Biophysical and physiological ecology of loggerhead turtle nests at Zakynthos and Kyparissia, Greece

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

BIOPHYSICAL AND PHYSIOLOGICAL ECOLOGY OF LOGGERHEAD TURTLE NESTS AT ZAKYNTHOS AND KYPARISSIA, GREECE

Jack S. Suss

Sea turtles bury large masses of eggs on beaches. Burying eggs deeply in sand provides a humid environment with fairly constant temperature, but also constrains the diffusion of gases. Zakynthos and Kyparissia, Greece host the largest loggerhead turtle rookeries in the Mediterranean, and the sand on the beaches visibly differs in texture. I used these differences as a natural experiment to determine how the physical characteristics of sand affect the physiology of loggerhead turtle nests in 2009 and 2010. Temperature at nest depth was lower on Marathonissi (26.7 ± 1.6°C) and Laganas (27.9 ± 0.8°C) than all other beaches (30.3 ± 1.5°C) and provided conditions for male hatchling production during this period. Beach temperatures on Kyparissia were higher than ambient weather conditions because topography of the beach allowed for increased absorbance of solar radiation. Based on soil water characteristic curves, eggs on the beaches of Greece were not under hydric stress. Diffusivity varied as a function of air-filled porosity in sand from sea turtle nesting beaches in Greece, Costa Rica and Equatorial Guinea. Conductivity varied as a function of air-filled porosity and median grain size. The oxygen concentration of sea turtle nests can be affected by both diffusion and bulk flow of gases, especially for beaches with large tidal amplitudes. Hatching success of nests was lowest on Kyparissia A (67.6 ± 4.5%) and maximum nest temperature was lowest on Marathonissi beach (29.4 ± 0.4°C). All other beaches had a hatching success of 83.0 ± 4.7% and a maximum temperature of 33.1 ± 0.4°C. Minimum oxygen concentration within the nest ranged from 17.1 to 20.1 kPa among all nests. Sand grain size characteristics, water content, clutch size, temperature, and ambient sand oxygen concentration predicted nest oxygen concentration when inactive pore space was consideredGas exchange may limit clutch size, and may be an important factor in the evolution of multiple nesting events in sea turtles. As beaches continue to be developed, eroded or renourished, the undisturbed state of the interstitial environment must be understood in order to properly mitigate and restore the beach ecosystem.

CHAPTER 1: INTRODUCTION

Background on Underground Nesting

Many reptiles bury clutches of eggs underground where they are incubated by the soil environment. Eggs contain the embryo and the resources, in the form of yolk and albumin, needed for growth and development, but must exchange heat, water and gases with the environment. The rates of these exchanges are limited in part by the egg shell and in part by the physical properties of the soil. Soil naturally exists in a matrix of solid, liquid and gas and the movement of materials through the matrix is dependent on the relative proportions of each of these phases. There are relatively few examples in the herpetological literature about how the incubation medium affects the physiology of egg clutches, but there is increasing awareness that climate can influence soil properties.

Sea turtle nests are large masses of metabolically active tissue buried in tropical and subtropical beaches worldwide. To survive from incubation through hatching, the egg clutch exchanges large and measurable amounts of heat, water and gases with the incubation environment. The biophysical properties of sand that influence these physiological properties of the developing embryos include thermal, hydric and gas transfer. Since sea turtles display philopatry and nest site fidelity, the beaches used for incubation tend to be favorable for incubation.

Temperature is important for sex determination of hatchlings and provides the thermal limitations for incubation. For loggerhead turtles (*Caretta caretta*) in the Mediterranean, females are produced when incubation temperatures are greater than

29.3°C (Mrosovsky et al. 2002). The upper thermal limits of hatchling emergence for leatherback turtle (*Dermochelys coriacea*) and Olive ridley turtle (*Lepidochelys olivacea*) are 36 and 37.5°C, respectively (Drake and Spotila 2002).

Water moves through the environment through gravitational and water potential gradients and the water potential of the incubation environment must be greater than the water potential of the egg contents for successful development. The turtle egg content water potential is -750 kPa (Booth 2005) and would lose water to any incubation environment drier than that.

Through metabolic processes, the clutch of eggs acts as an oxygen sink and a CO_2 source and provides a good model for studying gas exchange and gas movement through sand to understand the limitations of the environment as an incubator. If sand does not allow for adequate gas exchange, the nest can become hypoxic resulting in lower hatching success (Ackerman 1980, Garrett 2010). Gas exchange properties of beaches has been hypothesized to limit clutch size and influence nest site selection in sea turtles (Seymour 1980). It has been assumed that the dominant method of gas exchange is through diffusion (Ackerman 1977), but tidal ventilation of nests through bulk flow of gases may also be important (Wallace et al. 2004).

