

# Ecology of Marine Turtles under Climate Change

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

A handwritten signature in black ink, appearing to read 'Kimberley L. Stokes', is written over a horizontal dotted line.

Kimberley L. Stokes



### Abstract

Climate change threatens to disrupt biological systems around the globe, sparking debate over natural capacity for adaptation in a fragmented landscape. Marine turtles are evolutionarily ancient and have survived millions of years of prehistoric climate change, but are threatened by the rapidity of modern warming and a history of severe overexploitation that has left most populations depleted. This thesis explores a nesting aggregation of the green turtle (*Chelonia mydas*) in northern Cyprus, where a longitudinal programme of both intensive and extensive monitoring enables insight into individual and population level parameters and processes. Nesting on the two coastlines covered by this project is in the early stages of recovery, possibly in response to exhaustive nest protection efforts over the last twenty years. Saturation tagging at one key site allows us to confirm that recruitment of new breeders is an important driver of this trend, and that average clutch frequency has remained stable around three nests per female per year, validating nest-count derived abundance estimates at a regional scale. Concern has been raised, however, regarding recent changes in fishing practices which are impacting the local juvenile neritic phase, which may have a lagged effect on the recovery of this nesting population. A collaborative tracking effort including all other countries with major nesting in the Mediterranean allows us to identify major foraging grounds for this species, with two hotspots accounting for >50% of tracked individuals, as well as coastal and pelagic seasonal corridors of high use. Bycatch levels and mortality rates for turtles in these key areas are largely unknown and should be prioritised for investigation. Hatchling sex ratios from the main study beach are extremely female-biased (estimated 97% female for the twenty year period 1993-2012). A 1°C rise in average incubation temperatures threatens near complete hatchling feminisation on this beach, whilst a 2°C rise could reduce hatch success to less than 50%. Thermal effects on hatchling morphometrics are evident, with a 1°C rise in temperature reducing average length, width and weight by 1%, 2% and 3% respectively. More favourable incubation conditions were found early in the season, in deeper nests laid by larger females, and on beaches of lighter sand. In contrast, adult sex ratios at the main site are male-biased, posing questions regarding sex-specific survival rates and optimal hatchling sex ratios. A phenological shift towards earlier nesting is demonstrated for the first time in this species, and could potentially ameliorate warming effects. Carry-over climate forcing effects from the foraging ground influence the breeding frequency of individuals, driving population level responses in annual magnitude of nesting. This work emphasises the utility and necessity of long-term individual-based monitoring programmes in elucidating population trends and climate responses in iteroparous species with non-annual breeding.

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**Author's declaration and statement of co-authored papers**

All data analysis and writing in this thesis were completed by KLS under the guidance and supervision of BJG and ACB, with additional pre-submission comments from co-authors for Chapters I, II and III. Fieldwork in northern Cyprus was carried out by volunteers as part of the ongoing Marine Turtle Conservation Project set up in 1992 and managed by ACB and BJG, and was coordinated in 2010 and 2011 by KLS. Previous and subsequent coordinators include ACB, BJG, WJF, FG, KAR & RTS. AFC, OC, YL, AFR, GR & DT provided collaborative satellite tracking data from Turkey, Syria and Israel. All labwork was completed by KLS; CRT and GMK provided the use of laboratory space and equipment. Remote sensing data were provided by MJW, and DJH and IS provided guidance on aspects of scripting in Chapters I and II. Guidance on all aspects of work throughout was provided by BJG and ACB.

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### Introduction

Atmospheric concentrations of carbon dioxide, methane and nitrous oxide have increased by 40%, 150% and 20% since pre-industrial times, causing global mean land and sea surface warming of around 0.12°C per decade since 1951 (Stocker et al., 2013). Biological responses to recent climate change have been documented around the globe (Parmesan & Yohe, 2003), and projected further warming (0.3 to 4.8°C by the end of the century, Stocker et al., 2013) is expected to have serious consequences for the world's biodiversity (e.g. Thomas et al., 2004; Harley et al., 2006).

Global climate change is not new; extant species have persisted through and are adapted to coping with orbitally-paced changes in climate (Webb & Bartlein, 1992), but the synergistic effects of a drastically elevated rate of warming (Root & Schneider, 2006) occurring against a backdrop of other well established and ongoing anthropogenic impacts leave many plant and animal species with much reduced adaptive capacity. At particular risk are those species for which populations exist at heavily depleted levels, either through overexploitation or other sources of artificially-elevated mortality. Climate change impacts will necessitate the dramatic reappraisal of current conservation strategy, urgently commanding a deeper understanding of climatic threats and species responses.

Marine turtles are considered to be climate change indicator species (Hawkes et al., 2009; Newson et al., 2009). Migratory ectotherms with a complex life-history, they are dependent on widely separated marine and terrestrial habitats and are threatened by a multitude of climate effects (reviewed in Hawkes et al., 2009; Poloczanska et al., 2009). Life-history characteristics that include a capital breeding strategy with particularly high natural levels of adult stock, long generation times and aggregative migrations that are temporally and spatially predictable make this group both easily targeted and highly susceptible to fisheries mortality (Crouse, 1999; Musick, 1999). Most populations are severely depleted when compared to pre-exploitation levels (e.g. McClenachan et al., 2006; Seminoff & Shanker, 2008), although some have shown encouraging rebound capacity in response to protective legislation at sea and basic conservation measures at the nesting beach (e.g. Dutton et al., 2005; Chaloupka et al., 2008). Some species are regarded as habitat shapers (e.g. the green turtle *Chelonia mydas*: Jackson et al., 2001; hawksbill turtle *Eretmochelys imbricata*: León & Bjorndal, 2002), as their loss has cascading effects on the structure and function of ecological communities.

Sea turtles are evolutionarily ancient: the basic turtle body shape evolved over 200 Mya (Li et al., 2008) and the Chelonioidea are thought to have diverged from land and freshwater turtles over 100 Mya (Wang et al., 2013). Their highly mobile nature and circumglobal distribution equip them with a natural propensity for adaptation to changing climatic conditions: prehistoric range contractions and expansions track periods of past changes in climate and sea level (Bowen & Karl, 2007). As is also true for biodiversity more generally, the real threat arises from anthropogenic barriers to adaptation, which in this case include conversion of the coastline causing loss of historic, current and potential future nesting beach habitat, depressed population sizes in combination with ongoing fisheries/ bycatch/ poaching mortality, and the rapidity of modern climate change. Adaptive conservation strategy should ideally capitalise on sea turtles' natural capacity for adaptation rather than create ongoing reliance on intervention, although short-term solutions are often necessary to prevent population collapse whilst underlying causes are addressed.

To ensure the safeguarding of species of conservation concern into the future, conservation science must be informed by detailed understanding of population status, distribution and current threats, climatic threats and responses/ potential for adaptation. This thesis explores each of these in turn with regard to the Mediterranean green turtle, a model population with a history of severe overexploitation (Sella, 1995) threatened further and in the immediate future by increasing nesting beach temperatures (see Broderick et al., 2000).

**Chapter I** discusses sea turtle population monitoring techniques commonly employed around the world and the particular difficulties in monitoring population trends of this species. Drawing on a longitudinal study at a major nesting beach spanning twenty years, I describe the early stages of a recovery phase for this nesting rookery, and examine individual fecundity levels, breeding periodicity, and their implications for design of conservation monitoring schemes. This chapter has been published in *Animal Conservation* (2014, 17: 593-602), as included in Appendix I.

**Chapter II** combines data for all tracked females of this species in the Mediterranean to present an analysis of nesting, migratory and foraging habitat use, highlighting potential overlap with fisheries and areas where there are important gaps in knowledge of bycatch levels. This chapter is has been published in *Diversity and Distributions* (2015, 21: 665-674), as included in Appendix II.

**Chapter III** explores a single case of unusual tracking opportunity, and the potential implications for resilience to female sex ratio skew. This chapter formed part of a publication on green turtle mating patterns in *Proceedings of the Royal Society: B* (2012, 279: 2122-2127), included in Appendix III.

**Chapter IV** investigates the sex ratio of hatchlings produced at the main study beach and on the surrounding coast. The incubation temperature-sex ratio relationship (and incubation duration equivalent) is defined based on a subsample of natural mortality hatchlings over two breeding seasons, from which estimations are made covering two decades of monitored nests plus projected future climate increases. Temperature effects on fitness and hatch success are also examined.

**Chapter V** identifies climate forcing effects on the seasonality, periodicity and year-to-year magnitude of nesting for this rookery. Satellite-derived temperature data from foraging grounds identified in Chapter II are modelled against fecundity at the level of the individual and the nesting population. A phenological response to increasing pre-migration temperatures at the foraging ground signals a potential avenue of adaptation to climate change previously undetected in this species.

### The study system

Marine turtles are late-maturing, long-lived, and migrate between marine foraging grounds and terrestrial nesting beaches every few years throughout adulthood. The mating system is characterised by natal philopatry (Meylan et al., 1990), skipped breeding behaviour (Prince & Chaloupka, 2012), scramble polygamy (Jessop et al., 1999) and multiple paternity (Bowen & Karl, 2007). The majority of mating is thought to occur near nesting beaches (reviewed in Bell et al., 2009), and each female deposits multiple clutches of eggs within a single reproductive season (Miller, 1997). Hatchlings emerge after an incubation period of around two months (varying with species), crawl down to the sea during a frenzy period of elevated energy expenditure (Carr, 1962; Wyneken & Salmon 1992) and swim out to open water, where they live pelagically for an undefined number of years before returning to coastal waters as sub-adults to complete their development. Hatchling sex is determined by incubation temperature within the nest (temperature-dependent sex determination, TSD; Bull, 1980), and neonates are predated heavily on the beach and in the water, while adults have few natural predators. Once sexual maturity is reached, individual reproductive output is high, and increases with age

and size (Bjorndal & Carr, 1989). This life-history strategy leaves the group especially vulnerable to elevated adult mortality through over-harvesting and incidental bycatch (Lewison et al., 2004). Long generation times also leave them susceptible to modern climate change, as adaptive selection is unlikely to keep pace with the predicted rate of warming.

The seven species of sea turtle exhibit a diversity of foraging strategies. Leatherback and olive ridley turtles are oceanic feeders, spending a majority of their time meandering along the frontal systems that provide an abundance of pelagic prey. Neritic feeders (green, hawksbill and loggerhead turtles) in contrast usually return to specific foraging areas. Green turtles in particular exhibit high levels of fidelity to nesting beaches, foraging grounds and migratory routes (Limpus et al., 1992; Broderick et al., 2007), resulting in a shuttling migratory pattern between a predefined foraging ground and the natal nesting beach. Adult green turtles are herbivorous, feeding on sea grass or algae, depending on the specialisation of the microflora of the hindgut where fermentation takes place (Bjorndal, 1985).

Historically, green turtles have been heavily targeted for consumption of their meat and eggs, and many populations were reduced to a fraction of their former size or extirpated (Seminoff, 2004). Population trends show a strong rebound capacity where threats are ameliorated (Chaloupka et al., 2008), and perilous declines elsewhere (see Table 1 for a summary of global sea turtle population trends). Current threats in the Mediterranean include incidental bycatch, habitat loss and pollution; all exacerbate the looming additional and synergistic threat of climate change.

The green turtle makes an interesting model species for understanding the impacts of climate change on wild populations as it is thermally sensitive (being ectothermic with temperature-dependent sex determination) but slow-selecting (late maturing and long-lived), and its reliance on geographically disparate marine and terrestrial habitats renders it susceptible to climatic decoupling. It is a well studied species with circumglobal distribution, enabling comparisons across populations experiencing varying climate regimes. Its low trophic status enables the link between oceanic productivity and reproductive output to be examined with relative ease.

This thesis draws on data collected at the Marine Turtle Conservation Project (MTCP), which implements a long-term monitoring programme covering all nesting and hatching activity of green and loggerhead turtles on the north and west coasts of northern Cyprus. Day-time monitoring involves analysis of tracks left during nocturnal nesting activities, to locate new

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nests and record abandoned nesting attempts. Night-time monitoring requires intensive survey effort in order to observe each female as she nests, and is carried out at the main study site, Alagadi Beach. All hatched nests are excavated in order to quantify reproductive output. For further details see the Methods section that follows.

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**Table 1.** Sea turtle population trends by ocean basin. Statistics taken from the International Union for Conservation of Nature (IUCN) Red List of Threatened Species assessments (green turtle: Seminoff, 2004; hawksbill: Mortimer & Donnelly, 2008; leatherback: Wallace et al., 2013; olive ridley: Abreu-Grobois & Plotkin, 2008).

Region	Population trajectory	% change over three generations
<b>Green turtle <i>Chelonia mydas</i></b>		
Eastern Pacific Ocean	---	-89% to -94%
Central Pacific Ocean	++	+52%
Western Pacific Ocean	++	+56%
Southeast Asian Seas	---	-85% to -93%
Eastern Indian Ocean	---	-92% to -93%
Northern Indian Ocean	-	-23% to -31%
Western Indian Ocean	-	-32%
Mediterranean Sea	---	-93%
Eastern Atlantic Ocean	-	-25% to -26%
Central Atlantic Ocean	++	+39%
Western Atlantic Ocean and Caribbean Sea	++	+13% to +66%
<b>Hawksbill turtle <i>Eretmochelys imbricata</i></b>		
Pacific Ocean	---	-76%
Indian Ocean	---	-94%
Atlantic Ocean	---	-76%
<b>Leatherback turtle <i>Dermochelys coriacea</i></b>		
East Pacific Ocean	---	-97%
West Pacific Ocean	---	-83%
Northeast Indian Ocean	DD	
Southwest Indian Ocean	-	-6%
Southeast Atlantic Ocean	DD	
Southwest Atlantic Ocean	++++	+232%
Northwest Atlantic Ocean	+	+21%
<b>Olive Ridley turtle <i>Lepidochelys olivacea</i></b>		
East Pacific Ocean:		
Arribada rookeries	--	-32% to -55%
Non-arribada rookeries	---	-73% to -95%
Central and West Pacific Ocean	---	-92%
Eastern Indian Ocean:		
Arribada rookeries	stable	0%
Non-arribada rookeries	--	-58%
Western Indian Ocean	---	-60% to -98%
Western Atlantic Ocean:		
Arribada rookeries	---	-97% to -99%
Non arribada rookeries	++++	+364%
<b>Loggerhead turtle <i>Caretta caretta</i></b>		needs updating
<b>Kemp's Ridley turtle <i>Lepidochelys kempii</i></b>		needs updating
<b>Flatback turtle <i>Natator depressus</i></b>		DD

**Table 1 References**

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## Methods

### 1. Beach monitoring

Each summer, teams of volunteers from the Marine Turtle Conservation Project survey every nesting beach on the north and west coasts of northern Cyprus (excluding the Karpaz Peninsula, see Fig. 1, Chapter I for map) every 1-3 days for the duration of the breeding season (May - end September/ early October), recording and protecting new nests and, later in the season, performing hatched nest excavations.

#### i) Daywork

Early morning surveys record all new turtle emergences and ascertain the nature of nesting behaviour from the tracks left on the beach. Species is identified from the track pattern (loggerhead turtles walk on land using their fore flippers alternately whereas green turtles use both fore flippers simultaneously to heave themselves forward). Track width is measured from the outside edge of each rear flipper mark (perpendicular to direction of movement for both species - for loggerheads a line is drawn in the sand between the outside edges of two flipper imprints on one side so that the width can be measured across the track). The emergence is diagnosed as a False Crawl U-turn (FCU), False Crawl Activity (FCA; digging has occurred without oviposition) or Nest, and the location is triangulated using a series of numbered posts secured at the back of the beach for the duration of the season. The exact position of a nest is confirmed by digging down to the top of the egg chamber. A nest marker with unique ID is placed inside the egg chamber, and the nest depth is recorded three times using a tape measure and a straight stick laid at beach level. The nest is then recovered to its original depth, and a large piece of wide-mesh chicken wire is secured into the sand directly over the eggs using metal U-bend pegs to protect against predation by dogs and foxes. A centre stick is used to mark the location of the egg chamber, and the nest is labelled several times with its ID and the lay-date using masking tape. A plastic dome marker labelled with information for beach users is placed over the nest in order to prevent accidental disturbance. Tracks are scrubbed over using a wide zig-zag pattern in order to avoid re-recording the following day.

Nests are checked daily from 40 (loggerhead) or 50 (green) days post lay-date for signs of hatching such as a dipped centre (may be pre-emergence) and hatchling tracks. Hatched nests are excavated the day after hatching, unless only a few hatchlings are thought to have emerged. Hatchling tracks are always scrubbed over after recording so that new hatchling tracks are clearly visible. The entire remains of the nest (including nest marker tag) are dug up,

and the number of live full term (LFT) and dead full term (DFT) hatchlings are recorded. Hatched egg fragments are pieced roughly together and counted. Unhatched eggs that have died are opened and the contents checked for an embryo. Eggs with no embryo are recorded as 'yolk unfertilised' (YUF). Eggs with an embryo are recorded according to the stage of developmental arrest: dead in shell greater than, equal to or smaller than the remaining yolk sac (DIS>YS, DIS=YS, DIS<YS). Any unhatched eggs that may still be alive are reburied and kept until hatched or declared dead. Live hatchlings are kept in cool, dark conditions until they are released just above the shoreline after dark.

Loggerhead and green turtle nests are declared as having failed after 60 and 70 days respectively if no signs of hatching have been seen. Failed nests are excavated as above (with gloves!).

### ii) Nightwork

Alagadi Beach is patrolled throughout the night at sufficient regularity to encounter every single nesting turtle. Volunteers walk the shoreline of the four bays that make up the beach looking for new tracks to signal a turtle emergence. When tracks are found, the species is identified as described above and a signal is flashed using torchlight to the midpoint between bays, which is too hard for turtle nesting and where volunteers rest between patrols. A response flash indicates the signal has been received. The tracks are followed with caution so as to remain concealed from the turtle. The turtle is approached from behind and behaviour is recorded (ascending/ descending beach, digging a body pit - front and back flippers, digging an egg chamber - rear flippers only, laying). The time is recorded each time a new behaviour is started. Once a turtle is laying, it can be approached more closely and checked for external flipper tags, and PIT (passive integrated transponder) tags using a scanner. Three measures of both Curved Carapace Length (CCL) and Curved Carapace Width (CCW) are taken using a tape measure laid flat across the carapace, the former from the dip at the back of the neck to the notch at the tail end and the latter at the widest point across the carapace. The turtle's tag numbers are checked against a list to ascertain whether biopsy sampling is required or has been completed on a previous nesting occurrence. A nest tag with unique ID is placed inside the egg chamber with the eggs, and a temperature data logger is lowered gently into the centre of the clutch. Three sticks are used to triangulate the exact position of the nest so that it can be found after the female has covered up. Once she has finished laying, tags can be given if the female is a new nester or where old tags may have been lost. A biopsy sample is taken if required using a small blade and a pair of tweezers on the soft tissue between scales

at the rear facing edge of the flipper. Behaviour is recorded until a female leaves the beach (covering egg-chamber, covering body pit, descending beach, re-enters sea).

Once the female has re-entered the sea, the eggs are located by digging down to the top of the egg chamber, re-covered, and protected using wide-mesh chicken wire. Nest depth is recorded as for daywork. Nests are labelled and the position triangulated as for daywork. All tracks are carefully and clearly scrubbed as for daywork to avoid confusion in the dark when following other turtles later in the night.

All nests that are approaching their hatch date are checked on every patrol throughout the night. Fencing is placed around due nests so that emergent hatchlings can be collected, weighed and measured before being released to the sea. After the required number has been sampled, the ring fence is removed so that hatching can continue unabated. Hatched nests are excavated the following morning as for daywork. Temperature data loggers are recovered during nest excavation.

## 2. Satellite transmitter deployment

Transmitter attachment begins after successful oviposition, whilst the female covers her nest. A wooden 'box' is constructed around the female from four sides approx. 4m in length that slot together, in order to prevent re-entry before the glue has dried whilst minimising stress and energy expenditure. Transmitters are affixed on the second central carapace scute using two-part epoxy resin, following preparation of the surface using sand paper and acetone. The resin is sculpted over the transmitter into a hydrodynamic shape, and smoothed in order to minimise biofouling. Care is taken not to cover the two part salt water switch. The unit is switched on using a magnet before the turtle is allowed to make the descent to the sea. Fixes are obtained by the Argos satellite tracking system, and downloaded via the Satellite Tracking and Analysis Tool (STAT; Coyne & Godley 2005). For details of Platform Terminal Transmitter (PTT) models used, see Table S2, Chapter II.

## 3. Sexing of hatchlings

Full term hatchlings found dead on nest excavation at Alagadi Beach during the 2010 and 2011 hatching seasons were dissected, their gonads removed and preserved in formalin for 2-4 h

before transfer to ethanol for storage. Following the end of the breeding season, gonads were embedded in paraffin wax and sectioned transversely using a microtome at 4-10  $\mu\text{m}$ . Sections were mounted onto slides and stained using haematoxylin-eosin dye. Sex was recorded after inspection under a light microscope, the presence of a thickened cortex and lack of medullary structural organisation being diagnostic for females, and seminiferous tubules diagnostic for males. Illustrations from Yntema & Mrosovsky (1980) and Ceriani & Wyneken (2008) were used for guidance.

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## Chapter I

### **Detecting green shoots of recovery: the importance of long-term individual-based monitoring of marine turtles**

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## Abstract

Population monitoring is an essential part of evaluating the effectiveness of management interventions for conservation. Coastal breeding aggregations of marine vertebrate species that come ashore to pup or nest provide an opportunistic window of observation into otherwise widely dispersed populations. Green turtle (*Chelonia mydas*) nesting on the north and west coasts of northern Cyprus has been monitored consistently and exhaustively since 1993, with an intensive saturation tagging programme running at one key site for the same duration. This historically depleted nesting population is showing signs of recovery, possibly in response to nest protection approaching two decades, with increasing nest numbers and rising levels of recruitment. Strong correlation between year to year magnitude of nesting and the proportion of new breeders in the nesting cohort implies that recruitment of new individuals to the breeding population is an important driver of this recovery trend. Recent changes in fishing activities may be impacting the local juvenile neritic stage, however, which may hinder this potential recovery. Individuals returning to breed after two years laid fewer clutches than those returning after three or four years, demonstrating a trade-off between remigration interval and breeding output. Average clutch frequencies have remained stable around a median of three clutches a year per female despite the demographic shift towards new nesters, which typically lay fewer clutches in their first season. We show that where local fecundity has been adequately assessed, the use of average clutch frequencies can be a reliable method for deriving nester abundance from nest counts. Index sites where individual based monitoring is possible will be important in monitoring long-term climate driven changes in reproductive rates.

## Introduction

Population monitoring is integral to conservation biology (Goldsmith 1991), and forms an essential part of evaluating the effectiveness of active conservation management (Nichols & Williams 2006). Present-day conservation monitoring must not only endeavour to detect changes in population status, but also climate change driven alterations to reproductive rates, developmental biology (Milligan *et al.* 2009) and spatio-temporal displacements (Parmesan & Yohe 2003). For many marine, nocturnal or otherwise cryptic species, detection poses additional challenges, and direct monitoring may be difficult, impractical or impossible. Various indirect survey methods are used as indices of abundance, such as redd (nest) counts for salmonids (eg. Rieman & Myers 1997), egg-mass counts for pond breeding amphibians (eg. Raithel *et al.* 2011), acoustic monitoring for loquacious species (eg. anurans, Crouch & Paton 2002; whales, Simard *et al.* 2010), and camera trapping, live trapping, hair detection and road casualty data for terrestrial mammals (eg. George *et al.* 2011, Swan *et al.* 2013). For marine vertebrates, breeding aggregations are often monitored as an index of overall population status (eg. whales, Andriolo *et al.* 2010, Fretwell *et al.* 2014), and species that come ashore to pup or nest present a logistical opportunity to count individuals with greater accuracy and much reduced cost (e.g. sea lions, Pitcher *et al.* 2007).

The vast majority of marine turtle monitoring research is based at nesting beaches. The accessibility of females during this narrow window has made nester abundance a common response variable for sea turtle population trend monitoring (Heppell *et al.* 2003). Population assessments based solely on abundance of nesting females have drawn criticism (Bjorndal *et al.* 2010) and should ideally be combined with in-water foraging ground surveys, which are expensive and labour-intensive (Seminoff *et al.* 2003). Large discrepancies exist in levels of available funding, however, and nesting beach studies are often the only feasible approach to implement monitoring over long time frames at low expense (Meylan 1995; Gerrodette & Taylor 1999).

Studies of sea turtle reproductive ecology rely heavily on the practice of tagging individuals to elucidate breeding frequency and fidelity to nesting areas (Balazs 1999). In the past, tag loss has been a major confounding variable, with reports of 78% documented tag loss and upper retention estimates of just six years in early studies (Mortimer & Carr 1987). Tag retention rates have since been enhanced with improved tag design and the introduction of PIT (Passive Integrated Transponder) tags. These developments have increased the accuracy of neophyte/

remigrant classification, reducing uncertainty in the quantification of neophyte turtles and overall nester abundance (McDonald & Dutton 1996).

Most marine turtle populations display obligate skipped breeding behaviour due to the high energy demands of migration and reproduction (Prince & Chaloupka 2012), females laying a variable number of clutches within a breeding season (termed clutch frequency) every few years (the remigration interval). Individuals must attain a threshold body condition before embarking on a breeding migration, and so their remigration interval varies in response to fluctuations in environmental conditions (Solow *et al.* 2002). The low trophic status of the green turtle (*Chelonia mydas*) makes it particularly susceptible to environmental stochasticity, driving large inter-annual oscillations in numbers of nesting females (Limpus & Nicholls 1988; Broderick *et al.* 2001). The intrinsic variability characteristic of green turtle nesting makes longevity in monitoring programmes essential for identifying underlying population trends (Broderick *et al.* 2003; Heppell *et al.* 2003; Jackson *et al.* 2008). Individual plasticity and inter-population variation in clutch frequency add further uncertainty when deriving nesting population estimates from nest abundance counts (Van Buskirk & Crowder 1994; Rivalan *et al.* 2006). Many studies divide nest counts by an average value of clutch frequency to give estimated annual nester abundance or vice versa (e.g. Seminoff 2004; Troeng & Rankin 2005; Beggs *et al.* 2007). A simplistic model of stochastic nesting behaviour, applied to a loggerhead turtle nest count series with known nester abundance, indicated that this method has the potential to produce biased estimates of population trends (Mazaris *et al.* 2008). Thus more studies of individually marked populations are needed.

Green turtles in the Mediterranean have a history of severe exploitation (Sella 1995). Contemporary rookeries of modest size remain at a handful of sites in Turkey, Cyprus, Syria and Israel (Kasperek *et al.* 2001; Broderick *et al.* 2002; Canbolat 2004; Yalcin-Ozdilek 2007; Rees *et al.* 2008), with ca. 30% of Mediterranean nesting in Cyprus. Modern threats in the Mediterranean include fisheries bycatch and mass tourism (Casale & Margaritoulis 2010); this population has been highlighted as a conservation priority owing to its 'High Risk-High Threat' status (Wallace *et al.* 2011).

Since 1993, an extensive monitoring programme has conducted comprehensive surveys of the nesting beaches of the north and west coasts of northern Cyprus, located in the Eastern Mediterranean (for beach locations see Fig. 1). Intensive survey effort has been concentrated at Alagadi, where continual night patrols of this 2 km stretch of beach for the duration of the breeding season have allowed exhaustive tagging. Here, we examine the apparent recovery of

the population, and reveal the range of insights that long-term individual based monitoring can provide.

## Methods

The study system is a Mediterranean nesting rookery of female green turtles and their hatchlings, for which longitudinal data are available. Daytime monitoring of marine turtle nesting activity was conducted every 1-3 days on beaches with significant nesting on the north and west coasts of northern Cyprus for the duration of the breeding season (end of May to end of September) each year between 1993 and 2013 (less complete monitoring was undertaken in 1992; see Fig. 1 for beach locations). Daytime monitoring involves thorough examination of all nesting activity during the early morning, location of eggs if present, and protection from depredation by stray dogs and foxes using a wide mesh wire screen secured into the sand above the nest (carried out exhaustively since 1994). An intensive night monitoring and tagging programme has been conducted at Alagadi (comprising two coves 1.2 and 0.8 km in length) over the same time period (see Broderick *et al.* 2002, 2003 for detailed methods). Patrols are undertaken at sufficient frequency to encounter all females nesting at this beach. Internal PIT tags have been administered in addition to external flipper tags to all turtles nesting at this breeding site since 1997. Neophyte/ remigrant analyses were conducted on a subset of the data from 2000 onwards due to increased accuracy of neophyte classification three years (one full nesting cycle for most females) following the introduction of PIT tagging.

Long intervals between observed nesting events within a nesting season are indicative that a female has laid elsewhere on a nearby beach. Thus, the number of clutches laid per season at Alagadi by each marked individual (observed clutch frequency, OCF) is adjusted where turtles have interesting intervals of 20 days and over to give the expected clutch frequency (ECF; Frazer & Richardson 1985, see also Broderick *et al.* 2002 for bimodal distribution of interesting interval data). The remigration interval (RI) for remigrant turtles is calculated as the number of years since that individual was last recorded nesting at Alagadi.

Statistical tests and modelling were carried out using R version 2.14.2 (R Development Core Team 2012), and packages “nlme” (Pinheiro *et al.* 2012) and “lme4” (Bates *et al.* 2011). Tests of correlation were performed using Spearman’s rank order correlation coefficient. LOESS (locally weighted) regressions were fitted to RI and ECF time series data with degree one (linear) and a span of 0.75. Time series analyses of yearly nest counts were conducted using generalized least squares (GLS) modelling to account for temporal autocorrelation in the data.

Clutch frequencies were regressed against explanatory variables using generalized linear mixed modelling (GLMM), fitted using the Laplace approximation, restricted maximum likelihood estimates (REML) and stepwise model simplification. GLMMs allow statistical analysis of non-normal data with random effects, which quantify the variation across units/ grouping factors of the fixed effect parameters (Bolker *et al.* 2009). In this case, models had Poisson error structure and logarithmic link function, with zero-truncation. Explanatory variables included categorical fixed effects for neophytes (first time nesters; true or false) and remigration interval (two vs. three or four years), a fixed covariate of body size, and random effects for individual (to avoid pseudoreplication where females have returned to nest in subsequent years) and year (to account for interannual variation in magnitude of nesting arising from environmental stochasticity). GLMM was also used to regress body size against neophyte/remigrant nesters whilst accounting for pseudoreplication of individuals. The significance of removing model terms was assessed by likelihood ratio tests using maximum likelihood estimates (Crawley 2007), in order of least significance and with a threshold of  $p=0.05$ . Model residuals were checked for overdispersion, normality and homoscedasticity.

## Results

The annual green turtle nesting abundance for Alagadi and the total across the north and west coasts is shown in Figure 2. The high interannual variation typical of green turtle nesting is evident (combined nesting range: 35-335 nests per season, mean  $\pm$  standard deviation (SD):  $130 \pm 77.1$ ), following a two to three year pseudo-cyclical pattern. The coefficient of variation (CV=SD/mean: 0.59) lies within the range previously reported for this species by Broderick *et al.* (2001; 0.41-1.08). Nesting abundance on the two coasts is significantly correlated ( $r_{(19)}=0.72$ ,  $p<0.001$ ) showing a synchrony in reproductive cycles across this area. Comparison of nest count models at Alagadi and across the two coasts demonstrated significant autocorrelation at a time lag of one year (GLS, Alagadi:  $\phi=-0.729$ ,  $\chi^2_{(1)}=11.274$ ,  $p<0.001$ ; overall:  $\phi=-0.449$ ,  $\chi^2_{(1)}=4.224$ ,  $p=0.04$ ). Having accounted for this autocorrelation, nest counts showed a significant quadratic trajectory through time (Fig. 2; Alagadi linear slope:  $\beta=-10.663 \pm 2.790$ ; Alagadi quadratic slope:  $\beta=0.709 \pm 0.135$ ,  $\chi^2_{(1)}=17.471$ ,  $p<0.0001$ ; overall linear slope:  $\beta=-20.878 \pm 5.405$ ; overall quadratic slope:  $\beta=1.259 \pm 4.817$ ,  $\chi^2_{(1)}=14.379$ ,  $p=0.0001$ ). This indicates that nesting in the region has stabilised and may now be increasing. This trend was also significant for nester abundance at Alagadi (Fig. 3a; autocorrelation at one year:  $\phi=-0.743$ ,  $\chi^2_{(1)}=10.711$ ,  $p=0.001$ ; linear slope:  $\beta=-3.151 \pm 0.929$ ; quadratic slope:  $\beta=0.231 \pm 0.045$ ,  $\chi^2_{(1)}=17.079$ ,  $p<0.0001$ ). Recruitment (as measured by the proportion of nesters that are neophytes) has followed a similar quadratic trend (linear slope:  $\beta=-0.103 \pm 0.016$ ; quadratic slope:  $\beta=0.005 \pm 0.001$ ,  $\chi^2_{(1)}=22.005$ ,  $p<0.0001$ ), but with no significant autocorrelation. Record numbers of nests, nesters and neophytes were observed at Alagadi in 2013 (236 nests, 85 nesting females, 57 neophytes). There has been no trend in survey effort, detection probability (imperfect detection of nests or individuals), or detectability (beach fidelity) over the study period (see Supplementary Figure S1 and Pfaller *et al.* 2013).

We confirmed that the recent trajectory describes a significant increase in nests, nesters and recruitment, by considering the number of nests and nesters post-2000, which corresponds with the local minimum of all our quadratic fitted lines. Since 2001, there has been a significant increase through time in the number of nests across all beaches ( $\beta=15.993 \pm 3.063$ ,  $\chi^2_{(1)}=11.938$ ,  $p=0.0006$ ), the number of nests on Alagadi ( $\beta=9.799 \pm 1.605$ ,  $\chi^2_{(1)}=15.516$ ,  $p=0.0001$ ), the number of nesting females on Alagadi ( $\beta=3.493 \pm 0.606$ ,  $\chi^2_{(1)}=14.398$ ,  $p=0.0001$ ) and rates of recruitment ( $\beta=0.029 \pm 0.009$ ,  $\chi^2_{(1)}=8.399$ ,  $p=0.004$ ).

A comparison of observed and estimated nester abundance is shown in Figure 3a. Here, the known number of females nesting each year at Alagadi is used to test the accuracy of estimates derived using nest counts and average values of clutch frequency. Estimated nester abundance is taken as the quotient of annual nest abundance divided by an average clutch frequency of three (Seminoff 2004; also the overall mean and median clutch frequency from the current study). Estimated nester abundance and actual/ observed nester abundance were highly correlated ( $r_{(19)}=0.97$ ,  $p<0.0001$ ). Conclusions drawn from these abundance series about the population trend at this breeding aggregation would be analogous.

