1600 km) volcanic island located in the central south Atlantic (Chapter 3, Figure 1), is home to one of the largest green turtle rookeries in the world. This species has been legally protected at Ascension Island since 1957 (Huxley, 1999), and the population has grown considerably in the last few years (Weber et al., 2014). This population was the site of pioneering research into sea turtles in the 1970s (Carr et al., 1974; Carr and Coleman, 1974), and as such there is a good understanding of the general ecology of this rookery and considerable amount of data to build on (Bowen et al., 1989; Broderick et al., 2001; Carr, 1975; Carr et al., 1974; Carr and Coleman, 1974; Endres et al., 2016; Formia et al., 2007; B. Godley et al., 2002; B. J. Godley et al., 2002; Godley et al., 2001; Hays et al., 2003, 1999, 1995; Mortimer, 1990; Mortimer and Carr, 1987; Mortimer and Portier, 1989; Pintus et al., 2009; Weber et al., 2013, 2014, 2012, 2011).

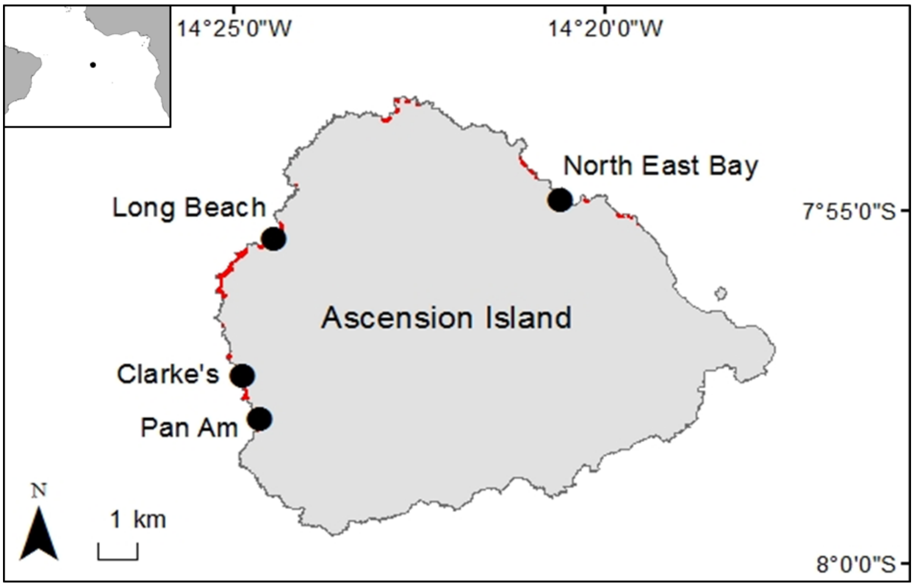

Figure 3: *Relative position of Ascension Island in the South Atlantic (inset), and distribution of nesting beaches on Ascension Island (red lines), and beaches used for this study. Long Beach, Clarke's and Pan Am have similar sand characteristics, whilst North East Bay has darker warmer sand.*

Turtles breeding at Ascension Island migrate every two to four years from foraging grounds off the coast of Brazil (Hays et al., 2002; Luschi et al., 1998). Annually, up to 4000 individuals (Weber et al., 2013) come to shore depositing over 20 000 clutches, and although one beach holds nearly 50% of all nests, there are 28 other beaches where nesting occurs on the island (Weber et al., 2014). Radio tracking of female turtles has shown they can lay up to eight clutches in a season, with exploratory behaviour between beaches, but also some high levels of fidelity to nesting areas (Weber et al., 2013). This reinforces the genetic analysis that revealed some weak but significant structure between nesting beaches (Formia et al., 2007). High levels of multiple paternity recorded in the population has not been shown to confer any advantages in reproductive success (Ireland et al., 2003; Lee and Hays, 2004) but may explain the weak genetic structure (Formia et al., 2007). Ascension Island offers beaches of different sand type and colour, creating highly variable incubation temperatures for clutches over small spatial scales (B. Godley et al., 2002; Hays et al., 1995; Weber et al., 2012). The different albedos make for different thermal incubation conditions, with dark sand beaches approximately 2.5°C warmer, throughout the nesting season, than pale sand beaches. Previous studies have shown there to be a high proportion of female offspring produced at Ascension Island, with temperatures on some beaches approaching the maximum thermal tolerance of embryos (Broderick et al., 2001; B. Godley et al., 2002; Hays et al., 2003). The most recent research at Ascension Island suggested there may be some thermal adaptation, with eggs from hotter beaches having greater hatch success at higher incubation temperatures (Weber et al., 2012), which may confer a degree of resilience on the population. The limited geographic range of the nesting grounds on Ascension Island, and the remoteness of the island provide little opportunity for a range shift. In the context of global climate change, and ESD, sea turtles will potentially be affected unless they can prove resilience and/or adaptation to climate change. This makes it a unique setting to study population-level responses to climate change and potentially apply findings to different nesting aggregations around the world.

Thesis layout

In this thesis, I present four chapters, written as independent units, in which I focus on the Ascension Island green turtle rookery. I investigated whether turtles may be able to adapt to climate change and the implications for the rookery and species as a whole.

In Chapter 2, “**Current knowledge of pivotal temperature and sex ratios of green turtles**”, I conducted a review of the literature on pivotal temperatures and primary sex ratios in green turtle rookeries around the world. These parameters are important to understand population dynamics, and how increasing temperatures may affect sea turtle populations. These have been identified as key questions for sea turtle conservation.

In Chapter 3, “**No evidence of fine scale thermal adaptation in green turtles**”, I assess the pivotal temperature and hatching success for the Ascension Island green turtle rookery. This experiment was carried out to determine if there was any form of localised adaptation, and therefore if site specificity conferred any advantage in the face of climate change. I first carried out a laboratory study looking at the impact of temperature on sex and hatching success, comparing eggs from different thermal backgrounds. I then widened the study to carry out similar experiments in field conditions.

In Chapter 4, “**Translocation of sea turtle clutches: effects on offspring phenotype and survival**”, I conducted a relocation experiment, cross incubating clutches from pale and dark sand beaches. These beaches have different thermal properties and thus, by carrying out these translocations I was able to directly compare how clutches from different origins performed under similar incubation conditions.

In Chapter 5, "**Nowhere to go – Modelling climate change impacts on a remote green turtle rookery**", using the results from chapter 3, and using well established IPCC climate forecast scenarios, I estimated historic and future offspring sex ratios for the entire island of Ascension.

Finally, in Chapter 6, I provide a synthesis of my findings and discuss their conservation implications for turtles and reptiles in the context of climate change. I suggest next steps to fully standardise methodology in TSD studies for sea turtles.

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CHAPTER 2: CURRENT KNOWLEDGE OF THE SEX RATIOS OF GREEN TURTLE OFFSPRING

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Green turtle; *Chelonia mydas*; TSD; TSP; Temperature dependent Sex Determination; Thermosensitive Period; Pivotal temperature; sex ratio

Abstract

Climate change poses a major threat to species that exhibit temperature-dependent sex determination (TSD), where sex of the offspring is determined by temperatures experienced during development. In sea turtles, female offspring are produced at higher incubation temperatures while males are produced at cooler temperatures. Understanding how temperature impacts offspring sex is therefore crucial to predict and/or mitigate the impacts of future climate change. Here, we review the literature pertaining to the green turtle (*Chelonia mydas*) to assess the current state of knowledge and identify gaps, and priorities. Few studies (n = 5) have established the pivotal temperature (at which a 1:1 sex ratio is produced under laboratory conditions) for a population, partially owing to the need to establish sex from histological examination of the gonads. Based on this limited sample however, there appears to be little variation in the pivotal temperatures among populations (range: 28.8°C - 29.5°C), although the eleven studies that have established field-pivotal temperatures (from *in situ* clutches) have a broader range (27.6°C - 30.3°C). Wide variation in offspring sex ratios is observed both among and within sites, with 35 (74%) of 47 studies reporting female biased sex ratios. Future studies should be carried out over a broader range of geographic locations, encompassing all Distinct Population Segments, and ensure spatial and temporal variation at sites are captured using standardised methods to ensure comparability. This field is currently restricted by the difficulties of determining sex of offspring sea turtles without euthanasia and the development of a marker for sex would revolutionise this area of research.

Introduction

Temperature dependent sex determination

The development of vertebrates into males or females is determined among species by a range of sex determining mechanisms. The genotypic background dictates morphological differentiation of the gonads in genotypic sex determination (GSD) species, whilst environmental cues experienced during the development of the embryo can influence the gonadal differentiation in species subject to environmental sex determination (ESD); in some rare cases both GSD and ESD contribute to the sex of offspring (Literman et al., 2018; Valenzuela et al., 2003; Valenzuela and Lance, 2004).

Temperature-dependent sex determination (TSD) is a form of ESD, where incubation temperatures determine the sex of the offspring. First described in the 1960’s by Charnier on Agama lizards (Charnier, 1966), it has since been shown to occur in species from all reptile clades (Warner, 2011). TSD can impact both oviparous and viviparous species, for instance the viviparous skink *Eulamprus tympanum* actively thermoregulates, allowing the mother to ‘select’ the sex of the offspring (Robert and Thompson, 2006), and some crocodilians are known to maintain their nests (e.g. adding building materials) which could influence incubation temperatures (López-Luna et al., 2015). Nevertheless, in clutches of eggs, developmental conditions are typically fully subject to the ambient environment and its fluctuations.

TSD in sea turtles was first described for green turtles (*Chelonia mydas*) with eggs sourced from the Cayman Island turtle farm by Owens *et al*. (Owens et al., 1978) and loggerhead turtles (*Caretta caretta*) in Georgia (USA) by Yntema & Mrosovsky (Yntema and Mrosovsky, 1980) with laboratory and field studies confirming it in all seven species of sea turtle: green

(Miller and Limpus, 1981), loggerhead (Yntema and Mrosovsky, 1982), olive ridley (*Lepidochelys olivacea*) (McCoy et al., 1983), hawksbill (*Eretmochelys imbricata*) (Dalrymple et al., 1985), leatherback (*Dermochelys coriacea*) (Rimblot et al., 1985), Kemp's ridley (*Lepidochelys kempii*) (Shaver et al., 1988), and flatback (*Natator depressus*) (Hewavisenthi and Parmenter, 2002).

The impact of temperature on sex determination

Species exhibiting TSD broadly conform to one of three different systems that are differentiated by the proportions of each sex produced across the normal thermal range of development: Type IA, females produced at warmer temperatures (e.g. all marine and most freshwater turtle species); Type IB, males produced at warmer temperatures (e.g. Tuatara (*Sphenodon punctatus*); or Type II with extremes producing females and intermediate temperatures producing males (some crocodilians, lizards and some freshwater turtles (Figure 1). Each pattern follows the same general rule of a thermal range of incubation beyond which embryonic development does not occur (extreme hot and cold temperatures), a transitional range(s) of temperatures (TRT) where a mixed proportion of each sex is obtained and a pivotal temperature(s), which produces a balanced sex ratio. It is important to note that pivotal temperature and TRT are defined for constant incubation temperature conditions, as opposed to the fluctuating thermal regimes that may be experienced *in situ* (see Box 1), although used in many field studies this is not correct use of this terminology and as result in this review we use the term field-pivotal to refer to those studies that estimate this value from *in situ* clutches. In sea turtles, the thermal range of development broadly spans 25°C to 35°C (Howard et al., 2014). There is evidence to suggest that increasing temperatures are detrimental to offspring development both in laboratory conditions (Tilley et al., 2019; Weber et al., 2012), and in field settings (Hays et al., 2017; Sim et al., 2015; Tilley et al., 2019), although constant incubation at high temperature may be more detrimental than increasing temperatures over the course of incubation (Howard et al., 2015; Tilley et al., 2019), with some suggestion of population level resilience observed in flatback turtles (Howard et al., 2015). The lower thermal range of development is seldom found in natural environments, but data from a hawksbill nest in Florida recorded incubation temperatures as low as 22°C during the early stages of development (Dalrymple et al., 1985).

Studies of TSD mechanisms in sea turtles are hampered by the fact that sex can only be easily assigned using external morphological characteristics in adults. In hatchlings, juveniles and sub-adults internal examination of the gonads is generally required, through histology, gross morphology or laparoscopy. For hatchling stages, this requires either euthanasia or collection of naturally occurring dead offspring (Ceriani and Wyneken, 2008; Wyneken and Lolavar, 2015).

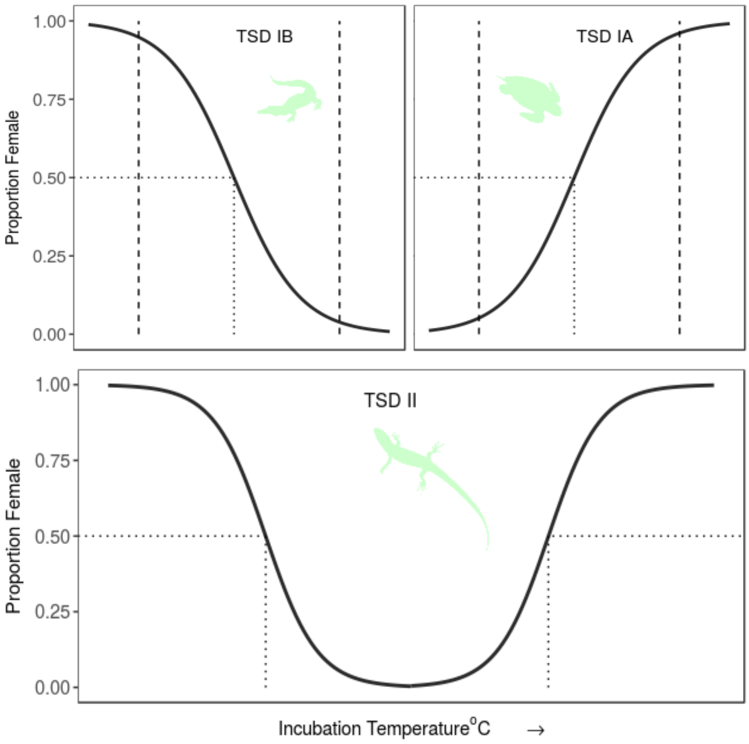

***Figure 1**: Temperature-dependent Sex Determination: Type IB, associated with some crocodilians, producing increasing proportion of males as temperatures increase. Type IA, associated with all sea turtles producing increasing proportion of females as temperature increase and Type II, associated with some crocodilians, agamid lizards and geckos, with females produced at extremes of temperatures and males in the middle range. All have pivotal temperatures (dotted lines) at which 50:50 sex ratio is produced, and a TRT (dashed lines).*

Box 1: *Terminology*	
Distinct Population Segment (DPS)	Population that is separated from other populations of the same taxon due to physical, physiological, ecological, or behavioural factors (Seminoff et al., 2015). Currently used by IUCN Marine Turtle Specialist Group to delineate sub-populations for regional Red List assessments.
Operational sex ratio	Ratio of sexually active males to receptive females in a population (Clutton-Brock and Parker, 1992)
Pivotal temperature (Pvt or PT):	Constant incubation temperature at which an equal proportion of individuals of each sex is produced (Mrosovsky and Pieau, 1991).
Field-Pivotal temperature (Field-Pvt or Field-PT):	Constant equivalent temperature obtained from the thermosensitive period at which an equal proportion of each sex is produced
Primary sex ratio	Sex ratio at hatching
Temperature-dependent Sex Determination (TSD):	Form of environmental sex determination where incubation temperature determines sex of the offspring (Bull, 1980)
Thermal Reaction Norm (TRN)	Phenotypic response to temperature
Thermosensitive period (TSP):	First stages of gonadal differentiation as determined by histology. The duration of TSP is usually associated with the middle third of incubation (Pieau et al., 1999)
Transitional range of temperature (TRT):	The range of temperatures that yields both sexes in variable proportions (Mrosovsky and Pieau, 1991; Warner, 2011). It is also referred to as incubation temperature limits producing between 5% and 95% of any one sex e.g. (Girondot, 1999; Godfrey et al., 2003).

The case of the green turtle

The green sea turtle, *Chelonia mydas*, has a circumglobal distribution with a thermally constrained range encompassing tropical and subtropical waters of approximately 140 nations, and nesting on the sandy beaches of approximately 80 (Groombridge and Luxmore, 1989). It is one of the most studied sea turtle species (e.g. (Casale et al., 2018; Jeffers and Godley, 2016)), and has been grouped into 11 Distinct Population Segments (DPS) each identified by a unique set of ecological, geopolitical and/or geographic characteristics (Seminoff et al., 2015) (Figure 2). As a highly migratory species with a complex developmental and reproductive cycle, green turtles occupy multiple habitats across their life stages (Bolten, 2003) and as such are exposed to a wide range of environmental conditions and stressors. Turtle nests are usually dug on sandy beaches, above the high water line, in which females deposit their clutches. Green turtle clutches hold around 110 eggs (Miller, 1997) (although there is considerable variation) that incubate between 40 to 70 days (Godley et al., 2002; Mrosovsky, 1980). In addition to sex ratios, development rate is determined by temperature with warmer clutches developing faster (Howard et al., 2014). Development and viability of eggs is also affected by abiotic factors such as humidity, oxygen levels, flooding and rainfall (Houghton et al., 2007).

After emerging from the nest, hatchlings leave the beach and disperse into the ocean. Dubbed the *lost years* because little is known about where they go and how they travel, recent work on neonates (Mansfield et al., 2014; Putman and Naro-Maciel, 2013) has started to shed light on this life stage. Currently very little information is available on neonate or young juvenile sex ratio estimates, although there are suggestions that male hatchlings have higher survival rate than females (Kobayashi et al., 2017), however, quantifying ratios after emergence is challenging (Jensen et al., 2016, 2018). After an initial post-hatching pelagic phase, juveniles start recruiting to foraging grounds when reaching approximately 25-40 cm (SCL: straight carapace length) (Pilcher, 2010; Reich et al., 2007), where they forage predominantly on seagrass and algae (Bjorndal, 1997; N Esteban et al., 2018). Foraging aggregations are typically comprised of individuals from multiple nesting grounds with differing characteristics (Jensen et al., 2018), making it difficult to infer primary sex ratios. Further genetic analysis of the stock may help determine the origin of the individuals (Casale et al., 2006; Jensen et al., 2016) and once that is established, informed sex ratio estimates can be established (Jensen et al., 2016, 2018).

Green turtles exhibit natal philopatry (Lee et al., 2007; Weber et al., 2013), and upon reaching sexual maturity (SCL : 60 - 90 cm (Avens and Snover, 2013)), return to nest in the region from which they themselves hatched. Females typically return every two to four years to nest, once they have accumulated sufficient energy reserves to sustain vitellogenesis and complete a breeding cycle (Solow et al., 2002), whilst males undertake annual (or at a more frequent rate than females) migrations to nesting grounds (Casale et al., 2013; Hays et al., 2010) and some may move between nesting aggregations (Lucy I Wright et al., 2012). This disparity in breeding frequency may allow for an operational sex ratio that is more balanced than the primary sex ratios (Lucy I. Wright et al., 2012); however in cases of extreme bias, the number of male turtles may become a limiting factor and impact on clutch fertility (Fuentes et al., 2011; Witt et al., 2010).

Green sea turtles are dependent on coastal ecosystems for both foraging and breeding; these habitats are sensitive to various threats, including anthropogenic disturbance and climate change (Fish et al., 2005; Hoegh-Guldberg and Bruno, 2010). As such the impacts of climate change on marine turtles are likely to be far reaching, and at the nesting beach, sea level rise (Fuentes et al., 2010; Patino-Martinez et al., 2014; Pike et al., 2015) and increasing temperatures are likely to have serious consequences for offspring sex ratios, and survivorship e.g. (Hays et al., 2017; Marco et al., 2018).

Plasticity

With fossil records spanning millions of years (Reisz and Head, 2008), sea turtles have persisted through large scale changes in sea level and temperatures in the global environment (Hamann et al., 2007; Poloczanska et al., 2009). The mechanisms behind how they coped are unclear. Potential adaptive responses to climate change, however, include nest site selection (Hays et al., 2001), developing new migratory routes (Poloczanska et al., 2009), adaptation of pivotal temperatures and/or thermal tolerance (Cheng et al., 2008; Davenport, 1997; Hawkes et al., 2007; Howard et al., 2014; Tilley et al., 2019; Weber et al., 2012), and shifts in phenology (Dalleau et al., 2012; Mazaris et al., 2013; Weishampel et al., 2010, 2004) as well as geographic range (Lenoir and Svenning, 2015; Moreno-Rueda et al., 2012). It could be hypothesised that, sea turtles are capable of adapting to climate change through a combination of different responses (Refsnider and Janzen, 2016). Whether anthropogenic factors such as exploitation and the rapid pace of change disrupt the coping mechanism is another question. Studies on green sea turtle populations have suggested that differing thermal sensitivities in embryological development may be due to localised adaptation (D. T. Booth and Astill, 2001; Stubbs and Mitchell, 2018; Weber et al., 2012), but this was not seen in laboratory and field studies carried out at Ascension Island (Tilley et al., 2019), whilst in the loggerhead sea turtle among female variation in hatching success was detected (Reneker and Kamel, 2016). Furthermore, differences in pivotal temperatures reported in flatback sea turtles have been attributed to two genetically differing populations (Stubbs et al., 2014). Research on other reptiles suggest some degree of plasticity and adaptive response to temperature fluctuations (synthesis in (Urban et al., 2013)). For instance, some skinks (*Bassiana duperreyi; Niveoscincus* spp.) have been shown to adjust nesting seasonality and nest depth (Telemeco et al., 2009), and basking behaviour in adults (Caldwell et al., 2017) in response to increasing temperatures. Moreover, horned lizards (*Phrynosoma hernandesi*) have been shown to alter thermoregulatory behaviour (Refsnider et al., 2018), whilst the painted turtle (*Chrysemys picta*) exhibits plasticity in nest site choice (Refsnider and Janzen, 2012), and may show individual variation in pivotal temperature (Refsnider and Janzen, 2016). This could give an insight into how turtles may respond to anthropogenic and natural changes in environmental conditions.

Climate change has been highlighted as a global research priority for the conservation of sea turtles (Hamann et al., 2010; Rees et al., 2016), with the impacts on primary sex ratios highlighted as an area in need of future research (Question 2 (Hamann et al., 2010; Rees et al., 2016)). Understanding variation in pivotal temperatures and offspring sex ratios of sea turtles is a key priority if conservation practitioners are to mitigate the impacts of climate change. Here we review the published literature on green turtle pivotal temperatures and offspring sex ratios and consider whether, given their wide geographic range, variation among populations may provide resilience to the predicted impacts of climate change.

Methods

Reviewing the literature

We reviewed all literature reporting green turtle pivotal temperatures and sex ratios published prior to January 1st 2019. We searched Web of Knowledge, Scopus, ScienceDirect and Google Scholar using the following search terms: "pivotal temperature" OR "sex ratio" AND "green turtle" OR "*Chelonia mydas*", sorting results by relevance and covering all years available. The search covered both title and abstract. We then filtered the search results manually, discarding any spurious results and duplicates. We supplemented the results by searching the reference lists of papers found in the web search process. We acknowledge a proportion of 'grey literature', that we only consider if it complements what has been published (e.g. Horikoshi 1992 (Horikoshi, 1991)); however the main focus is on articles published in the peer-reviewed literature and thus 'grey literature' is not presented in the table in supplementary material.

Literature analysis

Each article was sorted based on: 1) general theme (nest temperature, pivotal temperature, sex ratio, sexing); 2) setting (laboratory based, field based or both); 3) whether gonad histology was carried out; 4) whether the sex ratios presented used sex ratio curves from the study site; and 5) statistical model used. Articles were also classified by geographic area and Distinct Population Segment (DPS (Seminoff et al., 2015)).

Data Review

Data from papers presenting temperature and associated sex ratio or incubation duration models, and pivotal temperature data were retrieved; we did not attempt to reprocess the data. Information is presented either *verbatim* where possible or inferred from the available source. If ranges of temperatures were given, we synthesised them by using median values. We used raw non-aggregated data as opposed to summary statistics wherever this was made available in the published source. A number of publications provided data from hatcheries or relocated nests, however these are not considered in this review (e.g. (Leh, 1985)) as we are primarily interested in nests from 'natural' environments.

For spatial analyses data were mapped using ArcGIS 10.5, using terrestrial boundaries defined in (OpenStreetMap contributors, 2018), and DPS units established in (Seminoff et al., 2015). Graphical figures were produced using *'ggplot2'* (Wickham, 2009) and *'rphylopic'* (Chamberlain, 2018) packages in the statistical software R version 3.4.1 (R Core Team, 2017).