The loggerhead turtle nesting beaches at Zakynthos and Kyparissia, Greece, offer an opportunity to examine how the differences in biophysical properties affect nest physiology because of the visible differences in sand grain size distributions among beaches. At Zakynthos, *C. caretta* females bury about a hundred eggs to a depth of 25-50 cm below the surface of the sand (Margaritoulis et al. 2011). These eggs incubate for 6-12 weeks, depending on the temperature and generally more than two thirds of the eggs hatch (Margaritoulis 2005). The loggerhead turtle population in Zakynthos produces mostly female hatchlings (Zbinden 2007) because the nest temperatures are mainly above 29.3°C, the pivotal sex-determination temperature for loggerheads in Kyparissia (Mrosovsky et al. 2002), although early season nesting may contribute to male production more than previously thought (Katselidis et al. 2102). Together, Zakynthos and Kyparissia host the two largest loggerhead rookeries in the Mediterranean accounting for 857-2018 (N=16 years) and 286-927 (N=15 years) nests per year, respectively (Rees 2005).

Occasional records of interchange have been reported between these rookeries from external flipper tags of nesting females (Margaritoulis 1998) and telemetry (Schofield et al. 2010). Given the differences between sand characteristics of these beaches, it is not clear if turtle nesting behaviors are selected for laying their eggs on a particular beach. Loggerheads in Greece use sand characteristics for oviposition sites displaying preferences for sand grain size distributions that maximize aeration and water drainage and provide structural support during egg chamber construction (Karavas 2005, Mazaris et al. 2006).

Biophysical Ecology

Sea turtles bury large clutches of eggs on beaches worldwide. Each egg contains resources necessary for growth and development and exchanges heat, water and gases at a rate commensurate to the physical properties of the incubation environment (Ackerman 1977, Seymour 1980, Ackerman et al. 1985, Seymour et al. 1986).

The conditions within the sand that influence timing and survival are determined by the biophysical conditions experienced at the beach (Santidrian Tomillo et al. 2012). Temperature affects hatching success (Santidrian Tomillo et al. 2012), hatchling condition (Booth et al. 2004), hatchling sex ratio (Standora 1985), incubation duration (Georges 1989), hatchling emergence success (Santidrián Tomillo et al. 2009), and oxygen consumption (Reid et al. 2009). Turtles have temperature-dependent sex determination where females are produced at high temperatures, so maintaining variations in beach temperature on either side of the pivotal sex determining temperature is important for the population sex ratio and can have implications for species survival (Standora 1985). In Chapter 2, I compared the ambient weather conditions at Zakynthos and Kyparissia, Greece to beach environmental conditions of oxygen and temperature, and I described how the topography of the beach can contribute to the temperatures experienced by the nests.

Water is crucial for the formation of new tissue in embryos (Packard 1999). In buried eggs, the embryo can obtain water either directly from the yolk and albumin or through exchange with the environment (Ackerman 1997). The magnitude and direction of the water exchange between the environment and the egg contents are driven by a water potential gradient. Water is contained in a given environment dependent on the matric and osmotic potential of the material and the amount of water already present. Water potential increases as water content increases, thus increasing the potential for water to move out of the environment. For developing embryos, the water potential of the environment must be lower than the water potential of the egg contents or else the embryo loses water (Ackerman and Lott 2005). Environments with low water potential have lower hatching success (Packard 1999) and less fit hatchlings (Booth and Yu 2009). In Chapter 3, I examined the soil water characteristics curve of sands from beaches in Zakynthos and Kyparissia, Greece to determine the potential for hydric stress.

Physiological Ecology

Gas exchange through soils is vital for many organisms that reside there, from plants to micro- and macrofauna. The gas exchange of sea turtle eggs is limited by the physical properties of the sand surrounding the nest (Ackerman 1977). Diffusion is the dominant process for gas exchange (O'Connor et al. 2009) as oxygen concentrations in the nest are lower than in the surrounding sand resulting in a net diffusion of oxygen into the nest, at the same time CO_2 diffuses out (Ackerman 1977). Bulk flow of gases can occur through tidal movement, as the water table acts as a piston under the beach producing high and low pressures along with the tide, which can ventilate the nest (O'Connor et al. 2011). Bulk flow can also be generated through the metabolic gas sink environment in the nest where there is lower CO_2 production than O_2 consumption (Reid et al. 2009). The nest, while metabolizing is therefore an area of low pressure and the net convective flux of gas will be into the nest (O'Connor et al. 2011).

The structure of soil consists of solid, liquid and gas phases, and as such, a material moving through soils will follow the path of least resistance. Gas moves through sand mainly using the air-filled pore space (Currie 1961). Diffusion is the dominant force of gas exchange in the soil (Buckingham 1904) where the gas flux ($\mu g\ s^{-1}$) is governed by Fick’s first law:

$$F = AD\frac{\Delta C}{\ell} \tag{1}$$

where A is the cross-sectional area (cm^2), D is the diffusivity of the material ($cm^2\ s^{-1}$), ΔC is the concentration gradient ($\mu g\ cm^{-3}$) through the material over a distance ℓ (cm). Diffusive fluxes are therefore driven by the partial pressure differences, or differences in concentration, of a given substance. The differences in diffusive gas exchange through a variety of porous media can be described through the unique D of each substance.