The tagging programme based at Alagadi has revealed a strong correlation between the number of nests and the proportion of neophytes since 2000 ( $r_{(12)}=0.94$ ,  $p<0.0001$ ; Fig. 3b). This strong correlation between the proportion of neophytes in the nesting cohort and the magnitude of nesting implies that recruitment of new individuals into the breeding population is an important driver of year to year nester abundance, an encouraging sign of a population in recovery. The reduced correlation between the number of nesters and the proportion of neophytes seen prior to 2000 provides evidence that the introduction of PIT tagging has had a significant effect on the accuracy of neophyte/ remigrant identification. First time nesters at Alagadi are significantly smaller than remigrant nesters (GLMM,  $\chi^2_{(1)}=84.95$ ,  $p<0.0001$ ; mean CCL  $87.7 \pm 6.5$  cm for neophytes cf.  $92.0 \pm 5.9$  cm for remigrants), re-affirming their classification as true neophytes. The intensity of survey effort at this site has afforded near perfect attribution of nests to known females (98% since 2000, 93% since comprehensive monitoring began in 1993).

Figure 4a shows RIs observed for the marked green turtle population at Alagadi between 1994 and 2013. The majority of remigrants return after 2, 3 or 4 years [87%; median RI: 3, interquartile (IQ) range: 3-4,  $n=212$ ]. The low incidence of unusually long RIs most likely reflects individuals with lower site fidelity, who may have nested elsewhere in Cyprus, or further afield, undetected. The majority (78%) of remigrant turtles observed over three or more seasons varied their RI from one breeding season to the next ( $n=51$ , see Fig. 4b), exemplifying the high levels of modulated periodicity green turtles show in response to environmental stochasticity. Despite this, the annual average RI has remained relatively stable over the study period (see Fig. 5a), fluctuating mostly between three and four years. Lower RIs at the beginning of the time series are an artefact of time since tagging began; only those remigrants with lower than average remigration interval can be re-encountered within the first three years of monitoring.

Median ECF across all years and nesters was three (IQ range: 1-4, n=485). No long-term trend in median clutch frequency is apparent from the data (see LOESS smoother Fig. 5b). Instead, median ECF is correlated with the number of nesters present in a given season ( $r_{(19)}=0.52$ ,  $p=0.02$ ), with three of the four lowest nesting seasons having a low average ECF, indicating that females breeding in poor nesting years may be in suboptimal body condition. Median clutch frequency is more variable in the early part of the time series, stabilising as the number of females increases, effectively increasing the sample size and reducing susceptibility to skewed averages. ECF varies between neophytes and remigrants (see Fig. 6a-b), with neophytes most likely to lay a single clutch (40%, n=194), and the majority of remigrants laying three to five clutches (77%, n=212). GLMM showed the effect to be significant, with remigrants laying an average of 0.6 clutches more than neophytes, whilst accounting for individual and year to year variation ( $\chi^2_{(1)}=37.198$ ,  $p<0.0001$ ). Female body size had a statistically significant but biologically insignificant effect on clutch frequency ( $\chi^2_{(1)}=7.689$ ,  $p<0.01$ ), with a 10 cm increase in curved carapace length (CCL) increasing ECF by an average of 0.04.

RI was found to have a significant effect on clutch frequency, with short RIs of less than three years reducing ECF by almost a quarter (0.23), once variation across individuals and years was accounted for (GLMM,  $\chi^2_{(1)}=4.009$ ,  $p<0.01$ ). Body size did not have significant effect to be included in the model. Female nesters returning after a short interval of two years are most likely to lay three clutches (40%, n=48, Fig. 6c), whilst those returning after three or four years are more likely to lay four or five clutches (57%, n=136, Fig. 6d-e).

A total of 273 nesting females have been tagged at Alagadi since 1992. Forty percent of neophytes nesting between 2000 and 2008 (n=55) did not remigrate to this site in subsequent breeding seasons (we do not include 2009-2013 as these neophytes may yet return).

## Discussion

Evaluation of indirect survey method reliability is essential for accurate population monitoring. Validated indices are the primary tool for tracking changes in abundance of many cryptic species of conservation concern (eg. carnivore track counts and camera trap surveys, Balme *et al.* 2009). Long-term individual based monitoring of green turtles at Alagadi, northern Cyprus has provided fundamental and applied insights into sea turtle nesting ecology. Our data suggest that estimation of nesting population size from nest abundance data is reliable, provided that fecundity is adequately monitored at relevant localised index sites to provide the 'proportionality' information required to interpret these data (Gerrodette & Taylor 1999). Green turtles nesting at Ascension Island in the South Atlantic are larger in size, migrate further (~2300 km, Luschi *et al.* 1998) and have a longer period of suitable nesting conditions than those nesting in the Mediterranean, and thus perhaps unsurprisingly have a higher average clutch frequency of around 6 nests per season (Weber *et al.* 2013) compared to the average of 3 detected in the current study. Clutch frequencies derived through tagging efforts alone where complete survey is not possible or site fidelity is low will be underestimated, leading to inflated population assessments. Studies augmenting capture-mark-recapture methods with the use of tracking (Tucker 2010, Weber *et al.* 2013) and ultrasonography (Blanco *et al.* 2012) technologies can improve clutch frequency estimates in such cases. Breeding rates will likely be affected by long-term changes in foraging conditions, highlighting the importance of ongoing monitoring at index sites to ascertain multifaceted responses to climate change.

Saturation tagging at Alagadi has revealed clutch frequencies that are significantly different among groups (eg. neophytes vs. remigrants), but that are temporally stable across groups. Reduced clutch frequency in neophyte turtles as seen here has previously been reported in green turtles (Carr *et al.* 1978), as well as in leatherback (Tucker & Frazer 1991), loggerhead (Hawkes *et al.* 2005) and hawksbill turtles (Beggs *et al.* 2007). It is likely that this phenomenon is caused by both increasing physiological capacity with age, and changes in nesting behaviour such as site fidelity (Carr *et al.* 1978). Individual green turtles lay increasingly large clutches across (Bjorndal & Carr 1989) and within (Broderick *et al.* 2003) breeding seasons, indicating an increase in reproductive efficiency or capacity. Low subsequent remigration rates of neophytes tagged at Alagadi (this study) suggests lowered site fidelity in new breeders. Broderick *et al.* (2002) found that single-clutch neophyte females have a lower probability of remigrating to Alagadi in subsequent years than those with higher clutch frequencies (0.3 cf

0.8). Satellite telemetry of interesting loggerhead turtles in Florida has revealed a higher site fidelity in remigrants compared to new breeders (Tucker 2010). Such 'leaky' female nest site fidelity facilitates genetic mixing of the maternal lineage across nesting sites (Lee *et al.* 2007), and may promote resilience to loss of breeding sites through behavioural adaptation.

Our finding that females remigrating after three or four years lay extra clutches in comparison to those remigrating after two years supports the notion that suboptimal foraging conditions can be compensated for by building up energy reserves over a longer interval. A similar relationship between remigration interval and likely clutch frequency has been observed in leatherback turtles (Rivalan *et al.* 2005), and Van Buskirk and Crowder (1994) describe a comparable trade-off in interspecific reproductive effort resource allotment. Many iteroparous species may skip a breeding year if conditions are not favourable (eg. fat dormouse, Pilastro *et al.* 2003); this may partly be compensated for if a higher reproductive output can be attained in the following breeding season (eg. four-toed salamander, Harris & Ludwig 2004). The implications for population assessment are that short term fluctuations in breeding activity may be misinterpreted unless populations are monitored in the long term (Hays 2000), and that breeding frequency should be monitored at the individual level where possible in order to detect long term change in the scaling factors used for conversion of monitoring indices to population estimates.

The recent upward trend in nest numbers in northern Cyprus may signal the beginning of a recovery phase for this sub-population following the cessation of a heavy harvest and intensive screening of nests against unnaturally elevated predation levels. Recruitment can be viewed as a measure of cohort strength (Heppell *et al.* 2003), and rising numbers of neophytes as seen in this population are an early indication of population growth (Richardson *et al.* 2006). Similar nest protection schemes have had measurable success some 20 years later (Garduño-Andrade *et al.* 1999; Dutton *et al.* 2005). There is, however, considerable uncertainty surrounding the time it takes for green turtles to reach breeding age; published age at sexual maturity estimates for wild green turtles range from 27 (Frazer & Ladner 1986) to 40 (Limpus & Chaloupka 1997) years. Evidence from living tags, however, has shown that male and female green turtles released from the Cayman Island Turtle Farm (a conservation facility/ tourist attraction/ turtle meat supplier in the Caribbean) as hatchlings can breed at 19 and 17 years respectively (Bell *et al.* 2005). If this species can indeed reach sexual maturity at less than 20 years, then it is possible that sustained reduction in nest depredation across two decades has aided in the early stages of recovery of this historically depleted breeding aggregation.

Behavioural reproductive mechanisms such as natal philopatry and polyandry contribute to the resilience of sea turtles (Bell *et al.* 2009), which have shown encouraging recovery potential and rebound capacity in response to long-term protection (Garduño-Andrade *et al.* 1999; Broderick *et al.* 2006; Richardson *et al.* 2006; Marcovaldi & Chaloupka 2007). The complex life cycle of this group, and others involving multiple distinct habitats and delayed sexual maturity, makes adequate protection particularly challenging (Heppell *et al.* 2003); protective measures on the nesting beach will not be effective if threats at sea are not addressed (Dutton *et al.* 2005), and the potential for trophic uncoupling of resources and ability to shift ranges under changing climatic conditions are important considerations for such species (Møller *et al.* 2008; Robinson *et al.* 2009).

Nest count series should be used in conjunction with data regarding other life stages wherever possible (Bjorndal *et al.* 2010). Encouragingly, genetic studies at Alagadi have revealed a greater number of males than females in the breeding population (Wright *et al.* 2012a; Wright *et al.* 2012b), suggesting that the population increase observed here has occurred across demographic groups. However, a recent assessment of sea turtle bycatch in northern Cyprus (Snape *et al.* 2013) has found a high incidence of juvenile green turtle mortality. Potential increased fishing effort in the region following changes in trade regulation between northern Cyprus and southern Cyprus (Snape *pers. obs.*) may impede the recovery of this population.

Monitoring projects must be cost-effective in the long-term in order to ensure the longevity of data required to make meaningful estimations of population trends (Schroeder & Murphy 1999). Re-sampling assessments of extant data from comprehensively monitored nesting sites have found that temporal sub-sampling within the breeding season could save up to 50% of monitoring costs with little loss of statistical power (Jackson *et al.* 2008; Sims *et al.* 2008, Whiting *et al.* 2013). The efficacy of these more parsimonious sampling regimens is reliant on consistency in the temporal distribution of the nesting season, however, which has been shown to be variable in accordance with both long- (Weishampel *et al.* 2004) and short- (Hawkes *et al.* 2007) term fluctuations in sea surface temperature. Furthermore, complete sampling of the breeding season yields additional advantages in localities where remedial conservation measures such as nest protection and surveillance of illegal take are beneficial (eg. Bell *et al.* 2007).

A range of strategies is required to cover the breadth and depth necessary to detect changes in biological parameters and spatio-temporal distributions that are likely to occur in response to climate change. Index sites such as Alagadi, where long-term and consistent individual based

monitoring is possible, can offer valuable insights into survival and reproductive rates that other localities can use in converting more basic density indices into population estimates. Long-term datasets are vital in documenting change, but are often difficult to maintain with variable funding stability through time (Hays *et al.* 2005). Monitoring programmes with a core set of simple, robust and inexpensive measurements may have a greater likelihood of remaining consistent and sustainable in the long-term (Bennun 2001; Lovett *et al.* 2007).

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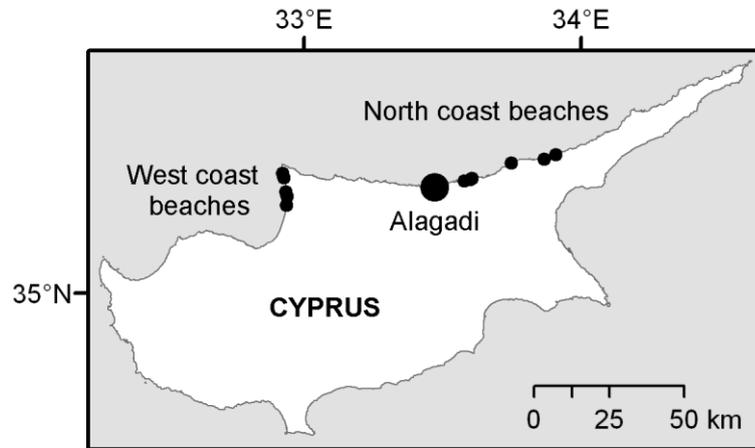
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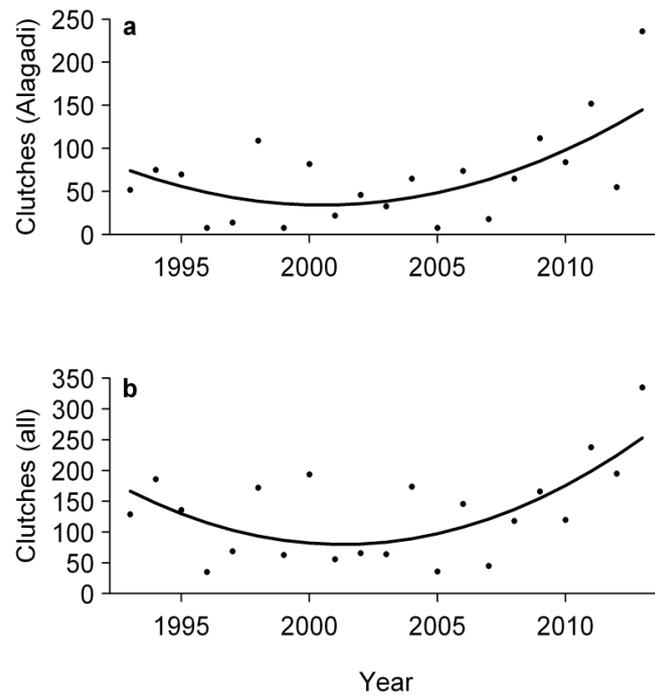
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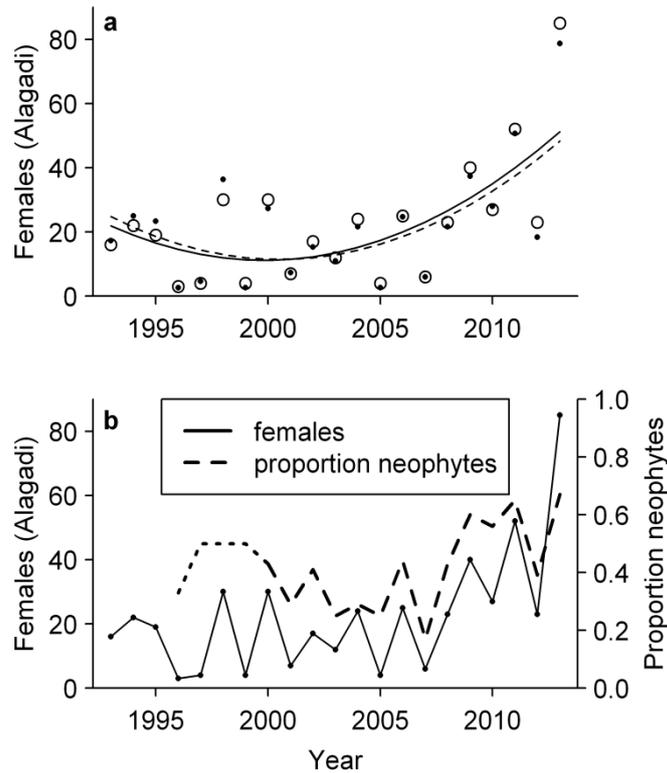
Figures



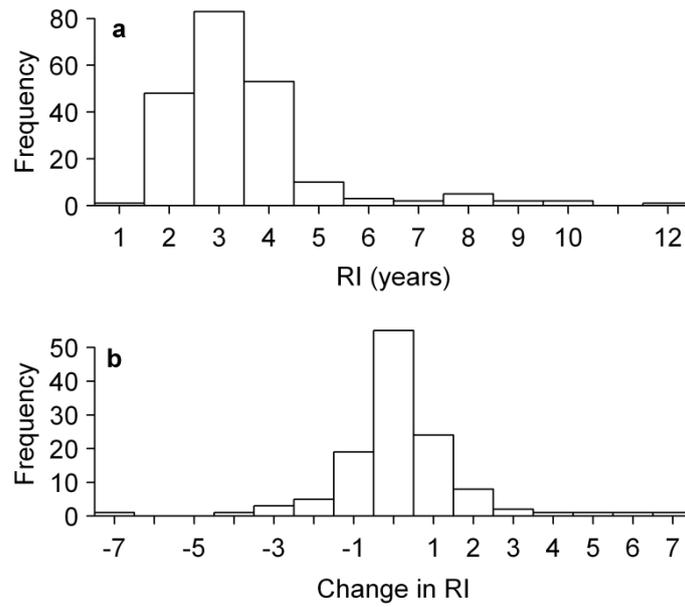
**Figure 1.** Turtle nesting beaches monitored in this study.  
Circle size is indicative of monitoring intensity.



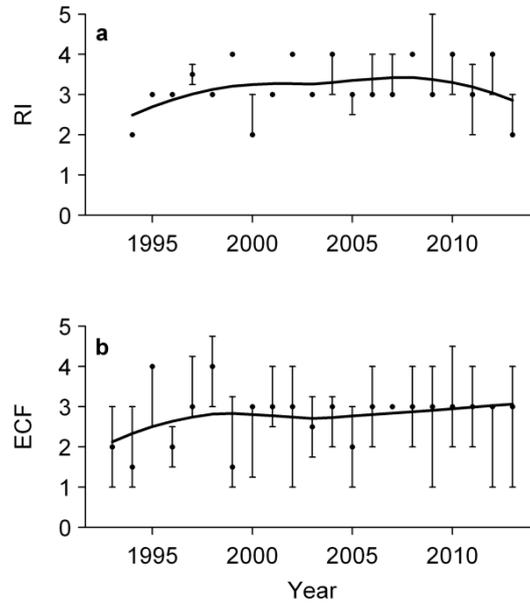
**Figure 2.** Green turtle clutches on **a)** Alagadi Beach and **b)** across all monitored sites against time, with quadratic trend lines (solid lines). These data build on the data 1993-2000 presented in Broderick *et al.* (2002).



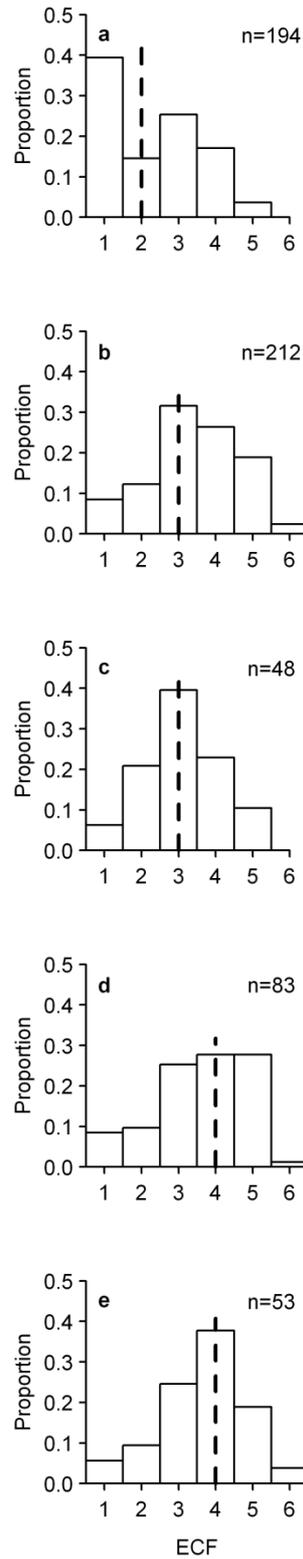
**Figure 3.** Green turtle nesting at Alagadi from 1993 to 2013. **a)** Number of females nesting at Alagadi as observed through intensive tagging effort (black dots; data for 1993-2000 previously presented in Broderick *et al.* (2002)), and as predicted by dividing annual nest counts by the grand mean expected clutch frequency for this population (3; open circles). Nesting population trends estimated using these two measures are almost identical (solid line: actual data, dashed line: predicted data). **b)** Number of nesting females (solid line), and the proportion of those that are first time nesters (dotted/ dashed line) at Alagadi over the same time period. The dotted portion indicates lower confidence in neophyte/ remigrant identification prior to 2000.



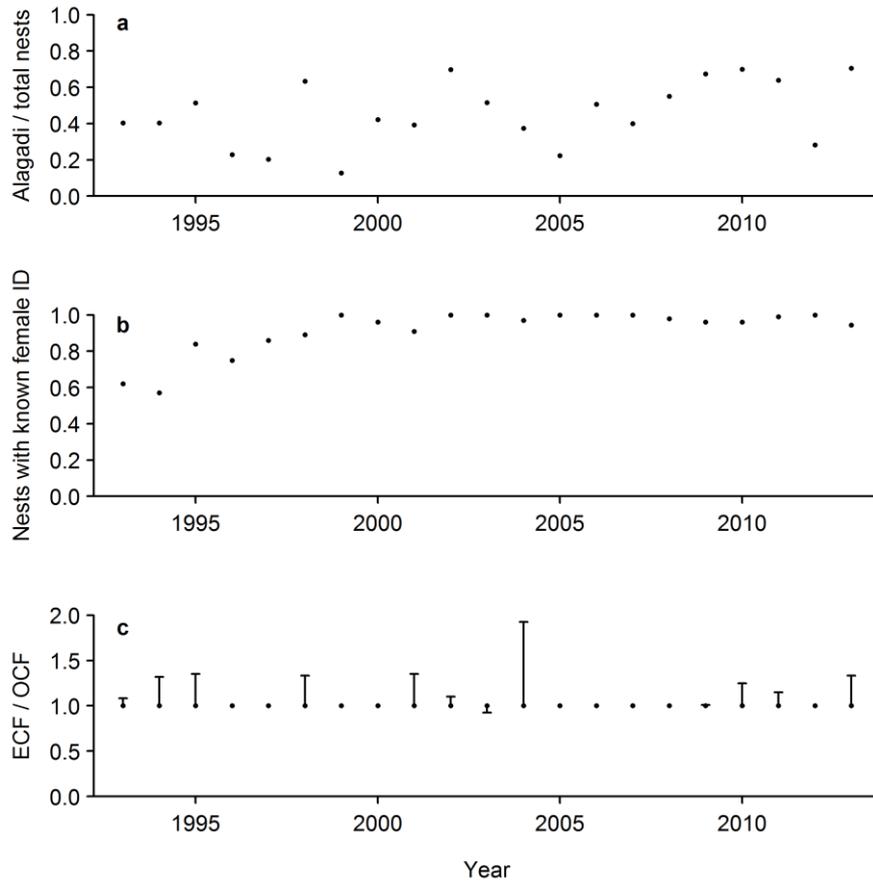
**Figure 4.** Remigration interval (RI) of green turtles returning to nest at Alagadi. **a)** Observed RIs (1994-2013). **b)** Change in RI for green turtles nesting at Alagadi during three or more seasons, taken as the increase/ decrease in RI compared to the previous RI recorded for each individual.



**Figure 5.** Breeding frequency of green turtles at Alagadi from 1993 to 2013. **a)** Yearly median and interquartile range for remigration interval (RI) and **b)** expected clutch frequency (ECF) for nesting at Alagadi, each with locally weighted regression line (LOESS smoother).



**Figure 6.** Expected clutch frequency (ECF) for **a)** neophyte (2000 - 2013), **b)** all remigrant, **c)** two year remigrant, **d)** three year remigrant and **e)** four year remigrant green turtles nesting at Alagadi (1994 to 2013). Dashed lines are median values.



**Figure S1.** Lack of trend in fidelity and detectability. **a)** Proportion of all nests recorded across the north and west coasts that are laid at Alagadi. **b)** Proportion of nests laid at Alagadi that are assigned to a particular female (through witnessing of oviposition). **c)** Yearly median ratio of OCF:ECF (observed clutch frequency : expected clutch frequency; ratios were calculated for each individual female), with 5<sup>th</sup> - 95<sup>th</sup> percentiles displayed as error bars.

## Chapter II

### **Migratory corridors and foraging hotspots: critical habitats identified for Mediterranean green turtles**

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### **Abstract**

**Aim:** Levels of sea turtle bycatch in the Mediterranean are thought to be unsustainable. We provide a comprehensive overview of adult green turtle (*Chelonia mydas*) distribution during nesting, migration and foraging phases, highlighting transitory as well as residential areas of high use in order to facilitate adequate protection for this long-lived, migratory species.

**Location:** Mediterranean Sea.

**Methods:** Thirty four females were satellite tracked from breeding grounds in the four countries with major nesting (Cyprus, Turkey, Israel and Syria) for a total of 8,521 (mean: 251) tracking days in a collaborative effort to summarise the most comprehensive set of distribution data thus far assembled for this species in the Mediterranean.

**Results:** Ten foraging grounds are identified, with two major hotspots in Libya accounting for >50% of turtles tracked to conclusive endpoints. The coastlines of Egypt and Libya contain high densities of migrating turtles following the nesting season, particularly July-September, and likely also pre-nesting (April-June). A high-use seasonal pelagic corridor running southwest from Turkey and Cyprus to Egypt is also evident, used by >50% of all tracked turtles.

**Main conclusions:** Bycatch levels and mortality rates for the key foraging areas and high density seasonal pathways identified here are largely unknown, and should be investigated as a priority. We recommend that the Gulf of Sirte in Libya be explored as a potential biodiversity hotspot and considered for proposal as a marine protected area (MPA). Green turtle fidelity to nesting beaches, foraging areas and migratory pathways renders them vulnerable to localised threats but enables targeted mitigation measures and protection.

### Introduction

The extensive movements of migratory species pose significant challenges to conservation. Aggregative behaviour and occurrence in geographically disparate habitats can expose migratory groups to diverse and often heightened threats in comparison to non-migratory species. Satellite telemetry studies have been revolutionary in facilitating the identification of widely separated critical habitats, as well as key elements of connectivity such as stopover sites (e.g. cranes, Kanai et al. 2002) and migration corridors (e.g. ungulates, Sawyer et al. 2009). Understanding such migratory connectivity is essential for the successful management of migrant species, not least in the marine realm where populations may be liable to unquantified threats from fisheries in multiple exclusive economic zones (EEZs) and in international waters. Knowledge of the spatiotemporal distribution of highly mobile species in relation to fisheries can be used to inform conservation management protocols, such as gear mitigation or time-area closures (Block et al., 2011).

Marine turtles undergo vast ontogenetic migrations between hatchling, juvenile and adult habitats, and subsequently enter into a cycle of reproductive migrations between foraging areas and suitable nesting beaches that continues throughout adulthood. Life history traits of delayed maturity and longevity leave the group particularly vulnerable when adult mortality levels are elevated (Lewison et al., 2004). Extreme levels of historical harvest have left most populations severely depleted (Seminoff & Shanker, 2008), and whilst some have shown encouraging rebound capacity (Chaloupka et al., 2008), incidental bycatch in fisheries has impeded recovery in other areas (Lewison et al., 2004). A robust understanding of marine turtle spatial ecology is crucial to the development of effective conservation strategies; satellite telemetry has been used to identify areas of high use (e.g. Shillinger et al. 2008), predict spatial distribution of marine turtle bycatch (e.g. Howell et al. 2008), and evaluate the potential effectiveness of conservation measures (e.g. Maxwell et al. 2011; Scott et al. 2012). Such tracking studies often highlight the need for coordinated, international approaches (e.g. Blumenthal et al. 2006), and in other cases have demonstrated the efficacy of unilateral protection (e.g. Moncada et al. 2012).

For species with dynamic prey landscapes such as loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) turtles, habitat modelling may be used to predict spatio-temporal probability of species occurrence (see Witt et al., 2007; Panigada et al., 2008; Zydalis et al., 2011) in order to reduce heavy crossover with fisheries (Howell et al., 2008; Hobday et

al., 2010, 2011). Fleet communication programmes have also been successfully implemented to provide real-time reporting of bycatch hotspots, reducing fleet-wide levels of bycatch (Gilman et al., 2006b; Alfaro-Shigueto et al., 2012). More static mitigation measures such as marine protected areas and seasonal fisheries closures can be particularly effective for neritic-feeding species with a predictable migratory pattern such as the green turtle (*Chelonia mydas*), with its high fidelity to nesting beaches, foraging grounds and migratory routes (Limpus et al. 1992; Broderick et al. 2007). Within the Mediterranean, the magnitude of marine turtle bycatch is considered unsustainable (Casale, 2011) and warrants urgent conservation action (Wallace et al., 2010). Two species nest in the region: loggerhead turtles in the central and eastern basins, and green turtles in the eastern (Levantine) basin only. Green turtles in the Mediterranean have suffered extreme declines in the past (Seminoff, 2004) due to heavy overharvesting during the twentieth century (Hornell, 1935; Sella, 1982), and significant rookeries remain only in Turkey, Cyprus and Syria (see Fig. 1 and Table 1; Canbolat, 2004; Rees et al., 2008; Stokes et al., 2014). Previous tracking studies have revealed green turtle foraging grounds within sheltered bays in Turkey, Egypt and Libya (Godley et al., 2002), and have demonstrated female fidelity to these areas both within and across seasons (Broderick et al., 2007). A large-scale tracking project for loggerhead turtles from Zakynthos, Greece, has revealed a more flexible foraging pattern, with cooler, more productive (Zbinden et al., 2011), foraging sites in the north of the central and eastern basins used as seasonal habitat during the summer months only, and year round foraging sites largely in the Gulf of Gabès and Ionian Sea in the central basin (Schofield et al., 2013). Here, comprehensive tracking efforts for green turtles in the Mediterranean are used to identify key foraging habitat and migratory corridors, allowing recommendations for further conservation.

### Methods

The study system is a Mediterranean nesting rookery of female green turtles and their hatchlings, for which longitudinal data are available. Thirty four post-nesting green turtles were tracked between 1998 and 2010 using Platform Terminal Transmitters (PTTs; for details see Table S1) from nesting beaches in northern Cyprus (n=22), Turkey (n=8), Israel (n=3) and Syria (n=1). Transmitters were attached using epoxy resin following the methodology of Godley et al. (2002). Four individuals were tracked during a second post-nesting migration (Broderick et al., 2007); for this analysis, only the first track showing a clear conclusive endpoint from each individual was included. Locations were obtained via the Argos satellite tracking system, and were downloaded, stored and managed using the Satellite Tracking and Analysis Tool (STAT; Coyne & Godley 2005).

Tracks were processed and mapped using R, ArcGIS, Geospatial Modelling Environment (GME), Quantum GIS (QGIS) and fTools. A Best Daily Location (BDL) filter was applied to the pre-filtered datasets (location classes 0 and Z, inferred speeds  $>5\text{km}^{-1}$  and turning angles  $<25^\circ$  excluded). Tracks were split into internesting, migrating and foraging stages using displacement plots and visual assessment (see Blumenthal et al., 2006). A post-nesting track was deemed to have conclusively reached a foraging ground if transmissions continued from the end destination for sufficient time to indicate residency (minimum, this study: 27 days). To approximate migratory density, we created a density raster of the number of tracks crossing each cell of a hexagonal grid ( $0.25^\circ$  by  $0.25^\circ$ ).

## Results

Transmissions lasted for  $251 \pm 184$  days (mean  $\pm$  SD; range: 22-714), and 29 out of the 34 turtles were tracked to a definitive foraging ground. Transmissions continued from within foraging grounds for  $227 \pm 165$  days (range: 27-650). Turtles from all four countries shared migratory routes and end destination foraging grounds (see Fig. 2).

Ten foraging destinations have been identified in Turkey, Cyprus, Lebanon, Egypt, Libya and Tunisia, ranging from 181 to 2,641 km minimum swimming distance from the breeding site (mean  $\pm$  SD:  $1283 \pm 825$ ). Two major foraging grounds in Libya, the Gulf of Bomba (marked C in Fig. 2d, n=8) and Gulf of Sirte (B, n=7), were used by 52% turtles tracked to conclusive end points. An additional foraging ground in the Gulf of Antalya, Turkey (I, n=4), accounts for a further 14%.

Post-nesting migrations lasted 6 - 80 days (mean  $\pm$  SD:  $36 \pm 23$ ), and took place between 27<sup>th</sup> June and 12<sup>th</sup> October (see Fig. 3). The majority of individuals (87%) completed their return migrations during the months of July - September. Tracked turtles spent an average of 84% of their migration following coastline ( $\pm$  11%, range 59-100%), making use of coastal waters around the eastern basin coastline from Cyprus and Turkey through Syria, Lebanon, Israel and the Gaza Strip to Egypt and across Libya. Particularly high densities of tracks (Fig. 2d) are seen between the Gulfs of Arab (E), Salum (D), Bomba (C) and Sirte (B), with 62% of all conclusive tracks converging on the approach to the Gulf of Salum (n=18), 59% continuing to Bomba (n=17), and 31% continuing past Bomba to the Gulf of Sirte (n=9). A high-use pelagic corridor is evident, running southwest from Turkey, across Cyprus, to North Africa. The width of this corridor, as defined by the most central 90% of tracks (n=16), ranges between  $<0.25^\circ$  longitude at the westernmost tip of Cyprus and  $3.5^\circ$  where it meets Egypt. More than half (53%, n=18) of all migrants (including those with inconclusive tracks) used this corridor.

Four individuals from Cyprus made secondary migratory movements ( $>100$  km) after prolonged stays (51, 93, 134 and 221 days) in their respective initial foraging grounds. Three of these were tracked to nearby foraging grounds (107, 390 and 475 km distant), and two later returned to their former foraging grounds after periods of 73 and 129 days.

### Discussion

Green turtles nesting on Mediterranean beaches disperse to widely separated foraging grounds in shallow coastal waters, which they share with conspecifics from other Mediterranean nesting rookeries. This collaborative tracking effort clearly emphasizes the utility of animal tracking in the elucidation of transitory areas of high use as well as residential hotspots. Tracking has revealed a clear migratory pattern, highlighting the coastal waters of the Levantine basin and a southwest pelagic corridor as being critical migratory habitat.

The use of a shared pelagic migration corridor by turtles tracked from beaches in Turkey and Cyprus indicates that this pathway is of critical importance during the months surrounding the Mediterranean nesting season. However, there is a disparity between tracking effort and rookery size (Fig. 1), suggesting that further tracking should be directed towards Turkey's major nesting beaches, which are used by the majority of the Mediterranean population. Two turtles tracked by Turkecan and Yerli (2011) from Akyatan, the largest single rookery in the Mediterranean, travelled to sites B and I (Gulfs of Sirte and Antalya) following similar routes as those described here, further highlighting the importance of these sites.