Results & Discussion

Summary of literature search

A combined 7844 results were obtained of which we retained the first 1263 publications (Table S1), with further reduction to 580 papers after filtering duplicates (same publication listed in different format/search engine or same data used in multiple publications). This list was further refined by removal of spurious papers that did not contain any of the search terms, resulting in a total of 52 published studies that presented metrics on pivotal temperature and/or sex ratios of green turtles.

Of these 52 studies, only six (11.5%) carried out incubation in controlled environments (Godfrey and Mrosovsky, 2006; Miller and Limpus, 1981; Mrosovsky et al., 1984; Tilley et al., 2019; Wood and Wood, 1982; Xia et al., 2011) (Table 1), which is a prerequisite for pivotal temperature studies (Mrosovsky and Pieau, 1991). We exclude Wood & Wood 1982 (Wood and Wood, 1982) as these observations were primarily a by-product of commercial operations and not designed to investigate pivotal temperature; these data are presented in Table S2 for reference. Seminal work by Miller & Limpus (Miller and Limpus, 1981) first described temperature dependent differentiation of the gonads in green turtles, and provided the first estimate of a pivotal temperature for this species. Despite research on TSD in sea turtles spanning nearly four decades, we found that only five (9.6%) of the 52 studies (Table 1) used established methods to correctly calculate pivotal temperature in green turtles. The majority of these studies based their estimates of pivotal temperature on a very small sample of clutches , or using few eggs from multiple clutches (Tilley et al., 2019). Thus, if within population variation exists, it is unlikely to be detected.

Synthesis of pivotal temperature studies

Reported pivotal temperatures for green sea turtles ranged from 28.7°C to 29.5°C (Table 1) whilst field pivotal temperatures ranged between 27.6°C and 30.3°C (central tendency 29°C) (Table S2). Field pivotal temperatures are all within the range reported for all other species of sea turtle of 27°C to 30°C (Wibbels, 2003). These data however can vary based on the mathematical model used and which data (e.g. middle third, TSP, mean nest temperature) are analysed to estimate pivotal temperatures (Table S2) (Fuentes et al., 2017).

Moreover, most studies to date use field temperature data to infer offspring sex, and although not necessarily incompatible with laboratory studies, the data need to be processed in the same manner, using standard proxies, and assumptions to have truly comparable data.

Plasticity of TSD mechanisms has been suggested as a way to cope with differing thermal conditions, where populations that nest in warmer locations may have a higher pivotal temperature (Howard et al., 2015; Limpus et al., 1985; Miller, 1997). For instance, pivotal temperatures were reported to vary from 27.6°C to 29.3°C between breeding populations of green turtles in Australia (Limpus, 2008), however, analysis from Ascension Island suggests no difference in pivotal temperature between beaches with differing thermal properties (Tilley et al., 2019). Further work to assess within population differences of pivotal temperature is thus necessary (Cheng and Wang, 2009). This would require large sample sizes both in number of eggs used and in number of unique nesting females sampled, with all of the logistical and ethical issues that this would entail. Comparing rookeries at a DPS level, using regions with highly contrasting sand temperature, may provide more insight into perceived differences, by allowing for comparison between genetically distinct populations. If it exists, such variation in pivotal temperatures may prove critical for sea turtles to adapt to increasing temperatures, and, from a methodological perspective, would limit the applicability of pivotal temperatures determined in one area as proxies for other populations.

Geographic disparity

Whilst green turtles have a wide distribution, research effort is strongly geographically biased towards a few populations. For example, publications describing TSD in the Mediterranean region primarily relate to nesting populations in Northern Cyprus and Turkey; globally important rookeries such as those of Tortuguero (Costa Rica), Heron Island (Australia), Ascension Island (UK), Suriname and Poilão (Guinea Bissau) are also relatively well represented in the literature (details in Table 2). Only four of the eleven DPS’s have had at least one study investigating pivotal temperatures, and only two have studies carried out in laboratory conditions (Table 1).

Hatchling sex ratios: global overview

This global overview reveals seasonal, annual, and geographic variation, suggesting that overall female biased sex ratios are common for green turtles (Table 2; Figure 2). There are notable exceptions with some sites strongly male biased (Esteban et al., 2016)In an effort to have a comprehensive understanding of primary sex ratios, it is necessary to 1) have seasonal analyses, carried out over multiple years, 2) account for nesting distribution, 3) account for microhabitats (Patrício et al., 2018).

For example, studies carried out at Poilão expose within site spatial and temporal variation. Gonad histology from dead hatchlings gave a range of sex ratio estimates for the differing seasons (85% female in 2008, 55% in 2009 (Rebelo et al., 2012)), and different habitat types within the beaches also accounted for variation e.g. 70.5% female in open sand to 9.7% female in the forest zone (Patrício et al., 2017). Similar variation in offspring sex ratios have been attributed to coastal vegetation at Tortuguero (Spotila et al., 1987; Standora and Spotila, 1985), Poilão (Patrício et al., 2017) or Chagos (Esteban et al., 2016), sand albedo on Ascension Island (e.g. from 53% female to 99% female (Broderick et al., 2001)), and within season temperature changes in the Caribbean (Laloë et al., 2016).

Table 1: *Reported pivotal temperatures determined in laboratory conditions for green sea turtles. Data are sorted by country and location, then by year the experiment was carried out. Statistical models are included as presented in the referenced studies. Sample size corresponds to the number of offspring sexed by gonad histology, with reference to the number of adult female turtles the eggs were taken from. Distinct Population Segment (DPS) value is extracted from Seminoff et al. (2015).*

State - Rookery	*Year*	*Pivotal T°C*	*Model*	*Sample size*		*DPS*	*Reference*
				Offspring	Clutches		
China - Guangdong Province	2011	29.5	Unclear	30	2	6	(Xia et al., 2011)
Suriname - Matapica Reserve	1983	28.8	Linear regression	108	3	3	(Mrosovsky et al., 1984)
Suriname - Matapica Reserve	1995	29.4 - 29.5	Logistic	78	2	3	(Godfrey and Mrosovsky, 2006)
Suriname - Matapica Reserve	1983; 1995	29.2 - 29.3[1]	Logistic	186	5[2]	3	(Godfrey and Mrosovsky, 2006)
UK - Ascension Island	2015-2016	29.3	Logistic	393	80	3	(Tilley et al., 2019)
Ningaloo - Australia	2017	29.2	Logistic	102	5	6	(Stubbs and Mitchell, 2018)

[1] Combined analysis of data presented in Mrosovsky et al. 1984 and Godfrey et al. 2006

[2] Combined number of offspring and females from Mrosovsky et al. 1984 and Godfrey et al. 2006

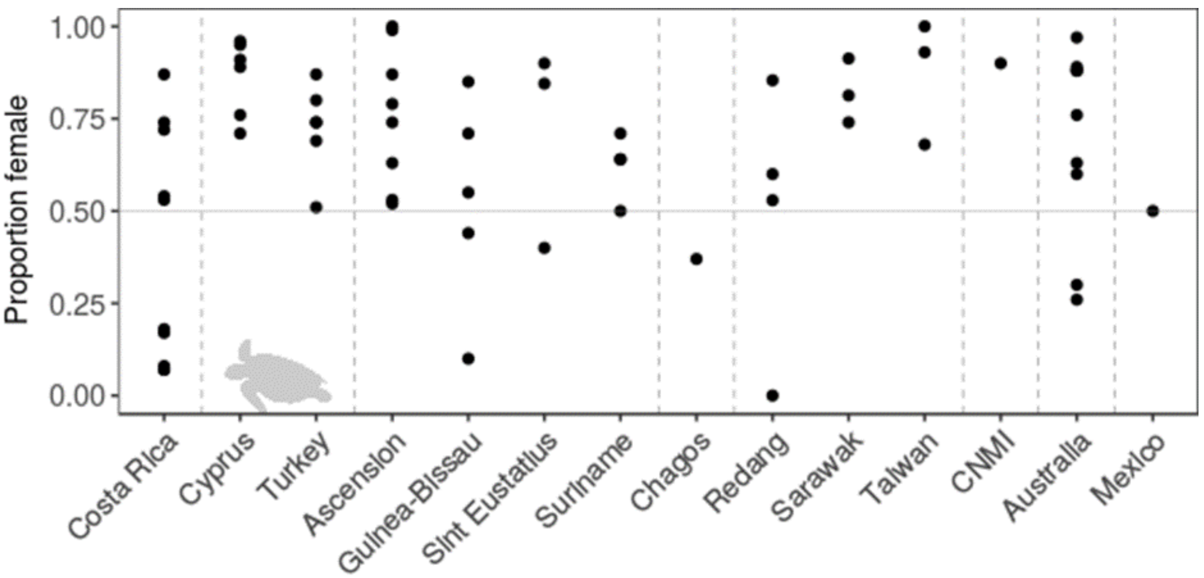

***Figure 2**: Different estimates for green sea turtle offspring sex ratios presented as proportion of females, based on all published data from each location presented in Table 2. These data are based on varying sample sizes, monitoring techniques, and estimating methods. They illustrate the wide ranges found within each region, based on seasonality, within and between beach location, and sampling rate, making overall assessments difficult. Vertical dashed lines are used to demarcate the different Distinct Population Segments (DPS - in order: 1, 2, 3, 4, 6, 7, 8, 11 as depicted in Figure 2). The horizontal line corresponds to an equal proportion of males and females. CNMI - The Commonwealth of Northern Mariana Islands.*

Given that pivotal temperature studies to date have found little variation, differences in sex ratio of offspring are likely due to local thermal conditions, and thus these examples highlight the importance of carrying out assessments over multiple nesting seasons, covering the range of habitats available, and accounting for within year seasonality. Nevertheless, high female output is not necessarily problematic; provided some males are produced within a population, a strong female bias may be the norm for sea turtles given their mating strategy and thus may pose little threat to population viability (Mitchell and Janzen, 2010; Wapstra et al., 2009). Limited male production could, however, impact populations through reduced female fertility (Boyle et al., 2014). Currently, adult sex ratios and operational sex ratios are far more balanced than primary sex ratios (Hawkes et al., 2013; Hays et al., 2017, 2010; Lucy I. Wright et al., 2012), but in some areas signs of extreme feminisation in juvenile and sub-adult aggregation may be an indication of future trends for these long lived species (e.g. (Jensen et al., 2018)).

Although controlled laboratory studies are needed to understand variation and the mechanism of TSD in sea turtles, given the many other factors that might influence sex ratios field studies are essential to understand the impact of fluctuating temperatures, for example diel variation (Georges, 1989), in addition to humidity (Lolavar and Wyneken, 2017), rainfall (Houghton et al., 2007; Lolavar and Wyneken, 2015), wind-cooling effects (Esteban et al., 2018) and sediment type amongst others, on offspring sex ratios. With current forecasts of climate change (Stocker et al., 2013), and current knowledge on TSD in turtles, it could be expected that sex ratios become increasingly female biased (e.g. (Patrício et al., 2018)), however, predicting changes in weather patterns for rainfall (Houghton et al., 2007) and nesting seasonality is challenging (Pike, 2009; Weishampel et al., 2004) and could influence sex ratio estimates. Added to this, climatic conditions may also lead to the development of coastal vegetation which in turn can have an impact on nest temperatures and therefore sex ratios (Kamel, 2013).

It is also important to standardise methods used to estimate sex ratios and in particular select a useful proxy for temperature in order for studies to be comparable, as this will influence results (e.g. using mean temperature from whole incubation period, temperature during the TSP or the mid third of incubation) (Fuentes et al., 2017; Girondot et al., 2018). Indeed, we need to understand the mechanism of TSD in order for more accurate estimates to be made, and to understand why at the pivotal temperature half of the embryos become male and half female.

Knowledge gaps: Assessment required

Although information on some of the key rookeries have been published in the peer reviewed literature, there is also a lot of information currently only available in conference proceedings or theses, which in turn highlight the missing information. It would be extremely valuable for this information to be published in the peer reviewed literature to gain a global understanding of primary sex ratios, and further highlight underrepresented regions that may require further assessment. Collating available data in a centralised database, in the same manner that mtDNA sequences are catalogued (https://accstr.ufl.edu/resources/mtdna-sequences/) or a global tracking tool such as STAT (http://www.seaturtle.org/stat/ (Coyne and Godley, 2005)), would facilitate future TSD studies, and would allow for coordination in methodology.

Assuming the current knowledge is representative of general trends worldwide (i.e. no variation in pivotal temperature), using metrics established in other populations is valid, or using mathematical modelling inferring different pivotal temperature scenarios (Laloë et al., 2014) may be sufficient to produce accurate estimates, however at the very least it is crucial to narrow down the temperature proxy used, as this can lead to very different results (Fuentes et al., 2017).

Harmonise methodology

Pivotal temperatures and the transitional range of temperatures are clearly defined for constant incubation conditions (see Box 1). Most authors use these metrics interchangeably -including in this review- for field or laboratory conditions (e.g. (Kaska et al., 1998; Patrício et al., 2017)). Carrying out laboratory based incubation is challenging for a number of reasons, including: (1) logistical constraints: not all field sites have laboratory facilities in proximity, and shipping eggs from nesting beaches to laboratories is not always feasible; (2) licensing: taking and shipping eggs for experimental studies requires permits that are not always easily obtainable; 3) ethics: for the data to be robust, hatchlings need to be sexed which usually requires euthanasia (although rearing of individuals in tanks until they reach a large enough size to sex by laparoscopy is possible (Lolavar and Wyneken, 2017) but post-study release to the wild is not always permitted (Paul and Sikes, 2013)). Many field studies therefore use defined pivotal temperatures obtained from the literature as proxies or as representative of the species so as to infer sex ratios. The assumption underpinning this method is that all populations have similar characteristics. The few studies carried out on pivotal temperatures tend to suggest this is the case, however the sample size is very small and methods not comparable.

The examples throughout this review emphasise the importance of: (1) collecting standardised temperature data from incubating nests; (2) carrying out artificial incubation using a span of temperatures that cover the full range of viable sea turtle egg temperatures; (3) using standardised statistical methodology for calculating field and laboratory based pivotal temperature; (4) ensuring studies cover entire field seasons, be representative of the different habitats available and be carried out over multiple seasons to get reliable seasonal trends; and (5) publishing the specifics of the research in order for results to be comparable to other studies. Furthermore, more research is needed into other factors that might influence sex ratios such as meteorological effects, including wind (Esteban et al., 2018), rainfall and humidity, especially in light of the predicted changes in weather (Lolavar and Wyneken, 2017, 2015).

We propose to expand the notions of pivotal temperature and TRT to field data, if and only if it is clearly stated e.g. Field-pivotal and Field-TRT. However, these metrics must be defined using a standard statistical method. Given that sex–temperature curves follow general ‘logistic’ patterns (among others see (Girondot, 1999; Godfrey et al., 2003; Hulin et al., 2009)), and that the proxy used has a clear impact on the result (Fuentes et al., 2017), when determining pivotal temperature and TRT, all analysis should use clearly established statistical methods (e.g. (Girondot and Kaska, 2014; Godfrey et al., 2003; Godfrey and Mrosovsky, 1999; Hulin et al., 2009)). These can be easily implemented with function *tsd* in package ‘embryogrowth’ (Girondot, 2016). Moreover, depending on the temperature data set used, results will vary considerably (Girondot, 2016), therefore, the thermosensitive period should be determined to obtain the time-weighted average temperature and use this as a proxy to define Field-Pivotal and Field-TRT (Girondot and Kaska, 2014).

Thus, by having a standardised approach, sex determining temperatures from across the world can be directly comparable and provide a better understanding of the potential implications of increasing temperatures on sex ratios.

Table 2: *Reported pivotal temperatures and sex ratio for green sea turtles from all available data. State- Rookery corresponds to where the study was carried out. Primary refers to whether the data analysed, or pivotal values used, in each publication is original or using methods from a different source. Source refers to any publication used for the analysis of the data. Season corresponds to the year in which data were collected. Pivotal temperature is reported verbatim with "Lab" referring to data from laboratory incubation, all values of percent female (**% ♀**) are presented specifying if the range is seasonal or spatial when applicable. Method corresponds to which proxy is used to determine pivotal or sex ratio with: histology GH; nest temperature NT; sand temperature ST; incubation duration ID; Estradiol-testosterone ratio E_2:T. If the corresponding time frame from which temperature data are used is noted (TSP: Thermosensitive Period, mid 3rd: middle third of incubation) specified temperature. Model corresponds to the mathematical model used to determine pivotal temperature. N clutches / sample size refers to the number of clutches, and/or number of offspring per clutch (when available) used for the analysis.*

State – Rookery	**DPS**	**Primary**	**Source**	**Season**	**Pivotal T**	**% ♀**	**Method**	**Model**	**Sample size**		**Reference**
									Offspring	**Clutch**	
Australia - Heron Island	8	Yes + others	(Miller and Limpus, 1981)	1998-1999		76	NT mid 3rd	linear 26-29°C		5	(David T Booth and Astill, 2001)
Australia - Heron Island	8	Yes + others	(David T Booth and Astill, 2001; Miller and Limpus, 1981)	2002-2003		94 (88 - 97)	NT mid 3rd	linear 26°C -29		14	(Booth and Freeman, 2006)
Australia - Heron Island	8	yes			<29		GH		121 eggs	1	(Miller and Limpus, 1981)
Australia - Heron Island	8	yes		1980-1981	<28.7[1]	63.1	GH	linear	130	13	(Limpus et al., 1983)[2]
Australia - Heron Island	8	yes		1980-1981	<28.7	29.5	GH	linear	120	12	(Limpus et al., 1983)
Australia - Heron Island	8	yes		02/1980		88	GH		60	6	(Limpus et al., 1984)

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[1] Because the measured *Caretta caretta* SDT$_{50}$ was 28.7°C and given that the northern beach of Heron Is. in the 1980-1981 nesting season was usually cooler than this, the *Chelonia mydas* sex ratio of 63.1% female from that beach in that season suggests that the *Chelonia mydas* SDT$_{50}$ could be lower than that of *Caretta caretta*

[2] The Capricorn Bunker cays provides a high hatching success and high probability of balanced sex ratio

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
Australia - Heron Island	8	yes		02/1980		26	GH		40	4	(Limpus et al., 1984)
Australia - Northern Great Barrier Reef / Raine Island	8	no	EPA Turtle Conservation Project unpublished data		29.3						(Limpus, 2008)
Australia - Heron Island	8	no	(Limpus et al., 1984, 1983) + unpublished Queensland data		27.6	♀ bias					(Limpus, 2008)
Australia - Ashmore, Bare Sand and Milman Islands; Moulter Cay; Bramble Cay	6	no	(Ackerman, 1997; Mrosovsky, 1994; Standora and Spotila, 1985)	2006-2008	29	mainly ♀	ST	Modelled ST			(Fuentes et al., 2009)
Australia - Ningaloo, North West Region	6	yes		2017	29.2 (Lab)		GH	Logistic	102	5	(Stubbs and Mitchell, 2018)
Cayman Islands	1	yes				2.7:1 - 1:3.3[3]	morphology		8452		(Wood and Wood, 1982)
China - Guangdong	6	yes		2006-2008	29.5 (Lab)		GH + E_2:T	Unclear	161[4]		(Xia et al., 2011)

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[3] incubated at 27.5° ± 0.5°C

[4] 30 GH + 131 E_2:T

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
CNMI - Saipan, Tinian and Rota Islands	7	no	(Ackerman, 1997; Godfrey and Mrosovsky, 2006; Mrosovsky, 1994; Standora and Spotila, 1985)	2006-2016	29	90>	mean NT	T value from literature[5]		174	(Summers et al., 2018)
Costa Rica - Tortuguero	1	yes			28< <29.5		GH + mean NT			19	(Morreale et al., 1982)
Costa Rica - Tortuguero	1	no	(Miller and Limpus, 1981; Morreale et al., 1982; Morreale, 1983)	1977;1980[6]	28.5-30.2	6 - 71 (spatial variation)	NT	linear regression		33	(Standora and Spotila, 1985)
Costa Rica - Tortuguero	1	yes		1980	28.5-30.3	8 - 74 (spatial variation) - 43 average	NT	exponential curve		15	(Spotila et al., 1987)
Costa Rica - Tortuguero	1	yes	reported in (Hirth, 1997)	1986; 1988	28.5-29[7]	40	GH + NT + ST		20 / clutch	55	(Horikoshi, 1991)
Costa Rica - Tortuguero	1	yes		1986-1989	29.4	35.5	GH + NT + ST	logistic on mean NT			(Horikoshi, 1992)
Northern Cyprus - Akdeniz Karpaz	2	yes		1995-1996	29	Average 76.25; top 91%, mid	NT			5	(Kaska et al., 1998)

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[5] This mean is above 29.0°C, the threshold beyond which a clutch becomes female biased (Standora and Spotila, 1985; Mrosovsky, 1994; Ackerman, 1997; Godfrey and Mrosovsky, 2006). Furthermore, it is above 30.3◦C, a temperature which produces a minimum of 90% females in green turtle nests (Standora and Spotila, 1985; Spotila et al., 1987).

[6] unclear

[7] ST

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
						83%, bottom 69%					
Northern Cyprus – Alagadi	2	yes		1993-1998	28.7[8]-29.2[9]	86 - 96	ID	linear regression		231	(Broderick et al., 2000)
Northern Cyprus - Alagadi	2	No	(Broderick et al., 2000)	2008		95	ID	SR: ID> pivotal ID		37	(Lucy I. Wright et al., 2012)
Northern Cyprus	2	yes		1993-1998	28.7[10]-29.2[11]	82	ID	linear regression		231	(Broderick et al., 2000)
Guinea Bissau - Poilão	3	yes		2013-2014	29.4	53.5	GH	logistic		27	(Patrício et al., 2017)
Guinea Bissau - Poilão	3	no		2013-2014	29.4	76-93	AT		modelled		(Patrício et al., 2018)
Guinea Bissau - Poilão	3	yes		2008-2009		85 -2008; 55-2009	GH		102		(Rebelo et al., 2012)
Indonesia	6	no	(Kaska et al., 1998; Morreale et al., 1982)	2013		♀ bias	NT	TRN; linear 26-29°C		7	(Tapilatu and Ballamu, 2015)
Malaysia - Sarawak Turtle Island	6	yes	(Standora and Spotila, 1985) analysis	1958		74	ID	Linear regression		328[8]	(Hendrickson, 1958)
Malaysia - Sarawak Turtle Island	6	yes	reported in (Hirth, 1997)	1984		81.3 - 91.3	GH + NT middle third			11[9]	(Leh, 1985)

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[8] Hatchery based clutches; overall sex ratio extrapolated to natural nests

[9] Hatchery based incubations

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
Malaysia	6	no	unpublished data/ (Whittier et al., 2003)		29.4±0.6[10]	♂ bias - 27.5 (0-60)	GH + NT	Linear regression for SR[11]			(Van De Merwe et al., 2005)
Malaysia - Sabah, Redang Island	6	yes		1998		52.9 - 85.4 (spatial variation)	GH+NT		20 / clutch	24	(Palaniappan et al., 2000)
Malaysia - Sabah, Redang Island	6	no	(Palaniappan et al., 2000; Whittier et al., 2003)		29.4±0.6	52.9 - 85.4 (spatial variation)	not original data				(Jensen et al., 2016)
Mexico - Michoacán	11	yes	reported in (Hirth, 1997)			50	ST (unclear) in Hirth 1997				(Alvarado and Figueroa, 1990)
Mexico – Yucatan	1	no	(Godfrey and Mrosovsky, 2006)	2011	29.2	balanced	NT (unclear)				(Comer Santos et al., 2015)
Mozambique - Vamizi Island	4	no	(Broderick et al., 2000; Godfrey and Mrosovsky, 2006)	2003-2010	29.2	bias ♂	ID	SR: ID> pivotal ID		687	(Anastácio et al., 2014)
		no	(Ackerman, 1997)		28.26			inverse linear regression			(Davenport, 1997)d
Netherlands - Sint Eustatius	1	no	(Ackerman, 1997)	2015	29	40 - 90 (season dependent) 84.5 - trend	ST	modelled	modelled		(Laloë et al., 2016)
NWHI - French Frigate Shoals	10	no	(Layton, 2011)	2003-2004;	29	♂ bias	NT + ST				(Balazs et al., 2015)

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[10] No detail on how this was established; the main reference is an abstract in conference proceedings with little detail

[11] Hatchery data

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
				2007-2009							
Suriname - Matapica Reserve	3	yes		1993-1994		63.8	GH		10 / clutch	79	(Godfrey et al., 1996)
Suriname - Wia-Wia Reserve	3	yes		1981-1982	<28.75?	64.1 beach	GH			12	(Mrosovsky, 1982)
Suriname - Matapica Reserve	3	yes			28.75 (Lab)	55.2[12] beach data	GH	linear regression	78 for PvT	113	(Mrosovsky et al., 1984)
Suriname - Matapica Reserve	3	yes		1995	29.4 - 29.5; 29.2-29.3[13] (Lab)		GH	logistic			(Godfrey and Mrosovsky, 2006)
Taiwan - Wan-an Island; Lanyu Island	6	yes				♀ bias	ID				(Cheng et al., 2008)
Taiwan	6	yes		2010-2011	29	68 - 100	GH	linear regression		26	(King et al., 2013)
Turkey – Akyatan	2	no	(Kaska et al., 1998)	1995-1996	29	♀ bias	ST				(Casale et al., 2000)
Turkey – Sugözü	2	no	(Kaska et al., 1998)	2005	28.9	74.24	NT			10	(Candan and Kolankaya, 2014)
Turkey – Sugözü	2	no	(Kaska et al., 1998)	2012	>28.9	70.5 - 93.5	GH		120		(Kılıç and Candan, 2014)

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[12] Weighted mean by nest frequency

[13] All available Suriname data

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
Turkey – Sugözü	2	yes		2008-2009	29	54.9 - 56.5 (season dependent)	GH + ID + NT	logistic	188	12 NT 103 GH	(Candan and Kolankaya, 2016)
Turkey – Sugözü	2	no	(Kaska et al., 1998; Mrosovsky, 1994)	2013	29	87.1	NT	regression based on Kaska 98		7	(Önder and Candan, 2016)
Turkey - Samandağ	2	no	(Broderick et al., 2000)	2003-2007		Average: 74; 39-79 season dependent; 46-94 within nest location	GH[14] + NT			14	(Yalçin Özdilek et al., 2016)
UK - Ascension Island	3	no	(Ackerman, 1997)a	1998-1999		53% - 99% beach dependent	ID	adding expected metabolic heating to ST	Predicted		(Broderick et al., 2001)
UK - Ascension Island	3	yes		1998-1999	28.8	3:1 ♀ (75)	GH+NT	Maximum likelihood / logistic		32[15]	(Godley et al., 2002)
UK - Ascension Island	3	no	(Godley et al., 2002)	2006	28.8	87	NT	Maximum likelihood / logistic		23[16]	(Pintus et al., 2009)
UK - Ascension Island	3	yes		2015-2016	29		GH+NT	Logistic	10 / clutch	25	(Tilley et al., 2019)
UK - Ascension Island	3	yes		2015-2016	29.3 (Lab)		GH	Logistic	393 o	393	(Tilley et al., 2019)

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[14] Some GH associated with NT, some with no apparent temperature data.