Many attempts have been made to clarify the relationship between the gas diffusion constant (D_s) and soil structure (van Bavel 1952, Freijer 1994). Generally, there is a positive relationship between diffusivity and air-filled porosity (ε_{air}) (Buckingham 1904, Penman 1940, Marshall 1959) which can be described as:

$$\frac{D_S}{D_0} = \alpha\varepsilon^x + \beta \tag{2}$$

where D_s is the diffusion coefficient in the soil ($cm^2\ s^{-1}$), D_0 is the diffusion coefficient in air ($cm^2\ s^{-1}$), ε is the air-filled porosity ($cm^3\ cm^{-3}$) and α,β, and x are factors that reflect the complexity of the soil media. Complexity factors can include tortuosity (Troeh et al. 1982, Ullman and Aller 1982, Freijer 1994), constrictivity (Freijer 1994), pore continuity (Bruckler et al. 1989), compaction (Currie 1984a, Fujikawa 2005), morphology (Bruckler et al. 1989), water content (Bakker and Hidding 1970) and water potential (Currie 1984b, Pereira and Cruciani 2001, Thorbjorn et al. 2008). While no single equation can adequately describe diffusion in all materials (Currie 1961), the general relationship that diffusivity depends on porosity has passed scientific scrutiny for more than a century.

Gas can also move through a soil via bulk flow driven by a total pressure gradient. This movement is driven by Darcy's Law:

$$Q = \frac{kA\Delta P}{\mu \ell} \tag{3}$$

where Q is the convective flux ($m^3\,s^{-1}$), k is the intrinsic permeability (m^2), A is the cross-sectional area (m^2), ΔP is the difference in total pressure (Pa), μ is the gas viscosity (Pa s) and ℓ is the length (m). Gas conductivity (m^3 s kg^{-1}) is $k\,\mu^{-1}$. Convective fluxes are driven by total pressure differences across a boundary. The differences in convective gas flow through a variety of porous media can be described through the unique conductivity of each substance.

Conductivities through soil are largely reported as a hydraulic conductivity, which includes a gravity component not applicable to gas flows in our system. Both hydraulic and gas conductivities through soil are affected by the pore radius (Campbell 1974) and intrinsic permeability (Olson et al. 2001). Different porosities, median grain size, and sorting factors affect the pore radius (Bezrukov et al. 2001). Air-filled porosity affects both diffusive and convective fluxes and varies with sorting but is independent of median grain size (Beard 1973, Bezrukov et al. 2001). Grain size distributions on natural beaches are mixtures of log-normally distributed populations of grain sizes produced by different sedimentation processes (Spencer 1963) and sorting, skewness and kurtosis are descriptors of the sum of the influence of each of these processes on the beach (Folk and Ward 1957). Median grain size and morphology describe the distance traveled and the maturity of the source population (Folk 1954, Pettijohn 1957).

In all sea turtle species, the porous eggs are adapted to the high humidity and low oxygen in the underground environment (Seymour 1980). Survival during this life stage requires eggs to respond to stressors physiologically. If the incubation medium does not allow for adequate gas exchange, the sea turtle nest can become chronically hypoxic resulting in lower hatching success (Ackerman 1980, Garrett 2010), or depressed growth and metabolism of the eggs in the clutch (Kam 1993). Embryos exposed to hypoxia have physiological plasticity in an increased ability to transport oxygen (Kam 1993, Crossley et al. 2003) that may reduce the response to future hypoxia (Eme et al. 2011b) but also result in cardiac stress (Eme et al. 2011a).

The sea turtle egg shell allows for greater gas and water exchange (Tracy et al. 1978) than in avian eggs (Wangensteen et al. 1970). The flexible and parchment-like shell (Packard et al. 1979) has gas permeability twice that of the chicken egg due to greater shell porosity (Ackerman and Prange 1972). In avian eggs, the resistance to gas exchange is through the hard shell that minimizes water loss in the air (Wangensteen et al. 1970). In the turtle egg the resistance to gas exchange is in the sand around the nest rather than in the porous shell (Ackerman and Prange 1972). Ackerman (1977) hypothesized that the sand surrounding the nest acts as an extension of the turtle eggshell, thereby decreasing the effective gas permeability to that of a hard-shelled avian egg. Gas exchange is ultimately limited by the physical characteristics of the beach (Ackerman 1975) and affects survivorship of eggs (Ackerman 1980, Wallace et al. 2004, Honarvar et al. 2008, Garrett 2010). Embryos at the center of the clutch may suffer greater mortality and grow more slowly and hatch later than the eggs at the periphery of the nest (Ackerman 1980, Wallace et al. 2004).