The range of seagrass beds in the Mediterranean is thought to be much reduced (Lipkin et al., 2003); previous damage by fisheries trawling in coastal areas may have contributed to the diminished extent of green turtle foraging grounds in the region. Foraging grounds highlighted in this research, and particularly those with relatively high densities of green turtles, may be indicators of remaining healthy seagrass habitat (Scott et al., 2012). The pelagic corridor identified here follows the direction of deep bathymetric contours and surface currents, which may aid in navigation (see Fig. S2a and b; see also Luschi et al., 1998, Hays et al., 1999). Green turtles may also be congregating along this path as a result of avoidance of cooler waters to the north west of the corridor (see Fig. S2b). Use of pelagic corridors has been observed previously in green turtles in the South Atlantic (Luschi et al., 1998), and in leatherback turtles in the Atlantic (Fossette et al., 2014) and Pacific (Eckert & Sarti, 1997; Shillinger et al., 2008). Seasonal closures may be appropriate in areas where migratory corridors lead to a high incidence of interactions with fisheries within a restricted season and area; however, such measures are limited to extreme cases due to the substantial associated economic impacts (Gilman et al., 2006a) and likelihood to displace fishing effort elsewhere (Lewison et al., 2004). Threats from fisheries vary with fishing gear type and sea turtle behaviour, and efforts should

be made to quantify bycatch levels specific to area and fishing practices, classified by species and age-class.

The highest-density migratory corridor habitat occurs within the exclusive economic zones (EEZs) of Cyprus, Egypt and Libya (see Fig. S1c), which have estimated marine turtle bycatch rates of around 3,700, 7,000 and 9,700 captures (species not given) per year respectively (Casale, 2011; Nada & Casale, 2011, see Table S2 for summarised bycatch data). Set netting has the highest mortality rate (60%), and makes up 97% of the turtle bycatch in Cyprus, compared with 41% in Egypt and just 3% in Libya, such that the total estimated deaths per year for these countries are more even at 2,200, 2,800, and 2,900 (Casale, 2011). Turkey and Tunisia have higher turtle bycatch figures of 12,900 and 17,600 respectively, resulting in 5,400 and 5,600 estimated turtle deaths per year (Casale, 2011). These rates are derived from official fleet statistics and are therefore minimum values.

During pelagic phases of migration, green turtles are most vulnerable to entanglement in drift nets, of which there remains a sizeable illegal fishery in the Mediterranean despite a total ban (EJF, 2007). Few data are available regarding this Illegal, Unreported and Unregulated (IUU) fishery, but it is not currently known to be a problem in the area of the pelagic corridor described in this study, with most vessels thought to operate in the western basin and the Aegean Sea. The reported incidence of green turtle bycatch in the Mediterranean from pelagic longlines is generally low, although it is impossible to tease apart the effects of improper species identification and a bias of studies to the western basin (Gerosa & Casale, 1999), where pelagic longlines are responsible for the majority of loggerhead turtle bycatch (Casale, 2011). The largely herbivorous diet of the adult green turtle may render it less susceptible to target baited longline hooks than the sympatric carnivorous loggerhead turtle, although opportunistic carnivory is known to occur (Bjorndal, 1997) and has been detected in young adults in the Mediterranean through stable isotope analysis (Cardona et al., 2010). However, pelagic longlines are responsible for a low proportion (6%) of estimated turtle deaths in the eastern Mediterranean countries in which green turtles have been observed in this study (for which data are available, Casale 2011; Table S2).

Coastal aggregation of both fishing vessels and green turtles puts this species at greater risk from nearshore fishing practices, of which bottom trawls, set nets (such as trammel nets and gill nets) and demersal longlines make up 40%, 30% and 20% respectively of the estimated 52,000 turtle captures (all species) per year (Cyprus, Egypt, Israel, Lebanon, Libya, Syria, Tunisia and Turkey; Casale 2011; Table S2). Bottom-set nets have the greatest impact due to

the high mortality rates associated with this gear type, accounting for 50% of the 20,000 estimated minimum turtle deaths per year (Cyprus, Egypt, Israel, Lebanon, Libya, Syria, Tunisia and Turkey; Casale 2011; Table S2).

Direct take of sea turtles for meat may still be a problem in some areas; there is still an active black market for turtle meat in Alexandria and other Egyptian ports (Nada & Casale, 2011). In addition, gear damage and perceived competition with local fishermen for depleted fish stocks can lead to intentional killings, evident through stranded carcasses either beheaded or with head trauma (e.g. Nada et al. 2013). Awareness campaigns and fishermen training programmes with repeated contact have proven successful in reducing post-release mortality rates of bycaught turtles, improving cooperation and attitudes towards sea turtles, and reducing motivation for intentional killing (e.g. Oruç 2001; Snape *pers. comm.*). Additionally, livelihood diversification interventions are needed in areas where poverty enforces reliance on dwindling fish stocks (Nada et al., 2013).

Additional threats to sea turtles in the region arise from oil and gas exploration and boat strike - the pelagic corridor highlighted here is crossed by paths of intense maritime activity, for example (Katsanevakis et al., 2015). Geopolitical instability across the region may cause delays to the successful implementation of new conservation measures, and transboundary collaboration is further complicated by socio-economic conflicts (Katsanevakis et al., 2015).

### Recommendations

The information available regarding marine turtle bycatch in the Levantine basin is spatially vague; further characterisation of turtle bycatch in the eastern Mediterranean should be prioritised as many data gaps exist, particularly from countries on the north African coast (Casale, 2011). Seasonally targeted quantification of bycatch from April to September (see Fig. 3) within transitory corridors of high use may illustrate the true cost of migration for such species. Post-release mortality rates specific to each fishery should also be further investigated due to the high variability in survival depending on practice (e.g. tow durations, soak times) and paucity of information, again from the eastern basin (Casale, 2011). Quantification of bycatch, associated mortality rates and intentional killings within the coastal foraging areas and seasonal migratory pathways highlighted here is urgently required so that remedial action can be implemented where required. Major knowledge gaps exist in relation to species identification of bycatch. Recommendations for fisheries management cannot be made until the threat to green turtles in the eastern basin from bycatch is quantified.

Monitoring within the Mediterranean is difficult due to the artisanal nature of much of the fishery (Casale, 2011), but is possible (see Snape et al. 2013).

Networks of marine protected areas (MPAs) can alleviate escalating pressure from fisheries on marine ecosystems, by protecting spawning stocks and vulnerable non-target species (Halpern & Warner 2002). Green turtle foraging sites have been described as potential indicators of quality tropical coastal marine ecosystems, therefore useful in the proposal of MPAs (Scott et al., 2012). Much of Libya's coastline has so far escaped over-exploitation and degradation; total fisheries catch is an order of magnitude lower than that of neighbouring Egypt and Tunisia, and vast stretches remain relatively unpopulated (Haddoud & Rawag, 2007). The rate of marine exploitation has accelerated, however, and implementation of conservation legislation has been delayed by political unrest (Badalamenti et al., 2011). The Gulf of Bomba (Fig. 2d site C), the most important green turtle foraging area identified here through satellite telemetry, is recognised as a biodiversity hotspot, and legislative framework for protection has been established through the Ain Gazala MPA (Badalamenti et al., 2011; see Fig. S1c for MPAs of the eastern Mediterranean). We recommend that the Gulf of Sirte (site B) also be investigated as a likely additional biodiversity hotspot, and thus a potential for MPA proposal. Protection of these two major foraging grounds should benefit a high proportion of the adult green turtle population in the Mediterranean. Site A at the Libya/ Tunisia border (as well as further offshore within the Gulf of Gabès shelf) is also a known year-round foraging site for male, female and juvenile loggerhead turtles from around six Mediterranean breeding populations (Broderick et al., 2007; Casale et al., 2007; Zbinden et al., 2011; Schofield et al., 2013); protection at this site would therefore afford benefits to both species. The green turtle foraging grounds at the Gulfs of Sirte (B), Bomba (C), Salum (D), Arab (E) and Tripoli, Lebanon (G) are also shared with foraging loggerheads (Broderick et al., 2007; Casale et al., 2007, 2013; Hochscheid et al., 2010; Schofield et al., 2013), although fewer individuals of the latter species have thus far been tracked to these sites.

Tracking studies targeting juvenile green turtles would be beneficial as the majority of bycaught turtles in the Mediterranean are small in size (Wallace et al., 2010), implying a higher degree of spatial overlap between fishing effort and habitat use of juveniles. Although bycatch data availability for the Mediterranean has a geographical bias to the western basin, the pattern in size-class may be consistent: a small-scale survey of coastal trawlers in Turkey (Mersin to İskenderun Bay) found that the majority of turtle bycatch was green turtle (77%), and 80% of bycaught turtles were juveniles (Oruç, 2001). Additionally, analysis of dead stranded turtles and registered by-catch in northern Cyprus (Snape et al., 2013) and eastern

Turkey (Türkozan et al., 2013) indicated that juvenile green and adult loggerhead turtles were at higher risk from local fisheries than adult green turtles.

Data from four of the individuals tracked in this study indicate that green turtles do not necessarily remain within a single foraging ground for the entirety of the non-breeding period, contrary to previous observations (Plotkin, 2003; Broderick et al., 2007). Transmitter deployments on turtles at foraging areas would be beneficial to determine the extent of this behaviour, to describe the timing of pre-nesting migrations and to confirm whether outbound breeding migrations match the return paths described here, all of which have implications for management of key migratory habitats. Tracks from Turkey and Syria have revealed two foraging bays that were not known from tracking efforts from Cyprus, despite close proximity and large sample size, demonstrating the importance of tracking from multiple sites. Tracking from Turkey in this study also highlights that as well as aspiring to large sample size (Schofield et al., 2013), it is advisable to collect tracking data across multiple years when building up a picture of dispersal patterns. This has previously been highlighted for leatherback turtles (Witt et al., 2011).

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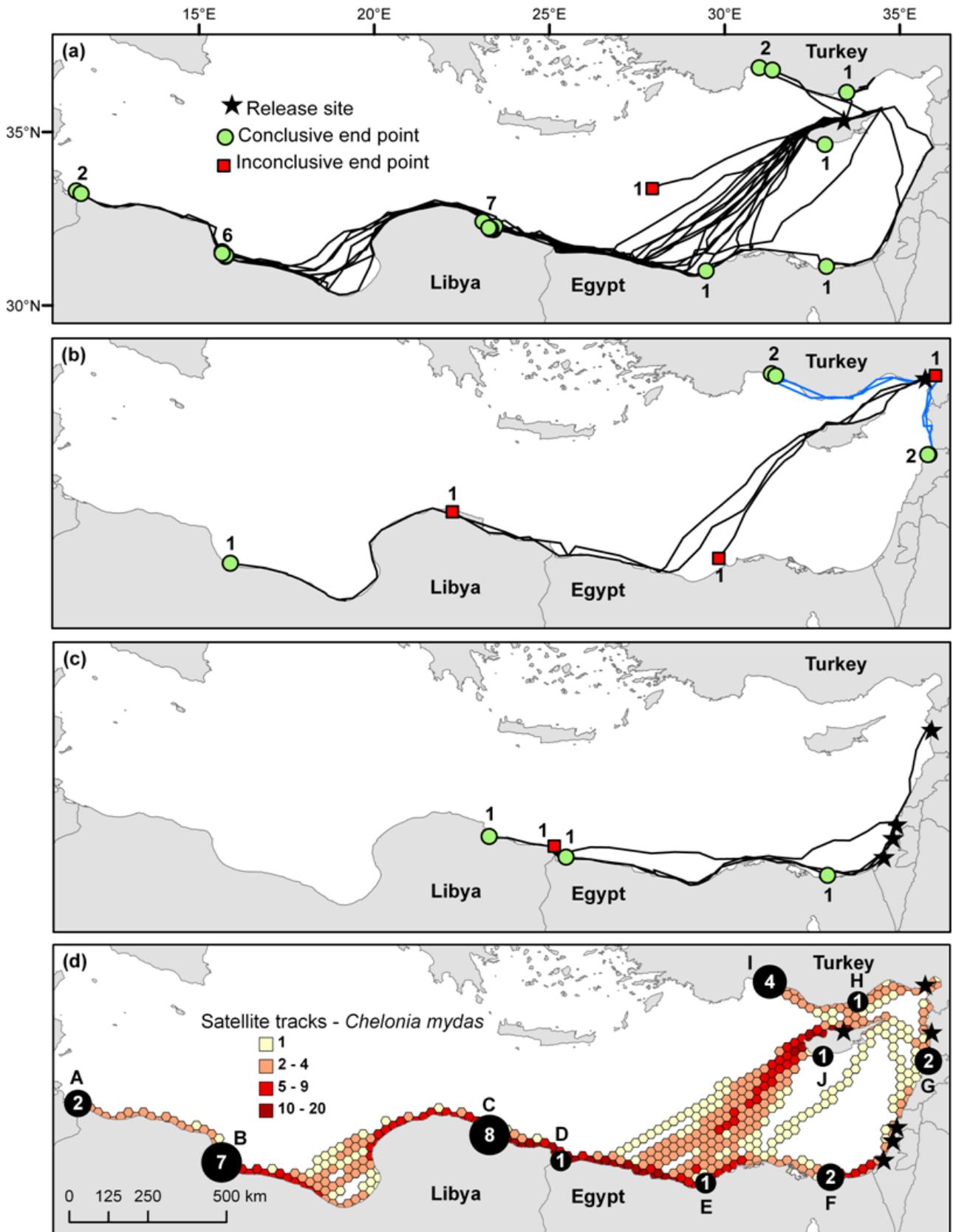
Zydelis R., Lewison R.L., Shaffer S.A., Moore J.E., Boustany A.M., Roberts J.J., Sims M., Dunn D.C., Best B.D., Tremblay Y., Kappes M.A., Halpin P.N., Costa D.P., & Crowder L.B. (2011) Dynamic habitat models: using telemetry data to project fisheries bycatch. *Proceedings of the Royal Society B*, 278, 3191–200.

Chapter II: green turtle migration

**Table 1.** Green turtle nesting beaches of the Mediterranean. Averages are means unless otherwise indicated (\*: medians taken for non-consecutive years)

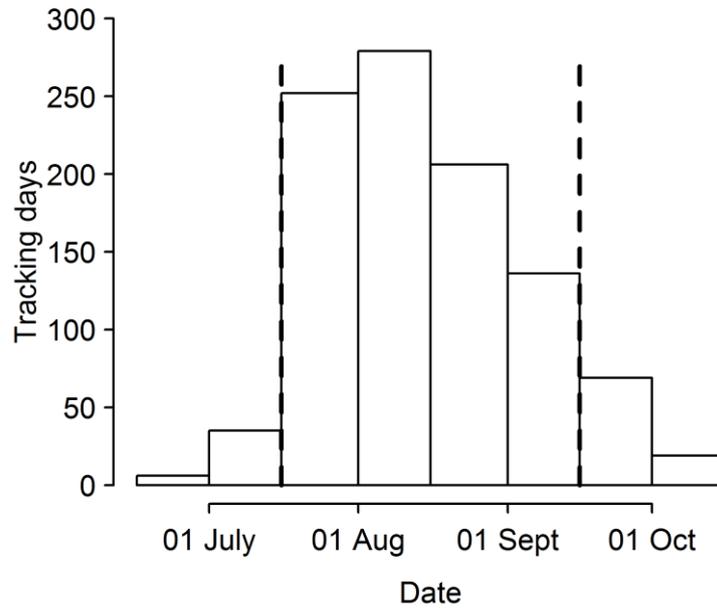
Country	Beach name	Max recorded no. nests	Year of max	Min recorded no. nests	Average no. nests/ year	No. years surveyed	Source	Tracks		
Cyprus	North Karpaz	179	2000	38	104	8	1	21		
	Alagadi	236	2013	8	66	21	1,2			
	Akamas Peninsula	114	2004	9	48	20	3			
	South Karpaz	107	1994	35	64	7	1			
	West Coast	125	2012	4	49	21	1,2			
	North Coast (excluding Alagadi)	37	2004	0	16	21	1,2			
	Akrotiri peninsula	7	1999, 2000	0	5	5	4			
Turkey	Akyatan	735	1998	108	223 *	11	1,5-16	8		
	Samandağ	440	2006	1	44 *	11	1,5,8,10,14,16-21			
	Kazanlı	403	2004	73	164 *	10	1,5,8,10,16,18,19,22-28			
	Sugözü	213	2004	213	213	1	16,29			
	Alata	198	2006	20	128 *	4	16,30,31			
	Göksu	20	1991	0	13 *	7	1,5,8,14,32-35			
	Yumurtalık	15	1988	1	3 *	3	5,14,16,17,36			
	Tuzla	9	2006	4	9 *	3	1,14,16,17,19,36			
	Belek	8	1998, 2000	1	4 *	8	1,5,8,14,16,35,37-40			
	Kumluca/ Fenike	7	1994	0	4 *	2	1,8,14,16,37			
	Ağyatan	4	1996	0	3 *	4	1,5,14-17,19			
	Kızılot	3	1993	0	1 *	3	1,8,14,16,37,41			
	Yelkoma	3	1988	2	3 *	2	1,5,14,16,17,19			
	Patara	2	2000	2	2	1	1,14,16,42			
	Syria	Latakia	273	2008	18	140	6		43,44	1
		Banias	15		1	9	6		44	
Wadi Kandil		13	Data not available	1	7	6	44			
Ras el Basit		11		0	4	5	44			
Um Toyour		7		0	3	3	44			
Lebanon	El-Mansouri, Tyre Nature Reserve, El Abbasiyeh	16	2004	0	7	5	45-52	3		
Israel	Nahariya, Gdor, Sharon, Ashkelon	20	2006	0	8	16	1,53,54			
Egypt	El Arish	3	2000	0	1	3	1,55-57			





**Figure 2.** Post nesting green turtle satellite tracks from a) Cyprus (n=22), b) Turkey (n=8), c) Syria (n=1) and Israel (n=3), and d) migratory corridor density map (conclusive tracks only; n=29). Numbers indicate the

number of individuals tracked conclusively to each foraging ground. In panel b, tracks in blue are from the first year of tracking (2004) and those in black are from the second year of tracking (2005). Colour in panel d is indicative of the number of satellite tracks that pass through each hexagonal grid cell. Movements to secondary foraging grounds after prolonged stays in initial foraging grounds are not included. Letters in d) indicate the following foraging grounds: A - Libya/Tunisia border, B - Gulf of Sirte, C - Gulf of Bomba, D - Gulf of Salum, E - Gulf of Arab, F - Lake Bardawil, G - Tripoli, Lebanon, H - Erdemli, I - Gulf of Antalya, J - Episkopi Bay.



**Figure 3.** Seasonality of post-nesting *Chelonia mydas* migrations tracked in this study. Eighty seven percent of all migratory tracking days took place between 15<sup>th</sup> July and 15<sup>th</sup> September (dashed lines). Outbound breeding migrations are estimated to take place from April to June.

**Appendix S1. Nesting data sources for Table 1.**

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**Table S1.** Summary of transmitter deployments

PTT	Release site	Country	Manufacturer	Model	Deploy date	Days tracked	Days in foraging ground	Result	Previous publication
4150	Alagadi	Cyprus	Wildlife Computers	SDR-SSC3	27/07/1998	295	264	Conclusive	1, 2
4149	Alagadi	Cyprus	Wildlife Computers	SDR-SSC3	29/07/1998	222	188	Conclusive	1, 2
4148	Alagadi	Cyprus	Wildlife Computers	SDR-SSC3	01/08/1998	288	278	Conclusive	1, 2
4405	Alagadi	Cyprus	Telonics	ST6	27/07/2002	404	383	Conclusive	2
36638	Alagadi	Cyprus	Sirtrack	Kiwisat 101	12/07/2003	348	256	Conclusive	2
36639	Alagadi	Cyprus	Sirtrack	Kiwisat 101	24/06/2004	385	309	Conclusive	2
49815	Alagadi	Cyprus	Sirtrack	Kiwisat 101	09/07/2004	60	38	Conclusive	2
49816	Alagadi	Cyprus	Sirtrack	Kiwisat 101	22/07/2004	359	308	Conclusive	2
49813	Alagadi	Cyprus	Sirtrack	Kiwisat 101	23/07/2004	312	237	Conclusive	2
95097	Alagadi	Cyprus	Sirtrack	Kiwisat 101	04/07/2009	487	420	Conclusive	
95101	Alagadi	Cyprus	Sirtrack	Kiwisat 101	05/07/2009	716	650	Conclusive	
95098	Alagadi	Cyprus	Sirtrack	Kiwisat 101	15/07/2009	117	51	Secondary*	
95100	Alagadi	Cyprus	Sirtrack	Kiwisat 101	15/07/2009	27	-	Inconclusive	
95102	Alagadi	Cyprus	Sirtrack	Kiwisat 101	24/07/2009	111	91	Conclusive	
52820	Alagadi	Cyprus	Sirtrack	Kiwisat 101	16/06/2010	752	538	Conclusive	
86898	Alagadi	Cyprus	Sirtrack	Kiwisat 101	26/06/2010	476	57	Conclusive	
52846	Alagadi	Cyprus	Sirtrack	Kiwisat 101	28/06/2010	349	93	Secondary*	
52827	Alagadi	Cyprus	Sirtrack	Kiwisat 101	01/07/2010	408	221	Secondary*	
52949	Alagadi	Cyprus	Sirtrack	Kiwisat 101	07/07/2010	479	440	Conclusive	
86900	Alagadi	Cyprus	Sirtrack	Kiwisat 101	13/07/2010	413	134	Secondary*	
52888	Alagadi	Cyprus	Sirtrack	Kiwisat 101	21/07/2010	123	40	Conclusive	
6598	Esentepe	Cyprus	Telonics	ST18	19/07/1999	243	179	Conclusive	1, 2
49822	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	15/07/2004	99	88	Conclusive	
49823	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	18/07/2004	113	103	Conclusive	
49824	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	19/07/2004	131	115	Conclusive	
49825	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	21/07/2004	53	41	Conclusive	
57388	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	13/07/2005	22	-	Inconclusive	
57387	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	16/07/2005	26	-	Inconclusive	
49826	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	17/07/2005	198	128	Conclusive	
57382	Yumurtalık (Sugözü)	Turkey	Sirtrack	Kiwisat 101	21/07/2005	50	-	Inconclusive	
86394	Gdor Nature Reserve	Israel	Sirtrack	Kiwisat 101	29/07/2008	22	-	Inconclusive	
93702	Sharon National Park	Israel	Sirtrack	Kiwisat 101	20/06/2009	355	308	Conclusive	
93699	Ashkelon National Park	Israel	Sirtrack	Kiwisat 101	27/06/2009	55	48	Conclusive	
49082	Latakia	Syria	Sirtrack	Kiwisat 101	09/07/2006	96	27	Conclusive	3

\* Secondary migratory movements were recorded following residency within initial foraging grounds

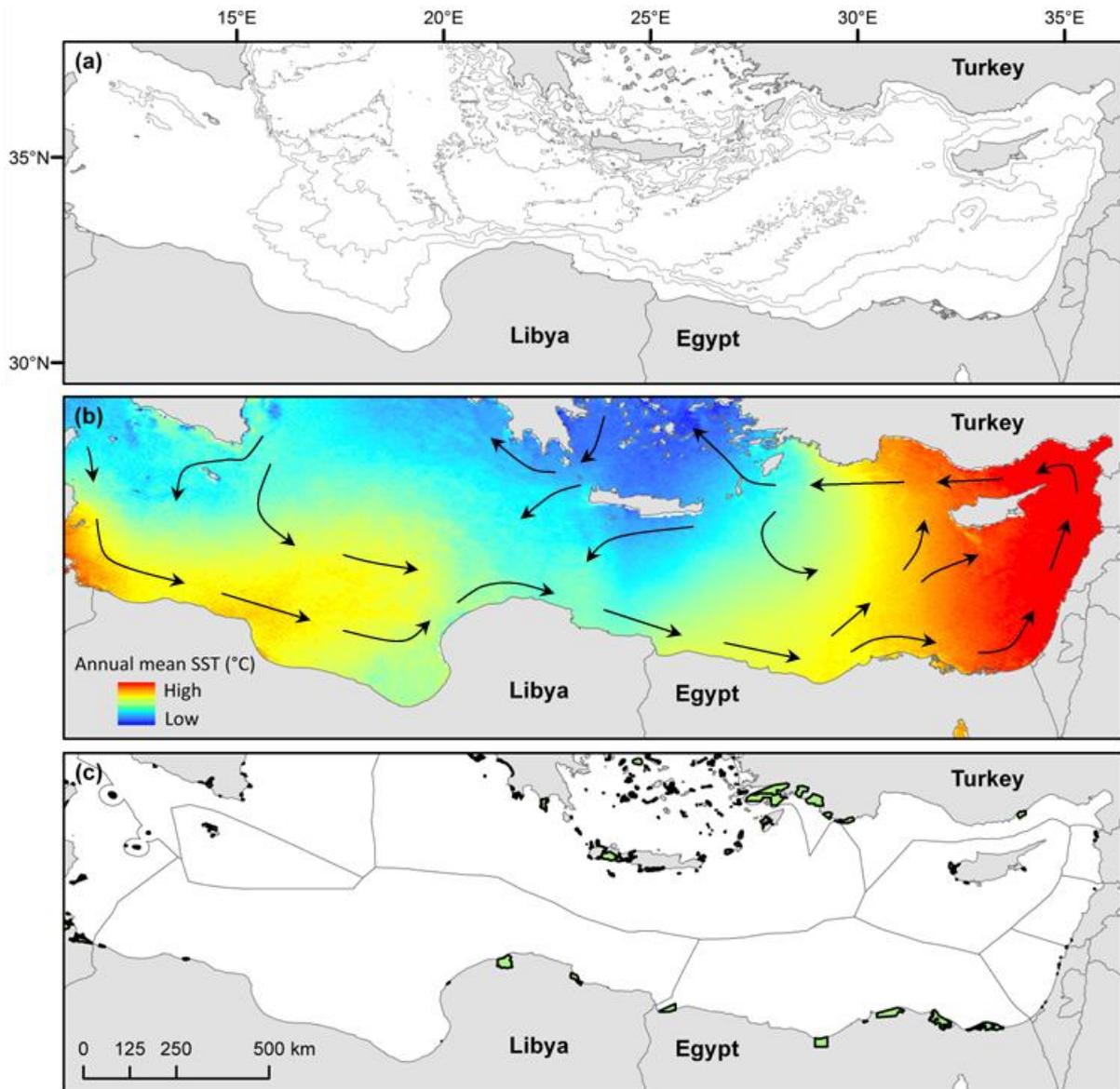
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**Table S2.** Marine turtle bycatch summary for eastern Mediterranean countries relevant to this study. BT: bottom trawl, 20% estimated average mortality rate (MR); PLL: pelagic longline, 30% MR; DLL: demersal longline, 40% MR; SN: set nets, 60% MR. Modified from Casale (2011).

	Captures per year					Deaths per year				
	BT	PLL	DLL	SN	Total	BT	PLL	DLL	SN	Total
Cyprus	100			3,600	3,700	20			2,160	2,180
Egypt	1,900	1,300	800	2,800	6,800	380	390	320	1,680	2,770
Israel	10				10	2				2
Lebanon										
Libya	4,700	1,400	3,300	300	9,700	940	420	1,320	180	2,860
Syria	200		300	900	1,400	40		120	540	700
Tunisia	10,900	1,000	1,500	4,200	17,600	2,180	300	600	2,520	5,600
Turkey	3,500		4,700	4,700	12,900	700		1,880	2,820	5,400
Total (eastern Mediterranean)	21,310	3,700	10,600	16,500	52,110	4,262	1,110	4,240	9,900	19,512
...of all methods	41%	7%	20%	32%		22%	6%	22%	51%	
Total (Mediterranean)	39,350	57,410	12,580	23,000	131,840	7,870	17,223	5,032	13,800	43,925
...of all methods	30%	44%	10%	17%		18%	39%	11%	31%	

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**Figure S1.** Oceanographic and political features of the eastern Mediterranean. **a)** Bathymetric depth derived from GEBCO (General Bathymetric Chart of the Oceans) - isopleths shown for every 1 km depth. **b)** Mean annual sea surface temperature (NOAA) / primary and secondary surface currents (arrows), as taken from Sverdrup et al. (1942) and Pujol (2006). See also Abudaya (2013). **c)** Fisheries exclusive economic zones (delineated in grey; Marineregions.org) and marine protected areas (shown in green with black borders; MAPAMED, 2014).

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### Chapter III

#### **Possible evidence for male-mediated gene flow in green turtles**

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**Abstract**

The study of mating systems and their impact on effective population size is fundamental to the assessment of species of conservation concern, and is particularly challenging in the marine realm. A rare opportunity allowed us to deploy a satellite transmitter on an adult male green turtle (*Chelonia mydas*) from a major nesting site in Cyprus. Upon release, the male travelled to two further nesting sites in Cyprus, before making a 350 km detour to Turkey. Here, the male spent 12 days in the coastal waters offshore from three major nesting beaches, undertaking behaviour patterns consistent with mate-seeking, before travelling to the north African coast. This diversion extended the distance travelled by 44% and took him within 20 km of six different rookeries, which together represent >50% of all conspecific nesting activity in the Mediterranean. Should this behaviour result in matings at multiple sites, it has considerable implications for the effective size of this population, representing a possible avenue for male-mediated gene flow and potentially ameliorating a significant female sex ratio bias in the face of climate change.

## Introduction

Natal homing behaviour has emerged as a fundamental life history trait in sea turtles (Bowen & Karl, 2007). Strong natal philopatry can lead to population structuring, and can therefore reduce effective population sizes dramatically (Chesser et al., 1993). Green turtles have been shown to exhibit 'complex population structure', evidenced by higher levels of genetic divergence at maternally inherited mitochondrial loci than at biparentally inherited nuclear loci (Bowen & Karl, 2007). Given that both males and females are thought to be philopatric to the natal site (Dizon & Balazs, 1982; FitzSimmons et al., 1997a), this has raised questions about where and when this male-mediated gene flow occurs. It is thought that extra-nesting aggregation matings occur at mixed feeding grounds or along shared migratory routes (Karl et al., 1992; Fitzsimmons et al., 1997b; Bowen & Karl, 2007). It is also possible, however, that males may move among rookeries. For species such as marine turtles with temperature-dependent sex determination (TSD) and female-biased primary sex ratios (Hawkes et al., 2009), such behaviour could increase effective population sizes (Wright, 1931), helping to explain how these highly skewed sex ratios persist.

Tracking studies have provided invaluable breakthroughs in our knowledge of marine turtle migratory behaviour and habitat use, but there has been a heavy bias towards the tracking of nesting females for logistical reasons (Godley et al., 2008). This inaccessibility of males has restricted our ability to fully elucidate the mating systems in operation (Karl et al., 1992). For the green turtle, we are aware of only three published studies that have tracked breeding adult males (Hawaii: Dizon & Balazs 1982, Balazs & Ellis 1998, Rice & Balazs 2008; Ascension Island: Hays *et al.* 2001), totalling ten individuals. Here we describe the movements of a breeding male green turtle in the Mediterranean, offering novel insight into possible mechanisms of male-mediated gene flow in this animal group.

## Methods

The study system is a Mediterranean nesting rookery of female green turtles and their hatchlings, for which longitudinal data are available. On 8<sup>th</sup> June 2009, a female green turtle was sighted emerging from the sea at Alagadi Beach (35°33'N, 33°47'E), Cyprus, with a male still in amplexus. A satellite transmitter (KiwiSat 101, Sirtrack, New Zealand) was attached to the male according to previously described protocol (Godley et al., 2002b). Locations were obtained using the Argos system and Satellite Tracking and Analysis Tool (STAT; Coyne & Godley 2005), and mapped in ArcGIS 9.3.1. The route was reconstructed using data of location classes 3, 2 and 1 (estimated errors of less than 1km, Witt *et al.* 2010), and a standard filter was applied to exclude locations inferring implausible speeds ( $>5 \text{ km h}^{-1}$ ) and turning angles ( $<25^\circ$ ). Minimum speeds were calculated assuming straight line travel between locations, and size of foraging ground home range was estimated using the minimum convex polygon (MCP; smallest polygon containing class 3, 2 and 1 point locations).

## Results

Over a period of 77 days, the male visited rookeries 200 km apart in Cyprus and Turkey before travelling to north Africa (Fig. 1). From Alagadi Beach (Fig. 1a site 3), he travelled east along the north coast of Cyprus, passing a smaller nesting site (site 4), to another major nesting site (arriving on 11<sup>th</sup> June; site 5). Although minimum speeds averaged 1.5 km h<sup>-1</sup> along the north coast, there was a demonstrable slowing to 0.7 km h<sup>-1</sup> at this rookery (see Fig. 1d). He then undertook the first pelagic crossing, travelling north at a minimum speed of 1.6 km h<sup>-1</sup> (bearing 350°, straightness index 0.83) to reach the Turkish coast by 14<sup>th</sup> June. Here, twelve days were spent travelling at low speeds (mean average 0.6 km h<sup>-1</sup>) within Mersin Bay in proximity to (< 20 km) the nesting sites of Alata, Kazanlı and Akyatan (sites 9-11; see Fig. 1b), the latter being the single most important rookery for this population (see Chapter II Table 1), in a pattern consistent with mate searching behaviour (see Fig. 1b).

On 26<sup>th</sup> June, the turtle embarked on a second pelagic crossing, this time heading SSE at a minimum of 2.5 km h<sup>-1</sup> (bearing 147°, straightness index 0.97) to reach Syria by 29<sup>th</sup> June. He then followed the Eastern Basin coastline (minimum average speed 1.8 km h<sup>-1</sup>) until arriving on the 12<sup>th</sup> July at Sabkhet el Bardawil saltwater lagoon, Bay of Tinah, Egypt, where he remained for a further 43 days of transmission travelling at low speeds (minimum average 0.1 km h<sup>-1</sup>) within a relatively large home range of 103 km<sup>2</sup> (Fig. 1c). The diversion to Turkey took a total of 19 days, adding 348 km to the journey, and increased the total journey length by 44% when compared to a beeline route (taken from the last location at the northeastern tip of Cyprus to the point at which the turtle arrived at the Syrian coast). The six rookeries visited account for 55% of green turtle nesting in the Mediterranean according to maximum nest numbers (see Chapter II Table 1).