[15] Includes histology and temperature based estimates

[16] Control clutches only

State – Rookery	DPS	Primary	Source	Season	Pivotal T	% ♀	Method	Model	Sample size		Reference
									Offspring	Clutch	
UK - Chagos Archipelago	4	no	(Mrosovsky, 1994; Mrosovsky and Pieau, 1991)	2013-2014	29	37 (projected)	ST projected		Modelled		(Esteban et al., 2016)

Ethics

The authors declare no ethical issues.

Data

Supplementary material data are stored on figshare [https://figshare.com/s/5dedc88cf5c23979634e].

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Competing interests

The authors declare that the research was carried out without any competing interests.

Authors contributions

ACB, BJG and SW conceived the study and were awarded a NERC iCase studentship. DT led the data collection and analysis and writing of the manuscript with assistance from all co-authors.

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CHAPTER 3: NO EVIDENCE OF FINE SCALE THERMAL ADAPTATION IN GREEN TURTLES

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Abstract

Adaptation to increasing temperatures may enable species to mitigate the long-term impacts of climate change. Sea turtles have temperature dependent sex determination (TSD), where the sex of the offspring is determined by incubation temperature. Variation in the pivotal temperature, at which a 1:1 offspring sex ratio is produced, has been suggested as a potential adaptive mechanism to rising global temperatures. Here, we investigate the sex ratio of green turtle *Chelonia mydas* offspring from nests on beaches with notable differences in their thermal properties, to look for evidence of localised adaptation. We compared pivotal temperatures and hatch success in both the laboratory and *in situ* using eggs laid on two nesting beaches (dark vs. pale sand) at Ascension Island that represent the extremes of the range of incubation temperatures experienced by this population. We found no effect of beach of origin on pivotal temperatures, hatch success, or hatchling size in the laboratory or the wild. This suggests that turtles from the same rookery are not locally adapted to different thermal conditions experienced during incubation. Under predicted climate change scenarios, this will result in reduced hatch success and an increased proportion of female offspring unless temporal or spatial range shifts occur.

Keywords

Chelonia mydas, pivotal temperature, hatching success, sea turtle, TSD, climate change

Introduction

Clear patterns of spatiotemporal shifts in biotic and abiotic trends have unequivocally been associated with a response to climate change (Laloë et al., 2014; Parmesan and Yohe, 2003; Rosenzweig et al., 2008), as species are forced to adapt, disperse or disappear (Parmesan, 2006). Broad scale responses include changes in phenologies (Scheffers et al., 2016), distributions (Poloczanska et al., 2013) and trophic mismatches (Edwards and Richardson, 2004). Species that exhibit temperature dependent sex determination (TSD) are highly sensitive to climatic variation (Refsnider and Janzen, 2016) and there are concerns that increasingly imbalanced sex ratios may affect the long term viability of some populations (Laloë et al., 2016; Nelson et al., 2004). Plasticity in the pivotal temperature at which a 1:1 sex ratio is produced has been suggested as one mechanism that may allow adaptation to changing climatic conditions. For instance, painted turtles (*Chrysemys picta*) inhabit a wide geographic area and are thought to exhibit some degree of heritability in pivotal temperature (Refsnider and Janzen, 2016). Marine turtles have inhabited the oceans for the last 100 million years (Naro-Maciel et al., 2008) and, as such, have had to contend with climate change over evolutionary timescales. So far, sea turtles have been shown to respond to climate change by changing the phenology of nesting (Weishampel et al., 2010), which may ensure clutches incubate under conditions within their thermal norm. Although not yet documented as a response to climate change, plasticity in nest site selection has also been observed with animals actively selecting nest sites with specific thermal conditions (e.g. (Doody et al., 2006; Warner and Shine, 2008)). In the short term, this behavioural plasticity may mitigate changes in thermal conditions, which, combined with restricted maternal gene flow through natal philopatry, could create the conditions that lead to localised adaptation. For instance loggerhead turtles (*Caretta caretta*) at the northern extreme of their range produce near balanced offspring sex ratios (Hawkes et al., 2007), but green turtles (*Chelonia mydas*) show a marked contrast in offspring sex ratio from the northern to southern end of the Australian Great Barrier Reef, likely as a result of differing incubation temperatures (Jensen et al., 2018).

Pivotal temperature and transitional range of temperature

For marine turtles, thermal tolerance limits (beyond which embryonic development and hatching is unlikely to occur) are thought to range occur around 25°C and 35°C (Howard et al., 2014), with a greater proportion of females produced at temperatures above the pivotal temperature. Although many nesting populations are yet to be assessed, pivotal temperatures for green turtles appear to be relatively consistent across the species range, and typically estimated to be between 28.5°C - 30°C (Broderick et al., 2000; Candan and Kolankaya, 2016; Godfrey and Mrosovsky, 2006; Godley et al., 2002; Kaska et al., 1998; King et al., 2013; Mrosovsky et al., 1984; Patrício et al., 2017; Spotila et al., 1987). The transitional range of temperature (TRT), during which a mixed proportion of offspring is produced (Mrosovsky and Pieau, 1991), has been found to span the pivotal by ≈ 1°C to 5°C (Godfrey and Mrosovsky, 2006; Hulin et al., 2009; Patrício et al., 2017). Furthermore, the TRT can be used to infer resilience in a population, as with greater ranges in temperature during which a mixed sex brood can be produced, there is more potential to respond to the changing thermal conditions and increase the chances of producing the rarer sex (Hulin et al., 2009). It is worth noting that the concepts of pivotal temperature and TRT were originally defined for constant incubation temperature conditions (Mrosovsky and Pieau, 1991; Yntema and Mrosovsky, 1982) and thus direct comparison between field and laboratory data need to be treated with caution, despite often being used as proxies.

Population growth, philopatry and thermal adaptation

The lack of parental care in many reptiles means that maternal investment of resources in eggs and abiotic properties of the nest environment are the dominant external influences on embryonic development (Deeming and Ferguson, 1988; Lolavar and Wyneken, 2017; Mitchell et al., 2015; Nelson et al., 2010). Various theories have been proposed to explain the occurrence of TSD in reptiles (e.g. (Shine, 1999)); one theory is that TSD may confer maternal fitness advantages by enabling the sexual differentiation of embryos best suited to the thermal conditions; combined with philopatry, adaptive fitness may be further enhanced (Shine, 1999). It has been suggested that fine scale philopatry can confer an adaptive advantage both for males and females, as it maintains genetic diversity and facilitates the retention of locally adapted genetic polymorphism (Stiebens et al., 2013). For example, at Ascension Island, Weber *et al.*, (2012) found that success of green turtle eggs, incubated under constant laboratory conditions, differed with beach of origin and hypothesised that philopatry combined with contrasting thermal regimes among nesting beaches may have facilitated local adaptation to specific beach conditions (Weber et al., 2012).

To build on this theory, we use laboratory conditions to assess how eggs from differing thermal backgrounds, due to female philopatry, perform under controlled thermal conditions. We then compare the output from clutches in field conditions. This allows us to investigate how the pivotal temperature of green turtles at Ascension Island may vary between beaches and among females which can provide an insight into mechanisms for adaptation to climate change.

Materials and Methods

Study site and species

Ascension Island (14°20’ W, 7°55’ S) is a volcanic island situated midway between the African and South American continents and home to one of the largest green turtle rookeries in the world (Broderick et al., 2006; Weber et al., 2014). The study beaches, Long Beach (LB) and North East Bay (NEB), collectively support over 55% of all nesting activity on the Island and exhibit widely differing sand characteristics (LB: ≈ 46% of nesting; pale, biogenic sand; NEB: ≈ 10% of nesting; dark grey volcanic sand (Stancyk and Ross, 1978; Weber et al., 2014). The different albedos of these beaches means that sand temperature on NEB is consistently ≈2°C warmer than LB (Hays et al., 1995; Weber et al., 2012) with conditions approaching the limit of known thermal tolerance. A more detailed description of the study site is available in (Broderick et al., 2001; Godley et al., 2002, 2001; Hays et al., 1999, 1995; Mortimer and Carr, 1987; Weber et al., 2014). Nesting at Ascension Island occurs from late December till June, with a peak in nesting around mid-March.

Beach incubation

During the 2015 and 2016 nesting seasons, a total of 88 clutches laid above the high tide line were selected at random across each study beach (2015: LB n = 23, NEB n = 21; 2016: LB n = 23, NEB n = 21). A Tinytag Plus 2 data logger (models: TGP4017 and TGP4500; Gemini Data Loggers Ltd, Chichester, UK) was placed in the centre of each clutch during laying (after approximately 50 eggs were deposited) and female curved carapace length (CCL notch to tip (Bolten, 1999)) was recorded. Once the turtle had finished covering the clutch, wooden stakes were positioned around the nest to prevent it being destroyed by other nesting turtles and GPS location recorded. After 40 (NEB) or 50 (LB) days of incubation, to encompass minimum previously recorded for each beach (Godley et al., 2002), a wooden corral (ESM1 - Figure S1) was placed on the surface of the sand above the clutch and checked daily at first light to monitor for hatching. A random sample of hatchlings (n = 10 per clutch) was collected from each hatched nest for measurement and histological sexing and the remainder released the following night. After hatching, nests were excavated and all contents were removed and classified as hatched or unhatched eggs. Unhatched eggs were opened to determine development stage as either early term embryo (embryo smaller than residual yolk) or late term embryo (embryo larger than residual yolk). Hatch success was defined as the number of hatched eggs divided by the clutch size (Miller, 1999).

Laboratory incubation

Incubation set up

For the duration of this study four sets of incubation were carried out using the following design. In 2015, two sets of incubation using eight incubation temperatures ranging from 26°C to 33°C at 1°C increments was carried out (except 26°C where n = 1 replicate; time constraints precluded a second round of incubation at that temperature). In 2016, two sets of incubation using a restricted range of three temperatures from 29°C to 31°C was carried out, with three replicates of each temperature.

All incubations were carried out in custom-made forced air incubators (ESM1 - Figure S2), set at different constant temperatures. Each incubator contained two boxes filled with humidified vermiculite (water:vermiculite ratio 1.7:1, ≈ -50kPa (Booth, 2004). Temperature was recorded using a Tinytag Plus 2 data logger at 30minute intervals. Prior to and after each season, each temperature datalogger was checked against a calibrated datalogger in a constant temperature room to verify accuracy and precision of readings. Any data logger varying by more than 0.3°C was excluded from the study.

A total of 528 eggs were sampled from 40 clutches over two nesting seasons, as follows. During the 2015 nesting season, 16 clutches were sampled (8 from LB and 8 from NEB) and eight eggs taken from each clutch at the point of laying. One egg from each study clutch was placed into each of the eight incubators (n = 1 egg / clutch / beach/ temperature treatment); note for replicate 2, only seven eggs per clutch were collected as only 7 temperature treatments were used; total of 240 eggs collected and incubated in 2015. During the 2016 nesting season, 24 clutches were sampled (12 from LB and 12 from NEB) and 12 eggs taken from each clutch at the point of laying. Four eggs from each study clutch were placed into each of the three temperature treatments (n = 4 eggs / clutch / beach / temperature treatment); total of 288 eggs collected and incubated in 2016.

Sample collection and management

In the field, eggs from each clutch were placed in labelled sample bags within an insulated box and transported back to the laboratory. They were brushed free of sand and organic material, patted dry and weighed to the nearest 0.01g (PGW 4502e Adam Scales, d = 0.01g). Each egg was randomly allocated to a box within an incubator. Eggs from each beach were distributed around the edge of each box and labelled with a pencil. The central position in each box was reserved for the data logger and thermostat or thermometer probe (Figure S2A&2B). Eggs were buried in the vermiculite to two thirds of their height, to avoid desiccation whilst enabling them to be monitored for fungal or bacterial growth. Placement in the incubator occurred within six hours of oviposition. Incubators were checked daily to ensure the temperature was adequate, opened to allow for ventilation, and to monitor the condition of the incubating eggs. From 40 days (or first sign of pipping) onwards, separators were placed between the eggs to isolate any hatchlings that emerged and inspected at up to four-hour intervals to monitor signs of hatchling emergence.

Sexing

All laboratory incubated hatchlings and ten hatchlings from each *in situ* study clutch were weighed to the nearest 0.01g (PGW 4502e Adam Scales, d = 0.01g) and measured (SCL: straight carapace length in mm) to the nearest 0.1mm with electronic callipers (Digitronics Caliper, Polycal Series). Hatchlings were then euthanised by pithing (destruction of the brain), using a modified version of Work and Balazs (2013) and dissected to excise the adrenal-kidney-gonad (AKG) complex and the yolk residue, with the latter weighed to the nearest 0.01g. The AKG was fixed in 10% formalin for a minimum of 48 hours, dehydrated in a series of alcohol baths and cleared in xylene, before embedding in paraffin wax, sectioning (at 3 to 10µm; Shandon Finesse 325 microtome; blade: MX35 ultra, 34°, 80mm) and staining. Sex was then determined using histological criteria by examining the sections under a light microscope. Male gonads were distinguished by a thin smooth cortex and the presence of immature seminiferous tubules in the medulla whereas female gonads exhibited a thickened and infolded cortex with a fairly homogenous medulla (Godfrey et al., 1999; Godfrey and Mrosovsky, 2006; Miller and Limpus, 2002; Yntema and Mrosovsky, 1980). If no sex could be determined, further sections and staining were carried out until a clear readable slide was obtained. Slides were read independently by two researchers and if they did not agree the slides were read again or the gonad reprocessed until a consistent result was obtained.

Statistical analysis

For *in situ* clutches, clutch sex ratio was evaluated at a clutch level (proportion female) using a binomial (logit link) generalised linear mixed model (GLMM) with incubation temperature during the thermosensitive period (TSP), beach of origin as a fixed effect with a random effect of female identity, starting with the temperature*beach interaction.

For the laboratory analysis, a generalised linear mixed model (GLMM) with a binomial error structure (logit link) was carried out to assess the importance of temperature (mean middle third of incubation), beach of origin (LB or NEB), adult female size (CCL), replicate (e.g. season 1 replicate 1), with female identity as random effect, as predictors of offspring sex, starting with a temperature*beach interaction effect.

The pivotal temperature and transitional range of temperatures for each beach were determined separately, and for the combined dataset, using function *tsd* in R package '*embryogrowth*' (Girondot, 2016). The sex-temperature curves and associated field pivotal and field transitional range of temperatures for *in situ* conditions were then produced using this same approach. Note, for beach data the temperature used was from the thermosensitive period obtained by modelling nest temperature data using package '*embryogrowth'* (Girondot et al., 2018); these TSP data are used to infer field pivotal temperature and field TRT.

For the laboratory study, hatch success was analysed at the egg level (hatched/unhatched) using a generalised linear mixed model (GLMM) with a binomial error structure (logit link) assessing the impact of temperature and beach of origin, starting with the interaction between these, with female identity as a random effect. For *in situ* clutches, hatch success was analysed at a clutch level (number of hatched eggs, number of eggs not hatched) using a binomial generalised linear mixed model (GLMM) with mean incubation temperature, beach of origin, and nest depth as a fixed effect with a random effect of female identity. Post hoc analysis of hatch success and mean incubation temperatures between beaches was evaluated with a Wilcoxon test for non-normal distribution.

For both the laboratory analysis and *in situ* clutches, we carried out a linear mixed model (LMER) to assess the importance of incubation temperature, beach of origin and sex on hatchling size (Straight Carapace Length), starting with the interaction between temperature and beach of origin.

Models were evaluated using information theoretic model selection, implemented with package 'MuMIn' (Bartoń, 2018), ranked by AIC value, and graphically checked for the relative importance of terms. All analyses were carried out in R version 3.4.1 (R Core Team, 2017).

Results

Beach incubation

For clutches incubated *in situ* from which we sampled hatchlings for sexing (n = 26 clutches; LB = 13, NEB = 13), we obtained a best fit model suggesting a field pivotal temperature of 28.9°C with a mixed proportion of sexes occurring between 27.1°C and 30.6°C for both LB and NEB combined (Figure 1 - A). Note, we could not fit the model for individual beaches as no NEB nests sampled produced male hatchlings. The result of the GLMM indicates a significant effect of temperature ($\chi^2_{(1)}$: 24.6, $p < 0.001$), but no temperature*beach interaction ($\chi^2_{(1)}$: 0, $p = 0.99$) or beach effect ($\chi^2_{(1)}$: 0.8, $p = 0.4$).

In situ clutches had a mean hatch success of 81% (SD 17%, n = 72 clutches), with clutches on LB having significantly higher success than nests on NEB (W = 1199, $p < 0.001$; LB: 91%, SD = 7%, n = 37, NEB: 71%, SD = 18%, n = 35) (Figure 2 - A), but also experiencing significantly cooler incubation temperatures (mean temperature: W = 77.5, p < 0.001; LB: 31°C, SD = 0.6°C, n = 37, NEB: 33°C, SD = 0.9°C, n = 35). The effect of temperature ($\chi^2_{(1)}$: 4.92, $p = 0.03$) and beach ($\chi^2_{(1)}$: 5.88, $p = 0.02$) had a significant negative impact on hatch success (Figure 2-A). There was however no effect of mean incubation temperature*beach interaction ($\chi^2_{(1)}$: 1.74, $p = 0.18$).

Further analysis of nest content revealed that late stage arrest is more common in clutches incubated on the warmer beach, NEB (W = 223.5, $p < 0.001$; LB: 3.6 embryos, SD = 6.1, n = 37 clutches, NEB: 20.9 embryos, SD = 24.1, n = 34 clutches).

Temperature had a negative effect on hatchling size ($\chi^2_{(1)}$: 8.62, $p < 0.005$), with no effect of beach of origin ($\chi^2_{(1)}$: 0.1, $p = 0.75$), sex ($\chi^2_{(1)}$: 0.25, $p = 0.61$), or mean incubation temperature*beach interaction ($\chi^2_{(1)}$: 0.01, $p = 0.9$) (Figure 3 - A).

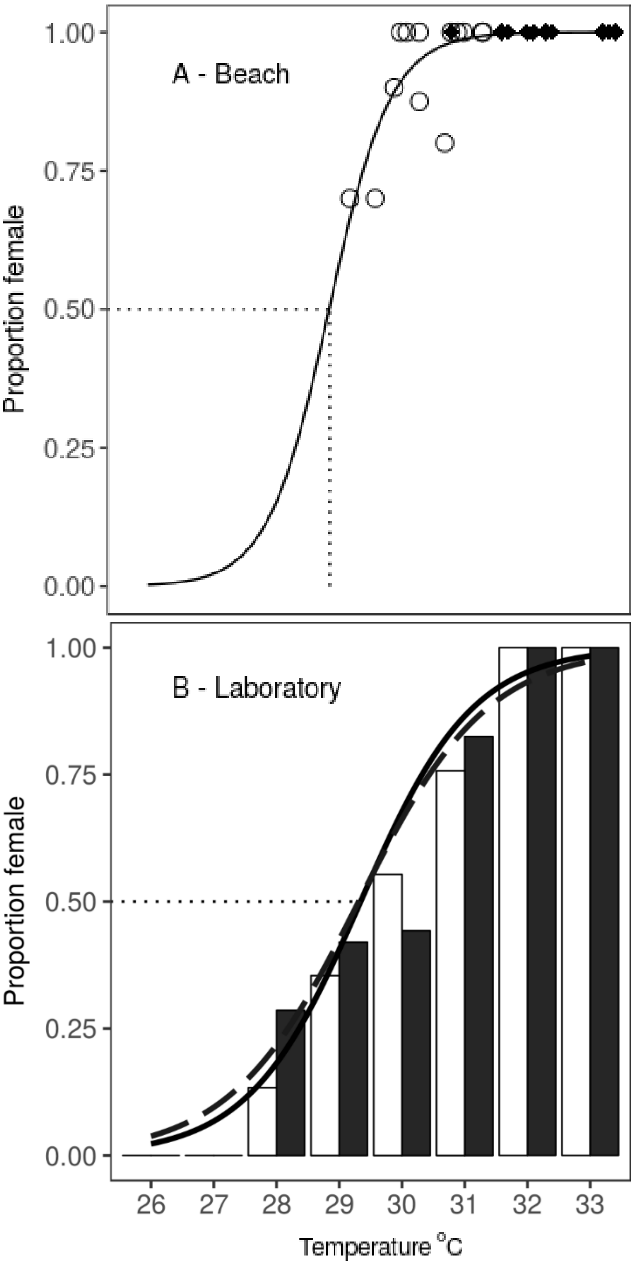

Figure 1*: Proportion of females obtained from eggs in relation to incubation temperature in field (A) and laboratory (B) conditions. A - Beach: Proportion of females obtained in clutches on Long Beach (LB; n = 128 offspring, 13 clutches, open circles) and the warmer North East Bay (NEB; n = 122 offspring, 13 clutches full diamonds) in relation to mean middle third of incubation temperature. The trend line is a binomial generalised linear model regression, where data were not split between beaches for lack of males in sampled nests on NEB. Dotted lines indicate temperature at which balanced sex ratio is obtained (field pivotal temperature). B - Laboratory: Proportion of females in relation to mean incubation temperature binned by 1°C increments for LB eggs (white fill, solid trend line) and NEB eggs (black fill, dashed trend line). The trend lines are based on a binomial generalised linear model. Dotted line indicates pivotal temperature, at which balanced sex ratio is obtained*

Laboratory incubation

In our laboratory incubated eggs, multi model inference determined that there was no statistical significance for the interaction between beach and temperature ($\chi^2_{(1)}$: 0.29, *p* = 0.56), group ($\chi^2_{(3)}$: 3.88, *p* = 0.27), or beach of origin ($\chi^2_{(1)}$: 0.03, *p* = 0.85) however there was a significant effect of incubation temperature ($\chi^2_{(1)}$: 132.0, $p < 0.001$), and a weak but significant effect of female size ($\chi^2_{(1)}$: 4.37, *p* = 0.04) on offspring sex (ESM2 - Table S1). However, 95% confidence intervals for female size encompass zero [-0.01; 0.5] suggesting that this is a spurious result (ESM1 - Table S2).

From our laboratory study we estimate the pivotal temperature to be 29.8°C [TRT 27.1°C – 32.4°C] and 29.7°C [TRT 26.7°C – 32.7°C] for LB and NEB respectively. Contrary to our hypothesis, there was no significant difference between beaches. Thus, we fitted the data in a single model without differentiating between beach of origin to obtain a pivotal temperature of 29.7°C [TRT 26.9°C – 32.6°C] for this population. Using the package *embryogrowth*, the best fit model produced a similar pivotal temperature of 29.7°C [TRT 26.9°C – 32.6°C].