Oxygen consumption of a clutch of eggs exhibits a peaked pattern, low at first, with exponential increase that may either flatten or decrease just before hatching (Prange and Ackerman 1974), which would allow for improved gas exchange in the final days of development (Kraemer and Richardson 1979). Increased embryonic growth rates are associated with increased oxygen consumption (Ackerman 1980). Oxygen concentration decreases throughout incubation reaching minimum O_2 and maximum CO_2 just before hatching (Ackerman 1977, Maloney et al. 1990, Miller 2008). The low-oxygen environment is due to increased oxygen consumption during embryonic development (Ackerman 1981, Reid et al. 2009), as well as the resistance to gas exchange of the nest environment (Ackerman 1977). The nests of megapode birds (Seymour et al. 1986) and crocodilians (Booth 2000) have similar oxygen profiles during development.

Generally, the minimum oxygen concentration in the nest corresponds to the number of developing embryos (Wallace et al. 2004), but if the nest becomes too hypoxic, hatching success can decrease (Honarvar et al. 2008, Garrett 2010). Sand characteristics explain differences in hatching success of *Chelonia mydas* nests (Maloney et al. 1990, Mortimer 1990), but have not explained variations in gas concentrations (Chen et al. 2010). Since changes in gas conductance affect growth and survival of eggs (Ackerman 1981), estimates of sand diffusivity have been made through modeling (Ackerman 1977), but diffusivity alone may not be adequate to explain nest gas concentrations especially on beaches with large tidal fluxuations (Wallace et al. 2004).

Diffusivity and conductivity contribute in different ways to the gas conductance of sand through the partial pressure and total pressure gradients, respectively and soil structure affects these factors. In Chapter 4, I report on the factors that affect gas movement through the sand of sea turtle nesting beaches. In Chapter 5, I examine the gas, temperature and hatching success of natural *C. caretta* nests in Zakynthos and Kyparissia, Greece and compare oxygen concentrations to predicted levels. These comparisons help to clarify the gas exchange relationship between eggs and sand.

Since adaptive behaviors are related to survival and reproductive success (Williams and Nichols 1984), the same sand characteristics that influence nest placement should influence the survival of the eggs within the nest (Hays and Speakman 1993). Sands on the beaches of Zakynthos and Kyparissia differ visually in grain size and color (personal observation). In this dissertation I describe these differences in sand characteristics and demonstrate the contribution of sand to the development of turtle eggs.

CHAPTER 2: BIOPHYSICAL ECOLOGY OF LOGGERHEAD TURTLE NESTING BEACHES IN GREECE

Introduction

Loggerhead turtles (*Caretta caretta*) lay their eggs in carefully constructed nests on beaches worldwide. On the Greek island of Zakynthos, *C. caretta* females bury an average of 106 eggs to a depth of 25-50 cm below the surface of the sand (Margaritoulis et al. 2011). These eggs incubate for 42-89 days, depending on the temperature and generally 71.5% of the eggs hatch (Margaritoulis 2005). The loggerhead turtle population in Greece produces mainly female hatchlings (Zbinden 2007) because the nest temperatures are usually above 29.3°C, the pivotal sex-determination temperature for loggerheads in Kyparissia (Mrosovsky et al. 2002), although early-season nesting produces males on all beaches in Zakynthos (Katselidis et al. 2102).

Conditions within the sand that influence incubation time and hatchling survival are determined by the weather experienced at the beach (Santidrian Tomillo et al. 2012). For instance, temperature affects hatching success (Santidrian Tomillo et al. 2012), hatchling condition (Booth et al. 2004), hatchling sex ratio (Mrosovsky et al. 2002), hatchling emergence success (Santidrián Tomillo et al. 2009), and oxygen consumption (Reid et al. 2009). Rain events can play an important role in environmental sand condition by cooling the beach (Standora 1985) and flushing the nest with oxygen (Prange and Ackerman 1974). Variations in beach temperature on either side of the pivotal sex determining temperature is important for the population sex ratio and can have implications for species survival (Standora 1985).

In this chapter, I report the weather and the ambient sand conditions at nest depth for two important loggerhead turtle nesting areas of Zakynthos and Kyparissia, Greece. Based on this information, I discuss the importance of these factors with respect to incubation conditions within loggerhead nests on those beaches.

Methods

I conducted this study on the Greek island of Zakynthos (Figure 2-1A) in the Ionian Sea and on the beach at Kyparissia Bay (Figure 2-1B) on the Peloponese Peninsula. Laganas Bay is located at the southern part of Zakynthos Island, and has six *C. caretta* nesting beaches: Marathonissi, Laganas, Kalamaki, Sekania (East, West and Thiafi), Daphni, and Gerakas. Laganas Bay is a NATURA 2000 site protected under the National Marine Park of Zakynthos (NMPZ), and the beaches have limited public access and strict regulations (Margaritoulis 2005). Kyparissia Bay is located on the western coast of Greece, with the beach, dunes and coastal forest comprising a NATURA 2000 site (Rees 2005). The nesting beach extends the entire 44 km length of the bay, but the majority of nesting occurs along the southern 9.5 km (Figure 2-1B). For this study, I only used sectors A and O, comprising the southernmost 5 km of the densest nesting habitat.