## Discussion

Green turtles exhibit scramble polygamy (Jessop et al., 1999) with varying levels of multiple paternity (Bowen & Karl, 2007), and it is thought that a high proportion of mating occurs near nesting areas (reviewed in Bell *et al.* 2009). Mating activity can overlap significantly with the nesting season (Godley et al., 2002a), and males are thought to be sexually active for a period of around one month (Limpus, 1993; Hays et al., 2001). Given the seasonality of nesting in the Mediterranean (see Broderick *et al.* 2002, Elmaz & Kalay 2006, and Chapter IV), it is possible that the male tracked in this study was seeking receptive females both on the northern coast of Cyprus and within Mersin Bay. There is a large body of evidence demonstrating the exceptional navigational abilities of sea turtles (Luschi et al., 2007; Lohmann et al., 2008), suggesting that the northward detour observed in this track arose out of such a strategy to access a greater number of females rather than due to a failure in orientation. Indeed, while female turtles tracked from Alagadi Beach undertook post-breeding migrations to Turkey, south Cyprus or north Africa, all individuals took relatively direct routes to their final locations, and none heading to north Africa routed via Turkey (see Chapter II).

This first evidence of a male sea turtle visiting multiple rookeries has implications for the mating systems of these species. Natal philopatry in conjunction with colonial breeding contributes to the resilience of marine turtle species by ensuring mate-finding even at low population densities, thus avoiding depensation (Bell et al., 2009). If males can display natal site philopatry and also visit additional nesting aggregations to mate, this could provide a significant mechanism for male-mediated gene flow and may explain in part the complex population structure observed in green turtle populations. This previously unknown behaviour, if shared by other males, will help confer resilience by slowing the loss of genetic variation through drift in a population much reduced from past exploitation (Broderick et al., 2002).

It has been demonstrated that unequal sex ratios can increase depensatory effects by reducing mate-finding ability (Stephens & Sutherland, 1999). Estimates of primary sex ratios at two major nesting beaches for green turtles in the Mediterranean were 92% female at Akyatan, Turkey (Casale et al., 2000) and 86-96% female at Alagadi, Cyprus (Broderick et al., 2000). Male movement among sites and more frequent migration to breeding grounds (as recorded in the loggerhead turtle *Caretta caretta*: Hays *et al.* 2010) have the potential to alleviate the effects of this bias. Male-mediated gene flow may become of greater importance with further skewing of sex ratios predicted under future climate change scenarios (Hawkes et al., 2009; Witt et al., 2010b).

### **Acknowledgements**

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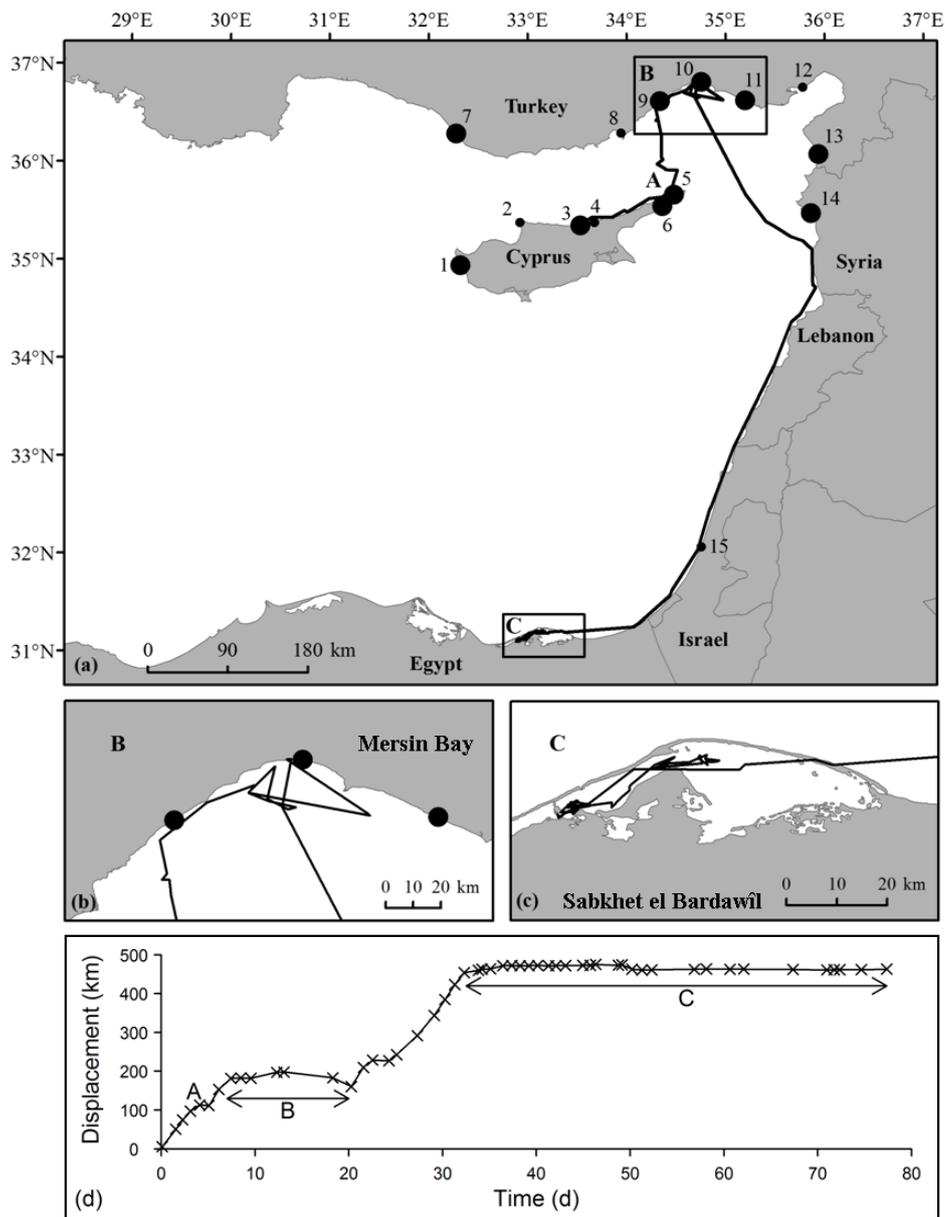
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**Figure 1.** Male green turtle tracked for 77 days from Alagadi, Cyprus to Sabkhet el Bardawil, Egypt. **a)** Full track from transmitter deployment to end point, showing known green turtle nesting sites in the Mediterranean (max recorded number of nests >10). Large dots represent nesting sites with >100 nests recorded in a single season, and smaller dots denote nesting sites with 10-100 maximum number of nests recorded in any one season. Nesting sites are as follows; Cyprus: 1. Akamas, 2. West Coast b, 3. Alagadi, 4. North Coast, 5. North Karpaz, 6. South Karpaz; Turkey: 7. Sugözü, 8. Göksu, 9. Alata, 10. Kazanlı, 11. Akyatan, 12. Yumurtalık, 13. Samandağ; Syria: 14. Latakia; Israel: 15. Hadera/ Netanya/ Ashgelon. For nesting data and sources, see Chapter II Table 1. **b)** Movements during eight days spent in Mersin Bay before commencing southerly migration. **c)** Movements within Sabkhet el Bardawil. **d)** Displacement plot for the duration of transmission showing time spent near the Karpaz rookery (A), within Mersin Bay (B) and at the final foraging ground (C).



## Chapter IV

### Predicting the impact of temperature rise on sea turtle offspring

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**Abstract**

Many reptiles with temperature-dependent sex determination face rapid contemporary climate change with depleted population sizes in a dramatically altered landscape. Establishing sex ratios of such groups has become a research priority, and detailed understanding of thermal effects on offspring development is essential for the long term conservation management of nesting grounds. We report sex ratios and morphometrics of green turtle (*Chelonia mydas*) offspring over a 20 year period on the Mediterranean island of Cyprus. An estimated 97% of offspring produced at the main study site, Alagadi Beach, since 1993 have been female (the sex produced at higher temperatures), with annual mean sex ratios ranging from 90-100% female. The few clutches that produced males were laid early in the season, at greater depth by larger females, or on beaches of lighter sand (range for northern Cyprus: 73-100% female, mean 93% female). Higher incubation temperatures also gave rise to smaller hatchlings, a 1°C rise in mean temperature causing a reduction of 1% in length, 2% in width and 3% in weight, with potential effects on fitness. Hatch success dropped sharply at mean temperatures over 33°C, and no clutches hatched above 34.2°C. If no compensatory mechanisms occur, a 1°C rise in nest temperatures is predicted to cause near complete hatchling feminisation at the main study site, and a 2°C rise could reduce hatch success to less than 50%. However, male-biased adult sex ratios at this site and others with heavily female-biased hatchling production raise questions about sex-specific survival rates and optimal hatchling sex ratios. Many further questions remain regarding the buffering effects and adaptive capacity of sea turtle reproduction.

## Introduction

The physical evidence for global climate change is now unequivocal (Stocker *et al.*, 2013), the biological signal is globally apparent (Parmesan & Yohe, 2003), and indirect effects have been implicated in species extinctions (e.g. Pounds *et al.*, 2006). The direct effects of rising temperatures will be suffered most acutely by ectotherms (Sinervo *et al.*, 2010), and susceptibility will depend on physiological sensitivity to thermal change (Deutsch *et al.*, 2008). Potential avenues of adaptation in macroscopic species are thought to be limited to spatio-temporal shifts in distribution and behavioural and physiological plasticity, as predictions suggest the rate of warming will be too fast for adaptation through microevolutionary processes (Harte *et al.*, 2004; Quintero & Wiens, 2013).

Many oviparous reptiles are already impacted by climate change due to the profound effects of incubation temperature on offspring development. Rising temperatures at nesting sites will affect the morphology, size and quality of emergent hatchlings (Deeming, 2004), and ultimately, hatch success (Saba *et al.*, 2012). Those species with temperature-dependent sex determination (TSD; all crocodylians, the tuatara, most turtles and some lizards; Janzen & Phillips, 2006), in which the sex of developing embryos is determined by thermal conditions during incubation rather than by genotype, are potentially susceptible to extreme sex ratio skew and even complete loss of one sex (Hulin *et al.*, 2009). As many studied populations yield females at higher temperatures, and most already show heavily female-biased primary sex ratios (Hawkes *et al.*, 2009), the concern is that raised temperatures could lead to complete feminisation and reproductive failure (Fuentes *et al.*, 2009; Patino-Martinez *et al.*, 2012). Monitoring of sex ratios is therefore a research priority for TSD groups (Gaze, 2001; Hawkes *et al.*, 2009; Hamann *et al.*, 2010).

Sex ratio estimation in sea turtles is complicated firstly because hatchlings are not sexually dimorphic, and secondly because reproductive females are much more easily accessible than reproductive males (Lasala *et al.*, 2013). Identification of hatchling sex involves microscopic inspection of gonadal tissue, requiring sacrificial sampling (Yntema & Mrosovsky, 1980), or use of offspring found dead post-hatching (e.g. Broderick *et al.*, 2000; Kaska *et al.*, 2006; Rebelo *et al.*, 2012; King *et al.*, 2013). Both methods have revealed very little variation in the pivotal temperature ( $P$ ), at which 50% of either sex is produced, within or between species ( $P \approx 29^{\circ}\text{C}$  across all studied green turtle populations, range:  $28\text{-}31^{\circ}\text{C}$  across all sea turtle species; Hawkes *et al.*, 2009). The transitional range of temperatures (TRT), conducive to mixed sex ratios

(Mrosovsky & Pieau, 1991), is variable among populations (Chevalier *et al.*, 1999; Hulin *et al.*, 2009) and determines the level of sensitivity in the sex ratio to small changes in incubation temperatures (Hulin *et al.*, 2009).

In addition to affecting the sex of offspring, raised temperatures will impact sea turtle hatchling survival in a number of ways. Warmer nests allow faster embryonic development and give rise to smaller hatchlings (Deeming, 2004), which may have lower survivorship due to limitations on locomotory speeds (Ischer *et al.*, 2009) and size-selective predation by gape-limited predators (Janzen, 1993; Gyuris, 2000; Rebelo *et al.*, 2012). Sea turtle hatchling survival is often proportional to the amount of time spent crossing high-risk predator zones on the beach or in shallow water (Gyuris, 1994), and the neonate 'frenzy period' serves to reduce the duration of this period of high vulnerability (Wyneken & Salmon, 1992). Studies of naturally incubated hatchlings have confirmed that cooler nests produce faster hatchlings (Mickelson & Downie, 2010; Booth & Evans, 2011; Booth *et al.*, 2012), although confounding results had previously been found for laboratory incubated hatchlings experiencing constant temperatures and controlled water potentials across treatments (Booth *et al.*, 2004; Burgess *et al.*, 2006). Additionally, very high developmental temperatures may cause morphological abnormalities (Telemeco *et al.*, 2013). Finally, increasing temperatures at beaches close to upper thermal limits will reduce hatch success and may eventually push many current nesting sites outside the thermal tolerance range for successful incubation (25 - 35°C: Ackerman, 1997), although poleward expansion into previously thermally unavailable nesting habitat may also become possible (Pike, 2013).

Regional determination of sex ratio curves allows the quantification of variation in the thermal response among populations and through time, and provides model curves and validation data on which proxy estimation methods using nest temperature and incubation duration can be based (Mrosovsky *et al.*, 2009). A large body of primary sex ratio estimations is based on indirect methods utilising temperature/ duration conversion curves from relatively few studies using direct histological sampling, often laboratory studies on a handful of clutches (Witt *et al.*, 2010). Our aims were to characterise thermal effects on sex ratio, hatchling size and hatch success in naturally incubating green turtle nests in northern Cyprus, and to predict future reproductive impacts of climate change on this vulnerable population.

## Methods

The study system is a Mediterranean nesting rookery of female green turtles and their hatchlings, for which longitudinal data are available. Green turtle reproductive activity on the beaches of northern Cyprus has been monitored since 1992. Data included in this study encompasses twenty nesting seasons (1993-2012) at Alagadi, the most intensively monitored site and the most important nesting beach on the north coast, sixteen seasons (1997-2012) on the wider north and west coasts, and four seasons (1997-1999, & 2008) on the Karpaz Peninsula, as determined by data availability and changes in methodology (for map see Fig. S1). Beaches were surveyed every 1-3 days throughout the nesting and hatching seasons. Comprehensive nocturnal patrols at Alagadi Beach (see Broderick *et al.*, 2003 for further details) allow exhaustive tagging of females and deployment of intra-nest temperature recording devices (1994-2012: Tinytag TGP-4017, Gemini Data Loggers, Chichester, UK,  $\pm 0.3^{\circ}\text{C}$  accuracy,  $0.1^{\circ}\text{C}$  resolution,  $n=370$ ; 2011-2012: Tinytag TGP-4500,  $\pm 0.4^{\circ}\text{C}$  accuracy,  $0.01^{\circ}\text{C}$  resolution,  $n=102$ ; sample sizes given for hatched nests only), which are lowered into the centre of the egg chamber approximately halfway through oviposition and recovered on excavation once a nest has either hatched or failed. Three measures of curved carapace length (CCL) and width (CCW) are taken using a tape measure at each nesting event.

Nest incubation duration (ID) is taken as the number of days between laying and first hatchling emergence, as determined through observation of nesting females and emergent hatchlings (Alagadi), or examination of tracks during early morning surveys (all other beaches). Clutch size (CS) and hatching success (HS) are determined through post-hatching nest excavation (Broderick *et al.*, 2003). Failed clutches were excluded from all analyses in order to isolate thermal effects on hatch success from other factors more commonly affecting whole clutches (e.g. waterlogging, predation, infestation). Clutches that had experienced disturbance of any kind (e.g. predation, translocation), or for which ID was not certain, were also excluded.

Full-term hatchlings found dead in hatched nests were dissected for microscopic inspection of the gonads for sex identification (Alagadi Beach only, 2010 & 2011), following standard histological techniques. Gonads were fixed in formalin for 2-4 h, transferred to ethanol for storage, embedded in paraffin wax, sectioned transversely at 4-10  $\mu\text{m}$ , mounted on slides and stained with haematoxylin-eosin. Sex was assigned using criteria following Yntema & Mrosovsky (1980) and further illustrations from Ceriani & Wyneken (2008). Females were identified through presence of a thickened cortex and lack of medullary structural

organisation. Males in contrast have a thin, smooth cortex and immature seminiferous tubules in the medulla.

The sex ratio of sexed hatchlings was modelled against both middle third (the thermosensitive period for sex determination in sea turtles, Mrosovsky & Pieau, 1991) mean incubation temperature and incubation length through logistic regression in R (R Development Core Team, 2012), using generalised (binomial) linear mixed-effects modelling (GLMM; package 'lme4', Bates *et al.*, 2011) to account for pseudoreplicated females (females laying multiple clutches across the sampling period). Within-nest temperature plots were used to determine the lay - hatch duration, termed hereon in as 'incubation period' (IP), for each clutch with sexed hatchlings (see Fig. 1 and Weber *et al. in prep.*), to eliminate the added variability of the hatch - emergence lag when modelling sex ratios against length of incubation. Sex ratios of emergent hatchlings from other clutches were then predicted using middle third mean temperature (where known) or IP, as estimated from ID using the average hatch - emergence lag (4 d) observed in clutches with sexed hatchlings (n=40, see also Godfrey & Mrosovsky, 1997). Error bars for IP and subsequent estimated sex ratios were calculated using the min and max observed hatch - emergence lag (0 and 7 d).

Further characteristics of females, nests and hatchlings were modelled using linear modelling and linear mixed-effects modelling in R, as above. Variance components analysis was conducted for mixed-effects models according to Nakagawa & Schielzeth (2013), using package 'MuMIn' (Barton, 2015).

A simple heuristic model was used to predict changes in estimated sex ratio and hatch success for future increases in nest site temperature. Average overall and middle third incubation temperatures were increased in increments of 0.5°C for each nest with known temperature from 2008 to 2012 (n=300, 74% of total nests laid at Alagadi over the same time period). Sex ratio was then re-estimated for each nest, and hatch success capped at 75% for average overall temperatures greater than 33.25°C, 50% for those over 33.5°C and 0% for 33.75°C and above, according to maximum hatch success observed at these temperatures in this study.

## Results

The mean average nest temperature recorded at Alagadi Beach over the study period was 31.2°C (SD: 1.07, range: 28.4-34.1, n=472, hatched nests only). Diel variation in nest temperature was recorded in nests with new model intra-nest temperature logging devices with improved resolution. Daily temperatures fluctuated on average by 0.14°C (range: 0.06-0.31°C, n=102).

Warmer average nest temperatures are associated with shorter incubation, lower maximum hatch success and lowered hatchling fitness (see Fig. 2). Temperature has a negative quadratic relationship with incubation duration (ID;  $f_{(1,469)}=64.252$ ,  $p<0.0001$ ,  $n=472$ ; Fig. 2a) and significant negative effect on average hatchling length (straight carapace length;  $f_{(1,435)}=68.629$ ,  $p<0.0001$ ), width (straight carapace width;  $f_{(1,435)}=120.66$ ,  $p<0.0001$ ) and weight ( $f_{(1,435)}=45.297$ ,  $p<0.0001$ ,  $n=437$  nests; Fig. 2c). A 1°C rise in overall mean incubation temperature caused a reduction in average hatchling size of around 1% (0.59 mm) in length, 2% (0.76 mm) in width and 3% (0.58 g) in weight. Maximum hatch success dropped abruptly at temperatures averaging above 33°C (Fig. 2b), and no nests hatched at  $\geq 34.2^\circ\text{C}$ . Nest depth had a significant negative effect on temperature ( $F_{(1,467)}=20.14$ ,  $p<0.0001$ ,  $n=472$ ), and was a function of female size (Curved Carapace Length;  $F_{(1,838)}=67.03$ ,  $p<0.0001$ ,  $n=840$ ). Larger females therefore laid nests with significantly higher hatch success rates ( $\chi^2_{(1)}=10.41$ ,  $p<0.01$ ,  $n=201$ ), although individual effects accounted for a greater proportion of the model variance than the size effect ( $R^2_m=0.02$ ,  $R^2_c=0.23$ ). Emergence lag was inversely related to the length of incubation (Fig. S2,  $\chi^2_{(1)}=34.188$ ,  $p<0.0001$ ,  $n=40$ ).

Sex was positively identified for 187 hatchlings from 40 nests, with only four nests yielding males. The nest with the greatest number of males identified through histology (six males, ten females) experienced cooler temperatures than average for Alagadi due to its position near an underground stream. The equations below detail the logistic functions describing (1) the temperature - sex ratio relationship and (2) the equivalent incubation period - sex ratio relationship (see Fig. 3):

$$\text{Proportion female} = 1 / ( 1 + \exp^{(78.770 - 2.735 * T)} ) \quad (1)$$

$$\text{Proportion female} = 1 / ( 1 + \exp^{(-38.889 + 0.707 * IP)} ) \quad (2)$$

where  $T$  is the mean temperature of the middle third of incubation ( $^{\circ}\text{C}$ ), and  $IP$  is the incubation period from lay date to hatch date (d).

The estimated pivotal temperature for this population is  $28.8^{\circ}\text{C}$  (95% confidence interval:  $27.8$ - $29.8^{\circ}\text{C}$ ), with the transitional range of temperatures extending from  $27.7$  to  $29.9^{\circ}\text{C}$ . The pivotal  $IP$  is 55 days (lay - hatch date; 95% confidence interval: 54-56 days), with equivalent lay - emergence duration ( $ID$ ) being 55-62 days. The transitional range of  $IP$  expected to give rise to both males and females is 51 to 59 days, with equivalent  $ID$  between 51 and 66 days.

Annual estimated primary sex ratios for Alagadi have remained consistently above 90% female over the study period (see Fig. 4 and Fig. S3; mean  $\pm$  SD:  $97 \pm 2$ , range: 90-100,  $n=20$  years/ 900 nests). Nests laid early in the season experience lower average temperatures than later nests, and have correspondingly longer incubation durations, less extreme sex ratio skew and result in larger hatchlings (see Fig. 5). Variation in estimated sex ratio is also apparent across the different beaches of the wider study area (see Fig. 6a). Beaches 2a and 6 (west coast and Karpaz Peninsula) both have a median  $ID$  of 55 days (the pivotal duration for balanced sex ratio), and durations above the pivotal have been recorded in lower numbers at all but one of the other beaches (Beach 4, north coast). Overall sex ratios ranged from 100% female (Beach 4, north coast, black sand) to 73% female (2a, west coast, white sand), with a mean of 93% female for the four seasons in which all beaches were monitored.

Figure 6b shows the effect of rising temperatures on hatchling production and estimated sex ratio at Alagadi Beach (projected). Current (2008-2012) estimated primary sex ratio at Alagadi is 97% female, with 73% hatch success (excluding clutches that failed entirely). Projected sex ratio exceeds 99% female with a  $1^{\circ}\text{C}$  rise in nest temperature, and hatch success drops to 66%. Further warming reduces hatch success to 55% at  $1.5^{\circ}\text{C}$ , and 42% at  $2^{\circ}\text{C}$ .

## Discussion

Whilst all extant species of marine turtle have survived past shifts in climate spanning millennia, the synergistic effects of the rapidity of contemporary climate change and other anthropogenic stressors threaten their natural ability to adapt (Poloczanska *et al.*, 2009). Nesting beaches around the world are managed to boost hatchling survival and recover population levels from overharvesting, but conservation management strategy is often hindered by a lack of data (Hamann *et al.*, 2010); a global assessment of threats to sea turtle conservation found that two thirds of Regional Management Units (RMUs) were data deficient for adequate assessment of current and future impacts of climate change (Wallace *et al.*, 2011). This study provides detailed information on the effects of incubation temperature on green turtle hatchling development in a Mediterranean nesting population, including estimated primary sex ratios over broad temporal and spatial scales, and thermal effects on hatch success and hatchling phenotype. The pivotal temperature observed here (28.8°C) is close to those of other studied green turtle populations (reviewed in Hawkes *et al.*, 2009), and while the transitional range of temperatures is narrower than elsewhere, the latter has rarely been defined for populations of this species (but see Godley *et al.*, 2002; Godfrey & Mrosovsky, 2006). Estimated primary sex ratios for green turtle populations elsewhere also show female bias (Costa Rica: 67% female, Spotila *et al.*, 1987; Ascension Island: 75% female, Godley *et al.*, 2002; Taiwan: 75-100% female, King *et al.*, 2013; Australia: 94% female, Booth & Freeman, 2006; Philippines: 100% female, De Ocampo & Jaojoco, 1998), except in localities experiencing considerable rainfall during part of the nesting season, making sex ratios more seasonally variable (Guinea-Bissau: 55-85% female, Rebelo *et al.*, 2012; Suriname: 20-90% female, Godfrey *et al.*, 1996).

Primary sex ratios at the main study beach show an extreme female-bias, and nest temperatures approach the upper thermal limit for this species. Future increases in temperature at this beach will lead to feminisation and reduction in hatch success, unless adequate adaptation can occur. Shifts in the seasonality of nesting in response to warming temperatures have been observed in other sea turtle populations (Weishampel *et al.*, 2004, 2010; Pike *et al.*, 2006; Mazaris *et al.*, 2008, 2013), and early results indicate that this may also be occurring at this site (Stokes *et al.* *in prep.*). Other mechanisms which may allow adaptation include maternal manipulation of steroid hormone levels present in egg yolk, which affect the TSD response (Bowden *et al.*, 2000; Navara, 2013), and, on a wider scale, shifts in the spatial distribution of nesting (Pike, 2013).

Molecular analysis allows assessment of operational sex ratios through genetic sampling of nesting females and emergent hatchlings to determine the number of breeding males fathering the offspring cohort. Secondary sex ratios for green turtles at this site (Wright *et al.*, 2012) and elsewhere (South Pacific, Chaloupka & Limpus, 2001; North Atlantic, Lasala *et al.*, 2013) were found to be male-biased, despite a heavy female bias in neonates. Juvenile and adult loggerhead turtles within the Mediterranean also show far less female bias than expected from hatchling sex ratios (Casale *et al.*, 2005, 2006, 2014; Maffucci *et al.*, 2013; Rees *et al.*, 2013), and a similar pattern has been found in an Atlantic population (Delgado *et al.*, 2010), as well as in leatherback turtles (Caribbean, Stewart & Dutton, 2014). Buffering against female skew may arise through inter-beach variation in incubation conditions (Hawkes *et al.*, 2007), mating behaviour (Hays *et al.*, 2010; Wright *et al.*, 2012), and differential mortality between the sexes (Burger & Zappalorti, 1988). Adult sex ratios in long lived animals such as sea turtles can be highly sensitive to slight differences in survival between males and females (Girondot & Pieau, 1993); low numbers of males produced during the cooler, early season and on cooler beaches may have higher survival probability due to favourable incubation conditions for increased body size and locomotory speeds. Rebelo *et al.* (2012) found no sexual dimorphism in histologically sexed green turtle hatchlings, although this was based on one hatchling per clutch, and maternal effects such as egg mass and quality have been shown to have a stronger influence on hatchling morphology while nest temperature has greater influence on locomotor ability (Booth *et al.*, 2012).

Although there is near to complete hatchling feminisation at Alagadi Beach, male producing refugia exist at the beginning of each breeding season, within microclimates on the beach, and at nearby nesting beaches with differing sand albedo and topography (see also Mrosovsky *et al.*, 1984; Spotila *et al.*, 1987). Our finding that of the histologically sampled nests, the least female-biased experienced cooler temperatures due to its proximity to an underground stream highlights the importance of microclimate for male hatchling production even on beaches with little or no shading. Sand albedo is a major determinant of nest temperature (Hays *et al.*, 2001; Weber *et al.*, 2012), and predicted sex ratios from the darker sand of the north coast beaches are far more female-skewed than those of the lighter sand of the Karpaz Peninsula and west coast, which are important male producing habitat. Two portions of beach (2a and 2b, west coast) show a significant discrepancy in estimated hatchling sex ratio (90% cf. 73% female) despite their close proximity and apparently similar sand type, demonstrating the probable additional effects that beach topography and tendency to washover have on sand temperatures.

The strong correlation of nest depth at Alagadi with female size indicates that in this population, females nest at the maximum depth possible for their reach (seemingly apparent from visual observation). Older, larger nesters are therefore of particular importance not only for their greater reproductive output (Wallace *et al.*, 2008), but also due to the increased depth and reduced temperatures of their nests, with positive effects on both hatch success and sex ratio. This effect may slow the recovery of this population, as the rising numbers of new nesters (Stokes *et al.*, 2014) will have relatively shallow nests and lower hatch success until they reach sufficiently large size for deeper nest excavation.

Primary sex ratio monitoring in threatened reptile populations will be of increasing importance as the world's climate continues to change. Use of natural mortality hatchlings to estimate the sex ratio of those that emerged is often employed in cases where lethal sampling is particularly undesirable, and may introduce a systematic bias if the sex ratio of dead hatchlings does not reflect that of successful emergents. This is particularly relevant in light of potential fitness differences between the sexes. Future investigations employing lethal sampling for sex ratio estimation and curve delineation from more abundant breeding aggregations should additionally sample dead full term hatchlings, for validation of non-lethal methods in existing and future research (see Rebelo *et al.*, 2012). A greater number of nests containing males would have strengthened our sex ratio models, as they suffer a bias of data towards the warm temperature/ short incubation end. This will always be problematic where naturally incubating nests from beaches towards the upper thermal limits of incubation are sampled, an effect exaggerated by the loss of a high proportion of samples to decomposition (it was not possible to reliably ascertain sex for 57% of samples processed in the current study).

Agreement between sex ratio predictions based on temperature and incubation length data was higher when the hatch-emergence lag was allowed for. Use of temperature data to better estimate the incubation period is ineffectual if the purpose is to broaden the scope of predictions beyond the number of temperature recording devices. However, using temperature-defined hatch dates for our experimental (histologically sampled) nests increased the accuracy of the sex ratio model curve, from which broader predictions were made. Estimations of incubation periods based on temperature data are therefore beneficial when defining relationships from a restricted sample to be used in making wider predictions.

Green turtle nests in the Mediterranean were not previously thought to experience diel variation in temperature (Kaska *et al.*, 1998; Broderick *et al.*, 2000), but improved temperature

logging device resolution has shown this does occur in turtles of this size, although it is slight. Average nest temperature is a simplified descriptor for naturally occurring temperature regimes, which contain varying degrees of fluctuation on a daily basis as well as over longer timescales, and the influence of thermal variance on sex ratios and hatchling phenotype is receiving increasing research attention (Ashmore & Janzen, 2003; Neuwald & Valenzuela, 2011; Georges, 2013; Refsnider, 2013). At our study site, nest temperatures were commonly reduced for up to 14 days following a washover event; such events affecting nests within the thermosensitive period are important in reducing temperatures over a sustained period, affecting fitness and sex ratio of developing embryos. An early study of map turtles (*Graptemys*, family Emydidae) found that males were more easily induced by isolated bursts of low temperatures than females were by bursts of high temperatures (Bull, 1980), indicating that even brief temperature reductions caused by washover may have substantial effect on female sex ratio skew. Washover was found to be an important masculinising factor for loggerhead turtle nests in Greece (Margaritoulis & Rees, 2006), although hatch success can be reduced depending on the level of overwash and drainage conditions (Caut *et al.*, 2010).

Species' ability to track suitable climate geographically is limited by anthropogenic conversion of the landscape, and the additive effects of habitat loss and rapid climate change should not be underestimated (Hof *et al.*, 2011). Conservation aims for sea turtles should be to restore depleted populations and prevent further habitat loss in order to increase their natural resilience to climate change. Identification of male producing nesting beaches will aid in conservation strategy (e.g. Fuentes *et al.*, 2010), particularly in regions such as the Mediterranean where highly female-biased primary sex ratios are the norm (Kaska *et al.*, 1998, 2006; Broderick *et al.*, 2000; Casale *et al.*, 2000; Godley *et al.*, 2001; Fuller *et al.*, 2013). Future sea turtle conservation efforts may require more active management in climate impact mitigation (Fuentes *et al.*, 2011; Patino-Martinez *et al.*, 2012), but crucial questions remain regarding optimal sex ratios, sex specific survival rates, and the buffering effects and adaptive capacity of sea turtle reproduction. All conservation endeavours are underpinned by a need to reverse the trend in greenhouse gas emissions.