Previous laboratory based studies suggest that, as a result of evaporative cooling, the core egg temperature is between 0.25°C and 0.5°C cooler than the air, thus a correction factor approximating to the mean difference of 0.4°o was applied (Mrosovsky et al., 2009), and we obtained a corrected pivotal temperature of 29.3°C [TRT 26.5°C – 32.2°C] (Figure 1 - B).

Mean incubation temperature had a significant negative effect on hatching success (GLM, $\chi^2_{(1,521)}$: 59.6, p<0.001). Neither beach of origin (GLM, $\chi^2_{(1,520)}$: 1.4, *p* = 0.24) nor the interaction between beach * temperature (GLM, $\chi^2_{(1,521)}$: 0.48, *p* = 0.49) had any effect (Figure 2 - B).

Temperature was found to have a negative effect on hatchling size ($\chi^2_{(1)}$: 70.9, $p < 0.001$), but with no effect of beach of origin ($\chi^2_{(1)}$: 2.76, *p* = 0.1), sex ($\chi^2_{(1)}$: 0.57, *p* = 0.45), or mean incubation temperature*beach interaction ($\chi^2_{(1)}$: 2.8, *p* = 0.1) (Figure 3 - B).

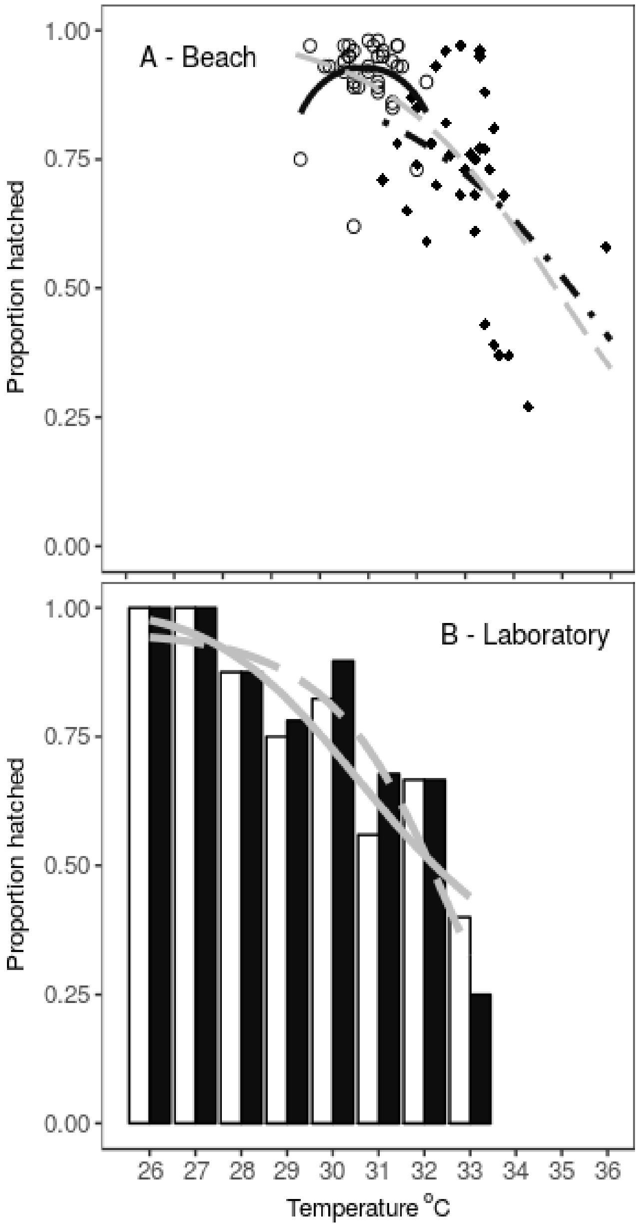

Figure 2: *Hatching success for eggs in relation to incubation temperature in field (A) and laboratory (B) conditions. A - Beach - Proportion of LB (n = 37 clutches, open circles, full black line) and NEB (n = 35 clutches, full diamonds, dash and dot line) eggs hatched in relation to mean incubation temperature. Pale dashed line is the combined hatching success, fit with a binomial general linear model. Trend lines start at coldest and end at the hottest recorded temperature, rounded to the closest full °C, on each beach (LB: 29.5°C – 32 °C, NEB: 31°C - 36°C). B - Laboratory - Proportion of LB (white bar, solid grey trend line) and NEB (black bar, dashed grey trend line) eggs hatched in relation to mean incubation temperature. Data are binned in 1 degree increments, fit with a binomial general linear model.*

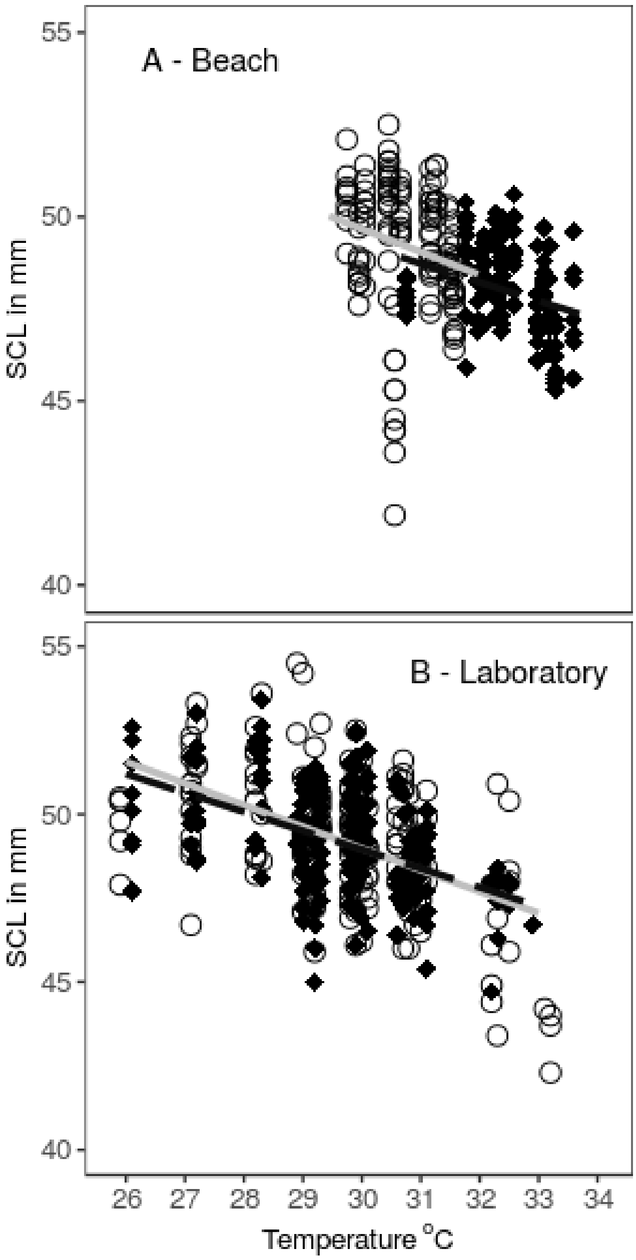

***Figure 3**: Straight carapace length (SCL) of offspring in relation to different incubation temperatures in field (A) and laboratory (B) conditions. A - Beach - Straight Carapace Length in mm of hatchlings from LB (n = 128 offspring, 13 clutches; open circles, full grey line) and NEB (n = 122 offspring, 13 clutches; full diamonds, black dashed line) in relation to mean incubation temperature. Trend lines start at coldest and end at the hottest recorded temperature, rounded to the closest full °C, on each beach (LB: 29.5°C – 32 °C, NEB: 31°C - 36°C). B - Laboratory - Straight Carapace Length (SCL) in mm of hatchlings from LB (n = 188; open circles, full black line) and NEB (n = 201; full diamonds, dashed line) in relation to mean incubation temperature.*

Discussion

Key findings

In this study we tested whether variation in pivotal temperatures in green turtles may provide a mechanism for adaptation to predicted rising temperatures. Comparing the pivotal temperatures obtained in laboratory condition for eggs from two beaches with different thermal conditions, we find them to be consistent between beaches. All differences in offspring sex, size and hatching success of clutches recorded between the two beaches were a result of incubation temperature.

Context

Over the last 150 years, or 3 to 5 sea turtle generations (Seminoff, 2004), sand temperatures on Ascension have progressively risen (Hays et al., 2003), along with global air temperatures (Stocker et al., 2013); The lack of difference in pivotal temperature that we recorded between the nesting beaches suggests adaptation to specific nesting beaches does not occur or that gene flow through paternal influence or maternal exploratory behaviour between the distinct nesting aggregations masks the specific long term adaptation. If there is a lack of natural plasticity in pivotal temperatures between greatly differing thermal environments, it may be more difficult for long lived species with extended generation times to deal with the rapid pace of contemporary climate change, as overall fewer males will be produced. The short geographic separation (≈ 7km straight line distance) between the beaches in our study may not lead to selective pressure on adaptation of pivotal temperatures as there is still a production of males from nearby beaches. Thus, we may expect isolated rookeries to be more labile as presumably increasing the production of the rarer sex would provide fitness benefits to the population. Recent studies suggest that differing thermal conditions in nesting grounds at the extremes of the Great Barrier Reef (Jensen et al., 2018) and in Malaysia through the use of shading in hatcheries (Jensen et al., 2016) are responsible for the different observed sex ratios on foraging grounds. Similarly, divergence in loggerhead populations using thermally distinct conditions in the Mediterranean has not led to any specific adaptation to local conditions (Monsinjon et al., 2017). Our findings support those results, but go against our hypothesis and the initial work by (Weber et al., 2012). The difference in findings between these studies may be due to differences in sample size and treatments (n = 40; temperature 29°C and 32.5°C (Weber et al., 2012) and n = 528; temperature 26°C to 33°C, this study). However it must be noted that male mediated gene flow has been shown to occur at ocean basin level (Roberts et al., 2004), and therefore selection of pivotal temperature may not be required, as males may prove to be less philopatric.

In the absence of any pre-existing genetic adaptation, turtles may need to respond to rapid climate change through range shifts, altered phenology, or nest site selection, although the former will be problematic for populations using isolated rookeries such as Ascension Island. It is the plasticity of each individual which in the long run may confer the adaptive potential of the population (capturing this among female variation would require sampling full clutches of multiple females which would be ethically and logistically challenging). Sea turtles inhabit all major temperate oceans, with different nesting aggregations found along vast geographic areas, and varied biotic and abiotic conditions (e.g. different thermal conditions, rainfall and vegetation levels between Florida, Ascension, Poilão, and the Eastern Mediterranean) thus it would be surprising if each rookery responded in the same manner and exhibited the same thermal tolerances.

Ectotherms are highly sensitive to thermal conditions and respond to changes in different ways; for instance brown anoles (*Anolis sagrei*) (Logan et al., 2018) and snow skinks (*Niveoscincus* species) (Caldwell et al., 2017) show strong phenotypic plasticity as an immediate response to changing thermal conditions. However the long term response in anoles is slow, meaning that they may not be able to keep up with the rate at which climate change is occurring (Logan et al., 2018), but the skinks may be showing signs of adaptive evolution (Caldwell et al., 2017). In contrast, *Pleurodema thaul*, a south American frog, exhibits a behavioural change to contend with thermal variation (Barria and Bacigalupe, 2017), and the Andean toad (*Rhinella spinulosa)* showed little response, and generally coped with a wide variation in temperatures (Riquelme et al., 2016). Conversely Asian sea bass (*Lates calcarifer*) exhibit underlying molecular adaptation to cooler temperatures (Newton et al., 2013). Sessile species, such as larch (*Larix gmelinii*) may face more pressures as spatial displacement is not possible for established individuals, leading to interesting situations where the trees are adapted to their local provenance, but acclimatise to current conditions (Xiankui and Chuankuan, 2018).

Thus it is clear that there is not a single response to increasing temperatures, and understanding the mechanisms driving the selection is complex; whether sea turtles truly adapt to climate change is yet to be conclusively determined, and whether they can on a rapid enough scale is debatable.

Conclusion

Our findings for the laboratory data are consistent with pivotal temperatures obtained from other green turtle rookeries using similar methodology (e.g. Suriname 29.2°C (Godfrey and Mrosovsky, 2006), China 29°C (Xia et al., 2011)). Field pivotal temperature for *in-situ* clutches was also consistent with what was previously found for Ascension (28.8°C (Godley et al., 2002)) nearly two decades earlier and may show slight variation from other sites (e.g. Poilão, Guinea Bissau 29.4°C (Patrício et al., 2017), Heron Island, Australia < 28.7°C (Limpus et al., 1983)).

Contrary to our hypothesis, and the findings of (Weber et al., 2012) that suggested there may be a genetic basis to heat tolerance of turtles nesting on NEB, we found no evidence of fine scale adaptation to thermal conditions. We do however find reduced hatch success at higher temperatures which suggests that increasing temperatures will have a detrimental impact on overall hatchling production in addition to skewing the already very highly female bias, as has been previously suggested (Broderick et al., 2001; Hawkes et al., 2007; Hays et al., 2017; Kobayashi et al., 2017). Therefore to contend with increasing temperatures, turtles on Ascension may need to shift spatially or temporally.

Ethics

Research was carried out under Ascension Island Government Conservation Department Research Permits #ERP-2014-11, #ERP-2015-17, and in accordance with the University of Exeter CLES-Ethics Committee # 2015/890

Data

Data are stored in the Ascension Island Government Conservation Department database. Data are available on figshare, under embargo until publication. Beach data: https://figshare.com/s/dc16fbd027a59560ce1d ; Lab data: https://figshare.com/s/24788e81a4140547b38b .

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Competing interests

The authors declare that the research was carried out without any competing interests.

Authors contributions

ACB, BJG and SW conceived the study and were awarded a NERC iCase studentship. DT conducted the field work with assistance and/or guidance from all co-authors. DT lead the data analysis and writing of the manuscript with assistance from all co-authors.

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Supplementary Material

Nest marking supplementary figures

***Figure S1**: Wooden stakes used to mark incubating sea turtle clutches, and wooden corral placed over incubating nest prior to emergence.*

Incubation methodology

Incubation was carried out at temperatures ranging, at 1°C increments, from 26° to 33°C.; internal temperature was recorded using TinyTag Plus 2 data logger (Gemini Data Loggers Ltd, Chichester, UK), tested against a UKAS calibrated data logger.

Incubators were made from expanded polystyrene boxes (n =8; approximately 800x360x300mm, 25mm thickness), sitting on 3 shelving units. Within each incubation box, there was a 600W HabiStat heat mat (HabiStat, Euro Rep, Hayes, UK), placed in the bottom of the incubator, connected to a manual HabiStat thermostat; a shelf was placed 40 mm above the heat mat supporting two BPA free plastic storage containers used as nest boxes (250x250x120), two trays of water and two Habistat Minifans (Figure S2). The fans are necessary to avoid thermal gradients.

Each nest box was pierced with eight ventilations holes (two on each side), and one in the lid to insert the thermostat probe into the incubation substrate; boxes not equipped with thermostat probes had a thermometer probe with external reading to easily monitor temperature in the incubators.

All of the equipment used in the incubators was washed, wiped down with 70% alcohol, and sprayed with Brinsea incubation disinfectant (Brinsea Products Ltd, Weston Super Mare, UK) to avoid fungal growth.

Humidified vermiculite was used as the incubating substrate due to its high water retention potential; boiled mineral water was mixed with vermiculite (ratio 1.7:1, ≈ -50kPa (Booth, 2004)) with 845g of the mix placed in each nest box (533g H_2O, 312g vermiculite). This volume of incubation medium allowed for the humidity levels of the substrate to remain adequate for the duration of the incubation, thus avoiding having to top up the substrate with water part way through the incubation.

Incubator layout supplementary figures

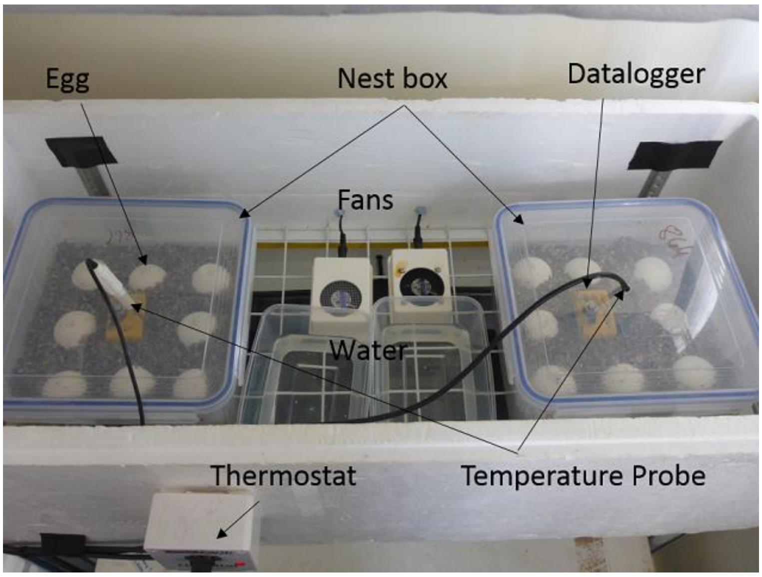

***Figure S2**: Layout within the incubator showing 2 nests boxes (on either side) containing 8 eggs, a data logger, and a thermal probe, the 2 water containers (middle front) and 2 fans (middle back).*

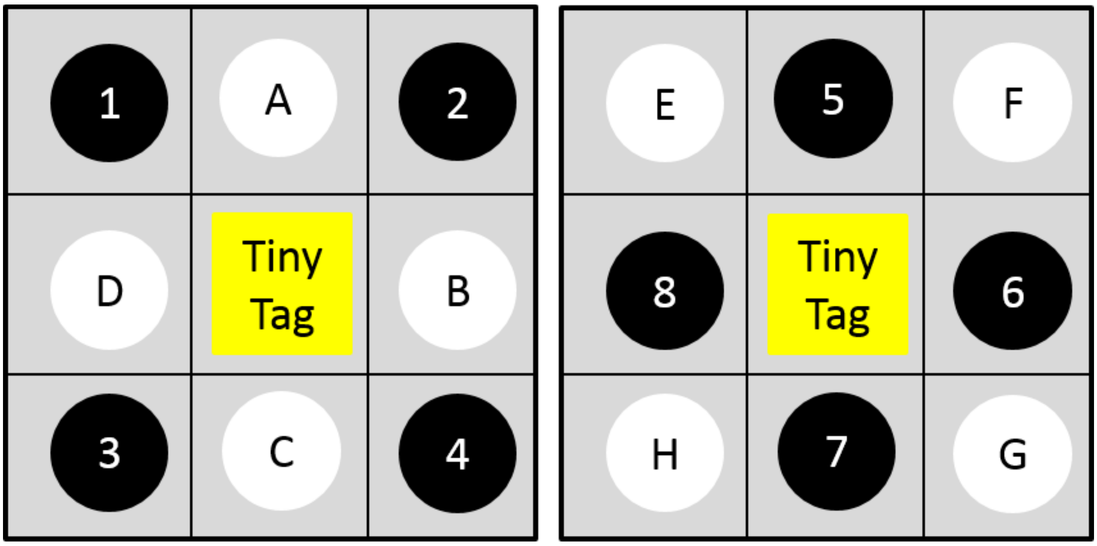

Figure S3A*: 2015 nesting season: Egg layout within an incubation box with numbers marking eggs from NEB and letters for Long Beach (middle). Each egg in each incubation box comes from a different clutch, with 1 egg per clutch per temperature treatment. This layout was replicated for each of the 8 temperature treatments.*

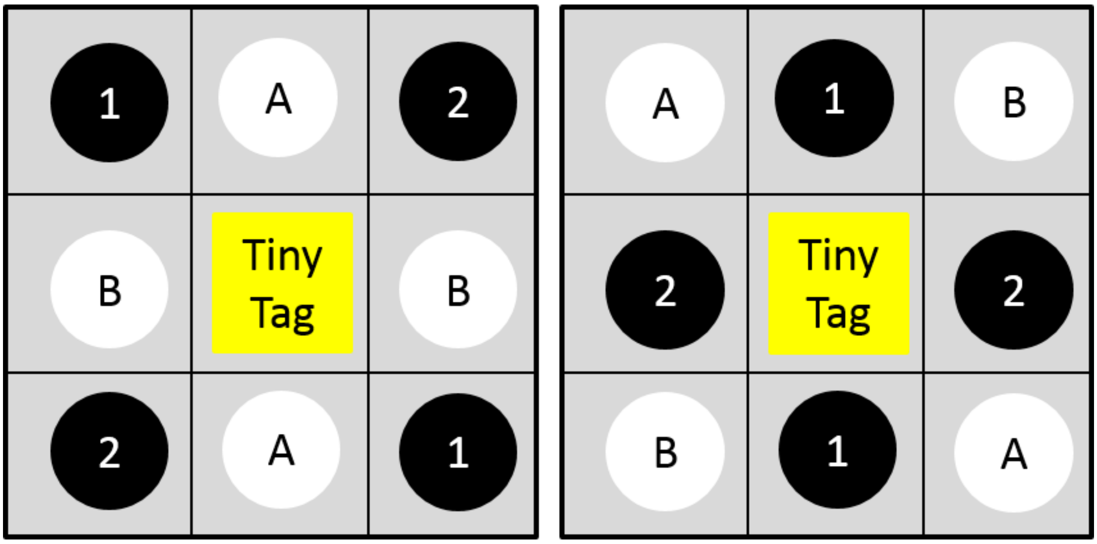

Figure S3B*: 2016 nesting season: Egg layout within an incubation box with numbers marking eggs from NEB and letters for Long Beach (middle). Each egg in each incubation box comes from a different clutch, with 1 egg per clutch per temperature treatment. This layout was replicate for each of three temperature treatments.*

Supplementary material 2

***Table S1**: Multimodel inference for laboratory data ranked by AIC value; factors are labelled "NA" when not included and with "+" to indicate when a factor is added to the 'best' model. Note: models b, c, and e can be deemed as more complex than the best fit model.*

	INTERCEPT	**BEACH**	**GROUP**	**CCL**	**T°C**	**BEACH:T°C**	**DF**	**LOGLIK**	**AIC**	**DELTA**	**WEIGHT**
A	-0.15	NA	NA	0.25	1.61	NA	4	-202.94	413.88	0.00	0.45
B	-0.12	+	NA	0.26	1.61	NA	5	-202.91	415.82	1.94	0.17
C	-0.53	NA	+	0.25	1.66	NA	7	-200.98	415.97	2.08	0.16
D	-0.14	NA	NA	NA	1.60	NA	3	-205.12	416.25	2.37	0.14
E	-0.13	+	NA	0.25	1.72	+	6	-202.76	417.52	3.64	0.07

***Table S2**: Bootstrapped confidence intervals for the estimators of the best fit model.*

	2.5%	**97.5%**
INTERCEPT	-0.51	0.25
TEMPERATURE	1.34	2.32
CCL	-0.01	0.50

Chapter 4: Sea turtle clutch translocation: effects on offspring phenotype and survival

This chapter has been submitted to Ecology and Evolution

Authors

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Abstract

The impacts of climate change on the natural environment are varied and considerable. A range of mitigation strategies for species have been proposed to counter the effects of climate change. Ectotherms, such as reptiles, that depend on abiotic factors for physiological development, in particular during clutch incubation, may find their thermal niche shifting leading to biased sex ratios and reduced hatchling output. For sea turtles, translocation of clutches has been suggested as a method to reduce the impacts of sea level rise and increasing temperatures on clutch development and success. Currently this strategy is widely used to move clutches within beaches to safer locations away from the sea and has been successful in seeding new beaches. There is a need however, to understand the importance of local adaptation and the effects this may have on offspring production and sex ratios. We investigated the impacts of translocation on clutches of green sea turtles (*Chelonia mydas*) from two beaches, on Ascension Island, with contrasting thermal conditions. We cross incubated clutches between beaches, moving them from 'cool' to 'warm' conditions and vice versa, to investigate the level of localised adaptation that has occurred. We found no effect of beach of origin on offspring phenotype or hatch success of clutches and thus no evidence of localised thermal adaptation. Thus offspring from clutches of eggs originating from differing thermal conditions had a similar phenotype when incubated in similar environments. Although sea turtles may shift spatially or temporally in response to rising temperatures, translocation of clutches to new locations can be considered an effective conservation tool regardless of beach of origin.

Keywords

Green sea turtle, *Chelonia mydas*, Temperature-dependent Sex Determination, translocation, mitigation, climate change

Article Impact Statement

Translocating sea turtle clutches between beaches does not impact offspring phenotype and could be a climate change mitigation tool.