I set up HOBO U30 weather stations to record weather variables behind the beaches of Sekania East (34.494735 °E, 41.75267 °N) and Kyparissia A (34.561918 °E, 41.30203 °N). Weather stations were assembled according to the manufacturer’s specifications, using a 3 meter tripod, such that the temperature and relative humidity sensor was shaded at 1.5 meter above the ground, the anemometer was calibrated to north, and the rain gauge and photosynthetically active radiation (PAR) sensor had direct access to the sky above. I buried the tripod feet 50 cm in the sand to ensure stability and leveled the tripod with guy lines attached to stakes. I attached the pole via copper wire to a copper lightning rod buried in the sand nearby. The HOBO computer collected and recorded temperature (°C), relative humidity (%), dew point (°C), rainfall (mm), solar radiation (Wm^{-2}), wind speed (ms^{-1}), gust speed (ms^{-1}), and wind direction (°) at 5 minute intervals. Relative humidity was calculated as the ratio of partial pressure of water vapor (kPa) per saturated water vapor pressure (kPa) at any given temperature. The Zakynthos weather station recorded data from 01 July 2009 (Julian date 182) at 12:03 to 10 September 2009 (Julian date 253) at 07:33 and from 30 July 2010 (Julian date 211) at 06:48 to 30 September 2010 (Julian date 273) at 16:48. The Kyparissia weather station recorded data from 24 June 2009 (Julian date 175) at 11:54 to 06 September 2009 (Julian date 249) at 09:34 and from 27 July 2010 (Julian date 208) at 10:56 to 09 September 2010 (Julian date 252) at 17:21. All times were GMT + 02:00. I configured the data using HOBOWare Pro.

In 2009 and 2010 I collected temperature and O_2 data from the ambient sand at nest depth (25-50 cm) on the beaches of Marathonissi, Laganas (2009 only), Kalamaki (2009 only), Sekania (East, West and Thiafi), and Gerakas at Zakynthos and sectors A and O (2010 only) at Kyparissia. For ambient sand measurements, I excavated control nests on the beaches similar to a turtle nest in a location that would be suitable for a turtle to nest. I determined suitable nesting sites by selecting locations that were surrounded by visible body pits or previously marked nests within 15 meters, and at an intermediate distance to the sea. I placed an O_2 sampling port constructed from a perforated film canister (volume ≈ 75-cm^3) at a depth of 40-cm similar to natural loggerhead nests (Margaritoulis 2005). A 70-mm length of Tygon tubing (2-mm internal diameter) extended to the surface to allow for gas sampling to the gas analyzing equipment (Wallace et al. 2004, Honarvar et al. 2008, Garrett 2010). I also placed Cu/Cn thermocouples to measure temperature at depths of 0, 10, 25, 40 and 50 cm for a thermal profile of the beach. I covered the hole and packed it with sand to approximate the conditions of a natural nest, but without eggs. All beaches had N=3 control nests, except Kyparissia A in 2009 (N=4) and Thiafi in 2009 and 2010 (N=2, both years)

I collected gas samples and recorded temperatures on individual beaches bi-weekly. For real-time measurements I used a Qubit Systems RP2LP-FCM High Ranges Respiration Package. I used a gas pump calibrated to a flow rate of 50 mL min^{-1} and withdrew air from the sand at nest depth for three minutes to obtain dependable gas measurements. I compared O_2 readings in the sand at nest depth to atmospheric readings immediately prior to sampling. Due to unreliable measurements, I do not report CO_2 concentrations. I used OMEGA HH200A thermocouple readers (±0.1) for real-time temperature measurements of the nests and beach thermal conditions. I calibrated thermocouples to ±0.05 °C. I report the temperature at nest depth as the grand mean temperatures of the beach from thermocouples at 25, 40, and 50 cm depth.

I analyzed the data in R. I generated descriptive hourly statistics for daily weather patterns and descriptive daily statistics for seasonal weather patterns. I present hourly descriptive statistics from the week of 01-07 August 2009 and 2010 because this was a time when most loggerhead nests had been laid and few had begun to hatch and therefore would have the largest influence on incubation conditions. I compared weather and beach temperatures to the pivotal temperature for loggerhead turtle sex determination in Kyparissia of 29.3°C (Mrosovsky et al. 2002) above which nests produce female hatchlings, and below, male hatchlings. I compared temperature and solar radiation measurements between Kyparissia and Zakynthos in the same year and at each location between years using paired T-tests of the measured variables matched to the nearest 5 minute period. I compared relative humidity and wind speed using Welch Two Sample T-tests in the same manner. I used T-tests instead of ANOVAs so that data recorded during the month of July were included in 2009 comparisons. Non-overlapping data were removed from analyses and the Julian dates considered were 182-249, 2009 and 211-252, 2010 for location comparison and 211-253 for Zakynthos and 208-249 for Kyparissia between years. I calculated length of day as the amount of time in each 24-hour period that the location was exposed to solar radiation above nighttime readings of 0.6 Wm^{-2}. I compared day length between beaches and years using a paired T-test as described before. I analyzed beach temperature and O_2 at nest depth using ANCOVAs with year and beach as factor variables and Julian date as a regressor. When applicable, I report means with errors of two standard deviations.