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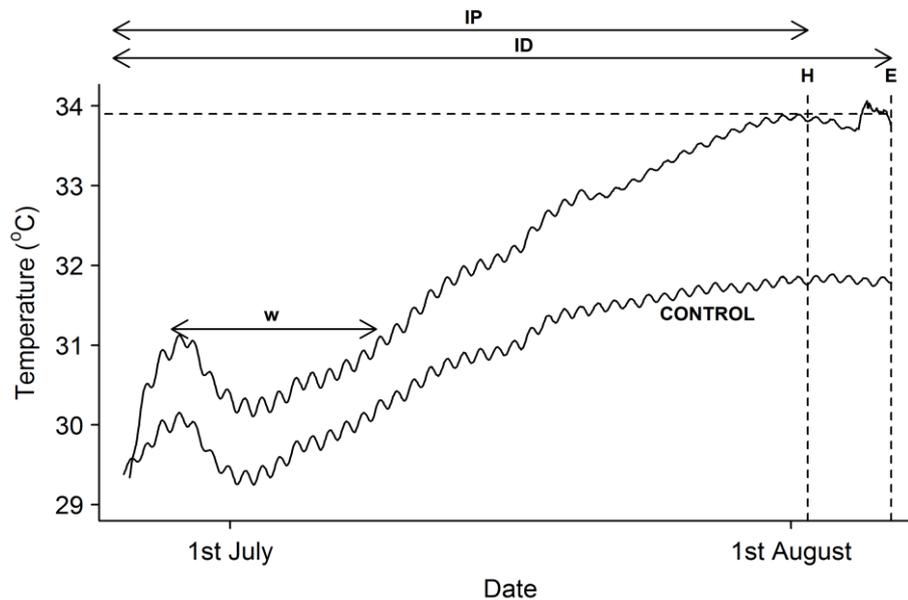
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#### Chapter IV: green turtle sex ratios

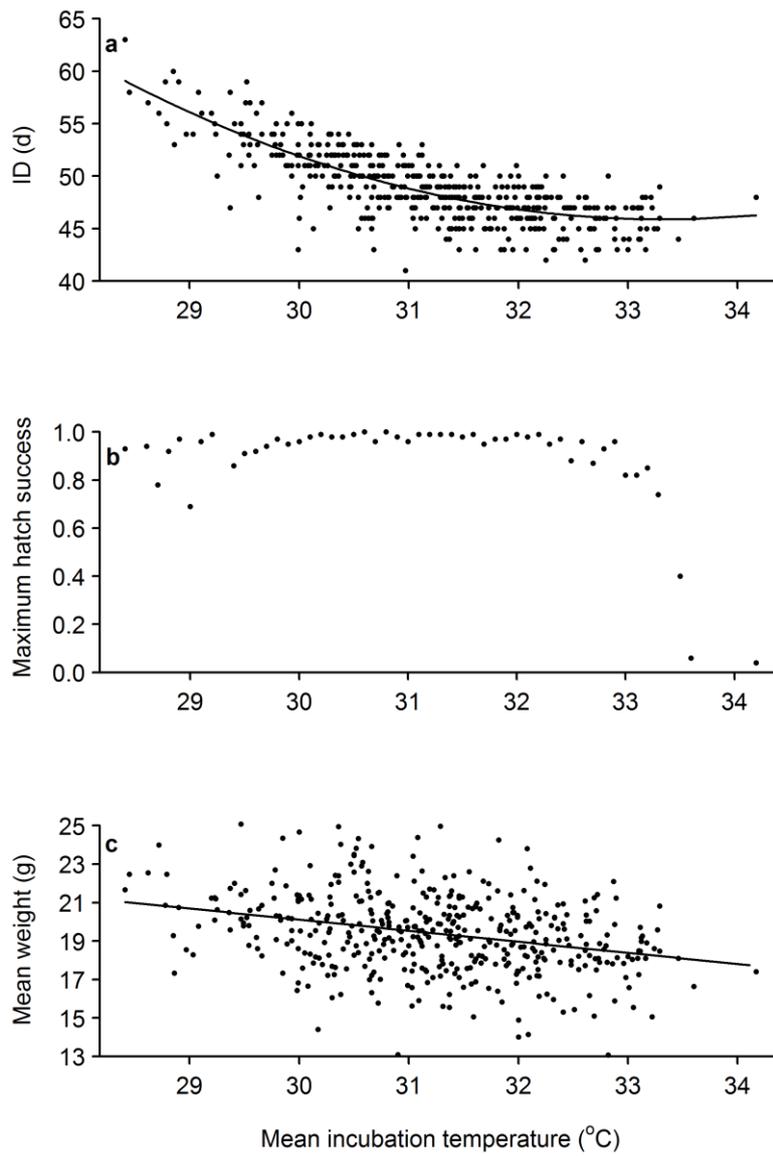
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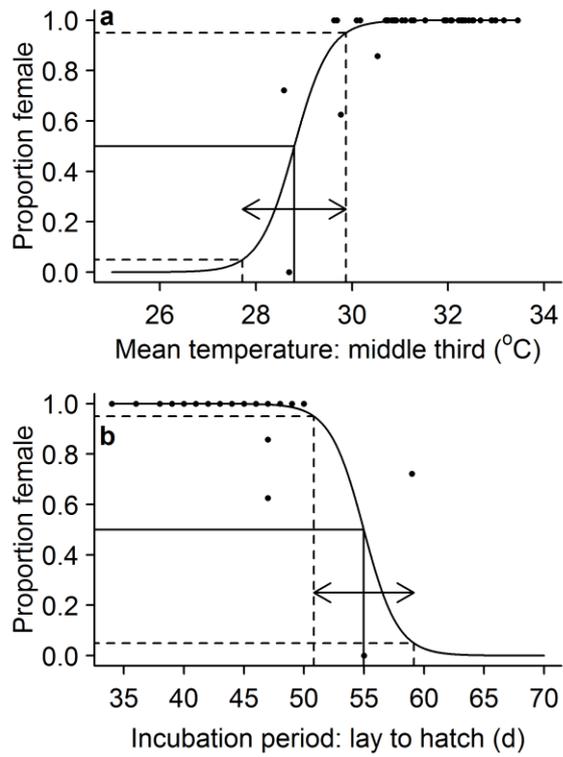
## Figures



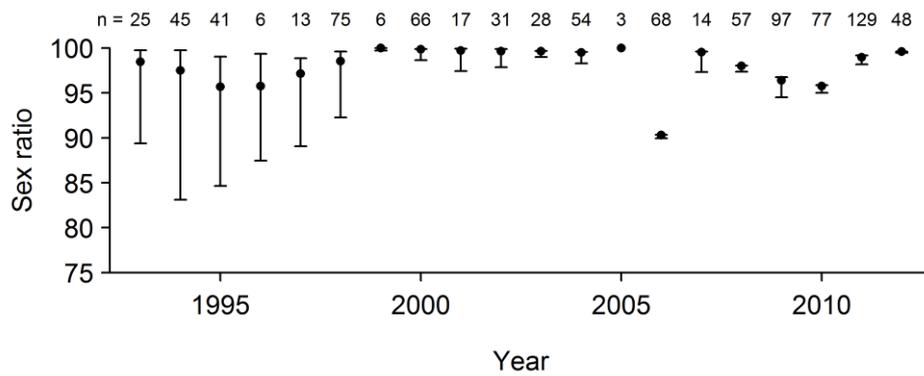
**Figure 1.** Example nest temperature graph showing diel variation, metabolic heating, washover, hatching and emergence characteristics (upper line). The lower line shows temperatures recorded over the same time period by a control temperature logger, buried at mean nest depth on the same beach. Hatch date (H) in this case is five days prior to emergence date (E), and is identified through the cessation of metabolic heating within the nest. The spike that usually follows arises from the flurry of activity as hatchlings scramble to rise up through the sand column away from the egg chamber. Incubation duration (ID) is commonly measured as lay date to emergence date, whereas the incubation period (IP) is the difference between the lay and hatch dates. Arrow labelled 'w' indicates period of cooler temperatures following a washover event.



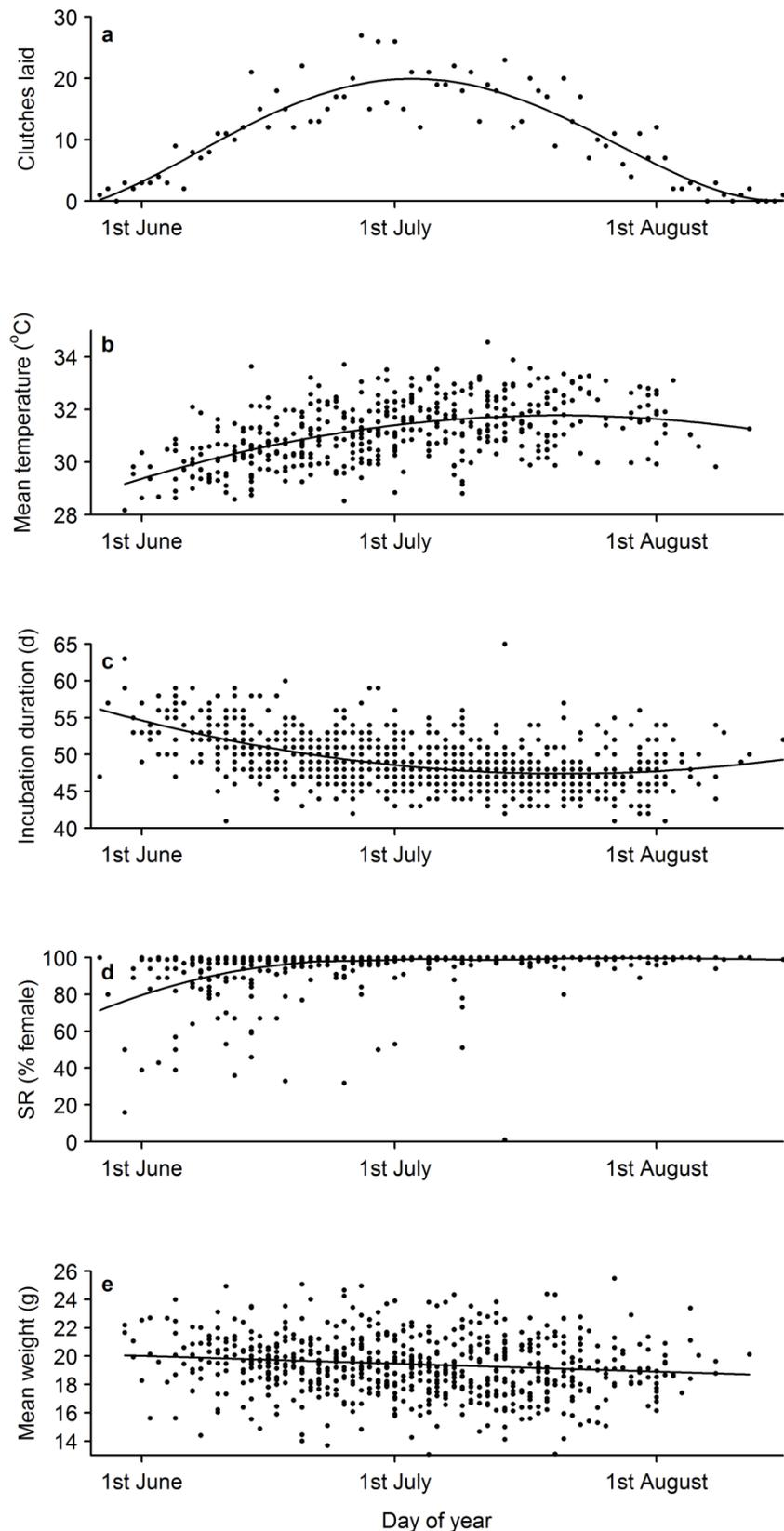
**Figure 2.** Effects of mean incubation temperature on **a)** incubation duration (ID; n=472), **b)** maximum hatch success (n=472) and **c)** mean weight of hatchlings (n=437), Alagadi Beach, 1995-2012.



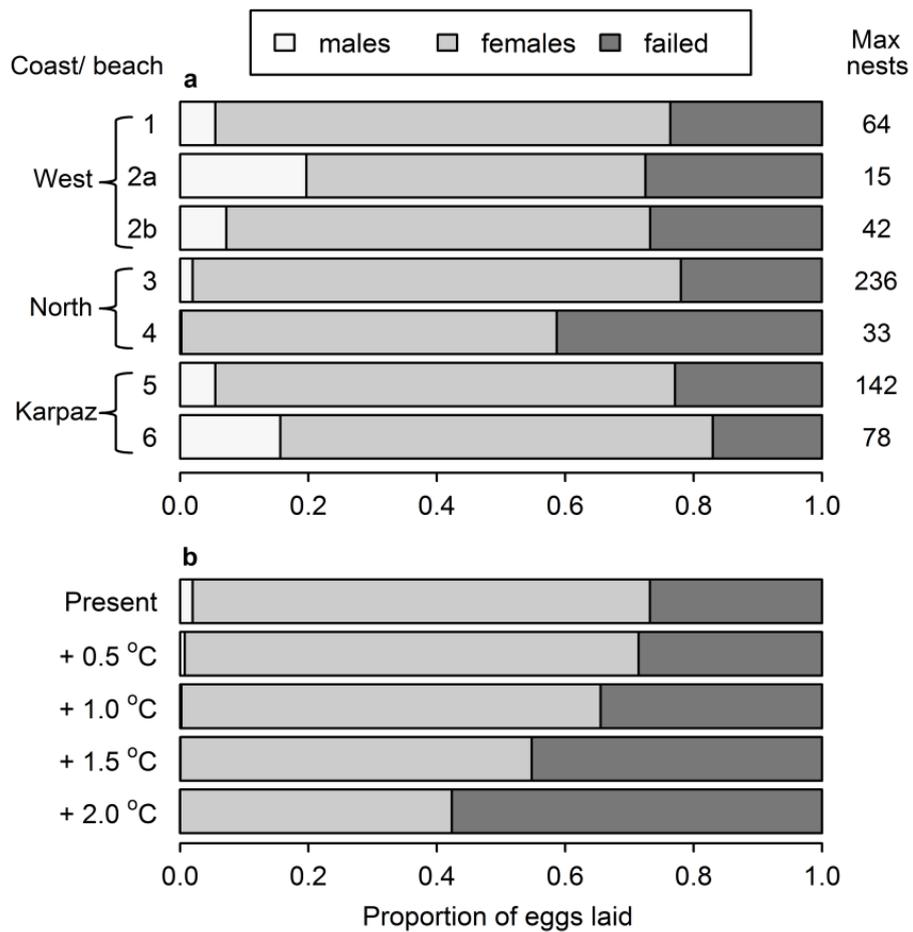
**Figure 3.** Hatchling sex ratio as a function of **a)** middle third mean temperature and **b)** incubation period. Solid lines show  $P$  (pivotal temperature or incubation period). Dashed lines and arrows indicate transitional range of temperatures or incubation periods.



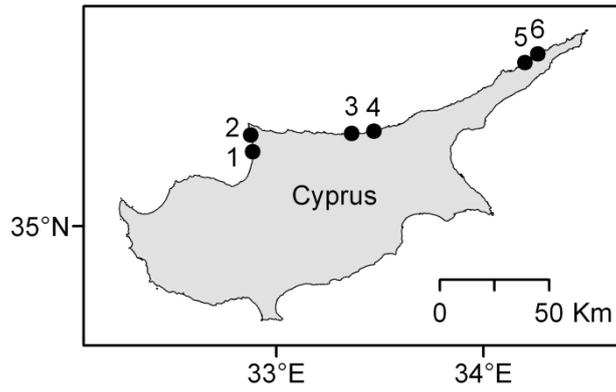
**Figure 4.** Estimated sex ratios (percentage female) of green turtle hatchlings at Alagadi Beach through time, predicted using temperature data for those nests in which intra-nest temperature was recorded (n = 472) and incubation duration of nests without temperature data (n = 428). See Fig. S3 for breakdown of data sources.



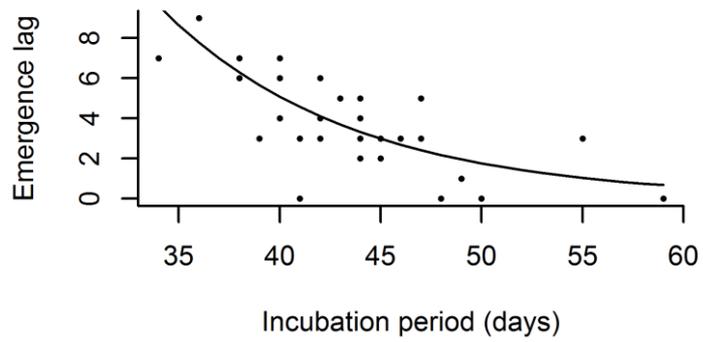
**Figure 5.** Effect of lay date on **a)** magnitude of nesting (n=900), **b)** mean intra-nest temperature (n=472), **c)** incubation duration (n=900), **d)** estimated sex ratio (n=900) and **e)** mean hatchling weight (n=678), Alagadi Beach, 1993-2012.



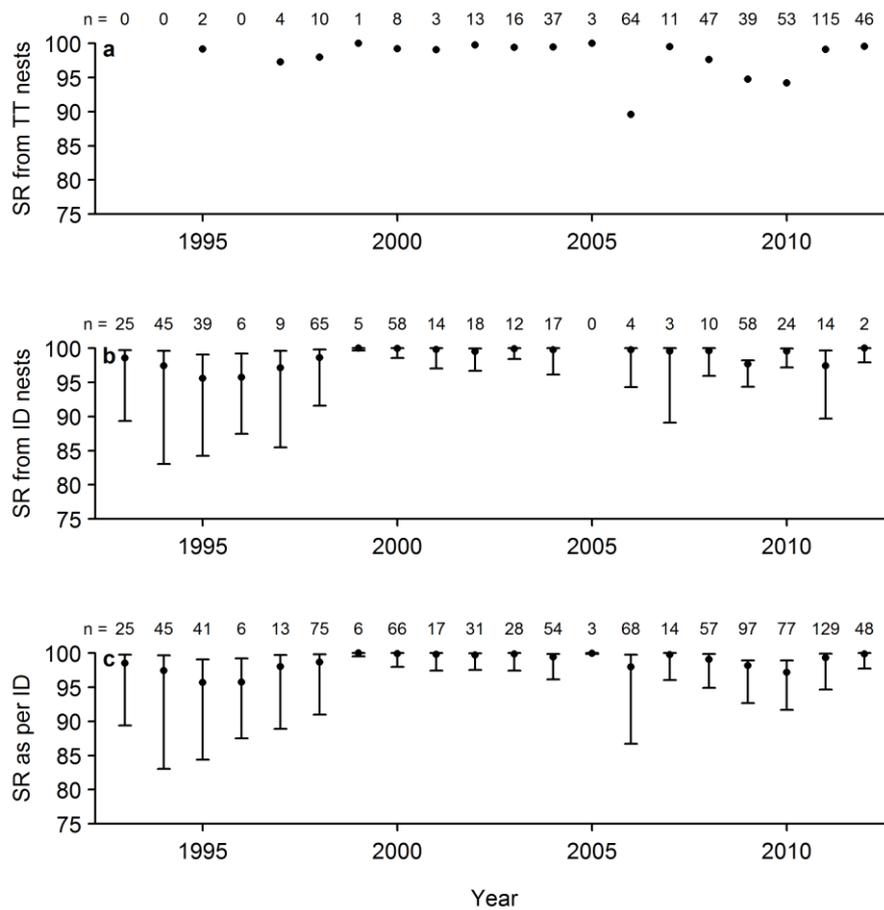
**Figure 6. a)** Variation in sex ratio and hatch success (hatched nests only) at the top six green turtle nesting beaches of northern Cyprus, and **b)** projected changes in sex ratio and hatch success under climate warming scenarios (nests with known temperature - Beach 3/ Alagadi only).



**Figure S1.** The six major green turtle nesting beaches of northern Cyprus. West coast: 1. West beaches, 2. Lost & Message; north coast: 3. Alagadi, 4. Esentepe; Karpaz Peninsula: 5. Ronnas, 6. Ayfilon.



**Figure S2.** The number of days between hatching and emergence is inversely related to the length of incubation (n=40).



**Figure S3.** Estimated sex ratios (percentage female) of green turtle hatchlings at Alagadi Beach over time, as predicted using **a)** temperature data for those clutches with intra-nest temperature recording devices ('TT nests', n=472), **b)** incubation duration data for those clutches for which temperature data is not available ('ID nests', n=428), and **c)** all clutches based on incubation duration data only (n=900). See Fig. 4 for combined results.

## Chapter V

### **Climate forcing affects reproductive frequency and phenology in a long distance marine migrant**

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**Abstract**

Biological systems around the globe are responding to climate warming through physiological changes and spatio-temporal shifts in distribution. Two decades of monitoring at an intensively surveyed site in combination with extensive satellite tracking effort allow us to relate foraging conditions to breeding output for a green turtle population in the Mediterranean, demonstrating carry-over climate forcing effects on sea turtle nesting dynamics at the individual and population level. Reproductive output in terms of both clutch frequency and remigration interval fluctuates in response to summer and winter temperatures at foraging grounds, driving annual magnitude of nesting at the breeding site. This variability in chelonid fecundity complicates the detection of population trends, and necessitates individual-based monitoring at regional index sites. Additionally, the onset, median date and final date of nesting are undergoing a phenological shift towards cooler temperatures earlier in the season in response to increasing pre-migratory temperatures at foraging sites. This may ameliorate the impact of nesting beach temperature rises that threaten to disrupt sex ratios and reduce hatch success and hatchling fitness, although it is unknown whether this buffering effect can keep pace with modern climate change. Both major findings emphasise the need for long-term monitoring of climate responses.

## Introduction

The world's climate has warmed by approximately 0.8°C over the last century, and is expected to continue to do so at an accelerated rate over the next century and beyond (Stocker et al., 2013). Ecological responses include alterations to physiology, productivity, behaviour, species distributions, abundance and community dynamics (e.g. Walther *et al.* 2002). Changes in phenology, or the timing of recurring seasonal activities and life-cycle events such as flowering, arrival of migrant species and egg laying, are particularly well documented across widespread regions, systems and taxa, and are overwhelmingly in the direction expected from recent global temperature rise (Parmesan & Yohe, 2003; Root et al., 2003; Menzel et al., 2006; Parmesan, 2007). Phenological shifts allow adaptation to a rapidly warming climate, but can also lead to the decoupling of favourable conditions or phenological relationships, causing mistimed migrations and breeding events (Miller-Rushing et al., 2010; Burthe et al., 2012). Changes in ecosystem productivity and individual physiology associated with climatic conditions can also alter reproductive frequency or brood size (e.g. Aebischer *et al.* 1990; Roy & Sparks 2000).

The marine realm may be particularly vulnerable to the deleterious effects of climate change because the great majority of energy accumulation in the global climate system is absorbed by the world's oceans (Stocker et al., 2013). In addition, the tendency for ocean warming to exacerbate consequences of anthropological mismanagement is pushing species and systems towards collapse (Walther et al., 2002). Long-lived species are particularly susceptible to overexploitation (Heppell et al., 2003) and also likely have fewer avenues of adaptation to climate change (Harte et al., 2004). Additionally, the nature of the marine environment complicates the monitoring of population dynamics due to the obvious difficulties in observing animals at sea, particularly those with extensive ranges and vast migratory movements. Marine species of commercial value or conservation concern are often monitored via proxies such as fisheries catch per unit effort or seasonal breeding aggregations (e.g. long-finned squid *Loligo forbesi*: Pierce *et al.* 1994; emperor penguin *Aptenodytes forsteri*: Barbraud & Weimerskirch 2001). For non-annual breeders, population indices derived from seasonal aggregations must be interpreted with caution: an individual's readiness to breed can be affected by conditions during the preceding non-breeding period (food availability, weather etc.), which can lead to population-level fluctuations in breeding output which do not reflect underlying demographic trends (Jenouvrier et al., 2005a, 2005b).

Sea turtles are ectothermic marine migrants characterised by longevity and multiple distinct life history stages, and are vulnerable to numerous climatic threats (Poloczanska et al., 2009), and many populations are recovering or still suffering from historical or current mismanagement through direct take or unsustainable levels of bycatch (e.g. McClenachan *et al.* 2006; Wallace *et al.* 2011). Sea turtle population size is often monitored via nest counts, which provide a simple, low cost index to estimate trends in the number of reproductively active females (Stokes et al., 2014). Most species undergo a lengthy, energetically expensive migration to reach breeding grounds and therefore must attain a threshold body condition before entering into a migration/ breeding phase (Broderick et al., 2001; Rivalan et al., 2005). Individuals lay between one and six clutches per season and may breed again after two to five years, and so a sound understanding of breeding frequency and output is necessary in order to interpret sea turtle nesting trends (Hays, 2000; Weber et al., 2013).

The low trophic status of the herbivorous green turtle presents a near direct link between environmental conditions and individual performance, driving large inter-annual variability in nest numbers (Broderick et al., 2001). This has as yet obscured detection of phenological shifting in this species (Pike, 2009; Weishampel et al., 2010; Dalleau et al., 2012). The use of an extensive tracking dataset in combination with long-term individual-based monitoring at a major nesting site in northern Cyprus allows us to examine the climate forcing effects of foraging conditions experienced during the interval between breeding seasons, and their influence on breeding frequency, output and seasonal timing.

## Methods

The study system is a Mediterranean nesting rookery of female green turtles and their hatchlings, for which longitudinal data are available. Twenty one post-nesting green turtles were tracked from Alagadi nesting beach in northern Cyprus between 1998 and 2010 via the Argos satellite tracking system, using Platform Terminal Transmitters (PTTs; Sirtrack: n=17, Wildlife Computers: n=3, Telonics: n=1; for further device/ deployment details see Chapter II Table S2; for details of transmitter attachment see Godley *et al.* 2002). Locations were downloaded and managed using the Satellite Tracking and Analysis Tool (STAT; Coyne & Godley 2005), and were processed and mapped using R version 2.14.2 (R Development Core Team, 2012), ArcGIS 10.1 and Geospatial Modelling Environment (GME). Tracks were filtered for unreliable location classes (O and Z), and fixes inferring implausible speeds ( $>5\text{km}^{-1}$ ) and turning angles ( $>25^\circ$ ; migration only). A Best Daily Location (BDL) was then taken for each track to remove any temporal bias.

Kernel Density Estimation (KDE) was used to delineate home ranges within foraging grounds for post-migratory tracks (n=20). Fifty percent isopleths were chosen with reference bandwidths (href) in order to highlight core areas of high use. Dominant foraging areas were defined according to Stokes *et al.* (2015, see Chapter II): foraging grounds in Bomba (Libya), Sirte (Libya) and Antalya (Turkey) account for 66% of tracked individuals (see Fig. 1).

Average monthly sea surface temperature (SST) was determined for kernel centroid locations from the Advanced Very High Resolution Radiometer (AVHRR) and Near Sea Surface Temperature (NSST) datasets available from the National Oceanic and Atmospheric Administration (NOAA)'s National Environmental Satellite, Data and Information Service (NESDIS). Yearly averages were taken from May-April, representing the twelve months prior to each breeding season, and seasonal averages were taken for summer (May-October), winter (November-February) and spring (March-April). Seasons were defined according to general weather patterns in Libya and Turkey, in combination with estimated outbound and documented return migration patterns for this rookery (see Chapter II).

SSTs were modelled against long term nesting datasets for Alagadi (see Stokes *et al.* 2014) using R (R Development Core Team, 2012) with packages 'lme4' (Bates et al., 2011) and 'nlme' (Pinheiro et al., 2012), and variance components analyses were conducted for mixed-effects models according to Nakagawa & Schielzeth (2013) using package 'MuMIn' (Barton, 2015).

Tracked individuals nesting sporadically at Alagadi (n=2, remigration intervals of eight and ten years indicating a high chance of additional undetected nesting elsewhere), or not returning to Alagadi in any subsequent breeding season (n=2), were excluded from the analysis. One individual nested regularly for three breeding seasons, and then returned for a fourth breeding season after a ten year remigration interval; in this case the first three seasons were used and the fourth excluded. Clutch frequencies were corrected where long internesting intervals indicate additional, undetected nesting elsewhere within a season according to Broderick *et al.* (2002; expected clutch frequency, ECF: additional clutches are counted where an individual re-nests after a period corresponding to multiple internesting intervals). ECF and remigration intervals were modelled using generalized linear mixed modelling (GLMM) with Poisson error structure and random effects to account for variation across individuals and foraging sites. Breeding output at the population level was modelled using generalized least squares (GLS) to account for temporal autocorrelation in the time series (see Stokes *et al.* 2014).

## Results

Data from a total of 76 breeding seasons were included in the models, an average of 4.5 seasons per individual (range: 3-8). The majority of remigrant breeding occurred after remigration intervals of three or four years (76%, n=63).

### Nesting phenology

There has been a clear increasing trend in April SST experienced by individuals at their respective foraging sites over the study period (mixed-effects model accounting for variation across sites;  $\chi^2_1 = 8.67$ ,  $p < 0.01$ ,  $n = 76$ ; see Fig 2a. The random effect 'site' accounted for around half of the variance explained by the model:  $R^2_m = 0.11$ ,  $R^2_c = 0.23$ ). There has been a corresponding shift towards earlier nesting of tracked individuals, with first lay date per individual significantly affected by this downward linear trend over time, April SST at the foraging site, neophyte/remigrant status and the interactions between all three of these variables (mixed-effects model accounting for variation across individual females and across foraging sites, zero truncated with poisson error structure due to poisson-shaped distribution of first lay date of tracked individuals;  $\chi^2_1 = 4.37$ ,  $p < 0.05$ ,  $n = 76$ ; see Fig 2b.  $R^2_m = 0.22$ ,  $R^2_c = 0.52$ ). Remigrant females ( $n = 63$  breeding seasons) commence each laying season on average ten days earlier than first time nesters ( $n = 13$  breeding seasons).

At the population level, the onset of nesting each year is correlated with the magnitude of nesting for that year, such that big nesting years start early (minimum lay date:  $r_{(19)} = -0.829$ ,  $p < 0.001$ ; fifth percentile lay date:  $r_{(19)} = -0.828$ ,  $p < 0.001$ ; median lay date:  $r_{(19)} = -0.654$ ,  $p < 0.01$ ). As a result, the oscillatory pattern seen in nesting numbers (Stokes et al., 2014) that is characteristic of green turtle nesting is also significant for the first lay date of the season (autocorrelation at one year time lag:  $\chi^2_1 = 16.3$ ,  $p < 0.001$ ). Taking this into account, there has been a significant linear downward trend in the date of first nest over the twenty year study period (see Fig. 2c), interacting with significant effects of April SST at the three major foraging sites ( $\chi^2_1 = 26.81$ ,  $p < 0.001$ ). This model is also significant for the median lay date (downward trend:  $\chi^2_1 = 44.0$ ,  $p < 0.001$ ; autocorrelation:  $\chi^2_1 = 39.4$ ,  $p < 0.001$ ), and final lay date of the season (downward trend:  $\chi^2_1 = 11.4$ ,  $p < 0.05$ ; autocorrelation:  $\chi^2_1 = 5.52$ ,  $p < 0.05$ ), indicating a shift towards earlier nesting rather than an extension of season length (no linear trend in season length:  $F_{(19,20)} = 1.21$ ,  $p = 0.3$ ; no autocorrelation in season length:  $\chi^2_1 < 0.001$ ,  $p = 1$ ).

### Reproductive output

Summer temperatures increased over the study period at two of the three major foraging sites (Sirte:  $F_{(18,19)}=4.86$ ,  $p<0.05$ ; Antalya:  $F_{(18,19)}=12.6$ ,  $p<0.01$ ). Average winter temperatures have increased only at Antalya ( $F_{(18,19)}=6.42$ ,  $p<0.05$ ).

Average temperature experienced by individual females during the second full summer of foraging has significant effect on remigration interval (RI:  $\chi^2_1=34.494$ ,  $p<0.001$ ) and expected clutch frequency (ECF:  $\chi^2_1=10.2$ ,  $p<0.01$ ), whilst accounting for variation across individuals and foraging sites (although these random effects accounted for zero variation in the models), with warmer temperatures shortening RI but also reducing ECF. A modelled average temperature increase of  $0.56^\circ\text{C}$  experienced by a female in her second full summer on the foraging ground was associated with increased probability of breeding the following summer (i.e. reducing RI from four to three years; mean  $\pm$  SD temperature based on raw data for four-year remigrants:  $24.4 \pm 0.67^\circ\text{C}$ , three-year remigrants:  $24.8 \pm 0.61^\circ\text{C}$ ). (NB: females may spend the bulk of the summer season of a breeding year away from the foraging ground. It must therefore be assumed that a female with an RI of three has only had two full foraging summers.) The corresponding reduction in ECF further demonstrates the reproductive frequency vs. output trade-off (see Rivalan *et al.* 2005, Stokes *et al.* 2014).

Summer temperatures at the three major foraging grounds and all interactions between them have significant effect on the overall number of nests at the study beach during the following nesting season ( $\chi^2_1=5.43$ ,  $p<0.05$ , see Fig. 3), whilst accounting for the increasing trajectory and temporal autocorrelation seen in this nesting population (see Stokes *et al.* 2014). This is also true of winter temperatures at the three main foraging sites ( $\chi^2_1=10.5$ ,  $p<0.01$ ).

## Discussion

With increasing access to an abundance of freely available remote sensing data, animal tracking can give insight into environmental conditions experienced by migratory populations during otherwise unseen lifecycle stages, allowing examination of climate forcing carry-over effects operating over vast distances (e.g. Inger *et al.* 2010). In this case, fine-scale home range delineation in combination with extensive tracking effort over many years allows us to describe effects at both the individual and population level, identifying the drivers behind nesting beach responses to directional and fluctuating climate forcing.

Understanding the multitude of climatic influences driving sea turtle demographics is fundamental for effective monitoring and management of threatened populations into the future. Despite the significant interannual variation characteristic of green turtle nesting dynamics, there are clearly thermal influences on both the magnitude and timing of nesting. Green turtle nesting phenology at this site reflects climate-forcing effects at the three major Mediterranean foraging grounds. A gradual shift towards earlier nesting is evident, with a corresponding increase in pre-migration temperatures at foraging sites likely acting as a cue or trigger for migration. Annual magnitude of nesting is determined in part by preceding summer and winter foraging conditions (Limpus & Nicholls, 1988), driven by an individual female's ability to vary the interval between breeding seasons according to the attainment of a threshold body condition required for the energetic investment of migration and reproduction (Solow *et al.*, 2002).

### Nesting phenology

Arguably the most immediate climatic threat to sea turtle populations is extreme sex ratio skew, complete feminisation of populations, and reduced hatch success from raised nesting beach sand temperatures (e.g. Patino-Martinez *et al.* 2012). The rapidity of contemporary climate change leaves long-lived species less likely to be able to respond through selective forces (Harte *et al.*, 2004), and the modern landscape presents much reduced opportunity for many species to adapt spatially. Phenological shifting therefore represents an important buffering mechanism for late maturing species dependent on particular habitats; shifts in the seasonality of nesting could potentially allow turtles to track suitable incubation conditions as global temperatures rise (Hawkes *et al.*, 2007). The link between nesting phenology and fluctuating temperatures has been well documented in the loggerhead turtle (*Caretta caretta*; Hawkes *et al.* 2007; Mazaris *et al.* 2009, 2013; Weishampel *et al.* 2010; Lamont & Fujisaki

2014), and a temporal shift in phenology towards earlier nesting over time has been documented in two loggerhead populations (Atlantic: Pike *et al.* 2006; Mediterranean: Mazaris *et al.* 2008). In green turtles, the previous lack of evidence for a shift in nesting phenology had raised concern that this species was unresponsive to changing environmental cues (Pike, 2009), although median nesting date has been found to be correlated with fluctuating sea surface temperature in an Atlantic population (Weishampel *et al.*, 2010), and a temperature-driven spatial pattern of nesting seasonality has been demonstrated in a regional comparative analysis in the South West Indian Ocean (Dalleau *et al.*, 2012). This first evidence of green turtle adaptation to changing climatic conditions highlights the need for further understanding of naturally occurring adaptive processes in chelonian reproduction. The seasonal shift observed here is in the opposing direction to that expected from the increasing proportion of new nesting females recruiting into this breeding population (see Stokes *et al.* 2014), further substantiating that this is indeed a phenological response to gradually increasing temperature. Modelling exercises have questioned the capacity of such compensatory mechanisms to enable this taxa to keep pace with contemporary rapid climate change (leatherback turtles *Dermochelys coriacea*: Saba *et al.* 2012; painted turtles *Chrysemys picta*: Telemeco *et al.* 2013); this phenological buffer may delay extreme feminisation and reduced hatch success, but it may or may not be sufficient to keep up with future temperature rises.

The occurrence of earlier laying in remigrant females further exaggerates the effect of increasing reproductive value with age, due to the more favourable conditions early in the season for higher hatch success and hatchling fitness (see Chapter IV: larger females lay deeper nests with higher hatch success). Seasonal and size differences in older females compared to new recruits also increase their output of male hatchlings, thus highlighting their importance in the face of rising beach temperatures.