Introduction

Climate change impacts on the physical environment by affecting temperature, weather patterns and sea levels, which in turn affects species, communities and ecosystems (Hoegh-Guldberg and Bruno, 2010; Scheffers et al., 2016; Stocker et al., 2013)

Species can respond to climate change by adapting to the new conditions by changing their behaviour or physiology. For instance, some corals (Rose et al., 2017) and some fish (Kovach et al., 2012) seem to show signs of physiological adaptation, whilst some plants (Cleland et al., 2007), birds (McDermott and DeGroote, 2016), reptiles (Weishampel et al., 2010) or insects (Forrest, 2016) have shifted phenology or range (Boyle et al., 2016). Philopatry may confer advantages through fidelity to foraging and nesting grounds which could lead to higher reproductive success (Refsnider and Janzen, 2010), which conversely could be a disadvantage if conditions at either site deteriorate.

Approximately half of all chelonian species are of conservation concern (Ihlow et al., 2012), as a result of overexploitation (Van Houtan and Kittinger, 2014), habitat degradation (Pikesley et al., 2013) and climatic variability (Fuentes and Porter, 2013). Therefore, understanding if and how chelonians can adapt to climate change is of critical importance for the long term conservation of these long lived species. Sea turtles, as ectotherms, are intricately linked to environmental conditions, with impacts on egg incubation (Spencer and Janzen, 2011), primary sex ratio (Patrício et al., 2018), hatchling dispersal (Booth and Evans, 2011), seasonal migrations (Hawkes et al., 2011), internesting interval (Hays et al., 2002) or phenology (Almpanidou et al., 2017; Weishampel et al., 2010). Ectothermic species which are particularly reliant on environmental conditions to maintain metabolic functions, may therefore be particularly affected by climate change (Ihlow et al., 2012; Paaijmans et al., 2013). Many reptilian species have temperature-dependent sex determination (TSD) (Pieau, 1996), a mechanism whereby the sex of the offspring is determined by the incubation temperature (Bull, 1980). Different patterns of TSD occur, but they can be characterised by a transitional range of temperatures producing mixed sexes, and a pivotal temperature producing an equal proportion of each sex, with extremes in temperature producing a majority of one sex or the other (Pieau, 1996). Beyond these extremes, embryonic development is unlikely to occur. Sexual differentiation occurs during the thermosensitive period (TSP), often associated with the middle third of incubation (Bull, 1980; Girondot et al., 2018). Sea turtles are highly philopatric (Bradshaw et al., 2018; Lee et al., 2007; Stiebens et al., 2013; Weber et al., 2013), although with some exploratory behaviour persisting (Carreras et al., 2018; Esteban et al., 2015; Mills and Allendorf, 1996; Stiebens et al., 2013), and typically return to nest on the beach from which they emerged. When considering impacts of climate change, incubating clutches are particularly vulnerable as they are completely reliant on maternal nest site selection. In all sea turtles, successful incubation occurs between 25°C and 35°C with higher incubation temperatures producing more females, and pivotal temperatures typically around 29°C (Ackerman, 1997; Howard et al., 2014). The pivotal temperature concept is defined for laboratory conditions (Mrosovsky and Pieau, 1991), thus for field conditions a constant temperature equivalent (CTE) needs to be determined to infer sex ratios (Georges et al., 1994). Under increasing temperatures, primary sex ratios are thus likely to become more female biased. In the short term, this could be beneficial as it could boost population size (Santidrián Tomillo et al., 2015), but in the longer term may result in reduced clutch success (Boyle et al., 2014). Furthermore, changes in weather patterns could increase storminess, and thus tidal inundation, and sea level rise could reduce available nesting habitat through coastal squeeze and coastal fortification (Fish et al., 2005; Mazaris et al., 2009).

Due to sea turtles being of conservation concern, management interventions are often put in place to increase recruitment to populations. A common strategy involves clutch translocation from vulnerable areas (e.g. human development, poaching, predation, flooding) to safer areas on the beach or into hatcheries (Mortimer, 1999). This can result in decreased hatching success (Pintus et al., 2009), and may alter the sex ratios of the offspring produced (Jensen et al., 2016; Sieg et al., 2011).

High levels of maternal philopatry and natal site fidelity in sea turtles (Bradshaw et al., 2018) mean that any advantage of microhabitat conditions potentially selected for by the mother may be lost in the translocation process. Preliminary work by Weber *et al.* (Weber et al., 2012) suggested some form of physiological adaptation, whereby eggs from a naturally warmer environment had a higher hatch success than those from a naturally cooler environment when incubated in the same warm conditions in the laboratory, however, a follow up study using a wider range of temperatures (Tilley et al., 2019a) did not support these findings. The difference in findings between these studies was unexpected, but could be due to the differences in experimental set ups (sample sizes, temperature treatments) (Tilley et al., 2019a; Weber et al., 2012). Therefore, understanding whether developing offspring are adapted to particular incubation conditions could inform translocation decisions in management strategies and determine their effectiveness.

We hypothesize that philopatry in turtles leads to localised adaptation and here we investigate whether maternal philopatry (determined by beach of origin) has an influence on offspring phenotype or whether environmental factors (determined by temperature) are the main driver. We carry out a cross incubation comparison by translocating clutches across beaches to expose them to different thermal conditions during incubation.

Study site

Ascension Island (7°56’S, 14°22’W), a UK Overseas Territory in the South Atlantic, is home to one of the largest green turtle (*Chelonia mydas*) rookeries in the world (Weber et al., 2014). We focus our study on two of the key index beaches that hold approximately 55% of all nesting activities on the island (Weber et al., 2014) and present widely differing sand characteristics (Long Beach (LB) – 46.5% of nesting; pale sand; North East Bay (NEB) – 9.8% of nesting dark grey sand (Weber et al., 2014)). The different albedo of these beaches means that the sand temperature and nest depth in NEB is ≈ 2°C warmer than LB (Hays et al., 1995; Weber et al., 2012) with the temperature often approaching the limit of known thermal tolerance (Ackerman, 1997) (Figure 1).

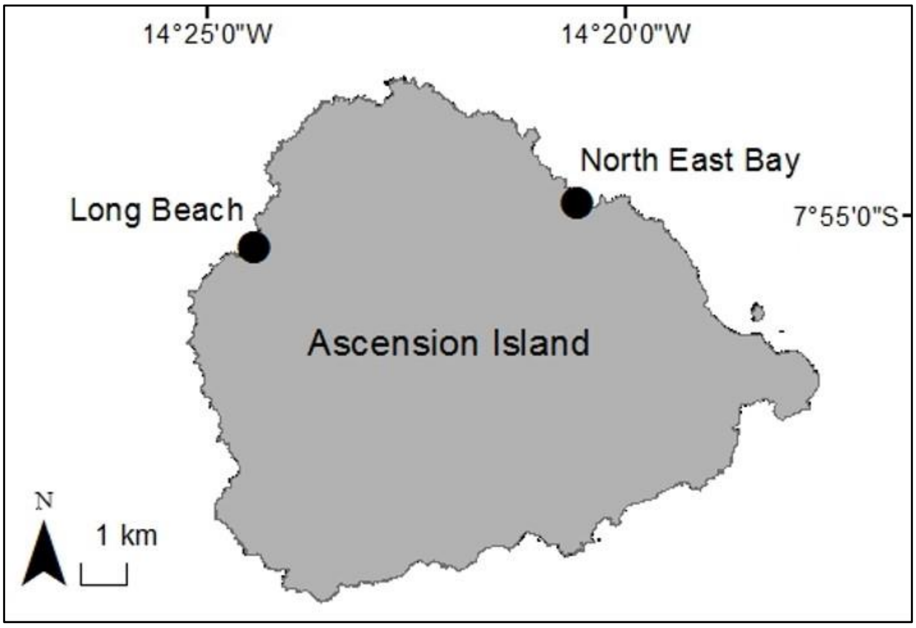

Figure 1: *Relative location of Long Beach and North East Bay on Ascension Island. The difference in sand characteristics of these beaches separated by ≈ 7km produces a wide divergence in thermal conditions. This divergence makes them ideal to test adaptation to local conditions.*

Methods

Data collection

To determine whether beach of origin influenced the outcome of offspring sex, size and overall hatching success, we carried out a cross fostering experiment translocating clutches of eggs deposited on Long Beach (LB) and North East Bay (NEB) and vice versa. Between January and April 2015, nocturnal patrols along the two study beaches were carried out to find nesting female turtles. Female turtles were selected at random (i.e. the first turtle found ready to lay was selected, no physical characteristics were used to select individuals). Upon encountering a female turtle about to deposit her clutch, the back of the nest chamber was excavated to facilitate access to the eggs for collection. Eggs were then collected directly into sample bags (n =2-6 bags/ clutch dependent on clutch size), which were then placed into an insulated box for transport. For each clutch translocated between beaches, a control clutch was relocated within beach to account for the effect of translocation, forming a pair of clutches. Thus a total of 72 paired clutches were relocated comprising of 36 controls (i.e. 18 clutches within beach) and 36 treatments (i.e. 18 clutches between beaches). Each pair was relocated on the same night. Two pairs (one on each beach) were carried out on each night on all but two occasions, where they were carried out within 48 hours of each other. Translocations were carried out at four to seven day intervals to obtain a temporal spread.

Clutches were relocated to hand excavated nests; the top 20 cm layer of dry sand was removed to get a base of humid sand allowing for the digging of a nest chamber 70 cm deep with an entrance column at least 20 cm in diameter. The final nest depth was similar to that of naturally dug nests (Hays et al., 1993). Eggs were carefully placed 2 to 4 at a time into the nest, with Tinytag data loggers (models: TGP4017 and TGP4500; Gemini Data Loggers Ltd, Chichester, UK) positioned after 50 eggs were placed into the chamber, to record incubation temperature at 30 minute intervals. For any clutch that had fewer than 80 eggs (n = 5), the data logger was placed after 30 eggs to ensure it was completely surrounded during the incubation. Once all eggs were placed the nest was carefully closed using moist sand, set aside during the excavation, and covered with dry sand to have a final depth to the bottom for all study nests of 90 cm. Although not to the same standard and size as a nest excavated by a turtle, all hand dug nests were to the same dimension to minimise and standardise the effect of translocation. Control and translocated clutches were placed side by side (1 m centre to centre), in similar sand and at the same distance from the high tide line. Most clutches were relocated within 90 minutes of deposition (n = 70), with six relocated between 2 and 4.5 hours after collection. To determine whether this time delay in relocating has an impact on hatching success, we included time retained prior to deposition in translocation site in the analysis. Each nest was surrounded by four wooden stakes, hammered 1m into the sand, and 50 cm from the centre of the clutch to prevent nesting turtles from disturbing the site.

After 40 (NEB) to 50 (LB) days (to make sure we encompass the minimum recorded incubation durations for each beach (Godley et al., 2002)), wooden frames covered with wire mesh, were placed above each nest and checked at dawn every morning for signs of hatching. Upon hatching, nests were excavated, content sorted, data loggers retrieved, and a subsample of 10 hatchlings collected and sexed after histology (Mrosovsky and Pieau, 1991; Tilley et al., 2019a). Hatching success was determined by the proportion of egg shells in relation to the total clutch size (Miller, 1999). Incubation duration was determined as the time difference in days between clutch deposition and first hatchling emergence. Hatchlings were measured (SCL: straight carapace length in mm) to the nearest 0.1 mm with electronic callipers (Digitronics Caliper, Polycal Series).

Statistical Analysis

To assess differences between pairs of clutches we carried out Wilcoxon signed-rank tests for paired samples to test the difference in incubation duration, mean incubation temperature, sex determining temperatures (TSP), hatching success, and sex ratio between control and translocated clutches.

To investigate effects of experimental treatment and environmental conditions on offspring sex ratio we used a binomial General Linear Model (GLM) with the proportion of females as response variable and incubation temperature during the TSP (°C), beach of origin, clutch treatment and the interaction between them as fixed effects. Clutch treatment was a four level factor: beach of origin - LB or NEB -, and beach of incubation - LB or NEB).

To investigate effects of experimental treatment, and environmental conditions on offspring hatching success we used a binomial GLM with the proportion of eggs hatched as response variable and mean incubation temperature (°C), beach of origin, clutch treatment, female size (CCL), time between deposition and translocation as fixed effects. Clutch treatment was a four level factor: beach of origin - LB or NEB -, and beach of incubation - LB or NEB).

To investigate effects of experimental treatment, and environmental conditions on incubation duration we used a GLM, with a Gaussian error structure, with incubation duration as a response variable and mean incubation temperature (°C), beach of origin, clutch treatment, female size (CCL), time between deposition and translocation as fixed effects. Clutch treatment was a four level factor: beach of origin - LB or NEB -, and beach of incubation - LB or NEB).

To investigate effects of treatment on offspring size, we used a Generalised Linear Mixed Model (GLMM) with a Gaussian error structure, with straight carapace length (SCL) in mm as a response variable with clutch treatment as a fixed effect and with nest ID and mean incubation temperature as random effects to account for multiple samples from each nest.

TSP mean temperature was determined by using the clutch and species specific thermal reaction norm with R package *embryogrowth* (version7.2.3) (Girondot, 2016, 2014; Kaska and Downie, 1999); GLM models were fit using package *lme4* (Bates et al., 2015).

Significance of fixed effects was assessed using likelihood ratio tests compared by dropping terms from the more complex model. All models were simplified by stepwise deletion of the least significant terms, starting with two way interactions, to obtain a minimal model containing only significant effects. All models were simplified by stepwise deletion of the least significant term ($p<0.05$); all data were analysed using statistical software R version 3.4.1 (R Core Team, 2017).

Results

Of the 36 paired clutches, 19 had usable temperature data, were not washed over or disrupted by other nesting turtles and produced offspring that were sexed. Ten clutches failed to hatch owing to tidal inundation or being destroyed by other nesting females and for a further 10, offspring were not collected as they emerged outside of the cage, thus for these latter clutches, data were included in analysis of hatching success but not for offspring sex ratios. In the first instance we used the paired clutch data to have direct comparison between pairs of clutches, then we used all available data from clutches that had usable temperature data, were not washed over or disrupted by other nesting turtles, irrespective of the outcome of the second clutch in the pair. Thus we consider, 52 clutches that hatched with offspring collected for sexing.

Paired clutch analysis

From our clutches for which both pairs hatched, we sampled 380 hatchlings, and obtained a positive sex identification for 371 hatchlings from 38 different clutches. Between control and treatment clutches, we found no difference in proportion of females ($W = 189$, $p = 0.62$) (Figure 2A), hatching success ($W = 188.5$, $p = 0.83$) (Figure 2B), incubation duration ($W = 180$, $p = 1$) (Figure 2C) or hatchling size ($W = 49$, $p = 0.77$) (Figure 2D).

Between control and treatment clutches there was no difference between mean incubation temperatures (W = 184, *p* = 0.93) and sex determining temperatures - TSP (W = 184, *p* = 0.19).

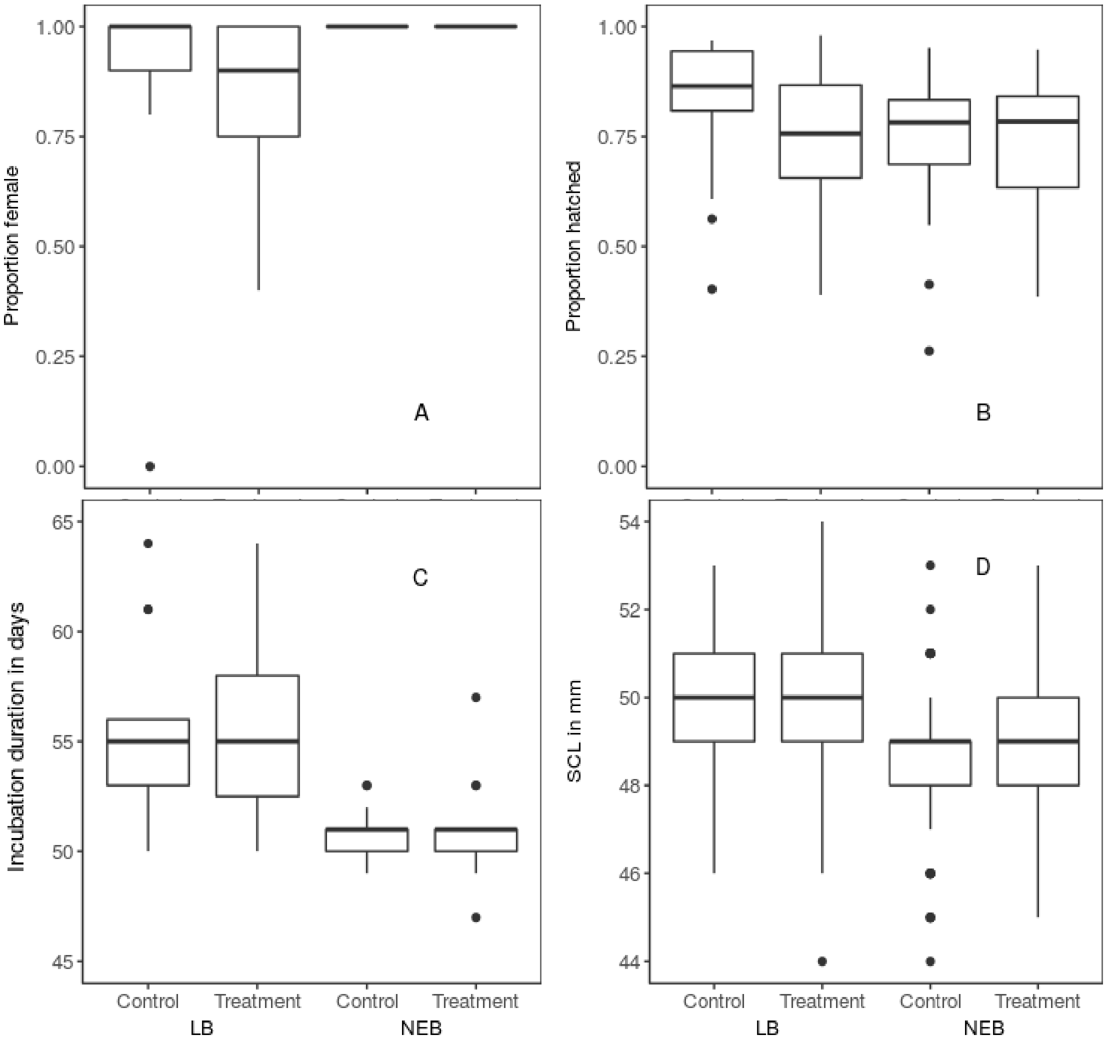

Figure* 2: Comparison between control and treatment clutches on LB and NEB for* **A***: No difference in proportion of females (W = 189, p = 0.62). Note the 'outlier' for a control clutch on LB and lack of males produced from NEB.* **B:** *No difference in proportion of hatched eggs (W = 188.5,* p *= 0.83).* **C:** *No difference in mean incubation duration (W = 180,* p *= 1) and* ***D:*** *No difference in mean straight carapace length (W = 49, p = 0.77) (SCL) in mm for hatchlings, with means and interquartile ranges presented in all cases, with individual points for outliers.*

Offspring Sex: all data

Since our paired clutch analysis revealed no difference between treatment and control, we considered all clutches that produced hatchlings (regardless of whether both clutches in a pair hatched), and were not affected by wash-over or disruption during incubation. From the 519 hatchlings sampled, we sexed 508 hatchlings from 52 clutches (n = 10 for 43 clutches, n = 9 for 8 clutches, and n = 6 for 1 clutch).

There was no effect of treatment ($\chi^2(1) = 2.27$, $p = 0.16$) (Figure 2 A) or interaction between temperature and treatment ($\chi^2(1) = 0.73$, $p = 0.44$) on sex of hatchlings; only incubation temperature had a positive effect ($\chi^2(1) = 92.3$, $p < 0.005$) on the proportion of females in a clutch. Given a full range of temperatures, eggs developed and produced both male and female offspring regardless of their origin (LB eggs: Figure 3-A; NEB eggs: Figure 3-B). A predicted CTE threshold temperature for all clutches used in this experiment was 29.3°C, with mixed proportion of sexes produced between27.6°C and 31.1°C; There was some variation when treating the incubation beaches separately (Figures 3A and 3B), but overall data were in line with what was expected from laboratory data (Tilley et al., 2019a) and previous studies of naturally incubated clutches on these beaches (28.8°C (Godley et al., 2002); 28.8°C (Tilley et al., 2019a)).

Hatching Success

We found no effect of time between deposition and translocation ($\chi^2(1) = 0.65$, $p = 0.42$), female size ($\chi^2(1) = 1.88$, $p = 0.17$), or treatment ($\chi^2(3) = 0.88$, $p = 0.83$) on hatching success. However both clutch size ($\chi^2(1) = 8.7$, $p < 0.01$) and temperature ($\chi^2(1) = 4.4$, $p < 0.05$) with its quadratic term ($\chi^2(1) = 4.6$, $p < 0.05$) had a negative effect on success, but with no interaction between clutch size and temperature ($\chi^2(1) = 0.48$, $p = 0.5$), and no interaction between clutch size and the quadratic effect of temperature ($\chi^2(1) = 0.05$, $p = 0.8$) (Figure 4 A).

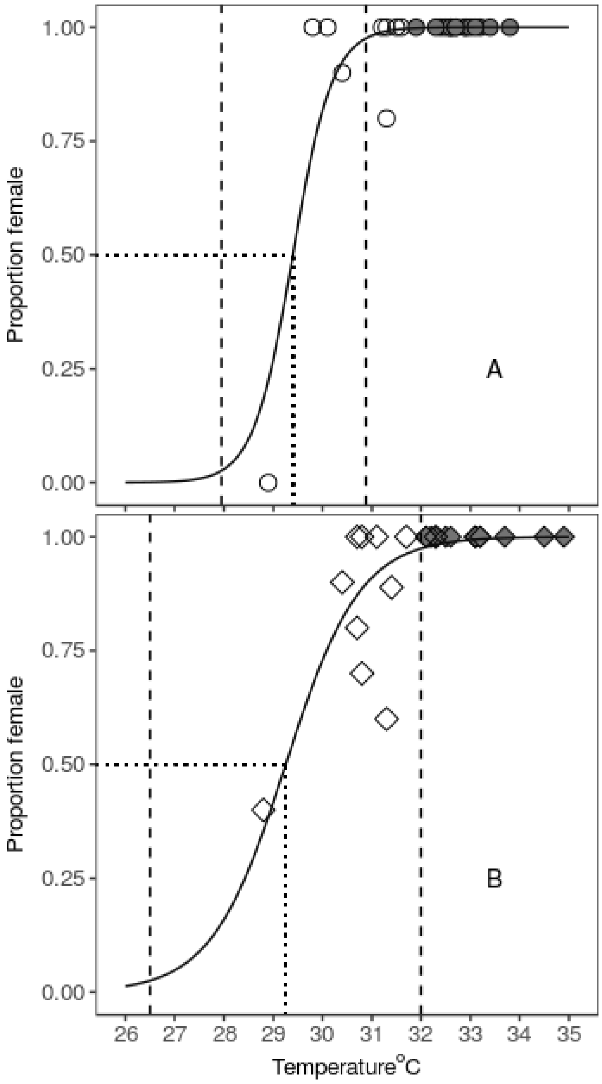

***Figure 3**: Proportion of females obtained in relation to mean incubation temperature during the TSP. Difference between clutches of eggs originating from **A:** Long Beach eggs incubated on Long Beach (open circles) and North East Bay (full circles). The dotted lines correspond to the CTE temperature producing equal proportion of each sex (29.4°C), with the dashed vertical lines corresponding to the range of temperatures producing mixed sexes [28.0°C – 30.8°C]. **B:** North East Bay eggs incubated on Long Beach (open diamonds) and North East Bay (black diamonds). The dotted lines correspond to the CTE temperature producing equal proportion of each sex (29.3°C), with the dashed vertical lines correspond to the range of temperatures producing mixed sexes [26. 5°C – 32°C].*

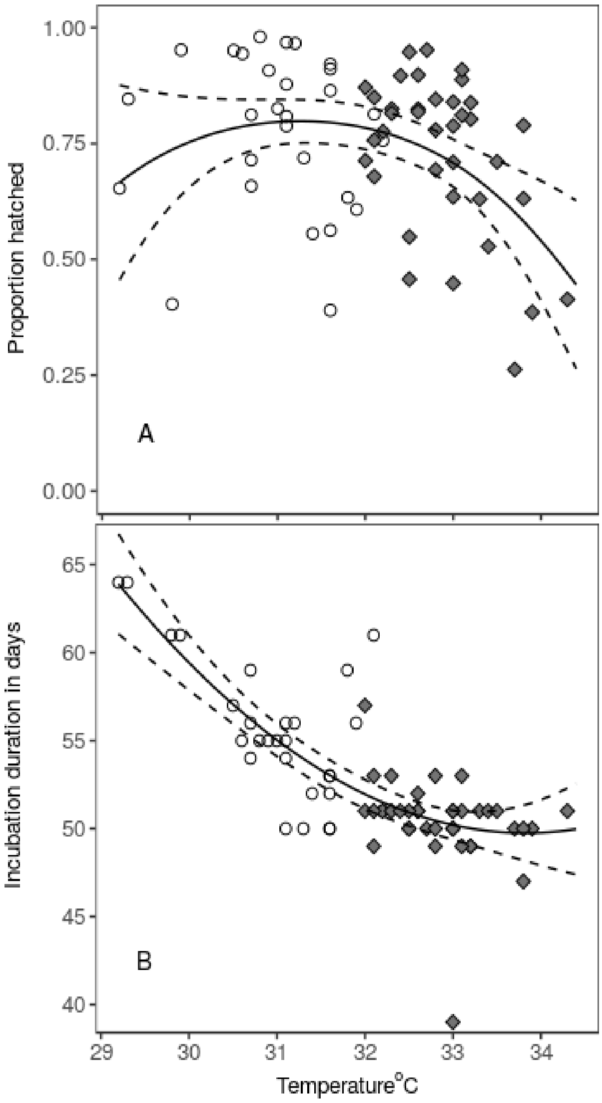

Figure 4 *:* ***A:*** *Proportion of eggs hatched for clutches incubated on Long Beach (open circles) and North East Bay (black diamonds) in relation to mean incubation temperature.* ***B****: Incubation duration in days as a function of mean incubation temperature in °C for all clutches. Full line is represents a predicted fit, dashed lines represent the 95% confidence intervals.*

Incubation duration

We found no difference in incubation duration between control or treatment clutches incubated on LB (W = 92.5, p = 0.83) nor between those incubated on NEB (W = 185, p = 0.89) (Figure 2 C). We found no effect of treatment ($\chi^2(3) = 2.04$, p = 0.56) or female size ($\chi^2(1) = 2.55$, p = 0.11) on incubation duration, but did find a negative effect of temperature ($\chi^2(1) = 11.6$, $p < 0.001$) and its quadratic term ($\chi^2(1) = 10.3$, $p < 0.001$) (Figure 4 B). It is worthy of note that whilst most data fit the curve, an apparent anomalous value (incubation duration: 39 days) was detected and kept for the analysis as it reflects the natural variation.