I used MATLAB functions (O’Connor, personal communication) derived from Gates (1980) and Oke (1992) to predict the amount of solar radiation on a flat beach, a sloped beach, and a sloped beach with exposed flat berm.

Results

Solar Radiation

Length of day decreased throughout the season (Figure 2-2) along with the seasonal decrease in maximum solar declination from the summer solstice to the autumn equinox. Day length was 1.2 to 3.9 minutes longer in 2009 than 2010 at Zakynthos (Paired T (40) = 3.8, $p < 0.001$), but there was no difference between years at Kyparissia (Paired T (38) = 0.9, $p > 0.05$). Day length was no different between Zakynthos and Kyparissia in 2009 (Paired T (64) = 0.5, $p > 0.05$), but was 0.3 to 2.7 minutes shorter at Zakynthos than at Kyparissia in 2010 (Paired T (39) = 2.5, $p < 0.05$). Solar radiation had a diurnal pattern with values following the cycle of solar declination, the angle of the sun relative to the horizon, throughout the day (Figure 2-3). Solar radiation was 5.79 to 7.87 W m^{-2} and 1.89 to 4.60 W m^{-2} lower at Zakynthos than Kyparissia in 2009 (Paired T (19266) = 12.9, $p < 0.0001$) and 2010 (Paired T (11935) = 4.7, $p < 0.0001$), respectively. Solar radiation was 11.57 to 14.47 W m^{-2} and 5.13 to 7.33 W m^{-2} lower in 2009 than 2010 at Zakynthos (Paired T (12105) = 17.6, $p < 0.0001$) and Kyparissia (Paired T (11792) = 11.1, $p < 0.0001$), respectively. Mean maximum solar radiation was 935.3 ± 160.1 W m^{-2} and 772.3 ± 55.3 W m^{-2} at Zakynthos in 2009 and 2010, and was 944.9 ± 144.0 W m^{-2} and 925.0 ± 94.8 W m^{-2} at Kyparissia in 2009 and 2010, respectively, and varied along with the seasonal solar declination.

Temperature

Temperature showed a diurnal pattern, with higher temperatures during the day than at night (Figure 2-4). The daily temperature range was 9.5 ± 4.6°C and 10.0 ± 3.4°C at Zakynthos in 2009 and 2010, and 10.0 ± 4.4°C and 10.0 ± 3.6°C at Kyparissia in 2009 and 2010, respectively (Figure 2-5). Temperatures were 2.1 to 2.2°C and 1.9 to 2.0°C warmer at Zakynthos than Kyparissia in 2009 (Paired T (19266) = 152.8, $p < 0.0001$) and 2010 (Paired T (11935) = 119.9, $p < 0.0001$), respectively. Temperatures were 0.4 to 0.5°C and 0.9 to 1.0°C warmer in 2009 than 2010 at Zakynthos (Paired T (12105) = 23.5, $p < 0.0001$) and Kyparissia (Paired T (11792) = 48.0, $p < 0.0001$), respectively. Sand temperature at nest depth was significantly different among beaches, years, and date (ANCOVA F(10, 182) = 86.5, $p < 0.0001$). Marathonissi had the lowest beach temperature (26.7 ± 1.6°C) followed by Laganas (27.9 ± 0.8°C). All other beaches were not significantly different from each other and above the pivotal temperature of 29.3°C for most of the season with a mean of 30.3 ± 1.5°C (Figure 2-5).

Rain

Kyparissia had a total of 16.2 and 0 mm of rain in 2009 and 2010, and Zakynthos had a total of 0.6 and 59.2 mm in 2009 and 2010 (Figure 2-6). Rainfall was unusual at these locations during July and August and began again during September in both years after I had disassembled the weather station at Kyparissia (both years) and Zakynthos (2009). The rainfall events of September 2010 in Zakynthos were typical for this time of year, although not recorded at the other stations. At this time, most loggerhead nests had hatched on all but the coolest beaches, such as Marathonissi.