#### Reproductive output

The responsiveness of individual remigration intervals to environmental stochasticity was originally proposed to explain the high interannual variability in green turtle nesting by Carr & Carr in 1970. Since then, warm El Niño events have been shown to boost nest numbers two years later for the Great Barrier Reef stock (Limpus & Nicholls, 1988, 2000), and nesting levels in Southeast Asian populations have been shown to follow fluctuations in the Southern Oscillation Index (SOI; Chaloupka 2001). An equivalent effect at the individual level showed higher SSTs in the second post-breeding winter to reduce remigration intervals in the green turtle nesting cohort at Tortuguero, Costa Rica (remigration intervals in this locality are predominantly two or three years; Solow *et al.* 2002). Leatherback and loggerhead turtles

have shown an opposing response, with cool El Niño Southern Oscillation (ENSO) events in the eastern Pacific reducing remigration intervals in leatherbacks (Saba et al., 2007; Reina et al., 2009) and high average sea surface temperatures one (Pacific: Chaloupka *et al.* 2008) or two years (Mediterranean: Mazaris *et al.* 2009) prior to the nesting season leading to reduced breeding output in loggerheads. Differences in the direction of climate forcing are due to the trophic level at which species forage, with warm temperatures indicating good general conditions for seagrass growth, and cool upwelling events and fronts associated with high productivity in loggerhead and leatherback prey. Similarly, Kitaysky & Golubora (2000) describe the opposing trends in reproductive success of planktivorous auklets and piscivorous puffins in response to changes in ocean temperatures in Siberia. Directional changes in prevailing environmental conditions associated with climate change have the potential to mask population trends where index monitoring is used to estimate abundance (Broderick et al., 2001; Chaloupka, 2001).

Climate change necessitates more frequent monitoring of both individual and population level parameters (Lepetz et al., 2009). The results found here reinforce the need for long-term monitoring schemes and individual-based monitoring at index sites to monitor reproductive frequency and output at the regional scale. This may be broadly applicable to conservation efforts for capital breeders with iteroparous breeding and/or modulated breeding periodicity.

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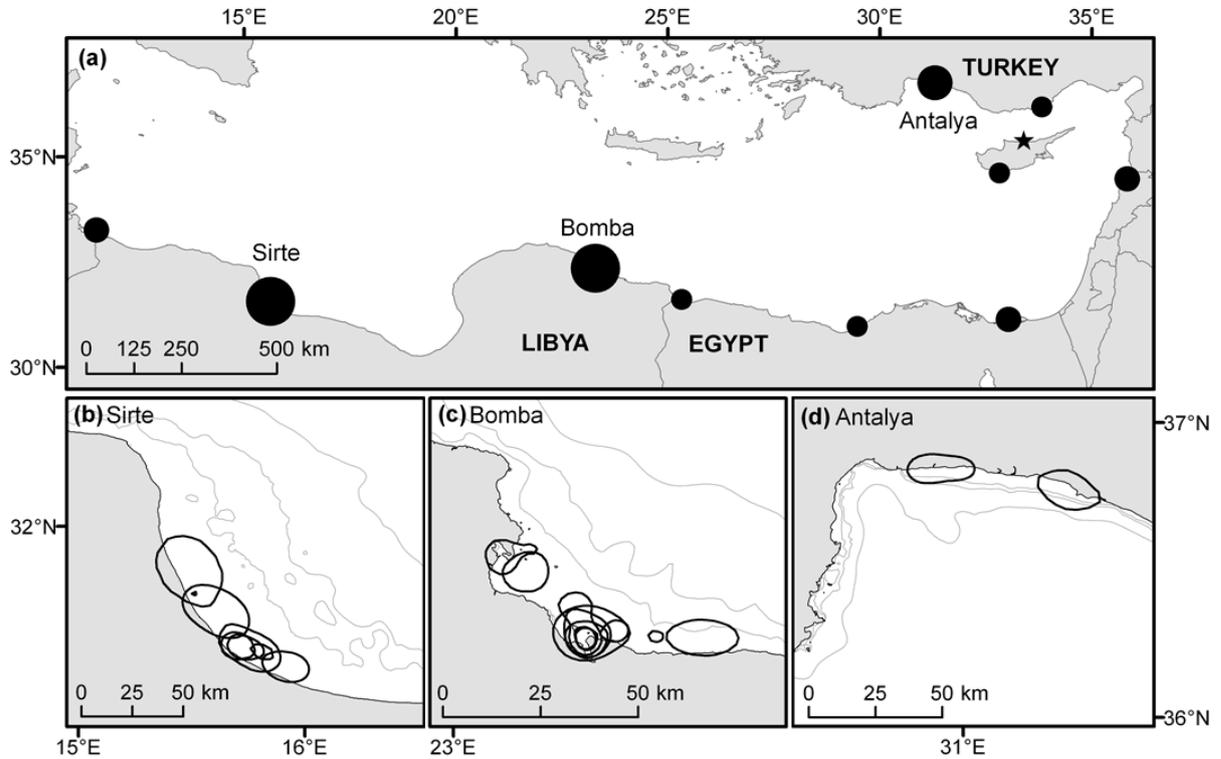
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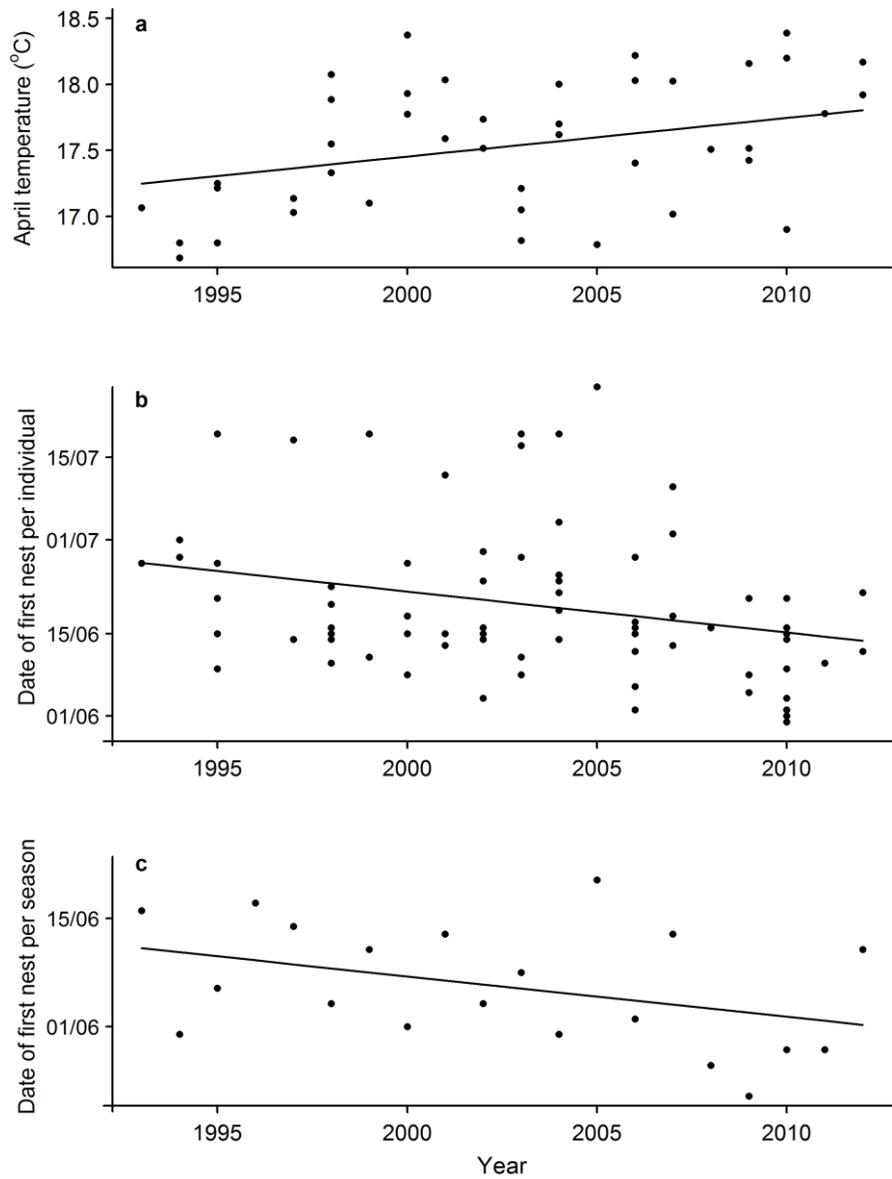
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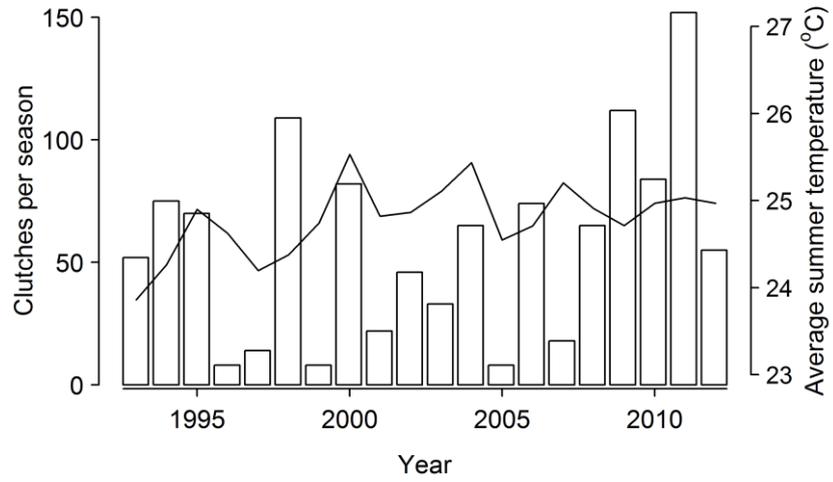
## Figures



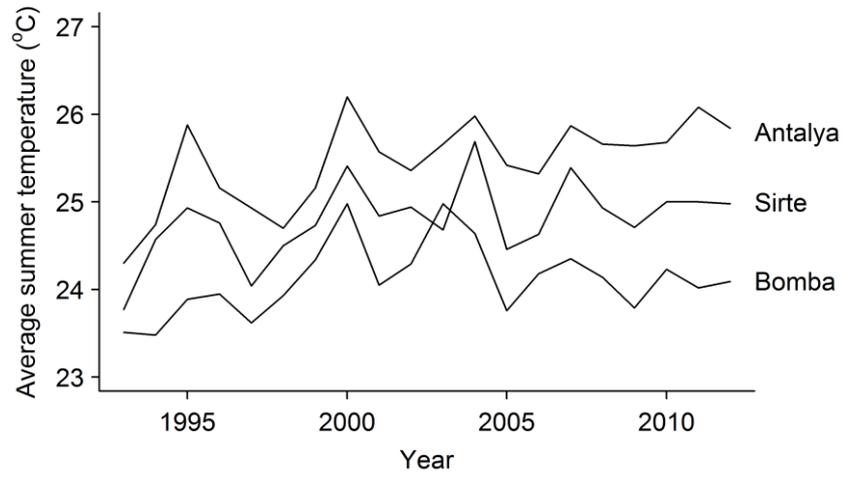
**Figure 1.** Green turtle foraging grounds of the Mediterranean. **a)** Size of circle is representative of number of turtles tracked to each site (range: 1-7; Data taken from Stokes *et al.*, 2015: see Chapter II). Star denotes Alagadi nesting beach. **b), c)** and **d)** The three major foraging bays, showing individual core home ranges (kernel density estimation with reference bandwidths, 50% isopleths - individual core ranges may be in two parts) of those females tracked from Alagadi (Sirte:  $n=6$ , Bomba:  $n=7$ , Antalya:  $n=2$ ). Bathymetric contour lines are 50m, 100m and 500m.



**Figure 2.** **a)** Increasing trend of April SSTs experienced by tracked individuals across foraging sites (n=76). **b)** Shift towards earlier nesting at the individual (n=76) and **c)** population level (n=20).



**Figure 3.** Climate forcing effect of summer foraging temperature on breeding output at Alagadi nesting beach. Summer temperature (which shows high correlation across sites, see Fig. S1) is averaged over the three major foraging sites for this nesting population, and corresponds to the summer previous to the nesting season.



**Figure S1.** Summer temperatures at the three major foraging sites are highly correlated (Principal Component Analysis: PC1 explains 82% of combined variance).



### Discussion

Climate change is likely to impact wild ectotherms through altered metabolic rates and disruption of reproductive systems. As seen in this study population, natural populations are responding to climate change through changes in reproductive rates and through behavioural mechanisms. Rather than demonstrating a response to selection, the shifting nesting phenology of green turtles in northern Cyprus is evident of a behavioural response to changing environmental cues at foraging grounds across the Mediterranean, which may or may not remain adaptive in the future. Marine turtle capacity to adapt through selection is hampered by long generation times, the accelerated rate of modern climate change, and vastly reduced population sizes relative to the pre-historical past.

While it is doubtful that selective forces in marine turtles can keep pace with modern climate change, less is known about how far behavioural and physiological adaptations can go in alleviating the immediate effects of warming. Major knowledge gaps remain regarding the resilience and adaptive potential of sea turtle reproduction in terms of optimal sex ratios, differential survival of the sexes and the capacity for phenological shifting to ameliorate rising nesting beach temperatures. Other mechanisms such as yolk hormone adjustment (affecting the TSD response) may prove to be adaptive. Additionally, the mobility and cosmopolitan distribution of this group leave it well equipped to adapt spatially through range adjustment. Leaky natal beach fidelity can facilitate the colonisation of new sites, particularly in combination with multiple paternity acting to broaden the gene pool of coloniser nests, provided that potentially suitable habitat is protected into the future. Conservation management strategy should therefore prioritise habitat protection and restoration of depleted population levels in order to facilitate natural adaptive abilities, as well as reduction of other threats and monitoring of population trends, reproductive output and sex ratios.

The findings herein reiterate the heightened need for long term monitoring, for accurate estimation of population trends and with regard to reproductive rates and changing climate regimes. This is broadly applicable to capital breeders with modulated breeding periodicity. Nesting habitat protection efforts should not only focus on those beaches with the greatest abundance of nests, but should aim to maintain a diversity of nesting beaches including those with lighter sand colouration and a range of beach topographies in order to retain a variety of incubation conditions into the future. Projects that relocate all nests in danger of washover should be aware of the role that temporary submergence plays in reducing temperatures

within those nests that survive the inundation. Conservation efforts that involve the use of hatcheries should make efforts to monitor temperatures within man-made nests vs. natural nests left in situ. And projects that employ artificial incubation methods should know that far more research into adult sex ratios and sex specific hatchling survival rates is required before we can begin to estimate optimal hatchling sex ratios for wild populations. Older, larger breeding females are of particular importance not only for their increasing reproductive output (clutch size and frequency), but also for their deeper, cooler nests and propensity to lay earlier in the season, contributing more male hatchlings than the younger nesters. This may be of relevance where sea turtle fisheries operate, due to the tendency of fishing pressure to reduce average body size as older individuals are gradually lost over time, and where incidental bycatch of turtles is high enough to exert similar pressures. Finally, the tendency for green turtles to converge along migratory corridors and foraging hotspots such as those identified in Chapter II highlights a need for further tracking studies in other parts of the world, to identify transitory and residential areas of high use which may need seasonal or year-round protection.

Findings from conservation research should be shared as widely as possible through multiple channels in order to ensure they filter down to conservation programmes and practitioners. This includes sharing new publications widely through social media networks, at global conferences (such as the annual International Sea Turtle Symposium, which is well attended by conservation practitioners) as well as localised conferences and workshops, and publishing through grey literature channels (e.g. SWOT - the State of the World's Sea Turtles magazine) as well as in academic journals. Equally, conservation project managers have a responsibility to read such literature and keep abreast of research and developments.

The increase in nesting activity seen in northern Cyprus adds to a growing body of evidence that recovery of vastly reduced sea turtle populations can be achieved with adequate protection over appropriate timescales (e.g. Chaloupka *et al.* 2008). Globally, sea turtles show a great deal of intra-specific variation in population trajectories (e.g. green turtles: Costa Rica, increasing, Troeng & Rankin 2005 vs. Mexico, decreasing, Seminoff *et al.* 2003; leatherback turtles: Atlantic, increasing, Dutton *et al.* 2005, Witt *et al.* 2009 vs. Pacific, decreasing, Spotila *et al.* 2000; Wallace *et al.* 2011). Whilst green turtles have remained classified as 'Endangered' on the IUCN red list of threatened species since their initial assessment in 1986 (Seminoff 2004; <<http://www.iucnredlist.org/details/4615/0>>), the problems associated with assigning a single status category to a globally distributed, long-lived species with genetically distinct subpopulations have generated much debate (e.g. Broderick *et al.* 2006). Many authors have

suggested that global listings are inappropriate and that threat categories should instead be assigned at a regional level (e.g. Godfrey & Godley 2008; Seminoff & Shanker 2008). Regardless, sea turtles worldwide are a conservation-dependent group, requiring maintenance of both large and small populations throughout their naturally occurring range for increased resilience to climatic threats and preservation of ecosystem function.

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## Ecology of marine turtles under climate change

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**Appendix I**

Stokes, K.L., Fuller, W.J., Glen, F., Godley, B.J., Hodgson, D.J., Rhodes, K.A., Snape, R.T.E. & Broderick, A.C. (2014) Detecting green shoots of recovery: the importance of long-term individual based monitoring of marine turtles. *Animal Conservation*, 17, 593-602.



# Detecting green shoots of recovery: the importance of long-term individual-based monitoring of marine turtles

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## Keywords

clutch frequency; green turtle; marine turtle; population monitoring; population recovery; recruitment; remigration interval; trade-off.

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## Abstract

Population monitoring is an essential part of evaluating the effectiveness of management interventions for conservation. Coastal breeding aggregations of marine vertebrate species that come ashore to pup or nest provide an opportunistic window of observation into otherwise widely dispersed populations. Green turtle (*Chelonia mydas*) nesting on the north and west coasts of northern Cyprus has been monitored consistently and exhaustively since 1993, with an intensive saturation tagging programme running at one key site for the same duration. This historically depleted nesting population is showing signs of recovery, possibly in response to nest protection approaching two decades, with increasing nest numbers and rising levels of recruitment. Strong correlation between year-to-year magnitude of nesting and the proportion of new breeders in the nesting cohort implies that recruitment of new individuals to the breeding population is an important driver of this recovery trend. Recent changes in fishing activities may be impacting the local juvenile neritic stage, however, which may hinder this potential recovery. Individuals returning to breed after two years laid fewer clutches than those returning after three or four years, demonstrating a trade-off between remigration interval and breeding output. Average clutch frequencies have remained stable around a median of three clutches a year per female despite the demographic shift towards new nesters, which typically lay fewer clutches in their first season. We show that where local fecundity has been adequately assessed, the use of average clutch frequencies can be a reliable method for deriving nester abundance from nest counts. Index sites where individual-based monitoring is possible will be important in monitoring long-term climate driven changes in reproductive rates.

## Introduction

Population monitoring is integral to conservation biology (Goldsmith, 1991), and forms an essential part of evaluating the effectiveness of active conservation management (Nichols & Williams, 2006). Present-day conservation monitoring must not only endeavour to detect changes in population status, but also climate change driven alterations to reproductive rates, developmental biology (Milligan, Holt & Lloyd, 2009) and spatio-temporal displacements (Parmesan & Yohe, 2003). For many marine, nocturnal or otherwise cryptic species, detection poses additional challenges, and direct monitoring may be difficult, impractical or impossible. Various indirect survey methods are used as indices of abundance, such as redd (nest) counts for salmonids (e.g. Rieman & Myers, 1997), egg-mass counts

for pond breeding amphibians (e.g. Raithel *et al.*, 2011), acoustic monitoring for loquacious species (e.g. anurans, Crouch & Paton, 2002; whales, Simard *et al.*, 2010) and camera trapping, live trapping, hair detection and road casualty data for terrestrial mammals (e.g. George *et al.*, 2011; Swan *et al.*, 2013). For marine vertebrates, breeding aggregations are often monitored as an index of overall population status (e.g. whales, Andriolo *et al.*, 2010; Fretwell *et al.*, 2014), and species that come ashore to pup or nest present a logistical opportunity to count individuals with greater accuracy and much reduced cost (e.g. sea lions, Pitcher *et al.*, 2007).

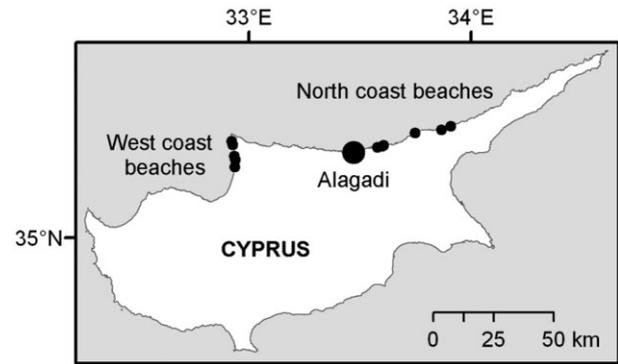
The vast majority of marine turtle monitoring research is based at nesting beaches. The accessibility of females during this narrow window has made nester abundance a common response variable for sea turtle population trend monitoring

(Heppell, Snover & Crowder, 2003). Population assessments based solely on abundance of nesting females have drawn criticism (Bjørndal *et al.*, 2010) and should ideally be combined with in-water foraging ground surveys, which are expensive and labour-intensive (Seminoff *et al.*, 2003). Large discrepancies exist in levels of available funding, however, and nesting beach studies are often the only feasible approach to implement monitoring over long time frames at low expense (Meylan, 1995; Gerrodette & Taylor, 1999).

Studies of sea turtle reproductive ecology rely heavily on the practice of tagging individuals to elucidate breeding frequency and fidelity to nesting areas (Balazs, 1999). In the past, tag loss has been a major confounding variable, with reports of 78% documented tag loss and upper retention estimates of just six years in early studies (Mortimer & Carr, 1987). Tag retention rates have since been enhanced with improved tag design and the introduction of PIT (Passive Integrated Transponder) tags. These developments have increased the accuracy of neophyte/remigrant classification, reducing uncertainty in the quantification of neophyte turtles and overall nester abundance (McDonald & Dutton, 1996).

Most marine turtle populations display obligate skipped breeding behaviour due to the high energy demands of migration and reproduction (Prince & Chaloupka, 2012), females laying a variable number of clutches within a breeding season (termed clutch frequency) every few years (the remigration interval). Individuals must attain a threshold body condition before embarking on a breeding migration, and so their remigration interval varies in response to fluctuations in environmental conditions (Solow, Bjørndal & Bolten, 2002). The low trophic status of the green turtle (*Chelonia mydas*) makes it particularly susceptible to environmental stochasticity, driving large inter-annual oscillations in numbers of nesting females (Limpus & Nicholls, 1988; Broderick, Godley & Hays, 2001). The intrinsic variability characteristic of green turtle nesting makes longevity in monitoring programmes essential for identifying underlying population trends (Broderick *et al.*, 2003; Heppell *et al.*, 2003; Jackson *et al.*, 2008). Individual plasticity and inter-population variation in clutch frequency add further uncertainty when deriving nesting population estimates from nest abundance counts (Van Buskirk & Crowder, 1994; Rivalan *et al.*, 2006). Many studies divide nest counts by an average value of clutch frequency to give estimated annual nester abundance or vice versa (e.g. Seminoff, 2004; Troeng & Rankin, 2005; Beggs, Horrocks & Krueger, 2007). A simplistic model of stochastic nesting behaviour, applied to a loggerhead turtle nest count series with known nester abundance, indicated that this method has the potential to produce biased estimates of population trends (Mazaris, Matsinos & Pantis, 2008). Thus, more studies of individually marked populations are needed.

Green turtles in the Mediterranean have a history of severe exploitation (Sella, 1995). Contemporary rookeries of modest size remain at a handful of sites in Turkey, Cyprus, Syria and Israel (Kasperek, Godley &



**Figure 1** Turtle nesting beaches monitored in the current study.

Broderick, 2001; Broderick *et al.*, 2002; Canbolat, 2004; Yalcin-Ozdilek, 2007; Rees, Saad & Jony, 2008), with *c.* 30% of Mediterranean nesting in Cyprus. Modern threats in the Mediterranean include fisheries by-catch and mass tourism (Casale & Margaritoulis, 2010); this population has been highlighted as a conservation priority owing to its 'High Risk-High Threat' status (Wallace *et al.*, 2011).

Since 1993, an extensive monitoring programme has conducted comprehensive surveys of the nesting beaches of the north and west coasts of northern Cyprus, located in the Eastern Mediterranean (for beach locations see Fig. 1). Intensive survey effort has been concentrated at Alagadi, where continual night patrols of this 2 km stretch of beach for the duration of the breeding season have allowed exhaustive tagging. Here, we examine the apparent recovery of the population, and reveal the range of insights that long-term individual-based monitoring can provide.

## Materials and methods

Daytime monitoring of marine turtle nesting activity was conducted every 1–3 days on beaches with significant nesting on the north and west coasts of northern Cyprus for the duration of the breeding season (end of May to end of September) each year between 1993 and 2013 (less complete monitoring was undertaken in 1992; see Fig. 1 for beach locations). Daytime monitoring involves thorough examination of all nesting activity during the early morning, location of eggs if present, and protection from depredation by stray dogs and foxes using a wide mesh wire screen secured into the sand above the nest (carried out exhaustively since 1994). An intensive night monitoring and tagging programme has been conducted at Alagadi (comprising two coves 1.2 and 0.8 km in length) over the same time period (see Broderick *et al.*, 2002, 2003 for detailed methods). Patrols are undertaken at sufficient frequency to encounter all females nesting at this beach. Internal PIT tags have been administered in addition to external flipper tags to all turtles nesting at this breeding site since 1997. Neophyte/remigrant analyses were conducted on a subset of the data from 2000 onwards due to increased accuracy of neophyte classification three years (one full nesting cycle for most females) following the introduction of PIT tagging.

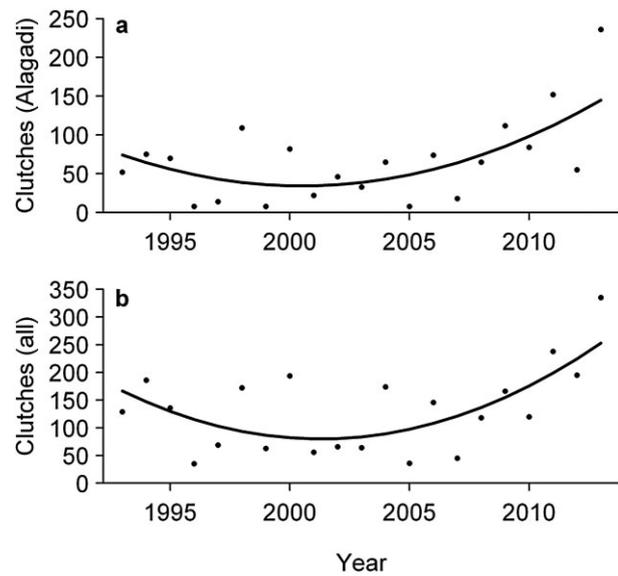
Long intervals between observed nesting events within a nesting season are indicative that a female has laid elsewhere on a nearby beach. Thus, the number of clutches laid per season at Alagadi by each marked individual (observed clutch frequency, OCF) is adjusted where turtles have interesting intervals of 20 days and over to give the expected clutch frequency (ECF; Frazer & Richardson, 1985, see also Broderick *et al.*, 2002 for bimodal distribution of internesting interval data). The remigration interval (RI) for remigrant turtles is calculated as the number of years since that individual was last recorded nesting at Alagadi.

Statistical tests and modelling were carried out using R version 2.14.2 (R Development Core Team, 2012), and packages 'nlme' (Pinheiro *et al.*, 2012) and 'lme4' (Bates, Maechler & Bolker, 2011). Tests of correlation were performed using Spearman's rank order correlation coefficient. Locally weighted regression lines (LOESS smoothers) were fitted to RI and ECF time series data with degree one (linear) and a span of 0.75. Time series analyses of yearly nest counts were conducted using generalized least squares (GLS) modelling to account for temporal autocorrelation in the data.

Clutch frequencies were regressed against explanatory variables using generalized linear mixed modelling (GLMM), fitted using the Laplace approximation, restricted maximum likelihood estimates and stepwise model simplification. GLMMs allow statistical analysis of non-normal data with random effects, which quantify the variation across units/grouping factors of the fixed effect parameters (Bolker *et al.*, 2009). In this case, models had Poisson error structure and logarithmic link function, with zero truncation. Explanatory variables included categorical fixed effects for neophytes (first-time nesters; true or false) and remigration interval (two vs. three or four years), a fixed covariate of body size, and random effects for individual (to avoid pseudoreplication where females have returned to nest in subsequent years) and year (to account for interannual variation in magnitude of nesting arising from environmental stochasticity). GLMM was also used to regress body size against neophyte/remigrant nesters while accounting for pseudoreplication of individuals. The significance of removing model terms was assessed by likelihood ratio tests using maximum likelihood estimates (Crawley, 2007), in order of least significance and with a threshold of  $P = 0.05$ . Model residuals were checked for overdispersion, normality and homoscedasticity.

## Results

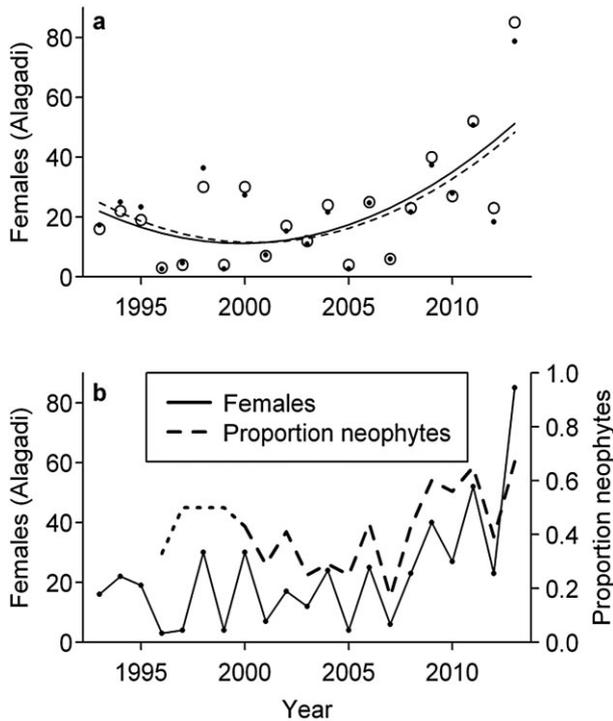
The annual green turtle nesting abundance for Alagadi and the total across the north and west coasts is shown in Fig. 2. The high interannual variation typical of green turtle nesting is evident (combined nesting range: 35–335 nests per season, mean  $\pm$  standard deviation (SD);  $13 \pm 77.1$ ), following a two- to three-year pseudo-cyclical pattern. The coefficient of variation (CV = SD/mean: 0.59) lies within the range previously reported for this species by Broderick *et al.* (2001; 0.41–1.08). Nesting abundance on the two coasts is



**Figure 2** Green turtle clutches on (a) Alagadi beach and (b) across all monitored sites against time, with quadratic trend lines (solid lines). These data build on the data 1993–2000 presented in Broderick *et al.* (2002).

significantly correlated ( $r_{(19)} = 0.72$ ,  $P < 0.001$ ) showing a synchrony in reproductive cycles across this area. Comparison of nest count models at Alagadi and across the two coasts demonstrated significant autocorrelation at a time lag of one year (GLS, Alagadi:  $\phi = -0.729$ ,  $\chi^2_{(1)} = 11.274$ ,  $P < 0.001$ ; overall:  $\phi = -0.449$ ,  $\chi^2_{(1)} = 4.224$ ,  $P = 0.04$ ). Having accounted for this autocorrelation, nest counts showed a significant quadratic trajectory through time (Fig. 2; Alagadi linear slope:  $\beta = -10.663 \pm 2.790$ ; Alagadi quadratic slope:  $\beta = 0.709 \pm 0.135$ ,  $\chi^2_{(1)} = 17.471$ ,  $P < 0.0001$ ; overall linear slope:  $\beta = -20.878 \pm 5.405$ ; overall quadratic slope:  $\beta = 1.259 \pm 4.817$ ,  $\chi^2_{(1)} = 14.379$ ,  $P = 0.0001$ ). This indicates that nesting in the region has stabilized and may now be increasing. This trend was also significant for nester abundance at Alagadi (Fig. 3a; autocorrelation at one year:  $\phi = -0.743$ ,  $\chi^2_{(1)} = 10.711$ ,  $P = 0.001$ ; linear slope:  $\beta = -3.151 \pm 0.929$ ; quadratic slope:  $\beta = 0.231 \pm 0.045$ ,  $\chi^2_{(1)} = 17.079$ ,  $P < 0.0001$ ). Recruitment (as measured by the proportion of nesters that are neophytes) has followed a similar quadratic trend (linear slope:  $\beta = -0.103 \pm 0.016$ ; quadratic slope:  $\beta = 0.005 \pm 0.001$ ,  $\chi^2_{(1)} = 22.005$ ,  $P < 0.0001$ ), but with no significant autocorrelation. Record numbers of nests, nesters and neophytes were observed at Alagadi in 2013 (236 nests, 85 nesting females, 57 neophytes). There has been no trend in survey effort, detection probability (imperfect detection of nests or individuals) or detectability (beach fidelity) over the study period (see Supporting Information Fig. S1 and Pfaller *et al.*, 2013).

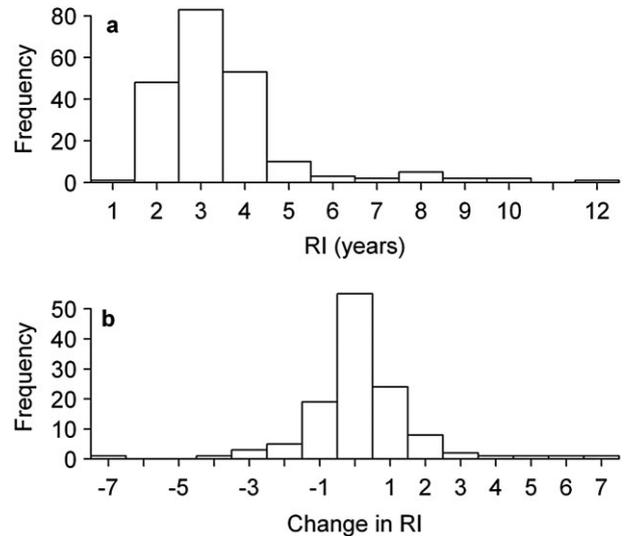
We confirmed that the recent trajectory describes a significant increase in nests, nesters and recruitment, by considering the number of nests and nesters post-2000, which corresponds with the local minimum of all our quadratic



**Figure 3** Green turtle nesting at Alagadi from 1993 to 2013. (a) Number of females nesting at Alagadi as observed through intensive tagging effort (black dots; data for 1993–2000 previously presented in Broderick *et al.*, 2002), and as predicted by dividing annual nest counts by the grand mean expected clutch frequency for this population (3; open circles). Nesting population trends estimated using these two measures are almost identical (solid line: actual data, dashed line: predicted data). (b) Correlation between the number of nesting females (solid line), and the proportion of those that are first time nesters (dotted/dashed line) at Alagadi over the same time period. The dotted portion indicates lower confidence in neophyte/remigrant identification prior to 2000.

fitted lines. Since 2001, there has been a significant increase through time in the number of nests across all beaches ( $\beta = 15.993 \pm 3.063$ ,  $\chi^2_{(1)} = 11.938$ ,  $P = 0.0006$ ), the number of nests on Alagadi ( $\beta = 9.799 \pm 1.605$ ,  $\chi^2_{(1)} = 15.516$ ,  $P = 0.0001$ ), the number of nesting females on Alagadi ( $\beta = 3.493 \pm 0.606$ ,  $\chi^2_{(1)} = 14.398$ ,  $P = 0.0001$ ) and rates of recruitment ( $\beta = 0.029 \pm 0.009$ ,  $\chi^2_{(1)} = 8.399$ ,  $P = 0.004$ ).