Hatchling size

We found no difference in hatchling size between control or treatment clutches incubated on LB (W = 37, p = 0.96) nor between those incubated on NEB (W = 63, p = 0.60). There was, however, a treatment effect ($\chi^2(3) = 23.7$, $p < 0.001$) with eggs incubated on LB producing larger offspring than those incubated on NEB (W = 492.5, $p < 0.001$) and a significant difference in incubation temperature on each beach ($\chi^2(1) = 10.9$, $p < 0.001$, mean difference = 2°C, LB = 30.7° (n =20); NEB = 32.7°C (n =32)).

Discussion

Key findings

Global climate change has the potential to impact species success and survival. Here we investigated whether philopatric association of sea turtles to their nesting beach confers any adaptive benefits to their offspring. We hypothesised that the benefits would be in the form of increased minor sex production, hatching success or hatchling size in warmer conditions. We found no differences of offspring sex or size or in clutch success or incubation duration other than those influenced by differing thermal conditions of the beaches. Contrary to initial findings which suggested localised thermal adaptation (Weber et al., 2012), our results do not support that hypothesis. However, these findings are in line with the laboratory based study showing no difference of sex ratio between beaches when incubation was carried out under a constant temperature environment (Tilley et al., 2019a). It therefore appears that, in this population, thermal adaptation is not, at the moment, a feasible mechanism to counter climate change linked increase in temperature. However, this does also mean that when translocation of clutches between beaches occurs, local adaptation is unlikely to be negatively impacted. Furthermore, using known pivotal temperatures for a different population may be a reliable method to estimate sex ratios for non-assessed population based on. Finally, the apparent negative effect of clutch size could be down to larger clutches producing more metabolic heating (Broderick et al., 2001), and reducing availability of oxygen for the developing embryos (Ackerman, 1981).

Temperature impact

Increasing temperatures linked to climate change are likely to cause a higher proportion of female offspring and for clutches to reach the limits of thermal tolerance. Therefore increasing temperatures could have a negative impact on offspring production. These findings are similar to some other recent studies (Hays et al., 2017; Kobayashi et al., 2017; Santidrián Tomillo et al., 2015), reinforcing the threat of climate change to incubating clutches and sea turtle populations. This could have serious consequences for the overall fitness and resilience of turtle populations in particular, and reptiles and other TSD species in general. Whilst TSD mechanisms may be resilient to extreme sex ratio bias, short term population growth through increased female output (Santidrián Tomillo et al., 2015), will lead to reduced output in the long term through decreased hatching, and lack of males reducing fecundity (Wright et al., 2012). Despite most studies finding female biased offspring sex ratios (Tilley et al., 2019b) even for those with balanced sex ratios (Patrício et al., 2017), long term forecasts indicate highly female bias population for the future (Patrício et al., 2018). Furthermore, sex ratio analysis at a population level on foraging grounds seems to indicate a feminisation of the population at some parts of the Great Barrier Reef, with few males being produced over the last 20 years, as a consequence of increasing temperatures (Jensen et al., 2018). These findings suggest that in order to contend with increasing temperatures, turtles will need to shift spatially to nest in cooler areas, or temporally to a cooler part of the year.

Egg translocation experiments in a scincid lizard (*Lampropholis guichenoti*) revealed the role of environmental variation in determining reptile phenotypes (Qualls and Shine, 1998), whilst eggs from *Bassiana duperreyi* (Shine, 2002)*, Oligosoma suteri* (Hare et al., 2004)*, and Sceloporus undulates* (Parker and Andrews, 2007) incubated outside of their natural thermal range had reduced hatching success and fitness. Furthermore, findings for tuatara (*Sphenodon punctatus*) suggested that temperature was a limiting factor for dispersal, but that suitable habitat may open up with climate change and increasing temperatures (Besson et al., 2012). Therefore, climate change may help expand the range of known nesting distribution for turtles (and other reptiles), and exploratory behaviour may help colonise these new sites (Carreras et al., 2018).

A potential caveat to this study is that we did not carry out any genetic analysis of the different individuals sampled and therefore it is possible that some of the turtle sampled are not highly philopatric (Formia et al., 2007); Exploratory behaviour from even only a few individuals is enough to mask any form of adaptation (Stiebens et al., 2013). Different thermal sensitivities in green turtle embryological development have been attributed to localised adaptation (Booth and Astill, 2001; Stubbs and Mitchell, 2018), with among female loggerhead sea turtle (*Caretta caretta*) variation detected in hatching success (Reneker and Kamel, 2016). Furthermore, variation in pivotal temperatures may occur in the red-eared slider (*Trachemys scripta*) (Dodd et al., 2006) and loggerhead turtle (Mrosovsky, 1988), and differences detected in two populations of flatback sea turtles (*Natator Depressus*) have been attributed to genetic differentiation between the populations (Stubbs et al., 2014). However, when comparing the thermal reaction norm of two loggerhead populations originating from contrasting thermal backgrounds, in the Mediterranean, no difference was found, implying no adaptation to local conditions (Monsinjon et al., 2017). Therefore, although we have not detected it, we cannot exclude the possibility of some form of genetic adaptation to localised conditions. Further genetic analysis may help distinguish fine scale separation and distribution (Bradshaw et al., 2018).

Management inference

Given the predicted effects of climate change on temperature, weather patterns and sea level rise it is likely that there will be impacts on coastal habitat quality and availability (Ahles and Milton, 2016) potentially subjecting sea turtle nesting beaches to increasing flooding (Varela et al., 2018), which could have devastating consequences for sea turtle productivity (Fuentes et al., 2010; Pike et al., 2015). Whilst it is difficult to predict how the coastlines will change and if new habitat will become available, to maintain future output and to balance/ limit the bias in primary sex ratios, it may be necessary to adopt an interventionist approach at some nesting sites. This may require providing shading/cooling or clutch translocation or both depending on the threat (Esteban et al., 2018). However, the scale of the rookery may dictate what sort of approach to take; for instance in places such as Ascension Island or in the Bijagós archipelago (Guinea Bissau), remote islands where thousands of nests are deposited every year [45,70,71], translocating clutches is not realistically feasible. The cost in time and effort would likely be prohibitive, and the logistics of where to relocate to need to be considered. In small rookeries, it could be argued that translocation to cooler beaches and those less prone to flooding could be a valid method of maintaining a healthy offspring output. On the other hand, shading nests has been proven to be an effective way to limit incubation temperatures (Esteban et al., 2018; Fuentes et al., 2012; Wood et al., 2014); in regions such as the Bijagós archipelago where maritime forest occurs, nesting habitat is comprised of both open beach and vegetated areas, with the shrubs and trees. These provide natural shading to the nests, which has been shown to increase the proportion of male offspring (Patrício et al., 2017). Therefore, management plans should take into consideration whether flooding is a risk or not; if it is translocating clutches should be considered. However, providing shading to beaches through restoration of coastal vegetation, could then be an effective way to mitigate against increasing temperatures (Wood et al., 2014), but also serve as coastal defence against sea level rise.

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Ethics

Research was carried out under Ascension Island Government Conservation Department Research Permits #ERP-2014-11, #ERP-2015-17, and in accordance with the University of Exeter CLES-Ethics Committee review # 2015/890.

Data

Data are stored in the Ascension Island Government Conservation Department database and on Figshare (under embargo until publication).

Competing interests

The authors declare that the research was carried out without any competing interests.

Authors contributions

ACB, BJG and SW conceived the study and were awarded a NERC iCase studentship. DT conducted the field work with assistance from all co-authors. DT led the data analysis and writing of the manuscript with assistance from all co-authors.

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CHAPTER 5: NOWHERE TO GO – MODELLING CLIMATE CHANGE IMPACTS ON A REMOTE GREEN TURTLE ROOKERY

This chapter is in preparation for Marine Ecology Progress Series

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Abstract

The climate is changing at an unprecedented rate, with profound implications for organisms and ecosystems alike. Increasing temperatures are of particular concern for species that exhibit temperature dependent sex determination as this could cause extreme bias in sex ratios. We use historic air temperature records, empirically-determined temperature response curves and Representative Concentration Pathway (RCP) scenarios from the Intergovernmental Panel on Climate Change (IPCC), to model long term trends in incubation conditions and offspring phenotype for the Ascension Island green sea turtle (*Chelonia mydas*) rookery. This remote island harbours the largest green sea turtle population in the South Atlantic, with nesting beaches covering a range of different thermal properties. On an island wide scale, we estimated offspring sex ratios and hatching success between 1923 and 2018 and found that Ascension Island has a near 90% (range 86.7% to 93%) female biased offspring output over this period, with variation between beaches. Assuming no change to current nesting patterns, sex ratios are predicted to rise to up to 98% female by the end of the 21st century, with hatching success on the hottest beaches reduced considerably. Although phenological shifts have been documented in some sea turtle populations, there has been no detectable shift in the seasonality of nesting at Ascension Island over the past 20 years of recording. Whether this rookery will adapt to new climatic conditions is unknown, however, the remoteness of Ascension Island and the limited opportunity to adapt through geographical range shifts make this population particularly vulnerable to changes in nesting conditions. Other impacts of climate change, such as sea level rise, are likely to compound the threat faced by this population and require further investigation.

Introduction

Climate change

The Anthropocene epoch is characterised by rising greenhouse gas emissions that are causing an increase in global mean temperatures (Crutzen, 2002; Stips et al., 2016). Along with changes in temperature, climate change is expected to impact rainfall patterns, storm intensity and frequency (M. Fuentes et al., 2010; Trenberth, 2011) and sea level rise through thermal expansion and the melting of ice caps (Rahmstorf, 2007). This changing climate is affecting species, communities, and ecosystems alike across all realms (Cleland et al., 2007; Forrest, 2016; Gian-Reto et al., 2002; Hoegh-Guldberg and Bruno, 2010; McDermott and DeGroote, 2016; Pacifici et al., 2017; Scheffers et al., 2016; Sunday et al., 2015), causing organisms to adapt or face extinction (Gian-Reto et al., 2002). Organismal responses range from genetic adaptation in some corals (Acroporidae (Rose et al., 2017), Poritidae (Kenkel and Matz, 2016)), fish (e.g. Salmonidae (Kovach et al., 2012; O'Malley and Banks, 2008)) and plants (e.g. Cyperaceae(Walker et al., 2019)), to shifts in range and/or phenology in some plants (Cleland et al., 2007), insects (Forrest, 2016; Maurer et al., 2018), reptiles (Boyle et al., 2016; Weishampel et al., 2010), birds (McDermott and DeGroote, 2016; Tomotani et al., 2018), fish (Cheung et al., 2015) and mammals (Schloss et al., 2012). Understanding the responses, adaptation or resilience of all species to climate change is crucial to put in place effective management plans to protect vulnerable systems.

Climate scenarios

Predicting long-term climate change outcomes is complicated by considerable uncertainty surrounding global political, economic and technological drivers that may influence future greenhouse gas emissions. To accommodate this uncertainty, the Intergovernmental Panel on Climate Change (IPCC) produces forecasts based on several Representative Concentration Pathways (RCPs) that make different assumptions concerning long-term trends in emission rates (Stocker et al., 2013). These scenarios range from a reduction in current emissions (RCP2.6), leading to a 1°C increase in global average temperatures by the end of the 21st century, to a business as usual scenario (RCP 8.5) that results in global average temperatures increasing 3°C by the end of the century (Nazarenko et al., 2015). Current data suggest that emissions are tracking above RCP 8.5, making this the most probable outcome unless a significant societal shift is achieved in the short to medium term (Sanford et al., 2014). These scenarios and associated change in temperatures vary between regions, with some areas affected more than others (Nazarenko et al., 2015). Depending on their habitat, behaviour and life history traits some species will also be more impacted.

Consequence for turtles

Like many reptiles, sea turtles exhibit temperature dependent sex determination (TSD), where the sex of the offspring is determined during egg incubation and female offspring are produced at higher temperatures (Godfrey and Mrosovsky, 2006; Tilley et al., 2019; Yntema and Mrosovsky, 1982). TSD mechanisms are characterised by a pivotal temperature, which under constant temperature conditions, produce an equal proportion of males and females, and sex is determined by the incubation temperature during the thermosensitive period (TSP) (Mrosovsky and Pieau, 1991). Furthermore, embryonic development is only known to occur within a window of temperatures ranging from 25°C to 35°C (Howard et al., 2014). Increasing temperatures therefore have the potential to both feminise populations and reduce hatching success (Hays et al., 2017). Paradoxically, increasingly female-biased sex ratios may enhance recruitment in the short term through increased offspring production (Boyle et al., 2014; Patrício et al., 2018b), but is ultimately expected to cause a long term population decrease owing to a shortage of males (Hays et al., 2017; Santidrián Tomillo et al., 2015). Most of the assessed sea turtle populations studied to date exhibit female-biased primary sex ratios (Broderick et al., 2001; M. M. P. B. Fuentes et al., 2010; Godfrey et al., 1999; B.J. Godley et al., 2001; Witt et al., 2010), although some populations are more balanced or have a high proportion of male output (Esteban et al., 2016; Patrício et al., 2017).

Whilst concerns over the long term impacts of climate change on sea turtle populations are justified, it is also true that this ancient group have persisted through multiple climate warming events across their evolutionary history, suggesting a high degree of plasticity or adaptability (Hamann et al., 2007; Poloczanska et al., 2009). Indeed, several studies have shown that contemporary sea turtle populations may already be responding to a changing climate through geographic range shifts and expansions (e.g. turtles recorded nesting in Spain (Carreras et al., 2018)) or through changes in breeding phenology (Weishampel et al., 2010). However, their ability to endure the rapid change predicted under some future climate scenarios against a backdrop of heavily human-modified coastal ecosystems is not yet well understood. Thus, assuming no change in phenology and no potential for adaptation, various IPCC scenarios have been used to hindcast and forecast trends in hatching success and sex ratios. For instance, beaches on Cape Verde are likely to become extremely female biased with forecast changes in air temperature (Laloë et al., 2014). When forecasting, it is important to take into account the variation within and among beaches in sand albedos, as this can result in significant differences in sand temperatures (Laloë et al., 2014) (but not meaningful at nest depth (Laloë et al., 2016) and microhabitats (Patrício et al., 2018a) as these may provide refugia and mitigate impacts of increasing temperature. Green turtles (*Chelonia mydas*) are currently considered to be endangered by the IUCN (Seminoff, 2004), although many populations, including our study site at Ascension Island, are now showing signs of recovery from historical exploitation (Balazs and Chaloupka, 2006; Stokes et al., 2014; Weber et al., 2014). Like all species of sea turtle, green turtles demonstrate natal philopatry (Bradshaw et al., 2018; Stiebens et al., 2013; Weber et al., 2013), tending to return to the same coastal area from which they hatched to breed (Lee et al., 2007). This species also has particularly high site fidelity, with the largest nesting rookeries found on remote islands and atolls (Weber et al., 2013). Such life history traits may constrain adaptation through range shifts, especially if there are no suitable alternative locations nearby. Lying more than 2000 km from the nearest alternative nesting sites, Ascension Island is typical of this remoteness, raising concerns that this isolated nesting population could be left with 'nowhere to go' as the climate warms.

In this study, we use historic air temperature records, empirically-determined temperature response curves and Representative Concentration Pathway (RCP) scenarios from the Intergovernmental Panel on Climate Change (IPCC), to model long term trends in incubation conditions and offspring phenotype for the globally important Ascension Island green sea turtle rookery. We evaluate the resilience of this rookery to predicted rising temperatures and we discuss the conservation implications for the population

Materials and methods

Study site

Ascension Island (14°20' W, 7°55' S) is a small volcanic island located west of the mid-Atlantic ridge in the South Atlantic Ocean. The Island has 31 nesting beaches (Mortimer and Carr, 1987) that vary in size and physical characteristics, although they are generally wide and lacking in supra-littoral vegetation. Three beaches receive over 70% of all nesting activity (Weber et al., 2014): Pan-Am (PAM ; 16.3%), Long Beach (LB ; 46.5%), and North East Bay (NEB ; 9.8%) (Weber et al., 2014). PAM and LB are composed of pale, biogenic sand, whilst NEB is dark grey with a high proportion of volcanic sediment. The lower albedo of the latter means that sand temperatures are consistently ≈ 2°C warmer than other primary nesting sites (Godley et al., 2002; Hays et al., 2003). Nesting starts sporadically from December, with the main activity concentrated between January and May, and a peak in nesting activity in mid-March. In this study we used temperature and histologically determined offspring sex ratio data from four beaches, Clarke's beach (beach 3 in (Mortimer and Carr, 1987)), LB, NEB, and PAM; NEB is the warmest nesting beach whilst the other three present similar thermal characteristics.

Nesting distribution and seasonality

Nest monitoring

Green turtle nesting activity on Ascension Island has been monitored intermittently since 1978 and annually since 1998 (Brendan J. Godley et al., 2001; Weber et al., 2014). Routine monitoring consists of weekly or fortnightly nest surveys on three main index beaches (LB, PAM & NEB), with island-wide censuses of all nesting beaches taking place approximately every 5 years [see (Weber et al., 2014) for details]. Prior to 2012, routine counts were limited to emergence tracks only (‘activities’), with numbers of successful nests quantified thereafter.

Seasonality

We used seasonal counts of recorded emergences covering the 2008-2018 nesting seasons to determine the overall temporal distribution of nesting effort. We fitted a generalised linear mixed model (GLMM) with a negative binomial error structure (implemented in package ‘*glmmADMB*’ (Bolker et al., 2012)) to the full time series of nesting activities for the three main beaches, with day of the year and its quadratic effect as explanatory variables and a hierarchical random effect structure of beach nested within season to account for different proportions of nesting on each beach. Individual models were also fitted for each nesting season with beach included as a random effect. For all analyses day of the year was set so that November 1st corresponds to day 1. For each fitted curve we extracted the date of peak nesting and calculated the interval between the 2.5th and 97.5th percentiles of cumulative nesting effort as an indication of season duration (i.e. the window during which 95% of nesting occurs). For purposes of assigning temperature windows to predict sex ratio and hatching success, we divided each month into halves and calculated average proportion of activities occurring in each period based on the general nesting curve. As our modelled nest distribution revealed that only 1% of activities occur prior to January, and 99% between January 1st and June 30th, we normalised the season to cover that period only.

Temperature effects on offspring phenotype

During the 2015 and 2016 nesting seasons, a subsample of clutches (2015: LB = 23, PAM = 21, NEB = 21, Beach 3 = 4; 2016: LB = 23, PAM = 23, NEB = 21), were individually monitored to gather baseline data on incubation temperature and hatching success. In each monitored clutch, a temperature datalogger (Tinytag, Gemini Data Loggers Ltd, Chichester, UK) was placed in the centre of the egg mass at the time of laying and programmed to record every hour throughout the incubation. Nest sites were marked using four wooden stakes to prevent them being disturbed by other nesting turtles. Upon emergence of hatchlings, nests were excavated and the contents categorised to determine the proportion of hatched eggs (see (Tilley et al., 2019) for full details).

Sex ratios

From the clutches monitored in 2015 and 2016, we sampled from 38 clutches (PAM: n = 10, LB: n = 13, NEB, n = 13, Beach 3 = 2), a total of 353 offspring (PAM: n = 100, LB: n = 128, NEB, n = 125, Beach 3 n =20) that were sexed through histology ((Tilley et al., 2019) for full details). We used the associated clutch temperature data to determine mean incubation temperature during the TSP using established methodology (Girondot and Kaska, 2014; Patrício et al., 2017; Tilley et al., 2019) to temperature-sex ratio curve. Sex ratios were obtained using function *tsd* in R package “embryogrowth”, using a logistic equation of the form:

$$sr(t) = \left(\frac{1}{1 + e^{\left(\frac{1}{S}(P-t)\right)}}\right)$$

where *sr* is the sex ratio, *P* is the pivotal temperature, *t* the incubation temperature and *S* is the shape of the transition from male to female producing temperatures (Girondot, 1999; Godfrey et al., 2003; Hulin et al., 2009).

Hatching success

Of the 136 clutches that were marked and monitored, we successfully obtained data from 103 undisturbed clutches that hatched (2015: PAM: n = 13, LB: n = 16, NEB: n = 16, Beach 3: n = 3; 2016: PAM: n = 15, LB: n = 21, NEB: n = 19) to model the relationship between sand temperature and hatching success. As the purpose of this analysis was to describe temperature effects on productivity independent of other sources of mortality, we excluded nests that were disturbed by other nesting turtles (15), flooded with seawater (n = 9), or when the datalogger failed (n = 9). Hatching success was determined as the proportion of hatched eggs in each clutch (Miller, 1999). We fitted a General

Linear Model (GLM) with a quasi-binomial distribution (hatched / not hatched) to model the effect of incubation temperature during the TSP on hatching success with a quadratic effect of temperature to account for non-linearity. We used all available data to produce a single curve representative of the range of incubation temperature occurring on Ascension Island (Tilley et al., 2019).

Air temperature data & climate forecasts

We obtained monthly mean air temperature data for Ascension Island from HadCRUT4, a global temperature dataset providing gridded coverage (5*5 degree resolution) developed by the Climatic Research Unit (University of East Anglia, UK) in conjunction with the Hadley Centre (UK Met Office), available from https://crudata.uea.ac.uk/cru/data/crutem/ge/ (last accessed January 13th 2019) (Jones et al., 2012; Osborn and Jones, 2014). Occasional gaps in the HadCRUT4 dataset (1973-1980) were bridged using data retrieved from the Global Historical Climatology Network monthly mean data set (GHCNM), to build a complete temperature time series from 1923 – 2018, maintained by NOAA (National Oceanic and Atmospheric Administration), and were downloaded from the Royal Netherlands Meteorological Institute (KNMI) Climate Explorer (https://climexp.knmi.nl/start.cgi; last accessed January 13th 2019). We used air temperature records covering the period from 1923 to 2018, as only 114 monthly records exist for the period from 1854 to 1922.

We fitted a Generalised Additive Model (GAM) implemented using the 'gamm' function in the R package '*mgcv*' to the full times series of annual mean air temperature with a corARMA1 autocorrelation structure to detect systematic trends (Wood and Wood, 2018).