Relative Humidity

Relative humidity had a diurnal pattern with higher values during the night and early morning than during the day. This pattern was more clearly defined at Kyparissia than at Zakynthos (Figure 2-7). Relative humidity was 11.4 to 12.0 % and 11.6 to 12.3 % lower at Zakynthos than Kyparissia in 2009 (Welch Two Sample T (37406) = 77.4, $p < 0.0001$) and 2010 (Welch Two Sample T (22942) = 68.1, $p < 0.0001$), respectively. Relative humidity was no different between years at Zakynthos (Welch Two Sample T (24190) = 0.6, $p > 0.05$) but was 3.8 to 4.4 % lower in 2009 than 2010 Kyparissia (Welch Two Sample T (23153) = 24.4, $p < 0.0001$), respectively. Mean relative humidity was 59.2 ± 23.4 % and 64.2 ± 21.0 % at Zakynthos in 2009 and 2010, and was 71.3 ± 20.9 % and 72.2 ± 17.1 % at Kyparissia in 2009 and 2010, respectively. There was no clear seasonal trend, although relative humidity did seem to have a regional effect especially evident where the peaks and troughs corresponded to the same dates between Zakynthos and Kyparissia in 2009 (Figure 2-8).

Wind Speed

Wind speed had a diurnal pattern with higher values during the day than at night that was more clearly defined at Kyparissia than at Zakynthos (Figure 2-9). Wind speed was 0.26 to 0.30 m s^{-1} and 0.18 to 0.23 m s^{-1} higher at Zakynthos than at Kyparissia in 2009 (Welch Two Sample T (38351) = 25.7, $p < 0.0001$) and 2010 (Welch Two Sample T (22831) = 14.0, $p < 0.0001$), respectively. Wind speed was no different between 2009 and 2010 at Zakynthos (Welch Two Sample T (23879) = 0.9, $p > 0.05$) or Kyparissia (Welch Two Sample T (21618) = 1.2, $p < 0.0001$), respectively. Mean wind speed was 1.85 ± 1.10 m s^{-1} and 1.75 ± 1.39 m s^{-1} at Zakynthos in 2009 and 2010, and was 1.59 ± 0.91 m s^{-1} and 1.57 ± 1.38 m s^{-1} at Kyparissia in 2009 and 2010, respectively. There was no clear seasonal trend in wind speed for Zakynthos and Kyparissia in 2009 and 2010 (Figure 2-10).

Oxygen

Oxygen concentration was lower on Thiafi than all other beaches in 2009 and 2010 (Figure 2-11). Thiafi was also the only beach that differed between years and had a trend across the season. Mean oxygen concentration was 20.7 ± 1.0 kPa on Thiafi and 21.1 ± 0.3 kPa on all other beaches for 2009 and 2010 combined.

Discussion

During the loggerhead turtle (*Caretta caretta*) nesting seasons of 2009 and 2010, the weather at Zakynthos was hotter, drier, and windier than at Kyparissia. Sand temperatures at nest depth were not different among most beaches at both sites, except that Laganas and Marathonissi at Zakynthos had lower temperatures. Oxygen content was only different on Thiafi compared to all other beaches.

Daily cycles of weather variables were more clearly defined in Kyparissia than Zakynthos especially for relative humidity, and wind speed. This was likely due to the topography of the locations. Kyparissia was a long beach (44 km) with a well-established dune system (Rees 2004). The weather station at Kyparissia was positioned without any visible taller obstructions for nearly 180° facing west and only low trees to the east, the nearest more than 100 m distance (Figure 2-1 B). Conversely, Zakynthos, and more specifically, Laganas Bay was 6 km wide at the mouth with occasional small beaches (Margaritoulis 1982). The weather station at Zakynthos was positioned in the small dunes behind Sekania beach between two dry river beds that during the rainy season drain the water from the steep slopes above. Although I placed the weather station at the most unobstructed place possible behind the beach, less than 90° facing southwest had no visible taller obstructions. There are also steep cliffs behind the beach, obstructing morning sun. While there were some differences in topography between individual beaches within Laganas Bay, the weather conditions experienced behind Sekania were likely very similar among the other beaches. The differences in topography did provide for differences between the Zakynthos and Kyparissia.

Oxygen content in the sand did not appear to vary with weather conditions. Oxygen did show an increasing trend on Thiafi in both years (Figure 2-11), but this was most likely due to the metabolic influence of hatching nearby nests. Due to the small size of suitable nesting habitat on Thiafi (approximately 100 m^2) and the relatively large number of nests on the beach (10-20 nests), the oxygen content at the control sites was most likely influenced by nearby metabolizing nests (Honarvar et al. 2008) or by microbial metabolism in decomposing nests from previous seasons (Clusella Trullas 2007). As nearby nests hatched, the amount of metabolic mass in the sand decreased, and the oxygen levels increased. There was very little rain during the incubation period (Figure 2-6), and since the effect of rain on oxygen concentration in the sand was brief (Prange and Ackerman 1974), I was unable to detected any change at the time of my measurements.