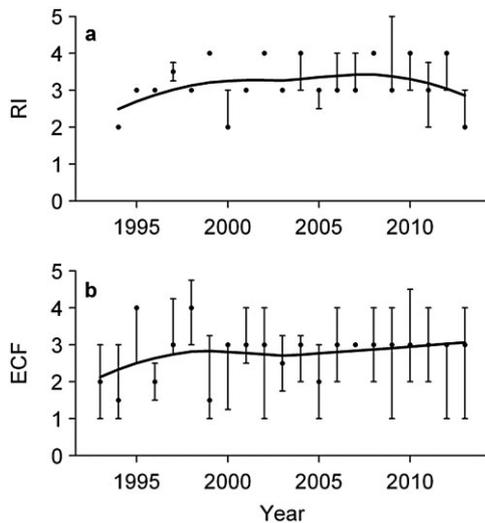
A comparison of observed and estimated nester abundance is shown in Fig. 3a. Here, the known number of females nesting each year at Alagadi is used to test the accuracy of estimates derived using nest counts and average values of clutch frequency. Estimated nester abundance is taken as the quotient of annual nest abundance divided by an average clutch frequency of three (Seminoff, 2004; also the overall mean and median clutch frequency from the current study). Estimated nester abundance and actual/observed nester abundance were highly correlated ( $r_{(19)} = 0.97$ ,  $P < 0.0001$ ). Conclusions drawn from these abundance series about the population trend at this breeding aggregation would be analogous.



**Figure 4** Remigration interval (RI) of green turtles returning to nest at Alagadi. (a) Observed RIs (1994–2013). (b) Change in RI for green turtles nesting at Alagadi during three or more seasons, taken as the increase/decrease in RI compared with the previous RI recorded for each individual.

The tagging programme based at Alagadi has revealed a strong correlation between the number of nests and the proportion of neophytes since 2000 ( $r_{(12)} = 0.94$ ,  $P < 0.0001$ ; Fig. 3b). This strong correlation between the proportion of neophytes in the nesting cohort and the magnitude of nesting implies that recruitment of new individuals into the breeding population is an important driver of year-to-year nester abundance, an encouraging sign of a population in recovery. The reduced correlation between the number of nesters and the proportion of neophytes seen prior to 2000 provides evidence that the introduction of PIT tagging has had a significant effect on the accuracy of neophyte/remigrant identification. First-time nesters at Alagadi are significantly smaller than remigrant nesters (GLMM,  $\chi^2_{(1)} = 84.95$ ,  $P < 0.0001$ ; mean CCL  $87.7 \pm 6.5$  cm for neophytes cf.  $92.0 \pm 5.9$  cm for remigrants), re-affirming their classification as true neophytes. The intensity of survey effort at this site has afforded near-perfect attribution of nests to known females (98% since 2000, 93% since comprehensive monitoring began in 1993).

Figure 4a shows RIs observed for the marked green turtle population at Alagadi between 1994 and 2013. The majority of remigrants return after two, three or four years [87%; median RI: 3, interquartile (IQ) range: 3–4,  $n = 212$ ]. The low incidence of unusually long RIs most likely reflects individuals with lower site fidelity, who may have nested elsewhere in Cyprus, or further afield, undetected. The majority (78%) of remigrant turtles observed over three or more seasons varied their RI from one breeding season to the next ( $n = 51$ , see Fig. 4b), exemplifying the high levels of modulated periodicity green turtles show in response to environmental stochasticity. Despite this, the annual average RI has remained relatively stable over the study

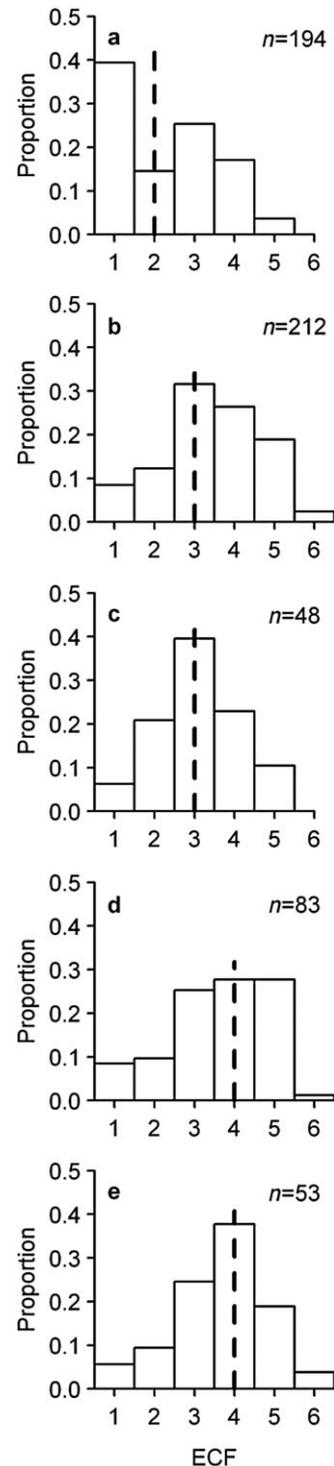


**Figure 5** Breeding frequency of green turtles at Alagadi from 1993 to 2013. (a) Yearly median and interquartile range for remigration interval (RI) and (b) expected clutch frequency (ECF) for nesting at Alagadi, each with locally weighted regression line (LOESS smoother).

period (see Fig. 5a), fluctuating mostly between three and four years. Lower RIs at the beginning of the time series are an artefact of time since tagging began; only those remigrants with lower than average remigration interval can be re-encountered within the first three years of monitoring.

Median ECF across all years and nesters was three (IQ range: 1–4,  $n = 485$ ). No long-term trend in median clutch frequency is apparent from the data (see LOESS smoother Fig. 5b). Instead, median ECF is correlated with the number of nesters present in a given season ( $r_{(19)} = 0.52$ ,  $P = 0.02$ ), with three of the four lowest nesting seasons having a low average ECF, indicating that females breeding in poor nesting years may be in suboptimal body condition. Median clutch frequency is more variable in the early part of the time series, stabilizing as the number of females increases, effectively increasing the sample size and reducing susceptibility to skewed averages. ECF varies between neophytes and remigrants (see Fig. 6a–b), with neophytes most likely to lay a single clutch (40%,  $n = 194$ ), and the majority of remigrants laying three to five clutches (77%,  $n = 212$ ). GLMM showed the effect to be significant, with remigrants laying an average of 0.6 clutches more than neophytes, while accounting for individual and year-to-year variation ( $\chi^2_{(1)} = 37.198$ ,  $P < 0.0001$ ). Female body size had a statistically significant but biologically insignificant effect on clutch frequency ( $\chi^2_{(1)} = 7.689$ ,  $P < 0.01$ ), with a 10 cm increase in curved carapace length (CCL) increasing ECF by an average of 0.04.

RI was found to have a significant effect on clutch frequency, with short RIs of less than three years reducing ECF by almost a quarter (0.23), once variation across individuals and years was accounted for (GLMM,  $\chi^2_{(1)} = 4.009$ ,  $P < 0.01$ ). Body size did not have significant effect to be included in the model. Female nesters returning after a short



**Figure 6** Expected clutch frequency (ECF) for (a) neophyte (2000–2013), (b) all remigrant, (c) two-year remigrant, (d) three-year remigrant and (e) four-year remigrant green turtles nesting at Alagadi (1994 to 2013). Dashed lines are median values.

interval of two years are most likely to lay three clutches (40%,  $n = 48$ , Fig. 6c), while those returning after three or four years are more likely to lay four or five clutches (57%,  $n = 136$ , Fig. 6d–e).

A total of 273 nesting females have been tagged at Alagadi since 1992. Forty percent of neophytes nesting between 2000 and 2008 ( $n = 55$ ) did not remigrate to this site in subsequent breeding seasons (we do not include 2009–2013 as these neophytes may yet return).

## Discussion

Evaluation of indirect survey method reliability is essential for accurate population monitoring. Validated indices are the primary tool for tracking changes in abundance of many cryptic species of conservation concern (e.g. carnivore track counts and camera trap surveys; Balme, Hunter & Slotow, 2009). Long-term individual-based monitoring of green turtles at Alagadi, northern Cyprus has provided fundamental and applied insights into sea turtle nesting ecology. Our data suggest that estimation of nesting population size from nest abundance data is reliable, provided that fecundity is adequately monitored at relevant localized index sites to provide the ‘proportionality’ information required to interpret these data (Gerrodette & Taylor, 1999). Green turtles nesting at Ascension Island in the South Atlantic are larger in size, migrate further (~2300 km, Luschi *et al.*, 1998) and have a longer period of suitable nesting conditions than those nesting in the Mediterranean, and thus perhaps unsurprisingly have a higher average clutch frequency of around six nests per season (Weber *et al.*, 2013) compared with the average of three detected in the current study. Clutch frequencies derived through tagging efforts alone where complete survey is not possible or site fidelity is low will be underestimated, leading to inflated population assessments. Studies augmenting capture-mark-recapture methods with the use of tracking (Tucker, 2010; Weber *et al.*, 2013) and ultrasonography (Blanco *et al.*, 2012) technologies can improve clutch frequency estimates in such cases. Breeding rates will likely be affected by long-term changes in foraging conditions, highlighting the importance of ongoing monitoring at index sites to ascertain multifaceted responses to climate change.

Saturation tagging at Alagadi has revealed clutch frequencies that are significantly different among groups (e.g. neophytes vs. remigrants), but that are temporally stable across groups. Reduced clutch frequency in neophyte turtles as seen here has previously been reported in green turtles (Carr, Carr & Meylan, 1978), as well as in leatherback (Tucker & Frazer, 1991), loggerhead (Hawkes *et al.*, 2005) and hawksbill turtles (Beggs *et al.*, 2007). It is likely that this phenomenon is caused by both increasing physiological capacity with age, and changes in nesting behaviour such as site fidelity (Carr *et al.*, 1978). Individual green turtles lay increasingly large clutches across (Bjorndal & Carr, 1989) and within (Broderick *et al.*, 2003) breeding seasons, indicating an increase in reproductive efficiency or capacity. Low subsequent remigration rates of neophytes

tagged at Alagadi (this study) suggest lowered site fidelity in new breeders. Broderick *et al.* (2002) found that single-clutch neophyte females have a lower probability of remigrating to Alagadi in subsequent years than those with higher clutch frequencies (0.3 cf. 0.8). Satellite telemetry of interesting loggerhead turtles in Florida has revealed a higher site fidelity in remigrants compared to new breeders (Tucker, 2010). Such ‘leaky’ female nest site fidelity facilitates genetic mixing of the maternal lineage across nesting sites (Lee, Luschi & Hays, 2007), and may promote resilience to loss of breeding sites through behavioural adaptation.

Our finding that females remigrating after three or four years lay extra clutches in comparison to those remigrating after two years supports the notion that suboptimal foraging conditions can be compensated for by building up energy reserves over a longer interval. A similar relationship between remigration interval and likely clutch frequency has been observed in leatherback turtles (Rivalan *et al.*, 2005), and Van Buskirk & Crowder (1994) describe a comparable trade-off in interspecific reproductive effort resource allotment. Many iteroparous species may skip a breeding year if conditions are not favourable (e.g. fat dormouse; Pilastro, Tavecchia & Marin, 2003); this may partly be compensated for if a higher reproductive output can be attained in the following breeding season (e.g. four-toed salamander, Harris & Ludwig, 2004). The implications for population assessment are that short-term fluctuations in breeding activity may be misinterpreted unless populations are monitored in the long term (Hays, 2000), and that breeding frequency should be monitored at the individual level where possible in order to detect long-term change in the scaling factors used for conversion of monitoring indices to population estimates.

The recent upward trend in nest numbers in northern Cyprus may signal the beginning of a recovery phase for this sub-population following the cessation of a heavy harvest and intensive screening of nests against unnaturally elevated predation levels. Recruitment can be viewed as a measure of cohort strength (Heppell *et al.*, 2003), and rising numbers of neophytes as seen in this population are an early indication of population growth (Richardson *et al.*, 2006). Similar nest protection schemes have had measurable success some 20 years later (Garduño-Andrade *et al.*, 1999; Dutton *et al.*, 2005). There is, however, considerable uncertainty surrounding the time it takes for green turtles to reach breeding age; published age at sexual maturity estimates for wild green turtles range from 27 (Frazer & Ladner, 1986) to 40 (Limpus & Chaloupka, 1997) years. Evidence from living tags, however, has shown that male and female green turtles released from the Cayman Island Turtle Farm (a conservation facility/tourist attraction/turtle meat supplier in the Caribbean) as hatchlings can breed at 19 and 17 years, respectively (Bell *et al.*, 2005). If this species can indeed reach sexual maturity at less than 20 years, then it is possible that sustained reduction in nest depredation across two decades has aided in the early stages of recovery of this historically depleted breeding aggregation.

Behavioural reproductive mechanisms such as natal philopatry and polyandry contribute to the resilience of sea turtles (Bell *et al.*, 2009), which have shown encouraging recovery potential and rebound capacity in response to long-term protection (Garduño-Andrade *et al.*, 1999; Broderick *et al.*, 2006; Richardson *et al.*, 2006; Marcovaldi & Chaloupka, 2007). The complex life cycle of this group, and others involving multiple distinct habitats and delayed sexual maturity, makes adequate protection particularly challenging (Heppell *et al.*, 2003); protective measures on the nesting beach will not be effective if threats at sea are not addressed (Dutton *et al.*, 2005), and the potential for trophic uncoupling of resources and ability to shift ranges under changing climatic conditions are important considerations for such species (Møller, Rubolini & Lehikoinen, 2008; Robinson *et al.*, 2009).

Nest count series should be used in conjunction with data regarding other life stages wherever possible (Bjorndal *et al.*, 2010). Encouragingly, genetic studies at Alagadi have revealed a greater number of males than females in the breeding population (Wright *et al.*, 2012*a,b*), suggesting that the population increase observed here has occurred across demographic groups. However, a recent assessment of sea turtle by-catch in northern Cyprus (Snape *et al.*, 2013) has found a high incidence of juvenile green turtle mortality. Potential increased fishing effort in the region following changes in trade regulation between northern Cyprus and southern Cyprus (Snape, pers. obs.) may impede the recovery of this population.

Monitoring projects must be cost-effective in the long term in order to ensure the longevity of data required to make meaningful estimations of population trends (Schroeder & Murphy, 1999). Re-sampling assessments of extant data from comprehensively monitored nesting sites have found that temporal sub-sampling within the breeding season could save up to 50% of monitoring costs with little loss of statistical power (Jackson *et al.*, 2008; Sims *et al.*, 2008; Whiting, Chaloupka & Limpus, 2013). The efficacy of these more parsimonious sampling regimens is reliant on consistency in the temporal distribution of the nesting season, however, which has been shown to be variable in accordance with both long- (Weishampel, Bagley & Ehrhart, 2004) and short- (Hawkes *et al.*, 2007) term fluctuations in sea surface temperature. Furthermore, complete sampling of the breeding season yields additional advantages in localities where remedial conservation measures such as nest protection and surveillance of illegal take are beneficial (e.g. Bell *et al.*, 2007).

A range of strategies is required to cover the breadth and depth necessary to detect changes in biological parameters and spatio-temporal distributions that are likely to occur in response to climate change. Index sites such as Alagadi, where long-term and consistent individual-based monitoring is possible, can offer valuable insights into survival and reproductive rates that other localities can use in converting more basic density indices into population estimates. Long-term datasets are vital in documenting change, but are often difficult to maintain with variable funding stability through

time (Hays, Richardson & Robinson, 2005). Monitoring programmes with a core set of simple, robust and inexpensive measurements may have a greater likelihood of remaining consistent and sustainable in the long term (Bennun, 2001; Lovett *et al.*, 2007).

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Lack of trend in fidelity and detectability. (a) Proportion of all nests recorded across the north and west coasts that are laid at Alagadi. (b) Proportion of nests laid at Alagadi that are assigned to a particular female (through witnessing of oviposition). (c) Yearly median ratio of OCF:ECF (ratios were calculated for each individual female), with 5th–95th percentiles displayed as error bars.

**Appendix II**

Stokes, K.L., Broderick, A.C., Canbolat, A.F., Candan, O., Fuller, W.J., Glen, F., Levy, Y., Rees, A.F., Rilov, G., Snape, R.T., Stott, I., Tchernov, D. & Godley, B.J. (2015) Migratory corridors and foraging hotspots: critical habitats identified for Mediterranean green turtles. *Diversity and Distributions*, 21, 665-674





# Migratory corridors and foraging hotspots: critical habitats identified for Mediterranean green turtles

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## ABSTRACT

**Aim** Levels of sea turtle bycatch in the Mediterranean are thought to be unsustainable. We provide a comprehensive overview of adult green turtle (*Chelonia mydas*) distribution during nesting, migration and foraging phases, highlighting transitory as well as residential areas of high use to facilitate adequate protection for this long-lived, migratory species.

**Location** Mediterranean Sea.

**Methods** Thirty-four females were satellite tracked from breeding grounds in the four countries with major nesting (Cyprus, Turkey, Israel and Syria) for a total of 8521 (mean: 251) tracking days in a collaborative effort to summarize the most comprehensive set of distribution data thus far assembled for this species in the Mediterranean.

**Results** Ten foraging grounds are identified, with two major hotspots in Libya accounting for >50% of turtles tracked to conclusive endpoints. The coastlines of Egypt and Libya contain high densities of migrating turtles following the nesting season, particularly July–September, and likely also pre-nesting (April–June). A high-use seasonal pelagic corridor running south-west from Turkey and Cyprus to Egypt is also evident, used by >50% of all tracked turtles.

**Main conclusions** Bycatch levels and mortality rates for the key foraging areas and high-density seasonal pathways identified here are largely unknown and should be investigated as a priority. We recommend that the Gulf of Sirte in Libya be explored as a potential biodiversity hotspot and considered for proposal as a marine protected area (MPA). Green turtle fidelity to nesting beaches, foraging areas and migratory pathways renders them vulnerable to localized threats but enables targeted mitigation measures and protection.

## Keywords

*Chelonia mydas*, conservation, density distribution, marine turtle, migration, satellite tracking.

## INTRODUCTION

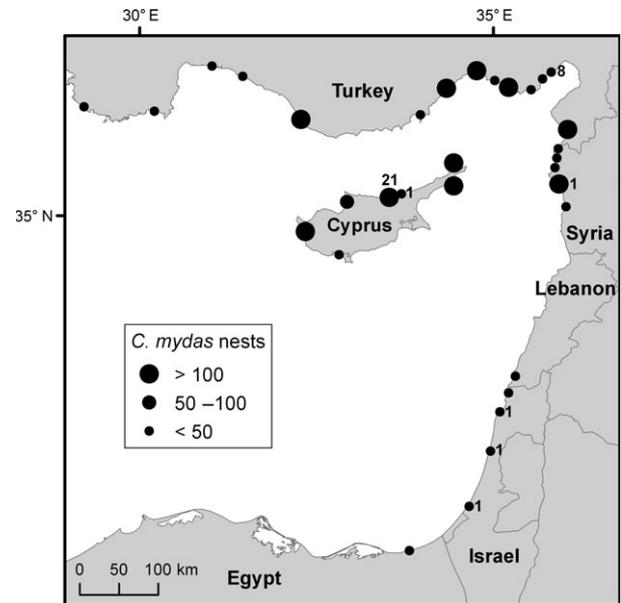
The extensive movements of migratory species pose significant challenges to conservation. Aggregative behaviour and occurrence in geographically disparate habitats can expose migratory groups to diverse and often heightened threats in comparison to non-migratory species. Satellite telemetry studies have been revolutionary in facilitating the identification of widely separated critical habitats, as well as key

elements of connectivity such as stopover sites (e.g. cranes, Kanai *et al.*, 2002) and migration corridors (e.g. ungulates, Sawyer *et al.*, 2009). Understanding such migratory connectivity is essential for the successful management of migrant species, not least in the marine realm where populations may be liable to unquantified threats from fisheries in multiple exclusive economic zones (EEZs) and in international waters. Knowledge of the spatio-temporal distribution of highly mobile species in relation to fisheries can be used to inform

conservation management protocols, such as gear mitigation or time-area closures (Block *et al.*, 2011).

Marine turtles undergo vast ontogenetic migrations between hatchling, juvenile and adult habitats, and subsequently enter into a cycle of reproductive migrations between foraging areas and suitable nesting beaches that continues throughout adulthood. Life history traits of delayed maturity and longevity leave the group particularly vulnerable when adult mortality levels are elevated (Lewison *et al.*, 2004). Extreme levels of historical harvest have left most populations severely depleted (Seminoff & Shanker, 2008), and whilst some have shown encouraging rebound capacity (Chaloupka *et al.*, 2008), incidental bycatch in fisheries has impeded recovery in other areas (Lewison *et al.*, 2004). A robust understanding of marine turtle spatial ecology is crucial to the development of effective conservation strategies; satellite telemetry has been used to identify areas of high use (e.g. Shillinger *et al.*, 2008), predict spatial distribution of marine turtle bycatch (e.g. Howell *et al.*, 2008), and evaluate the potential effectiveness of conservation measures (e.g. Maxwell *et al.*, 2011; Scott *et al.*, 2012). Such tracking studies often highlight the need for coordinated, international approaches (e.g. Blumenthal *et al.*, 2006), and in other cases have demonstrated the efficacy of unilateral protection (e.g. Moncada *et al.*, 2012).

For species with dynamic prey landscapes such as loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) turtles, habitat modelling may be used to predict spatio-temporal probability of species occurrence (see Witt *et al.*, 2007; Panigada *et al.*, 2008; Zydalis *et al.*, 2011) to reduce heavy crossover with fisheries (Howell *et al.*, 2008; Hobday *et al.*, 2010, 2011). Fleet communication programmes have also been successfully implemented to provide real-time reporting of bycatch hotspots, reducing fleet-wide levels of bycatch (Gilman *et al.*, 2006b; Alfaro-Shigueto *et al.*, 2012). More static mitigation measures such as marine protected areas and seasonal fisheries closures can be particularly effective for neritic-feeding species with a predictable migratory pattern such as the green turtle (*Chelonia mydas*), with its high fidelity to nesting beaches, foraging grounds and migratory routes (Limpus *et al.*, 1992; Broderick *et al.*, 2007). Within the Mediterranean, the magnitude of marine turtle bycatch is considered unsustainable (Casale, 2011) and warrants urgent conservation action (Wallace *et al.*, 2010). Two species nest in the region: loggerhead turtles in the central and eastern basins, and green turtles in the eastern (Levantine) basin only. Green turtles in the Mediterranean have suffered extreme declines in the past (Seminoff, 2004) due to heavy overharvesting during the twentieth century (Hornell, 1935; Sella, 1982), and significant rookeries remain only in Turkey, Cyprus and Syria (see Fig. 1 and Table 1; Canbolat, 2004; Rees *et al.*, 2008; Stokes *et al.*, 2014). Previous tracking studies have revealed green turtle foraging grounds within sheltered bays in Turkey, Egypt and Libya (Godley *et al.*, 2002), and have demonstrated female fidelity to these areas both within and across seasons (Broderick *et al.*, 2007). A large-scale



**Figure 1** Green turtle nesting beaches of the Mediterranean. Circle size represents magnitude of nesting at each site (maximum number of nests recorded in a season). Numbers indicate the sample size of individual females tracked from each nesting beach ( $n = 34$ ). For nesting data and sources, see Table 1 and Appendix S1 in Supporting Information.

tracking project for loggerhead turtles from Zakynthos, Greece, has revealed a more flexible foraging pattern, with cooler, more productive (Zbinden *et al.*, 2011), foraging sites in the north of the central and eastern basins used as seasonal habitat during the summer months only, and year-round foraging sites largely in the Gulf of Gabès and Ionian Sea in the central basin (Schofield *et al.*, 2013). Here, comprehensive tracking efforts for green turtles in the Mediterranean are used to identify key foraging habitat and migratory corridors, allowing recommendations for further conservation.

## METHODS

Thirty four post-nesting green turtles were tracked between 1998 and 2010 using Platform Terminal Transmitters (PTTs; for details see Table S1 in Supporting Information) from nesting beaches in northern Cyprus ( $n = 22$ ), Turkey ( $n = 8$ ), Israel ( $n = 3$ ) and Syria ( $n = 1$ ). Transmitters were attached using epoxy resin following the methodology of Godley *et al.* (2002). Four individuals were tracked during a second post-nesting migration (Broderick *et al.*, 2007); for this analysis, only the first track showing a clear conclusive endpoint from each individual was included. Locations were obtained via the Argos satellite tracking system, and were downloaded, stored and managed using the Satellite Tracking and Analysis Tool (STAT; Coyne & Godley, 2005).

Tracks were processed and mapped using R, ArcGIS, Geospatial Modelling Environment (GME), Quantum GIS (QGIS) and fTools. A Best Daily Location (BDL) filter was applied to the pre-filtered datasets (location classes 0 and Z,

**Table 1** Green turtle nesting beaches of the Mediterranean. For data sources, see Appendix S1. Averages are means unless otherwise indicated (\*)

Country	Beach name	Max recorded		Min recorded no. nests	Average no. nests/year	No. years surveyed	Source (see Appendix S1)	Tracks
		no. nests	Year of max					
Cyprus	North Karpaz	179	2000	38	104	8	1	21
	Alagadi	236	2013	8	66	21	1, 2	
	Akamas Peninsula	114	2004	9	48	20	3	
	South Karpaz	107	1994	35	64	7	1	
	West Coast	125	2012	4	49	21	1, 2	
	North Coast (excluding Alagadi)	37	2004	0	16	21	1, 2	
Turkey	Akrotiri peninsula	7	1999, 2000	0	5	5	4	8
	Akyatan	735	1998	108	223*	11	1, 5–16	
	Samandağ	440	2006	1	44*	11	1, 5, 8, 10, 14, 16–21	
	Kazanlı	403	2004	73	164*	10	1, 5, 8, 10, 16, 18, 19, 22–28	
	Sugözü	213	2004	213	213	1	16, 29	
	Alata	198	2006	20	128*	4	16, 30, 31	
	Göksu	20	1991	0	13*	7	1, 5, 8, 14, 32–35	
	Yumurtalık	15	1988	1	3*	3	5, 14, 16, 17, 36	
	Tuzla	9	2006	4	9*	3	1, 14, 16, 17, 19, 36	
	Belek	8	1998, 2000	1	4*	8	1, 5, 8, 14, 16, 35, 37–40	
	Kumluca/Fenike	7	1994	0	4*	2	1, 8, 14, 16, 37	
	Ağyatan	4	1996	0	3*	4	1, 5, 14–17, 19	
	Kızılot	3	1993	0	1*	3	1, 8, 14, 16, 37, 41	
	Yelkoma	3	1988	2	3*	2	1, 5, 14, 16, 17, 19	
Patara	2	2000	2	2	1	1, 14, 16, 42		
Syria	Latakia	273	2008	18	140	6	43, 44	1
	Banias	15		1	9	6	44	
Lebanon	Wadi Kandil	13	Data not available	1	7	6	44	3
	Ras el Basit	11		0	4	5	44	
	Um Toyour	7		0	3	3	44	
	El-Mansouri, Tyre Nature Reserve, El Abbasiyeh	16	2004	0	7	5	45–52	
Israel	Nahariya, Gdor, Sharon, Ashkelon	20	2006	0	8	16	1, 53, 54	3
Egypt	El Arish	3	2000	0	1	3	1, 55–57	

\*Medians are used where surveyed seasons are not consecutive.

inferred speeds  $>5 \text{ km}^{-1}$  and turning angles  $<25^\circ$  excluded). Tracks were split into internesting, migrating and foraging stages using displacement plots and visual assessment (see Blumenthal *et al.*, 2006). A post-nesting track was deemed to have conclusively reached a foraging ground if transmissions continued from the end destination for sufficient time to indicate residency (minimum, this study: 27 days). To approximate migratory density, we created a density raster of the number of tracks crossing each cell of a hexagonal grid ( $0.25^\circ$  by  $0.25^\circ$ ).

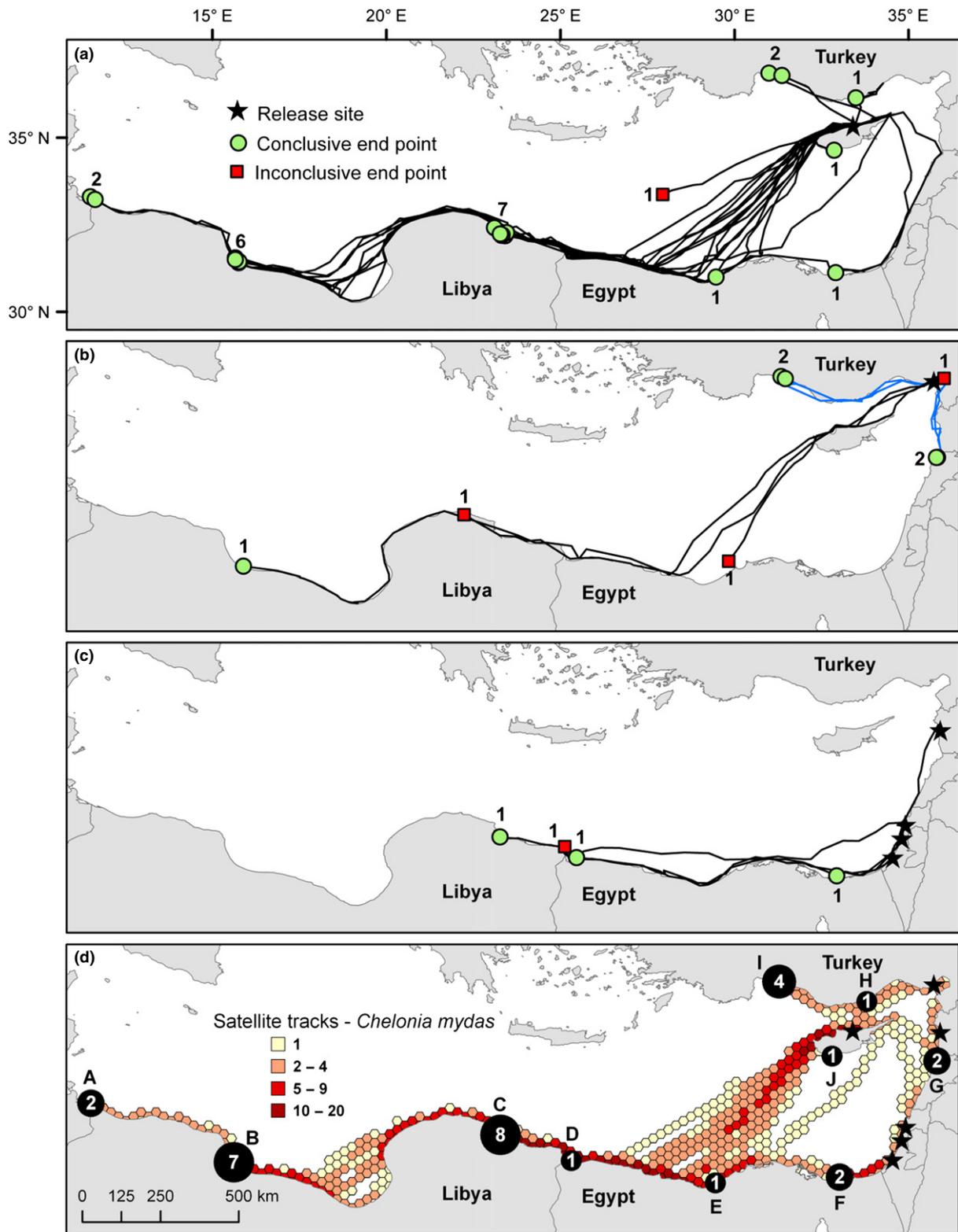
## RESULTS

Transmissions lasted for  $251 \pm 184$  days (mean  $\pm$  SD; range: 22–714), and 29 of the 34 turtles were tracked to a

definitive foraging ground. Transmissions continued from within foraging grounds for  $227 \pm 165$  days (range: 27–650). Turtles from all four countries shared migratory routes and end destination foraging grounds (see Fig. 2).

Ten foraging destinations have been identified in Turkey, Cyprus, Lebanon, Egypt, Libya and Tunisia, ranging from 181 to 2641 km minimum swimming distance from the breeding site (mean  $\pm$  SD:  $1283 \pm 825$ ). Two major foraging grounds in Libya, the Gulf of Bomba (marked C in Fig. 2d,  $n = 8$ ) and Gulf of Sirte (B,  $n = 7$ ), were used by 52% turtles tracked to conclusive end points. An additional foraging ground in the Gulf of Antalya, Turkey (I,  $n = 4$ ), accounts for a further 14%.

Post-nesting migrations lasted 6–80 days (mean  $\pm$  SD:  $36 \pm 23$ ), and took place between 27th June and 12th



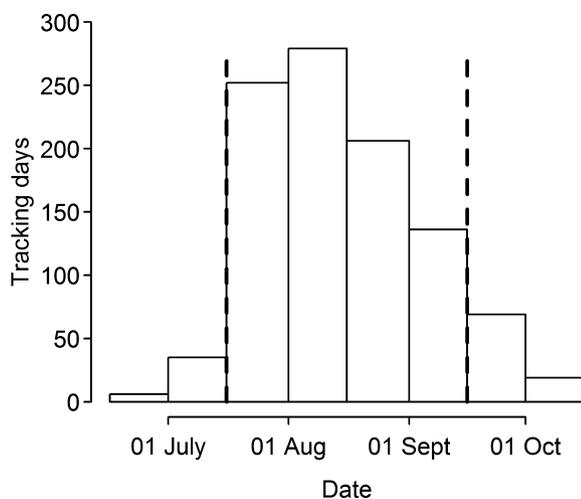
**Figure 2** Post-nesting green turtle satellite tracks from (a) Cyprus ( $n = 22$ ), (b) Turkey ( $n = 8$ ), (c) Syria ( $n = 1$ ) and Israel ( $n = 3$ ), and (d) migratory corridor density map (conclusive tracks only;  $n = 29$ ). Numbers indicate the number of individuals tracked conclusively to each foraging ground. In panel b, tracks in blue are from the first year of tracking (2004) and those in black are from the second year of tracking (2005). Colour in panel d is indicative of the number of satellite tracks that pass through each hexagonal grid cell. Movements to secondary foraging grounds after prolonged stays in initial foraging grounds are not included. Letters in (d) indicate the following foraging grounds: A – Libya/Tunisia border, B – Gulf of Sirte, C – Gulf of Bomba, D – Gulf of Salum, E – Gulf of Arab, F – Lake Bardawil, G – Tripoli, Lebanon, H – Erdemli, I – Gulf of Antalya, J – Episkopi Bay.