Following standard Met Office procedures (Osborn and Jones, 2014), we calculated the monthly mean air temperature during the reference period of 1961-1990. We downloaded from KNMI using Climate Explorer (last accessed January 13th 2019), the forecast air temperature anomalies based on the Coupled Model Intercomparison Project Phase 5 CMIP5 (Taylor et al., 2012), following three of the four Representative Concentration Pathways (RCP) scenarios: RCP 2.6, RCP 4.5 and RCP 8.5. These scenarios are low, medium and extreme impact, based on forecast increases in global temperatures of respectively 1°C, 1.9°C and 3.5°C by the end of this century (Moss et al., 2010; Nazarenko et al., 2015). RCP anomalies were then added to monthly mean air temperatures at Ascension Island over the reference period to recreate modelled temperature for the 1923 - 2100 period under different scenarios.

Relationship between air temperature and clutch temperature

Describing the relationship between air temperature and incubation temperatures experienced by developing embryos is a necessary step in linking climate predictions to phenotypic outcomes in sea turtles. We used daily minimum and maximum air temperatures obtained from the meteorological office on Ascension Island to calculate mean air temperatures during the TSP of clutches monitored during the 2015 and 2016 nesting seasons following standard methodology (Lawrimore et al., 2011). A GLMM was then used to model the relationship between mean incubation temperature of monitored clutches during the TSP and mean air temperature over the same period. Beach was held as a random factor to account for natural variation in temperatures between them.

For prediction purposes, we assigned each beach to one of five different thermal clusters based on the mean difference in temperatures compared to LB, following Godley et al 2002 (Godley et al., 2002). The difference in thermal conditions between clusters ranged from -0.5°C to +2.9°C (Table 1) primarily based on sand albedo (Hays et al., 1995).

Prediction models

We used the empirically-established relationship between air and clutch temperature to forecast incubation temperatures during the TSP for the entire 1923 - 2100 period. We then used the established clutch temperature - sex ratio curve to determine offspring sex ratio over the same period. From this we determined seasonal sex ratio trends. We also used our model of clutch temperature and hatching success to determine hatching success based on forecast air temperature for the 1923-2100 period. Each month of the nesting season was divided into two; the first and second half of the month; each clutch deposited in the first half of the month was assigned air temperatures for that month, whereas each clutch deposited in the second half is assigned the air temperature of the following month. For both sex ratio and hatching success, we account for spatial (among beaches, Table 1) and temporal (throughout the season, Figure S1) clutch distribution, by determining the output from each half month period for each beach cluster (cluster A to E) and add them together to determine island wide seasonal trends.

All statistical analyses were carried out in R 3.4.1 (R Core Team, 2017)

Results

Seasonality and air temperature

For the period 2008 – 2018, we found no significant relationship between year and Julian date of peak nesting (median date March 12th, range March 6th and 20th, $F_{1,9} = 0.0$, $p = 0.99$, Figure S1), and no change in the overall season length (mean = 126 days, range 119-133 days, $F_{1,9} = 0.0$, $p = 0.98$ Figure S2).

There were no detectable long-term trends in the mean annual air temperature over the 1923-2018 period ($F = 0.118$, $p = 0.73$, Figure 1 A) with temperature variation throughout the year (Figure 1 B). Mean annual air temperature for the 1961-1990 reference period was 25.6°C, with mean monthly temperatures ranging from 24.4°C in September to 27.6°C in March.

Each RCP scenario followed the same general pattern until *ca.*2020 from when the diverging models led to significant differences in predicted annual mean temperatures by 2100 RCP2.6: 26.6°C, RCP4.5: 27.3°C, RCP8.5: 29.1°C (Figure 2).

Air to clutch temperature

As expected, air temperature and incubation temperature of monitored clutches during the TSP were strongly correlated (GLMM: $F_{1,120} = 10.1$, $p < 0.005$, conditional $R^2=0.66$) and related by the equation:

$$T_{clutch} = 0.4845 * T_{air} + 17.4635 + \Delta T_{LB}$$

where ΔT_{LB} is the difference in mean sand temperature to relative to LB

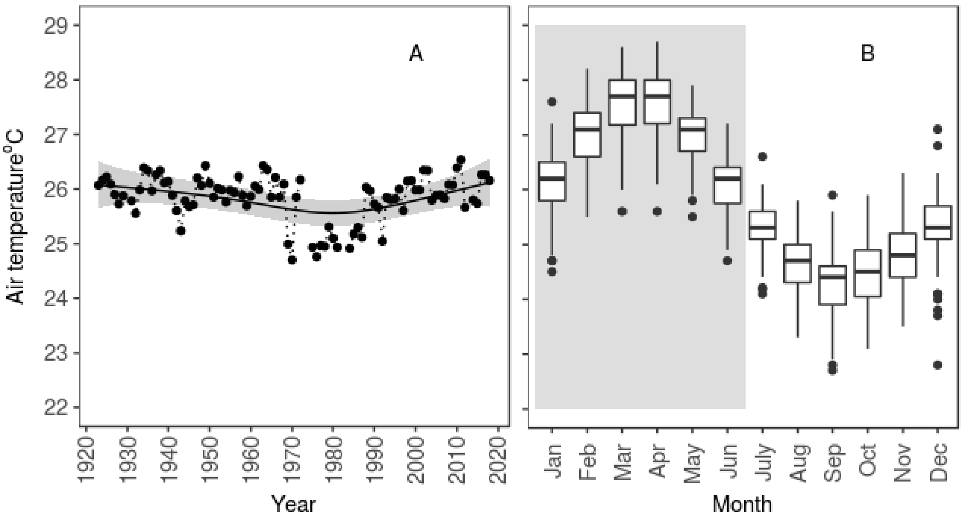

***Figure 1**: **A)** Trend in mean annual air temperature recorded at sea level on Ascension Island between 1923 and 2018 modelled using a thin plate spline regression (solid line) with associated 95% confidence interval (shaded ribbon). **B)** Seasonal variation in air temperatures at Ascension Island based on all available data for the period 1923 - 2018. Box and whisker plots represent monthly minima and maxima (whiskers), 25th to 75th percentile (box) and median values (black bars). Individual points represent outliers. The shaded polygon corresponds to the nesting season for green turtles on Ascension Island. Both plots, share a common y-axis scaled to encompass the full range of temperatures recorded on Ascension Island.*

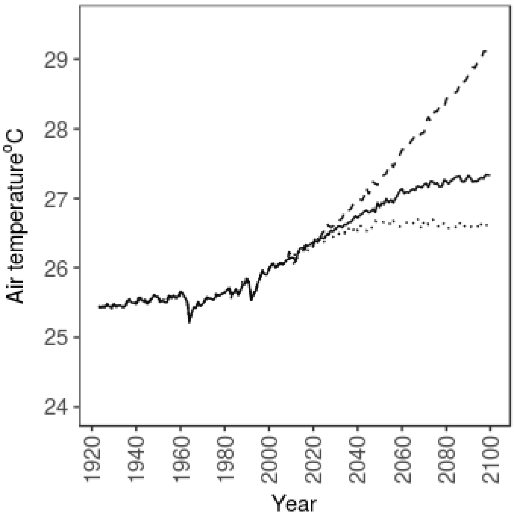

***Figure 2**: Reconstructed mean annual air temperature at Ascension Island for 1923 - 2100 based on three different IPCC scenarios: RCP 2.6 (dotted line), RCP 4.5 (full black line), and RCP 8.5 (dashed line). Note the y-axis is scaled from 24°C to 29°C to allow for direct comparison with recorded data. Temperatures are calculated by applying predicted anomalies to mean air temperature at Ascension over the 1961-1990 reference period.*

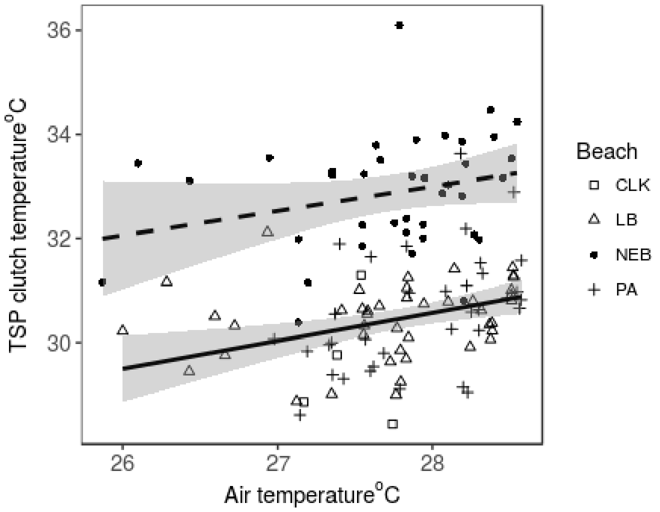

***Figure 3**: Relationship between air temperature and mean clutch temperature during the thermosensitive period (TSP) for Clarke's Beach (CLK), Long Beach (LB), North East Bay (NEB), and Pan Am (PA). Data are fitted with a linear regression and 95% confidence interval (shaded), grouping beaches based on thermal characteristics (NEB dashed line) from Table S1.*

Sex ratio

Based on all available data, the field pivotal temperature was estimated at 28.7°C (Figure 4 A) which is consistent with previous findings for this population (Godley et al., 2002; Tilley et al., 2019). Data from histological analysis suggest that clutches laid on beaches representative of cluster B were 87.9% female (LB: 91% female, PAM: 88% females and Beach 3: 60% female) which compares well with the modelled proportion of 87.5% based on 2015 and 2016 air temperature data. All hatchlings sampled from NEB clutches were female which is also consistent with model predictions over the same period (99.6%).

Assuming the 2017 nesting distribution would be representative of general annual trends, and based on predicted air temperature data, we estimate that overall primary sex ratios remained relatively stable on Ascension Island between 1923 and 2018 and were consistently female biased (mean = 89.7%, range 86.7% to 93%; Figure 5). This situation is predicted to change towards the end of the century, with an increasing proportion of female offspring predicted under all RCPs (proportion female in 2100, RCP 2.6:94.1% (CI: 91.1-96.8%); RCP 4.5: 96.2 % (CI: 93.9-98.2%); and RCP 8.5: 98.5% (CI:

97.3-99.3%) female (Figure 6). Sex ratios for beaches in cluster D and E, which account for ≈ 9% of all nesting activities at Ascension Island, were consistently highly female biased (99 - 100%) showing little to no variation over time (Figure S3). Beaches from cluster A, which also account for ≈ 9% of all nesting activities, show a lower proportion of females than average (Figure S3). Using recorded air temperature data yielded similar proportions for the mean proportion of female offspring over the 1923-2018 period (mean = 89.4% (CI 84.7-93.1%), but with greater variation ranging from 75.5% (CI 62.7-91.9%) to 94.5% (CI 91.9-97.1%) (Figure S3).

Hatching success

Clutch temperature during the TSP had a significant effect on hatching success ($F_{1,64}$ = 17.8, $p < 0.005$), with the quadratic effect of temperature also significant ($F_{1,64}$ = 18.7, $p < 0.005$), indicating that hatching success decreased as temperatures increased (Figure 4 B).

Based on hindcast air temperature we estimated that overall hatching success averaged approximately 84% (range in annual averages 82% to 85%) over the period 1923-2018, and this was not predicted to decline significantly by the end of the century under the RCP2.6 and RCP4.5 scenarios (Figure 6). The more pessimistic RCP8.5 scenario was predicted to result in a small decrease in hatching success to around 80% in 2100 (Figure 7), but with strong disparity among beach groups (Figure S3). For instance, the hottest beaches during the hottest month are forecast to reach 34.9°C, reducing hatching success to ≈ 38%, whilst the coolest ones would reach 31.4°C, which maintains hatching success at ≈ 83% (Figure S4). The modelled hatching success would reach towards 0% when clutch temperatures reach 36°C.

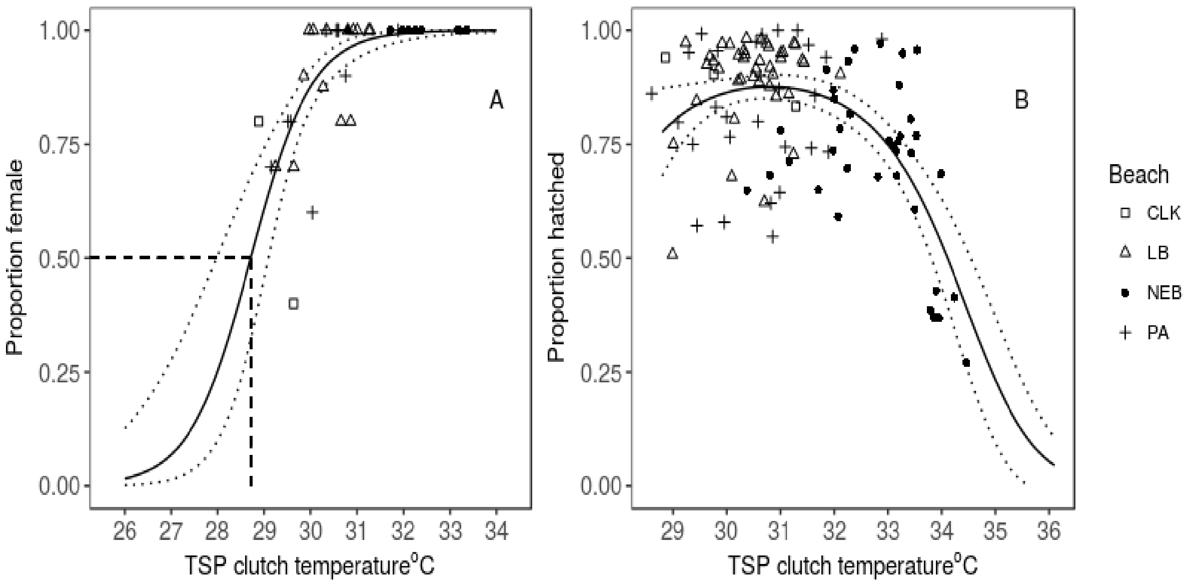

Figure 4: *Relationship between mean incubation temperature during the TSP and:* ***A)*** *the proportion of female offspring produced in clutches incubated in situ (n = 39). The data were fit with a logistic regression, with dotted lines representing the 95% confidence interval. Dashed lines indicate constant temperature equivalent during the TSP at which balanced sex ratio is obtained (field pivotal temperature: 28.7°C);* ***B)*** *the proportion of eggs hatched on for Clarke's Beach (CLK), Long Beach (LB), North East Bay (NEB), and Pan Am (PA) in relation to mean temperature during the thermosensitive period (TSP). The data were fit with a logistic regression including a quadratic effect of temperature, with dotted lines representing the 95% confidence interval.*

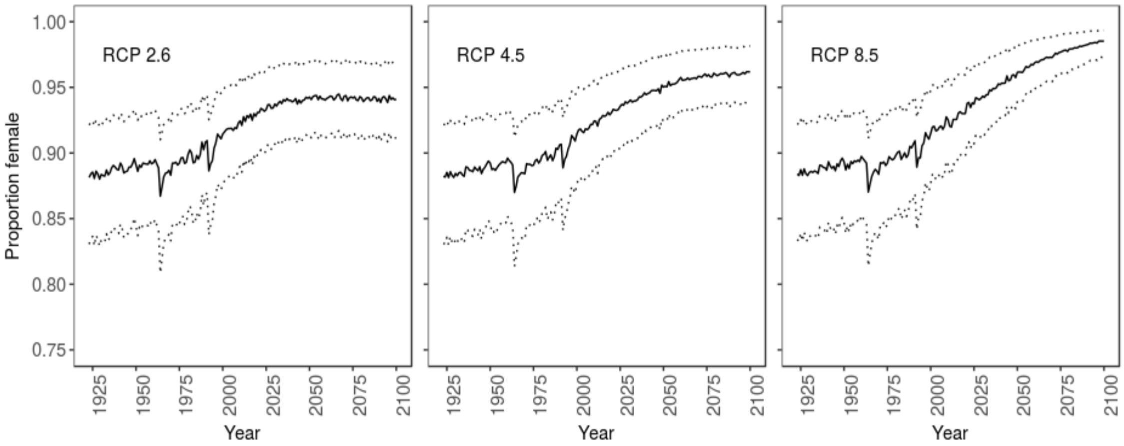

Figure 5: *Modelled mean annual proportion of female offspring produced on Ascension Island between 1923 and 2100, under three different Representative Concentration Pathways (RCPs) after correcting for the seasonal and spatial distribution of nesting effort (solid lines). Dotted lines represent the 95% confidence interval. Note that the y-axis is truncated at 0.75 to better illustrate annual variation and predicted trends. Air temperature was reconstructed based on predicted monthly anomalies added to the mean monthly temperature for the 1961-1990 reference period.*

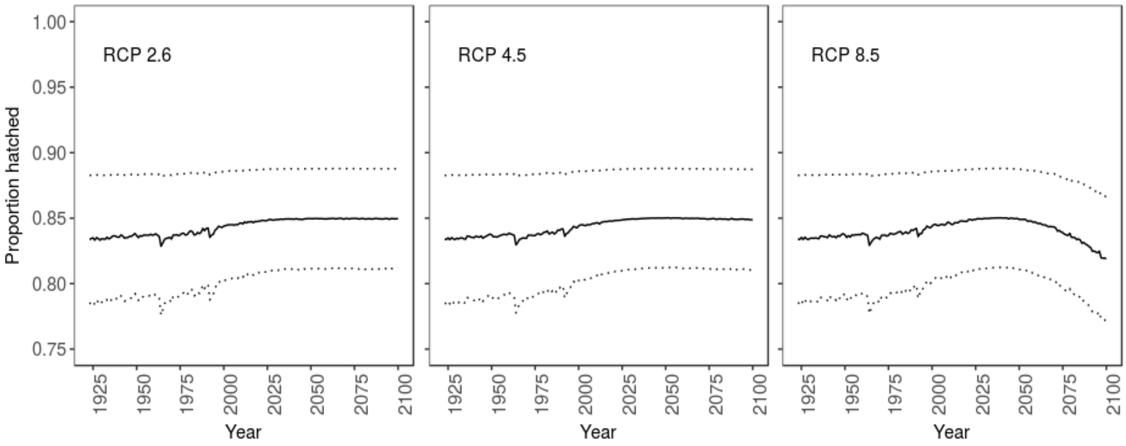

Figure 6*: Modelled mean annual hatching success produced on Ascension Island between 1923 and 2100, under three different representative concentration pathways (RCPs) after correcting for the seasonal and spatial distribution of nesting effort (solid lines). Dotted lines represent the 95% confidence interval. Note that the y-axis is truncated at 0.75 to better illustrate annual variation and predicted trends.*

Discussion

Key findings

This study aimed to model long-term trends in hatchling output and primary sex ratio for the globally-important green turtle rookery at Ascension Island under a range of possible climate change scenarios. We estimate that over the last century, the primary sex ratio at Ascension Island has likely been strongly female biased (≈ 90% female) and is expected to become increasingly skewed under even the most optimistic climate change scenarios, averaging between 94 and 98% by 2100 (Figure 5). In contrast, hatching success is predicted to remain relatively stable under more modest climate warming forecasts and was only marginally reduced under the most extreme that scenario considered. Historically, lethal thermal limits are unlikely to have been regularly exceeded on any of Ascension's beaches, but this will become increasingly common on the hottest, dark sand beaches under more severe projections. These beaches currently account for approximately 9% of total nesting and thus the impact on the overall hatchling output may not be significant; however, all are predicted to become increasingly unsuitable as nesting habitat in the future.

This contrasts with a previous estimate, of 75% female for the Ascension population (Godley et al., 2002) that may be partly attributable to methodological differences between studies (using actual clutch temperature measured during the TSP in this study compared to reconstructed incubation temperature using sand and metabolic heating (Godley et al., 2002)).

Historic perspective

Analysis of the last *ca.* 100 years of air temperature data for Ascension Island revealed no significant trend in temperature through time, but predictable seasonal variation in temperature between the hottest months (March and April) and the coldest month (September). Furthermore, natural inter-annual fluctuations mean some years are cooler than average whilst others are warmer. These fluctuations play an important role in the variation of offspring output and sex ratios produced. Our data suggest that Ascension Island has likely been producing female biased primary sex ratios over the last century, but with some inter annual variation. There is currently little evidence of this being detrimental to this green sea turtle population, which is rebounding strongly as a result of conservation measures introduced at its nesting and foraging grounds.

Most oviparous species use nest site selection to favour microhabitats that will reduce incubation duration and optimise hatching success (Huang and Pike, 2011; Mitchell et al., 2008; Pike, 2014; Telemeco et al., 2013, 2009). Currently the nesting seasonality of this rookery corresponds with the hottest months of the year, as during the coldest season, sand temperature at nest depth drops to below 26°C (Brendan J. Godley et al., 2001), which would lead to longer incubation durations (Mrosovsky, 1980) increasing the risk to the incubating clutches.

Although no discernible trend in temperature change was detected in the past, long term air temperature may rise on Ascension, in line with global forecasts. The current temperature rises predicted by the IPCC would have a moderate impact on hatching success but further the sex ratio bias, and that it will have consequences on recruitment.

Shifting nesting seasonality is well documented in many sea turtle populations (Dalleau et al., 2012; Mazaris et al., 2009; Neeman et al., 2015; Weishampel et al., 2010, 2004) and could represent a resilience strategy to overcome the effects of increasing temperatures. We find no evidence of such a shift at Ascension Island over past decade, which may not be unexpected given stable temperatures over period. However, the thermal window during which turtles currently nest becomes increasingly compressed under all RCP scenarios, potentially leading to a shortened or split nesting season (Figure S5). Furthermore, biological constraints imposed by the life history of the species, and the considerable distance and environmental differences between breeding and foraging grounds may also inhibit a rapid change in seasonality of the magnitude needed to offset climate impacts (Refsnider and Janzen, 2016).

Global impact

This case study focuses on a single, regionally-important green turtle rookery, but its implications are global in scope. Our findings are indicative of pressures that are likely to affect other marine turtle rookeries around the world, as well as other species exhibiting TSD. Indeed, increasing feminisation and reduced hatching success have already been documented in and forecast for rookeries such as in Australia (Butt et al., 2016) Cape Verde (Laloë et al., 2014; Perez et al., 2016), the Caribbean (Laloë et al., 2016), Poilão (Patrício et al., 2018a), and Colombia (Patino-Martinez et al., 2012). Primary sex ratios do not necessarily reflect those found in adult or sub adult aggregations (Hawkes et al., 2013; Rees et al., 2013; Wright et al., 2012). However, the bias seen in primary sex ratios can also be found on foraging grounds (Jensen et al., 2016). For instance, mixed stock analysis of turtles on a foraging ground on the Great Barrier Reef revealed a disparity in origin and in sex ratios of different life stages; this led the authors to suggest that the disparity in incubation temperature across nesting sites was responsible for the strong dichotomy in sex ratios seen in the adult and sub adult populations (Jensen et al., 2018). Furthermore, beach albedo has an influence on sand and clutch temperature, with dark sand beaches being warmer than pale sand beaches (Table 2). Dark sand beaches may already be at the upper limit of thermal tolerance and producing offspring that is near 100% female (Figure S3) (Hays et al., 2003; Laloë et al., 2014); if these are predominant nesting areas, this could have an impact on the overall offspring output. In contrast, pale beaches could provide refugia, but this would require changes in nesting distribution to increase the proportion of clutches deposited on the cooler beaches.

Further work and concluding remarks

Climate change will not only impact mean global air temperature, but also potentially causing changes in wider weather patterns. Rising temperatures are causing glaciers to melt and thermal expansion contributing to sea level rise (SLR). In turn, increased fresh water input into the oceans could have an impact on ocean circulation patterns (Maier et al., 2018) and therefore on sediment movement (Kang et al., 2017). Thus, the impacts on nesting beaches are difficult to evaluate and predict. SLR may cause nest flooding and destruction of habitat, with changes in weather patterns potentially compounding the problem. However, exactly how sediment movement may affect the formation of new beaches or exacerbate the potential destruction caused by SLR is not currently understood. For instance, the occasional large storms that hit Ascension deposit large amounts of sand, raising the beach platform and therefore reducing the coastal washover area (Pers. Obs.). Whilst storm frequency may be reduced under future climate change scenarios (Bacmeister et al., 2018; Fuentes and Abbs, 2010), extremes in rainfall may be increased (Trenberth, 2011). How this will impact a small isolated island is yet unquantified, however increased rainfall has an effect on incubating clutches (Houghton et al., 2007; Lolavar and Wyneken, 2015). However, sea turtle life history traits (multi annual remigration interval, multiple clutches per nesting season) may be well adapted to extreme weather events as only a fraction of the population would be affected in case of a storm (Dewald and Pike, 2014). Therefore, modelling the impacts of SLR and changes in weather patterns would be the next logical step to further assess resilience to climate change (Patrício et al., 2018a; Varela et al., 2018).