October (see Fig. 3). The majority of individuals (97%) completed their return migrations during the months of July – September. Tracked turtles spent an average of 84% of their migration following coastline ( $\pm 11\%$ , range 59–100%), making use of coastal waters around the eastern basin coastline from Cyprus and Turkey through Syria, Lebanon, Israel and the Gaza Strip to Egypt and across Libya. Particularly high densities of tracks (Fig. 2d) are seen between the Gulfs of Arab (E), Salum (D), Bomba (C) and Sirte (B), with 62% of all conclusive tracks converging on the approach to the Gulf of Salum ( $n = 18$ ), 59% continuing to Bomba ( $n = 17$ ), and 31% continuing past Bomba to the Gulf of Sirte ( $n = 9$ ). A high-use pelagic corridor is evident, running south-west from Turkey, across Cyprus, to North Africa. The width of this corridor, as defined by the most central 90% of tracks ( $n = 16$ ), ranges between  $<0.25^\circ$  longitude at the westernmost tip of Cyprus and  $3.5^\circ$  where it meets Egypt. More than half (53%,  $n = 18$ ) of all migrants (including those with inconclusive tracks) used this corridor.

Four individuals from Cyprus made secondary migratory movements ( $>100$  km) after prolonged stays (51, 93, 134 and 221 days) in their respective initial foraging grounds. Three of these were tracked to nearby foraging grounds (107, 390 and 475 km distant), and two later returned to their former foraging grounds after periods of 73 and 129 days.

## DISCUSSION

Green turtles nesting on Mediterranean beaches disperse to widely separated foraging grounds in shallow coastal waters, which they share with conspecifics from other Mediterranean nesting rookeries. This collaborative tracking effort clearly emphasizes the utility of animal tracking in the elucidation



**Figure 3** Seasonality of post-nesting *Chelonia mydas* migrations tracked in this study. Eighty-seven percent of all migratory tracking days took place between 15th July and 15th September (dashed lines). Outbound breeding migrations are estimated to take place from April to June.

of transitory areas of high use as well as residential hotspots. Tracking has revealed a clear migratory pattern, highlighting the coastal waters of the Levantine basin and a south-west pelagic corridor as being critical migratory habitat.

The use of a shared pelagic migration corridor by turtles tracked from beaches in Turkey and Cyprus indicates that this pathway is of critical importance during the months surrounding the Mediterranean nesting season. However, there is a disparity between tracking effort and rookery size (Fig. 1), suggesting that further tracking should be directed towards Turkey's major nesting beaches, which are used by the majority of the Mediterranean population. Two turtles tracked by Türkecan & Yerli (2011) from Akyatan, the largest single rookery in the Mediterranean, travelled to sites B and I (Gulfs of Sirte and Antalya) following similar routes to those described here, further highlighting the importance of these sites.

The range of seagrass beds in the Mediterranean is thought to be much reduced (Lipkin *et al.*, 2003); previous damage by fisheries trawling in coastal areas may have contributed to the diminished extent of green turtle foraging grounds in the region. Foraging grounds highlighted in this research, and particularly those with relatively high densities of green turtles, may be indicators of remaining healthy seagrass habitat (Scott *et al.*, 2012). The pelagic corridor identified here follows the direction of deep bathymetric contours and surface currents, which may aid in navigation (see Fig. S1a and b in Supporting Information; see also Luschi *et al.*, 1998; Hays *et al.*, 1999). Green turtles may also be congregating along this path as a result of avoidance of cooler waters to the north-west of the corridor (see Fig. S1b). Use of pelagic corridors has been observed previously in green turtles in the South Atlantic (Luschi *et al.*, 1998), and in leatherback turtles in the Atlantic (Fossette *et al.*, 2014) and Pacific (Eckert & Sarti, 1997; Shillinger *et al.*, 2008). Seasonal closures may be appropriate in areas where migratory corridors lead to a high incidence of interactions with fisheries within a restricted season and area; however, such measures are limited to extreme cases due to the substantial associated economic impacts (Gilman *et al.*, 2006a) and likelihood to displace fishing effort elsewhere (Lewison *et al.*, 2004). Threats from fisheries vary with fishing gear type and sea turtle behaviour, and efforts should be made to quantify bycatch levels specific to area and fishing practices, classified by species and age class.

The highest density migratory corridor habitat occurs within the exclusive economic zones (EEZs) of Cyprus, Egypt and Libya (see Fig. S1c in Supporting Information), which have estimated marine turtle bycatch rates of around 3700, 7000 and 9700 captures (species not given) per year, respectively (Casale, 2011; Nada & Casale, 2011; see Table S2 in Supporting Information for summarized bycatch data). Set netting has the highest mortality rate (60%), and makes up 97% of the turtle bycatch in Cyprus, compared with 41% in Egypt and just 3% in Libya, such that the total estimated deaths per year for these countries are more even at 2200,

2800, and 2900 (Casale, 2011). Turkey and Tunisia have higher turtle bycatch figures of 12,900 and 17,600, respectively, resulting in 5400 and 5600 estimated turtle deaths per year (Casale, 2011). These rates are derived from official fleet statistics and are therefore minimum values.

During pelagic phases of migration, green turtles are most vulnerable to entanglement in drift nets, of which there remains a sizeable illegal fishery in the Mediterranean despite a total ban (EJF, 2007). Few data are available regarding this Illegal, Unreported and Unregulated (IUU) fishery, but it is not currently known to be a problem in the area of the pelagic corridor described in this study, with most vessels thought to operate in the western basin and the Aegean Sea. The reported incidence of green turtle bycatch in the Mediterranean from pelagic longlines is generally low, although it is impossible to tease apart the effects of improper species identification and a bias of studies to the western basin (Gerosa & Casale, 1999), where pelagic longlines are responsible for the majority of loggerhead turtle bycatch (Casale, 2011). The largely herbivorous diet of the adult green turtle may render it less susceptible to target baited longline hooks than the sympatric carnivorous loggerhead turtle, although opportunistic carnivory is known to occur (Bjorndal, 1997) and has been detected in young adults in the Mediterranean through stable isotope analysis (Cardona *et al.*, 2010). However, pelagic longlines are responsible for a low proportion (6%) of estimated turtle deaths in the eastern Mediterranean countries in which green turtles have been observed in this study (for which data are available, Casale, 2011; Table S2).

Coastal aggregation of both fishing vessels and green turtles puts this species at greater risk from nearshore fishing practices, of which bottom trawls, set nets (such as trammel nets and gill nets) and demersal longlines make up 40%, 30% and 20%, respectively, of the estimated 52,000 turtle captures (all species) per year (Cyprus, Egypt, Israel, Lebanon, Libya, Syria, Tunisia and Turkey; Casale, 2011; Table S2). Bottom-set nets have the greatest impact due to the high mortality rates associated with this gear type, accounting for 50% of the 20,000 estimated minimum turtle deaths per year (Cyprus, Egypt, Israel, Lebanon, Libya, Syria, Tunisia and Turkey; Casale, 2011; Table S2).

Direct take of sea turtles for meat may still be a problem in some areas; there is still an active black market for turtle meat in Alexandria and other Egyptian ports (Nada & Casale, 2011). In addition, gear damage and perceived competition with local fishermen for depleted fish stocks can lead to intentional killings, evident through stranded carcasses either beheaded or with head trauma (e.g. Nada *et al.*, 2013). Awareness campaigns and fishermen training programmes with repeated contact have proven successful in reducing post-release mortality rates of bycaught turtles, improving cooperation and attitudes towards sea turtles and reducing motivation for intentional killing (e.g. Oruç, 2001; Snape *pers. comm.*). Additionally, livelihood diversification interventions are needed in areas where poverty enforces reliance on dwindling fish stocks (Nada *et al.*, 2013).

Additional threats to sea turtles in the region arise from oil and gas exploration and boat strike – the pelagic corridor highlighted here is crossed by paths of intense maritime activity, for example (Katsanevakis *et al.*, 2015). Geopolitical instability across the region may cause delays to the successful implementation of new conservation measures, and transboundary collaboration is further complicated by socio-economic conflicts (Katsanevakis *et al.*, 2015).

## Recommendations

The information available regarding marine turtle bycatch in the Levantine basin is spatially vague. Further characterization of turtle bycatch in the eastern Mediterranean should be prioritized as many data gaps exist, particularly from countries on the north African coast (Casale, 2011). Seasonally targeted quantification of bycatch from April to September (see Fig. 3) within transitory corridors of high use may illustrate the true cost of migration for such species. Post-release mortality rates specific to each fishery should also be further investigated due to the high variability in survival depending on practice (e.g. tow durations, soak times) and paucity of information, again from the eastern basin (Casale, 2011). Quantification of bycatch, associated mortality rates and intentional killings within the coastal foraging areas and seasonal migratory pathways highlighted here is urgently required so that remedial action can be implemented where required. Major knowledge gaps exist in relation to species identification of bycatch. Recommendations for fisheries management cannot be made until the threat to green turtles in the eastern basin from bycatch is quantified. Monitoring within the Mediterranean is difficult due to the artisanal nature of much of the fishery (Casale, 2011), but is possible (see Snape *et al.*, 2013).

Networks of marine protected areas (MPAs) can alleviate escalating pressure from fisheries on marine ecosystems, by protecting spawning stocks and vulnerable non-target species (Halpern & Warner, 2002). Green turtle foraging sites have been described as potential indicators of quality tropical coastal marine ecosystems, therefore useful in the proposal of MPAs (Scott *et al.*, 2012). Much of Libya's coastline has so far escaped over-exploitation and degradation; total fisheries catch is an order of magnitude lower than that of neighbouring Egypt and Tunisia, and vast stretches remain relatively unpopulated (Haddoud & Rawag, 2007). The rate of marine exploitation has accelerated, however, and implementation of conservation legislation has been delayed by political unrest (Badalamenti *et al.*, 2011). The Gulf of Bomba (Fig. 2d site C), the most important green turtle foraging area identified here through satellite telemetry, is recognized as a biodiversity hotspot, and legislative framework for protection has been established through the Ain Gazala MPA (Badalamenti *et al.*, 2011; see Fig. S1c for MPAs of the eastern Mediterranean). We recommend that the Gulf of Sirte (site B) also be investigated as a likely additional biodiversity hotspot, and thus a potential for MPA proposal. Protection of these two major foraging grounds should

benefit a high proportion of the adult green turtle population in the Mediterranean. Site A at the Libya/Tunisia border (as well as further offshore within the Gulf of Gabès shelf) is also a known year-round foraging site for male, female and juvenile loggerhead turtles from around six Mediterranean breeding populations (Broderick *et al.*, 2007; Casale *et al.*, 2007; Zbinden *et al.*, 2011; Schofield *et al.*, 2013); protection at this site would therefore afford benefits to both species. The green turtle foraging grounds at the Gulfs of Sirte (B), Bomba (C), Salum (D), Arab (E) and Tripoli, Lebanon (G) are also shared with foraging loggerheads (Broderick *et al.*, 2007; Casale *et al.*, 2007, 2013; Hochscheid *et al.*, 2010; Schofield *et al.*, 2013), although fewer individuals of the latter species have thus far been tracked to these sites.

Tracking studies targeting juvenile green turtles would be beneficial as the majority of bycaught turtles in the Mediterranean are small in size (Wallace *et al.*, 2010), implying a higher degree of spatial overlap between fishing effort and habitat use of juveniles. Although bycatch data availability for the Mediterranean has a geographical bias to the western basin, the pattern in size-class may be consistent: a small-scale survey of coastal trawlers in Turkey (Mersin to İskenderun Bay) found that the majority of turtle bycatch was green turtle (77%), and 80% of bycaught turtles were juveniles (Oruç, 2001). Additionally, analysis of dead stranded turtles and registered by-catch in northern Cyprus (Snape *et al.*, 2013) and eastern Turkey (Türkozan *et al.*, 2013) indicated that juvenile green and adult loggerhead turtles were at higher risk from local fisheries than adult green turtles.

Data from four of the individuals tracked in this study indicate that green turtles do not necessarily remain within a single foraging ground for the entirety of the non-breeding period, contrary to previous observations (Plotkin, 2003; Broderick *et al.*, 2007). Transmitter deployments on turtles at foraging areas would be beneficial to determine the extent of this behaviour, to describe the timing of pre-nesting migrations and to confirm whether outbound breeding migrations match the return paths described here, all of which have implications for management of key migratory habitats. Tracks from Turkey and Syria have revealed two foraging bays that were not known from tracking efforts from Cyprus, despite close proximity and large sample size, demonstrating the importance of tracking from multiple sites. Tracking from Turkey in this study also highlights that as well as aspiring to large sample size (Schofield *et al.*, 2013), it is advisable to collect tracking data across multiple years when building up a picture of dispersal patterns. This has previously been highlighted for leatherback turtles (Witt *et al.*, 2011).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Nesting data sources for Table 1.

**Figure S1.** Bathymetry, sea surface temperature, surface currents, fisheries Exclusive Economic Zones and Marine Protected Areas of the eastern Mediterranean.

**Table S1.** Summary of satellite transmitter deployments and data.

**Table S2.** Summary of marine turtle bycatch data available for the eastern Mediterranean countries relevant to this study (modified from Casale, 2011).

## BIOSKETCH

**Kimberley L. Stokes** is a marine ecologist interested in research led conservation and is part of the Marine Turtle Research Group (MTRG). This work constituted part of her

doctoral thesis with BJG and ACB at the University of Exeter. Further information about the MTRG can be found at [www.seaturtle.org.uk/mtrg/](http://www.seaturtle.org.uk/mtrg/).

Author contributions: ACB, BJG and KLS conceived the ideas; KLS, ACB, AFC, OC, WF, FG, YL, AFR, RTS and BJG collected the data; KLS analysed the data with contribution from IS; KLS, BJG and ACB led the writing, with contributions from all authors.

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**Appendix III**

Wright, L.I., Stokes, K.L., Fuller, W.J., Godley, B.J., McGowan, A., Snape, R., Tregenza, T. & Broderick, A.C. (2012) Turtle mating patterns buffer against disruptive effects of climate change. *Proceedings of the Royal Society: B*, 279 (1736), 2122-2127



# Turtle mating patterns buffer against disruptive effects of climate change

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For organisms with temperature-dependent sex determination (TSD), skewed offspring sex ratios are common. However, climate warming poses the unique threat of producing extreme sex ratio biases that could ultimately lead to population extinctions. In marine turtles, highly female-skewed hatchling sex ratios already occur and predicted increases in global temperatures are expected to exacerbate this trend, unless species can adapt. However, it is not known whether offspring sex ratios persist into adulthood, or whether variation in male mating success intensifies the impact of a shortage of males on effective population size. Here, we use parentage analysis to show that in a rookery of the endangered green turtle (*Chelonia mydas*), despite an offspring sex ratio of 95 per cent females, there were at least 1.4 reproductive males to every breeding female. Our results suggest that male reproductive intervals may be shorter than the 2–4 years typical for females, and/or that males move between aggregations of receptive females, an inference supported by our satellite tracking, which shows that male turtles may visit multiple rookeries. We suggest that male mating patterns have the potential to buffer the disruptive effects of climate change on marine turtle populations, many of which are already seriously threatened.

**Keywords:** marine turtle; temperature-dependent sex determination; climate change; sex ratio; mating patterns; *Chelonia mydas*

## 1. INTRODUCTION

Understanding and predicting how climate change impacts species with temperature-dependent sex determination (TSD) are critical for their conservation. In these species, sex is determined by the temperature regime experienced during embryonic development and even small temperature changes can produce offspring sex ratios that are heavily biased [1,2]. Many populations of species with TSD already exhibit offspring sex ratios skewed towards the sex produced at warmer temperatures, e.g. males in tuatara [3] and females in marine and freshwater turtle populations [4,5]. Future climate change scenarios are predicted to increase these sex ratio biases, with implications for population viability [2,3,6,7]. Potential consequences include a reduction in effective population size ( $N_e$ ) that will exacerbate the negative effects of inbreeding and increase genetic drift in small populations [8], the inability to find mates leading to reduced fecundity or female infertility [9], and, under more extreme climate projections, the production of single sex cohorts [3,7].

In principle, TSD species could adapt to a warming climate through various mechanisms including evolution of the pivotal temperature (at which 50% of either sex is

produced) and/or the transitional range of temperatures at which there is a mixed sex ratio, and behavioural change in nesting phenology or nest-site selection [2,10]. Although maternal nest-site choice has been shown to compensate for geographical differences in nest temperature in a lizard [11], and marine turtles have shown climate-related phenological nesting responses [12] (but see [13]), evidence suggests that these mechanisms may not be adequate to compensate for climate effects on sex ratio, especially in long-lived and late-maturing reptiles [10]. For example, the evolution of nest-site choice and threshold temperature (above which female offspring are produced) are predicted to be slow in response to climate warming in a freshwater turtle, and unlikely to effectively offset sex ratio bias resulting from rapid climate change [10]. Furthermore, earlier nesting by females, owing to individual plasticity in the timing of first nesting, is predicted to have a modest compensatory effect on offspring sex ratios compared with the perturbing effect of even small increases in summer temperature [14]. Although extant species with TSD have clearly survived and responded to substantial historical temperature fluctuations [15] and have adapted to geographical temperature variation [11,16], it is uncertain whether they will be able to keep pace with anticipated rates of future climate change.

All species of marine turtles have TSD, with females being produced at higher temperatures, males at lower temperatures and 50 per cent of either sex at around 29°C (reviewed in Hawkes *et al.* [4]). Hatchling sex ratios

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biased towards females are typical and in all green turtle populations studied to date, estimates of hatchling sex ratios range from 67 to 100 per cent female (reviewed in Hawkes *et al.* [4]). The few data available regarding juvenile and adult sex ratios in marine turtles suggest that the female biases seen at hatching are maintained at older life stages ([17,18] but see [19]), hence, a climate-induced increase in female-biased primary sex ratios could threaten the viability of marine turtle populations through a reduction in  $N_e$  and associated genetic effects, and potential reproductive failure owing to scarcity of males. These consequences are expected to be particularly deleterious in small populations, where the number of males could conceivably be reduced to below a critical minimum required to maintain a fertile population [9], and in areas where incubation temperatures already result in extremely female-biased offspring production [7]. Larger populations, and those that encompass rookeries at nesting range extremes where more males are produced, may be more robust to offspring sex ratio skews [4], except under the most extreme climate-change scenarios. Any reductions in  $N_e$  owing to lack of males will be further intensified if, as seen in most animals, variation among individuals in reproductive success results in only a small proportion of the available males siring most of the offspring in subsequent generations.

There is currently a scarcity of information regarding mating behaviour in male turtles. Operational sex ratios (OSRs) are poorly understood [7] (but see [20] for information relating to OSR) and data on the reproductive success of individual males are lacking in marine turtle species, despite the influence of these parameters on population dynamics and  $N_e$  [8,21]. These gaps in our knowledge currently prevent an accurate evaluation of the potential impacts of climate change on marine turtle populations [4]. To clarify whether the mating patterns of marine turtle populations increase or decrease their vulnerability to climate change and better understand the male contribution to the gene pool, we determined the number of males successfully breeding in a green turtle rookery in northern Cyprus that consists of approximately 100 nesting females [22] and already exhibits a highly female-skewed hatchling sex ratio (86–96% female [23]). There are an estimated 300–400 female green turtles nesting annually in the Mediterranean, with nesting restricted to the eastern basin, occurring mostly in Cyprus and Turkey [22]. The proximity of nesting areas in Cyprus and Turkey probably results in similar incubation temperature regimes and indeed other major Mediterranean rookeries also produce highly female-biased hatchling sex ratios (e.g. 92% female at Akyatan, Turkey [24]). We hypothesized that the sex ratio of breeding adults in our study rookery would, therefore, also be female-biased, with fewer males than females contributing to reproduction.

Unlike female marine turtles, males rarely come ashore and the difficulty in catching them at sea limits access to them. We have overcome this problem by intensively sampling and genotyping mothers and offspring and employing sibship reconstruction and parentage inference methods to estimate the number of males successfully siring offspring and detect any skew in male reproductive success that might further reduce  $N_e$ . In addition, we report satellite tracking results from our study site that

Table 1. Characterization of microsatellite loci in green turtles (*Chelonia mydas*) at Alagadi, northern Cyprus.  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity.

locus	original source for locus development	no.		$H_O$	$H_E$
		$n$	alleles		
A6	[26]	60	5	0.733	0.711
B103	[26]	60	5	0.617	0.702
B123	[26]	59	5	0.627	0.636
C102	[26]	60	5	0.550	0.550
D105	[26]	60	8	0.683	0.784
D2	[26]	59	10	0.797	0.733
Cm3	[27]	60	7	0.550	0.523
Cm58	[27]	60	7	0.833	0.806
Klk314	[28]	60	4	0.433	0.491
Or7	[29]	60	5	0.717	0.656
Cc2	[30]	60	8	0.717	0.759
Cc28	[30]	60	4	0.650	0.716
CcP7D04	[31]	59	8	0.847	0.794
CcP7E11	[32]	60	4	0.550	0.496

provide new insights into male mate-searching behaviour and lend support to the inferences drawn from our parentage assignments.

## 2. MATERIAL AND METHODS

The study was conducted in a wild population of green turtles at Alagadi beach, northern Cyprus during the 2008 breeding season (May–October). Tissue samples were taken from 20 nesting females of known identity (representing 91% of females that successfully nested at this site in 2008) and up to 23 (mean  $\pm$  s.d. =  $21.9 \pm 1.55$ , range 15–23) offspring from one or more clutches per female. The final dataset comprised 809 offspring from 37 clutches. Offspring sex was estimated for the 2008 nesting season from incubation durations (as previously described in Broderick *et al.* [23]) and includes all clutches that successfully hatched at the site in 2008.

### (a) Genotyping

Genomic DNA was extracted from tissue samples using a standard ammonium acetate precipitation method [25]. Samples were genotyped at 14 polymorphic microsatellite loci designed for use in sea turtles (table 1). Primers were labelled with fluorescent dyes (6-FAM, HEX or NED). PCR amplification was carried out in an MJ Research model PTC DNA Engine Tetrad thermal cycler according to the following schedule: 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 58°C for 90 s and 72°C for 60 s, and finally one cycle of 60°C for 30 min. Allele sizes were assigned using an internal size standard (Genescan-500-ROX, Applied Biosystems), an ABI 3730 DNA Analyser and ABI GeneMapper 3.7 software (Applied Biosystems). Samples that failed to amplify at all loci, or that displayed unexpected alleles, were re-amplified and re-scored. Any samples that still failed to amplify at a minimum of eight loci were excluded from further analyses. However, multi-locus genotypes were complete at all 14 loci for more than 90 per cent of individuals.

### (b) Characterization of microsatellite loci

Samples from 60 adult turtles (all collected from females that nested at the study site between 2007 and 2009) were used to

determine population allele frequencies and assess the suitability of the microsatellite markers for paternity analysis. Allele frequency analysis and tests of deviation from Hardy–Weinberg equilibrium (HWE) were performed in the program CERVUS v. 2.0 [33]. Linkage disequilibrium between all pairs of loci was tested using a Markov chain method in GENEPOP v. 4.0.10 [34]. Probability of detecting multiple paternity was assessed using PRDM software [35] (downloaded 2009), assuming both equal and skewed (10 : 90%) paternal contributions to clutches.

### (c) Paternity analysis

Sibship and parentage inference were carried out in COLONY v. 2.0 [36] assuming an error rate of 0.01 for allelic dropout and 0.02 for genotyping error. COLONY uses a maximum-likelihood model to assign sibship and parentage relationships. Individuals are clustered based on full-sib, half-sib (maternal and paternal) and parent–offspring relationships, candidate parents are assigned to full-sib groups and genotypes of unknown parents are inferred [36,37]. Ten replicate runs of ‘medium’ length were conducted on the same dataset. Each of the 10 replicate runs used different random number seeds to initiate the simulated annealing process. Maternity of all offspring was known. All genotyped offspring were analysed together in order to identify any paternal half-sibs, which would indicate males that sired offspring with more than one female.

### (d) Satellite tracking

In 2009, a satellite transmitter (KiwiSat 101, Sirtrack, New Zealand) was attached to a male turtle from the same study site according to a previously described protocol [38]. The male was tracked via satellite for 81 days. Locations were obtained using the Argos system and Satellite Tracking and Analysis Tool (STAT) [39], and mapped in ARCGIS v. 9.3.1. (for full details, see the electronic supplementary material).

## 3. RESULTS

### (a) Offspring sex ratio

Incubation durations of clutches in 2008 ranged from 43 to 59 days (mean  $48.3 \pm 3.48$  (s.d.),  $n = 57$ ), with only three clutches exceeding the pivotal incubation duration (at which 50 : 50 sex ratio is found) of 56 days [23]. Based on incubation durations, using the methodology of Broderick *et al.* [23], we estimate that the overall offspring sex ratio at this site in 2008 was 95 per cent female.

### (b) Paternity analysis

Parentage analysis of more than 800 offspring revealed that a minimum of 28 unique males sired offspring from 20 nesting females, demonstrating an unexpected sex ratio of breeders of at least 1.4 males to each female (figure 1). There was a high degree of convergence between the 10 COLONY runs (see electronic supplementary material, table S1). All runs identified 20 family clusters, all of which contained a single mother and all her offspring with one or multiple fathers (i.e. all offspring in the clusters were full-sibs or maternal half-sibs). Because the number of clusters was equal to the number of females included in the analysis, and no paternal half-sibs were identified, the results indicate that no male sired offspring across females. The analysis revealed 28–30 full-sib families and the total number of unique fathers contributing offspring to these full-sibling

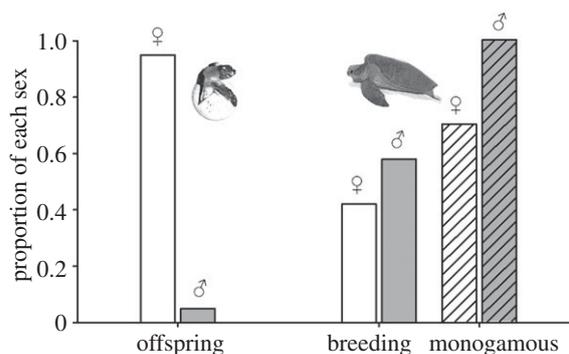


Figure 1. Sex ratios and monogamy of green turtles. The figure shows the proportions of female (un-shaded) and male (shaded) offspring and breeding adults for the 2008 nesting season at our study site. Also shown (hashed) are the proportions of breeding adults that were monogamous at the study site, i.e. the proportion of females that had a single sire for their offspring and the proportion of males that only sired offspring from one female at this rookery (note: use of the term ‘monogamy’ in this figure relates only to the Alagadi rookery; males may have mated with additional females at other nesting sites within the wider Mediterranean population). Offspring sex is estimated from incubation durations [23] and includes all clutches that hatched successfully at this site in 2008.

groups also ranged from 28 to 30 across the 10 runs, with 28 fathers being most probable in five runs.

Thirty per cent (six out of 20) of females in this study produced clutches with multiple paternity (see electronic supplementary material, table S2), but interestingly, we found no evidence that any single male sired offspring with more than one female at this rookery. This is evidenced by the lack of paternal half-sibs in the COLONY analysis, despite near complete sampling (more than 90% of females that successfully nested at Alagadi in 2008 were included in the study).

### (c) Characterization of microsatellite loci

All loci conformed to expectations of HWE ( $p > 0.05$ ), showed low probability of null alleles and showed no evidence of genotypic linkage disequilibrium after correction for multiple tests [40]. Combined exclusion probability (second parent) for all 14 loci was greater than 0.99, and the probability of detecting multiple paternity, assuming two fathers with skewed paternal contributions (10 : 90%) and 20 offspring sampled per clutch, was 0.876.

### (d) Satellite tracking

The male turtle tracked from the study site travelled in proximity to multiple nesting beaches in Cyprus and Turkey before travelling to North Africa (figure 2), in a pattern consistent with mate-searching behaviour (see the electronic supplementary material). The breeding sites within 20 km of the route account for 58 per cent of green turtle nesting in the Mediterranean according to maximum nest numbers taken from the literature [22,41–43].

## 4. DISCUSSION

Our finding that more males than females contributed to reproduction in this study was contrary to our expectations, considering the extremely female-skewed

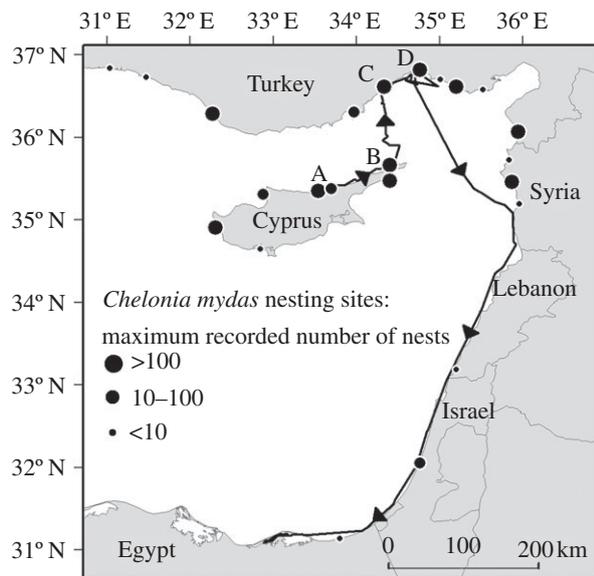


Figure 2. Route of an adult male green turtle that was released post-breeding at Alagadi Beach, Cyprus and tracked to Egypt via the Turkish coast. Major green turtle nesting beaches along the route are labelled (A–D): (A) Alagadi and the Cyprus North beaches, 8–9th June. (B) North Karpaz beaches, 10–11th June. (C) Alata, 14th June. (D) Kazanlı, 15–26th June. Data for nesting numbers are taken from the literature [22,41–43].

hatchling sex ratio reported at this rookery (this study, [23]). OSRs [44] depend not only on adult sex ratio but also on the potential reproductive rate of each sex [45]. Our results might reflect more frequent breeding periodicity in males than females, resulting in sex ratios of adults on breeding grounds that are much less female-biased than would be expected based on offspring sex ratios alone, as recently demonstrated in loggerhead turtles (*Caretta caretta*) [46]. A higher breeding frequency of males compared with females would help to explain the persistence of female-biased populations, by ensuring mate finding and the maintenance of marine turtle fertility even at low population size [47]. More frequent breeding by males will not, however, ameliorate the effects of low  $N_e$ , and populations with few males will still suffer negative effects of inbreeding and loss of genetic variation. Alternative explanations for our results may be that females are able to store sperm from previous breeding seasons to produce viable offspring, as has been recorded in freshwater turtles that breed annually [48], or that there are sex differences in mortality rates leading to an adult sex ratio that does not reflect that seen at primary life stages.

Additionally, some of the males breeding at our study site may originate from (thus far unidentified) rookeries elsewhere in the Mediterranean that produce more balanced offspring sex ratios, although nesting in this population is limited to the eastern Mediterranean where nest incubation temperatures are probably similar to or higher than those in Cyprus. Natal philopatry is a central life-history component in marine turtles and has been documented in both males and females, although the precision with which either sex returns to natal sites is not clear [49]. Lower levels of genetic divergence at

nuclear compared with mitochondrial DNA (mtDNA) markers is consistent with male-mediated gene flow between rookeries and suggests that males are more plastic in their philopatric behaviour [50]. Additionally, although courtship and mating are thought to occur close to the nesting beach at this colony [51], in some populations, mating occurs at regional courtship areas from which females disperse to nesting rookeries that can be distant from the mating site, hence males are associated with regional nesting populations rather than specific rookeries [20].

Our satellite tracking of a post-breeding male turtle from our study site in 2009 supports the conjecture that males might mate at multiple breeding grounds. In contrast to post-breeding female turtles, which travel from this site directly to foraging grounds in Turkey, Syria or North Africa [52], the male took a 348 km diversion to Turkey before travelling to the North African coast, passing in proximity to multiple green turtle nesting beaches [22,41–43]. The exceptional navigational abilities of marine turtles are well documented [53] and tentatively suggest that the observed detour was strategic. Mating activity in marine turtles can overlap significantly with the nesting season [54] and males are typically sexually active for a period of around one month [20]. Given the seasonality of nesting in the Mediterranean [22], it is possible that the male tracked in this study was seeking receptive females both in Cyprus and Turkey. It is not known whether there is maternal population structure among the major nesting sites for this species in the Mediterranean. In addition to ensuring mate finding and maintaining equal OSRs on mating grounds, if aggregations of nesting females are distinct (in terms of mtDNA), then the movement of males between breeding grounds will contribute to nuclear gene flow between rookeries and reduce inbreeding and loss of genetic variation that would occur if very small numbers of males were reproducing at each breeding site.

Although polyandry has been documented across marine turtle species (reviewed in Lee [55]), and was recorded in 30 per cent of females in this study, the observation that males only sired offspring with a single female at the Alagadi rookery (figure 1) is surprising and is in contrast to observations of polygynous behaviour at other green turtle breeding grounds [20]. In aggregate breeding systems with no parental care, regardless of whether or not males defend females or resources, multiple mating opportunities for both males and females are expected [44]. It is clear that males may have mated with additional females at other breeding sites; however, the genetic mating pattern observed at this rookery allows more males to participate in reproduction compared with a strictly monogamous system [21,56], further contributing to the maintenance of genetic variation in this population.

While previous work has focused on the potential of plasticity in maternal nesting behaviour to counter the sex ratio biases induced by climate warming [10,11], this study highlights the role of mating behaviour in maintaining relatively equal operational sex ratios despite highly female-biased hatchling sex ratios. Whether the breeding pattern observed at this nesting site results from males breeding more frequently than females, males moving between aggregations of receptive females, or from other processes leading to a more equal OSR than

expected based on offspring sex ratios warrants further investigation owing to the potential implications for  $N_e$ . It is important to note that the sex ratio of breeders observed in this study reflects hatchling sex ratios approximately 30 years ago (owing to late age at maturity in green turtles, reviewed in Heppell *et al.* [57]). However, sea surface temperatures at this site have risen by, on average, less than 1°C over the past 50 years, suggesting a female bias in offspring produced at that time [7]. Nonetheless, future adult sex ratios could be much more female-biased than at present. Current mating patterns will help to preserve genetic variation that may be critical if marine turtles are to adapt behaviourally or physiologically to a warming climate and have, no doubt, contributed to their persistence through historical climatic upheaval.

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