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Ethics

Research was carried out under Ascension Island Government Conservation Department Research Permits #ERP-2014-11, #ERP-2015-17, and in accordance with the University of Exeter CLES-Ethics Committee review # 2015/890.

Data

Data are stored in the Ascension Island Government Conservation Department database and on Figshare (under embargo until publication).

Competing interests

The authors declare that the research was carried out without any competing interests.

Authors contributions

ACB, BJG and SW conceived the study and were awarded a NERC iCase studentship. DT conducted the field work with assistance from all co-authors. DT led the data analysis and writing of the manuscript with assistance from all co-authors.

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Supplementary figures and tables

***Table S1**: Proportion of total nests occurring on five beach clusters during the 2012 and 2017 nesting seasons (island-wide census years). Beaches are grouped according to the mean difference in incubation temperature when compared to Long Beach as a reference site (ΔT_{LB}) and were adapted from Godley et al 2002* (Godley et al., 2002) *and from in situ sand temperature data recorded between July 2013 and May 2018 (Table S2). Beaches with T_{LB} within 0.3°C of one another were grouped and a mean calculated for the cluster. Beach numbers are defined in Mortimer & Carr 1987* (Mortimer and Carr, 1987).

CLUSTER	**BEACHES**	**ΔT_{LB}(°C)**	**PROP NESTING (%)**	
			2012	2017
A	2; 6-11; 14-17; 21-25	-0.5	24	20
B	1; 3; 4; 12	0	63	69
C	26; 29	0.7	2	2
D	27-28	1.7	11	8
E	30-31	2.9	0	1

***Table S2**: Mean (±SD), minimum and maximum sand temperature (n = 49 monthly readings), recorded for, Long Beach and North East Bay between July 2013 and January 2018.*

	TEMPERATURE(°C)	
	Long Beach	North East Bay
MEAN	28.1 (±1.5)	29.9 (±1.1)
MINIMUM	25.8	28.3
MAXIMUM	31.5	32.3

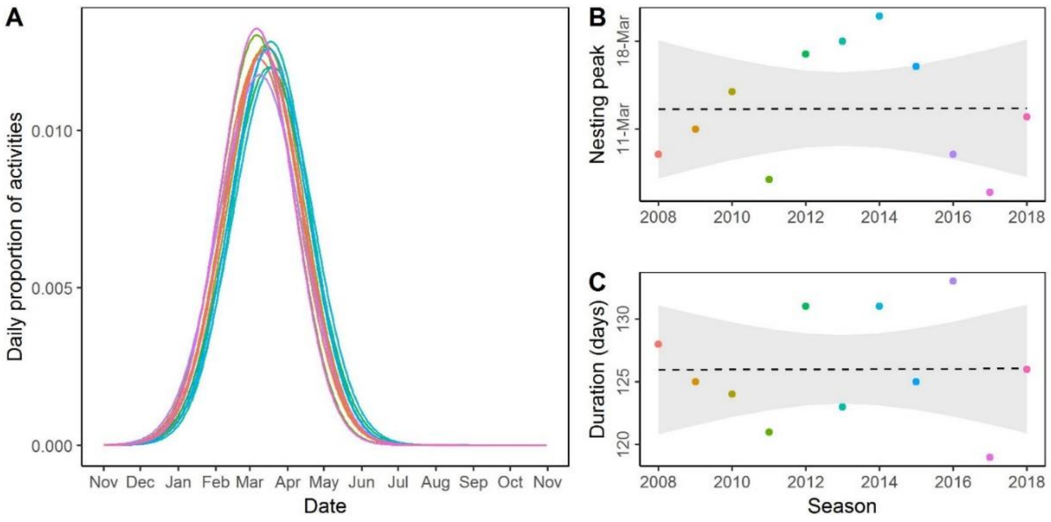

***Figure S1: A)** Seasonal distribution of nesting activities at Ascension Island for the 2008 - 2018 nesting seasons, with each colour representing a different year. Curves were fitted to daily count data using negative binomial GLMs with a quadratic date term and normalised to place all seasons on a common scale. **B)** Trend in date of modelled peak in nesting **C)** Trend in modelled duration of the nesting season. Both B and C are fit with a linear regression (dashed line), with 95% confidence intervals (shaded grey).*

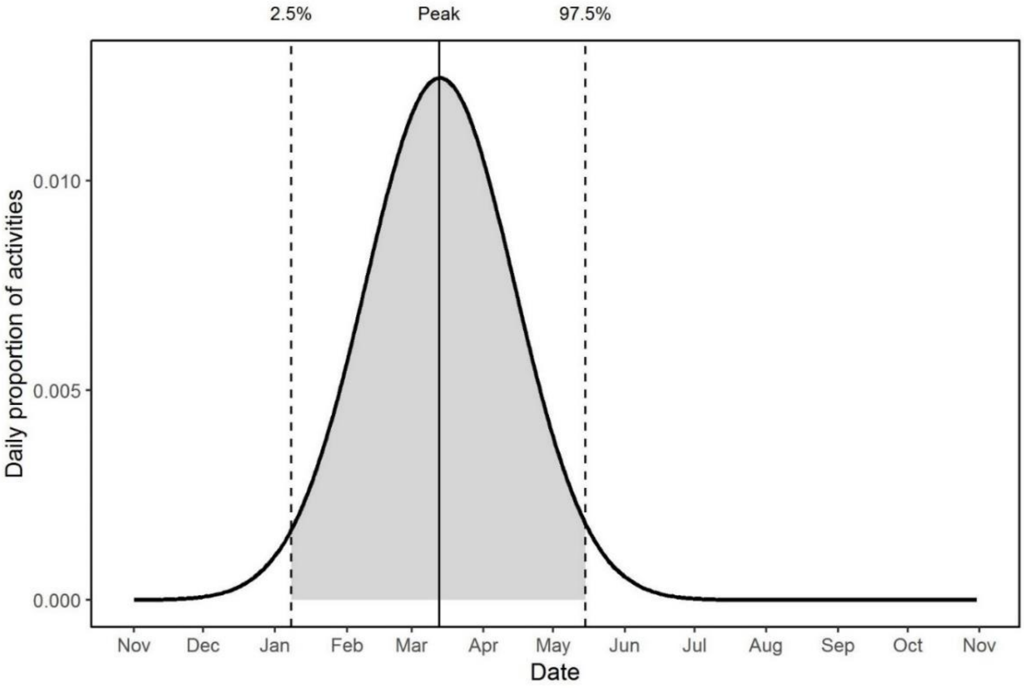

Figure S2: *Overall seasonal distribution of nesting activity at Ascension Island based on an integration of daily count data from all monitored beaches between 2008 and 2018. The seasonal curve was fitted using a negative binomial GLMM with a quadratic date term and a hierarchical random effects structure of beach nested within season. Season duration is defined as the period falling between the 2.5th and 97.5th percentiles of cumulative nesting activity (broken vertical lines).*

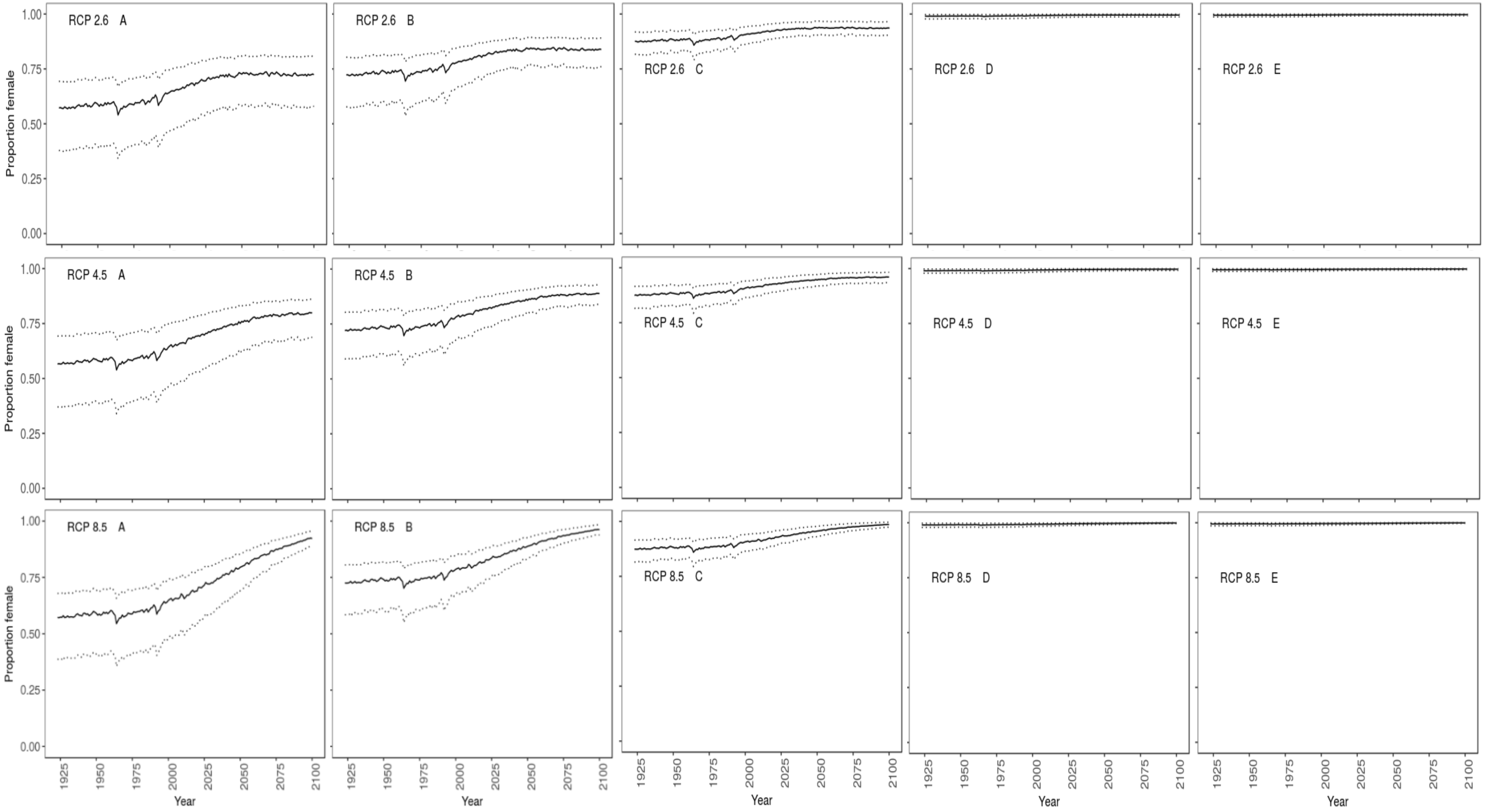

Figure S3: *Modelled mean annual proportion of female offspring produced from each beach cluster (A-E: Table 1) at Ascension Island between 1923 and 2100, under three different representative concentration pathways (RCPs). Dotted lines represent the 95% confidence interval.*

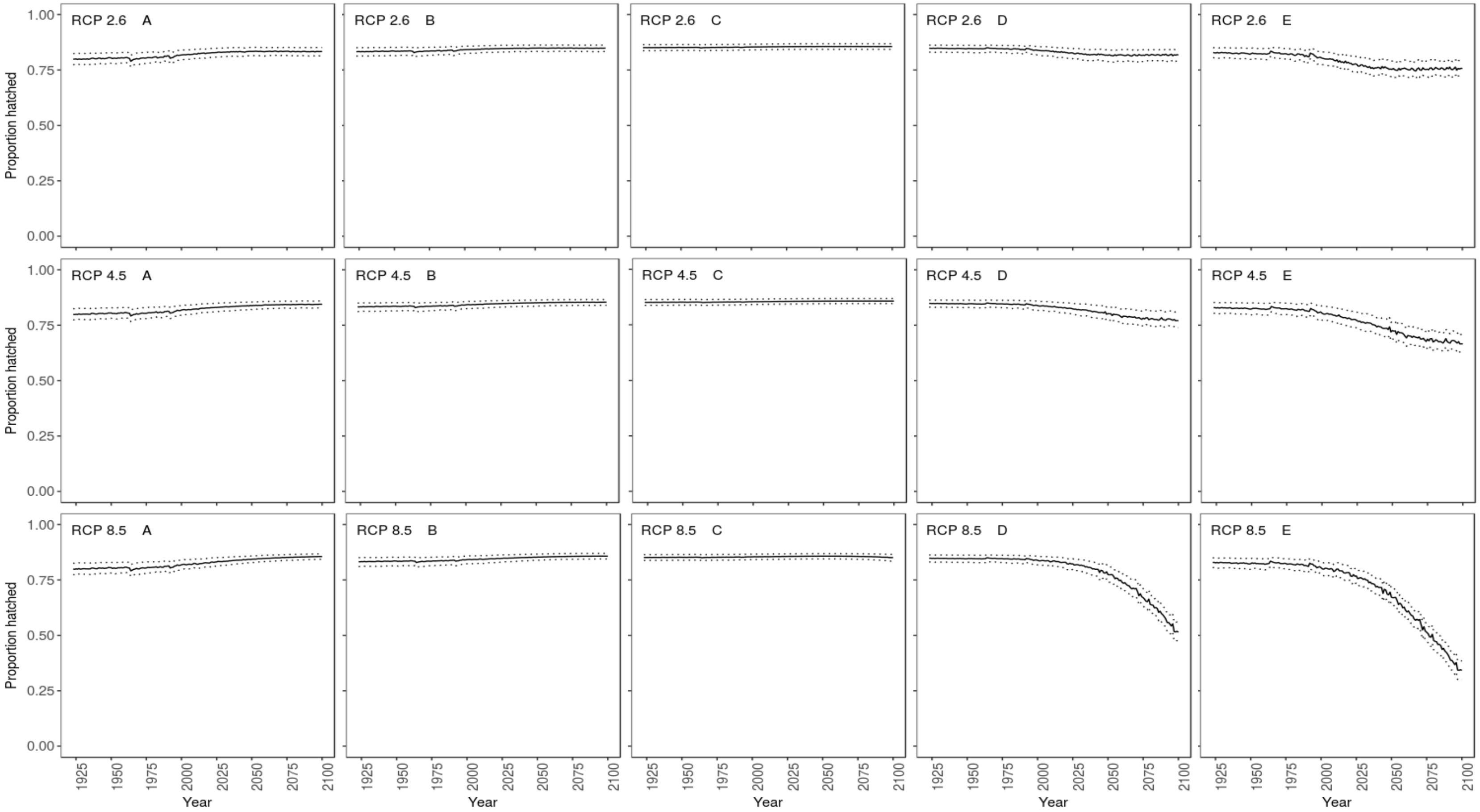

Figure S4: *Modelled mean annual hatching success from each beach cluster (A-E: Table 1) at Ascension Island between 1923 and 2100, under three different representative concentration pathways (RCPs). Dotted lines represent the 95% confidence interval.*

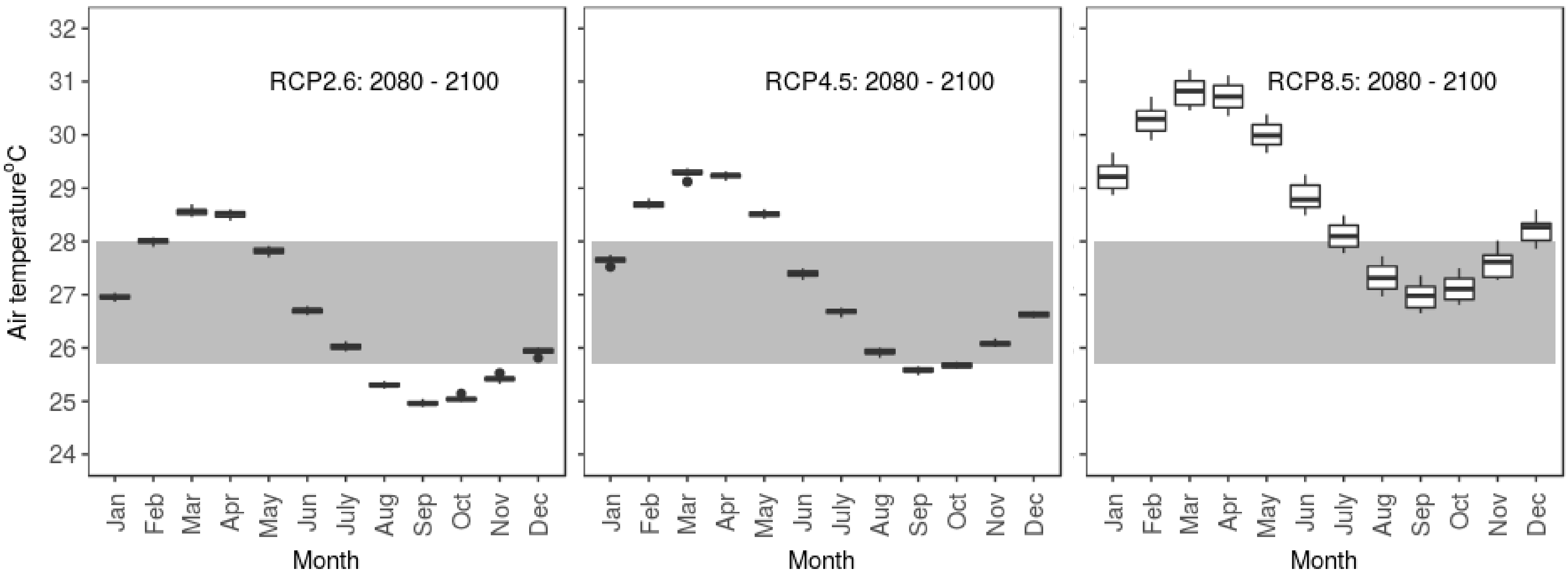

Figure S5: *Modelled mean monthly air temperature for the period 2080-2100 at Ascension Island, under three different RCPs. Shaded area corresponds to the current minimum (25.5°C) and maximum (28°C) interquartile range of monthly temperatures occuring during the nesting season.*

CHAPTER 6: GENERAL DISCUSSION

A broad body of literature on sea turtles in general and green sea turtles in particular has been developed since the days of the pioneering work of Archie Carr (Carr, 1967). Despite decades of work, some fundamental questions still remain unresolved (Hamann et al., 2010; Rees et al., 2016), which I try to address in this thesis. I investigated the potential for sea turtle adaptation and resilience to climate change, which provides some further knowledge on answering questions 2, 3, and 12 (Rees et al., 2016).

In Chapter 2, "**Current knowledge of pivotal temperature and sex ratios of green turtles**", I reviewed the current knowledge on pivotal temperature and primary sex ratio for different rookeries across the world. I find that generally sex ratios are female biased, and that pivotal temperature is seemingly conserved across the different nesting grounds. However, pivotal temperature and transitional range of temperatures are specifically defined based on constant incubation conditions and most studies do not follow this assumption. Instead, the terms are used interchangeably between field and laboratory studies, and more importantly very few published studies have assessed pivotal temperature for the different green sea turtle rookeries around the world. Furthermore, various methods are used (incubation duration, incubation temperature, mean middle third) to assess population level metrics. I reiterate that standardised methods, using incubation temperature during the thermosensitive period as a proxy to infer offspring sex ratios, and using similar statistical models (logistic regressions), be used in order to have comparable studies and results. This review highlights that very few studies have been carried out investigating population level metrics. Instead general information from one population is used as reference for others, without having clear knowledge on whether site specific adaptations exist.

In Chapter 3, "**No evidence of fine scale thermal adaptation in green turtles**", I determine pivotal temperature, field-pivotal temperature and hatching success for the Ascension Island green turtle rookery. This work has shown that, contrary to our initial hypothesis, there was no sign of localised adaptation. This work provides information to inform some key questions for sea turtle conservation.

In Chapter 4, "**Translocation of sea turtle clutches: effects on offspring phenotype and survival**", I carried out a translocation experiment between beaches of different thermal properties, and thus, we can directly compare how clutches from different origins perform under similar conditions. Similarly to results from the laboratory (Chapter 2), I find no signs of localised adaptation. Thus inferring sex ratios for non-assessed population based on known pivotal temperature for a different population may be a reliable method. Furthermore, a physiological adaptation to climate change is unlikely to occur and instead physical change may be required.

Finally, in Chapter 5, "**Nowhere to go – Modelling climate change impacts on a remote green turtle rookery**", I build on results from Chapter 2 and 3 to assess the overall output from this globally important rookery. This investigation reveals that Ascension has likely been female biased over the last century with no significant effects on hatching success. However, under extreme climate scenarios forecast temperatures could increase the sex ratio bias to female and start hatching success to decline. Assuming that findings for Chapter 2 apply to sea turtles in general, and TSD species more broadly, this implies that to contend with increasing temperatures species will have to adapt in time and space.

Further research

An aspect of climate change that we did not assess here is the impact of climate change on nest site availability, and flooding of nesting grounds. Similarly to studies from Cyprus and Poilão (Guinea Bissau) (Patrício et al., 2018; Varela et al., 2018), where sea level rise has been shown to be a threat to sea turtle nests, work is being carried out to assess how the nesting beaches on Ascension Island may be affected. This combined with the general findings of this thesis will provide data to help develop the biodiversity action plans on island. Over their evolutionary life time, turtles have had to contend with changing nesting grounds, therefore sea level rise may be detrimental but also may provide new opportunities. Rising sea levels may bring more biogenic sediment which is the basis of the pale sand beach, potentially attenuating the impacts / reducing the amount of dark sand beach availability; this would limit the negative impacts of increasing temperatures on dark sand beaches. Moreover, potential changes in precipitation levels (e.g. increasing rainfall) associated with changing weather patterns may provide a cooling effect on nests. Thus understanding seasonality of weather patterns could provide further knowledge on incubation conditions and therefore offspring output. Although the outlook seems negative, it is very difficult to predict the exact implications of climate change on this population and sea turtles in particular, making it difficult to predict the full impact of climate change. Species that have smaller overall populations, or more restricted geographic ranges may be more at risk (e.g. Kemp's Ridley or Flatback turtles), as the loss of nesting grounds may have a proportionally larger impact.

It would be beneficial to fully assess key rookeries using standardised methods and equipment to produce comparable results and estimates for pivotal temperatures. For instance, collecting eggs from various sites around the world and incubating them in a series of laboratories set up with similar equipment. Thus providing a comparable analyses of the effect of temperature on egg incubation, and possibly discerning regional variations. Whilst this would be logistically challenging, a minimum would be to have key indicator sites representative of local regions that would then be used as reference points for locations which receive fewer nesting turtles.

Long term monitoring effort

Long lived and slow maturing species need long term conservation efforts to be able to assess the population, understand the dynamics and implement management strategies if required. It is not sufficient to assume that what holds true for one population, necessarily holds true for another. For instance, phenology is a well-known adaptation to increasing temperatures, and seen in various sea turtle populations across different regions, with however varying degrees of response. As a response to increasing sea surface temperature, green and loggerhead turtles (*Caretta caretta*), in Florida, nested earlier (Pike, 2009; Pike et al., 2006; Weishampel et al., 2010, 2004). However, (Pike, 2009) didn't detect any shift for Florida nesting green turtles. In the Indian Ocean, green turtles have a delayed onset of nesting (Dalleau et al., 2012). Loggerheads in North Carolina also showed signs of earlier nesting, with longer nesting seasons (Hawkes et al., 2007); but the nesting season for this species seems to be getting shorter in Florida (Pike et al., 2006). Leatherback turtles (*Dermochelys coriacea*) nesting on the Pacific coast of Costa Rica have shown no sign of shift in phenology in response to changing temperatures (Neeman et al., 2015; Robinson et al., 2014), however those in the Atlantic seem to be nesting earlier (Robinson et al., 2014). On Ascension, no changes in nesting seasonality have yet to occur. Therefore, if we are to understand how species behave, we must test the hypothesis on multiple populations. Phylogenetic analyses of loggerhead turtles from the east coast of the United States suggest a wide genetic diversity and possible subdivision into distinct genetic groups (Shamblin et al., 2012). Similarly, genetic differences have been attributed to perceived variation in flatback turtles (*Natator depressus*) response to increasing temperatures (Howard et al., 2015; Stubbs et al., 2014). Thus the perceived link between latitudinal gradient and response to increasing SST (Mazaris et al., 2013), may also be underlined by genetic factors. Whilst the disparity between Pacific and Atlantic leatherbacks has been attributed to population size and structure (Robinson et al., 2014), with the Pacific leatherback population crashing (Spotila et al., 2000) and those in the Atlantic region increasing (Stewart et al., 2014). Therefore, the perceived lack of adaptation found on Ascension may be due to undetected genetic factors combined with population growth and not all turtles being philopatric. In view of this, it is crucial to keep up monitoring efforts and to integrate different techniques to get a better understanding of the mechanisms that may help in the conservation of species in the face of climate change.

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