Sand temperature was lower on the beaches of Marathonissi and Laganas at Zakynthos than all other beaches (Figure 2-5). Since loggerheads, and most turtles, have temperature-dependent sex determination with females produced at high temperatures, these beaches are important to the preservation of the population because the temperatures are conducive to producing males (Zbinden 2007). Climate change predictions for the Mediterranean show higher temperatures that suggest a female skewed population sex ratio for *C. caretta* (Godley et al. 2001), but turtles nesting early in the season (Katselidis et al. 2102) and on Marathonissi and Laganas (Zbinden 2007) produce more males at Zakynthos, which could slow this skewing. In addition to the sand temperature differences among the beaches of Zakynthos, the sand temperatures on sectors A and O of Kyparissia were higher than ambient air temperatures most days (Figure 2-5). This can be explained through the profile of the beach influencing the exposure of the sand to solar radiation.

At Zakynthos, the beaches have a slight lip at the water's edge, then continue at a gradual slope until they become steeper at the back of the beach where most loggerhead turtle nests occurred. The beaches of Sekania East, Sekania West, Thiafi, Gerakas, were predominantly south-west facing while Laganas, and Kalamaki were south facing. Marathonissi had exposures to both north and east, and lighter color sand. Behind all of these beaches were either steep clay cliffs or a dune system with vegetation, or a combination of both. Turtles did not nest behind the beach, so these areas were not considered, except that there may have been additional obstructions to solar radiation. The topography of Kyparissia sector A consisted of a west-facing slope above the wave zone continuing to a berm that extended horizontally with very little vegetation until it reached the dunes behind the beach. Kyparissia sector O was similar in topography to sector A, except that instead of dunes, there was an asphalt road and a stone wall. The turtles nested at or near the crest of the berm.

To approximate the profile for the beaches at Zakynthos, I used a plate with 30° west-facing slope and a horizontal plate. For Kyparissia I used a horizontal plate attached to the top of a 45° west-facing slope. Both locations were at 40°N Latitude. I assumed clear skies (1 dust particle cm-3 and 0.01 m water) at sea level for sand with albedo 0.2. I used biophysical equations (Gates 1980, Oke 1992) converted into MATLAB functions (M.P. O'Connor, personal communication) to determine the total (direct and diffuse) solar radiation at the equinox and summer solstice for each profile.

For the Zakynthos beach profiles, a horizontal flat plate had a longer exposure to direct solar radiation at both the equinox (Figure 2-12) and summer solstice (Figure 2-13) than the west-facing slope. The sloped surface had a higher peak than the horizontal flat plate at the equinox, but was equal at the solstice. The total solar radiation (area under the curve) would be 6137 and 9379 W hr m^{-2} for the horizontal plate and 5703 and 8545 W hr m^{-2} for the 30° west-facing slope for the equinox and the summer solstice, respectively. Not shown in the graphs, a 30° south-facing slope would have a daily total solar radiation of 7599 and 8644 W hr m^{-2} and a 30° north-facing slope would have 3293 and 7966 W hr m^{-2} at the equinox and summer solstice respectively.

For the Kyparissia beach profile, the solar radiation throughout the day would be the maximum value of all plates from horizontal through 45° west-facing. The solid line shows the integration of all of these slopes for the equinox (Figure 2-14) and summer solstice (Figure 2-15). The total solar radiation (area under the curve) for the beach profile of Kyparissia would be 7381 and 10984 W hr m^{-2} at the equinox and summer solstice respectively.

The curves in Figures 2-12, 2-13, 2-14 and 2-15 describe the solar radiation at the surface of each of the beach profiles. Differences in sand temperatures at nest depth among similarly profiled beaches could be due to thermal conductivity of the sand and the temperature and distance of the water table below the beach. The curves show that the sand at the crest of the berm at Kyparissia receives more solar radiation than at any of the Zakynthos profiles, which would allow for solar heating which is transferred through conduction to nest depth. Additional heat loading at the surface and conduction to nest depth could account for sand temperature at Kyparissia being higher than the ambient air temperature.

There were two beaches at Zakynthos that had lower beach temperatures at nest depth, Marathonissi and Laganas (Figure 2-5). Marathonissi beach consisted of a spit of sand that had a steep north-facing slope and a gradual east-facing slope. North-facing slopes at a latitude greater than 23.5°N, such as on Marathonissi, decrease the amount of solar radiation by increasing the solar angle of incidence from normal to slope (Gates 1980), so the north slope of Marathonissi beach received less direct solar radiation during the incubation seasons, but the east-facing gradual slope would have received solar radiation similar to a mirror image of the west-facing slope in Figures (2-12 and 2-13). Additionally for Marathonissi, the sand was a lighter color which may decrease the absorptance of solar radiation (Gates 1980). However, the explanation for lower temperatures on Laganas cannot be from solar radiation alone. Laganas beach had a near-horizontal south-facing slope, and the sand had similar color to most other beaches (except Marathonissi) so the daily solar radiation profile for Laganas would be similar to the curve for the flat plate in Figures (2-12 and 2-13). Furthermore, in 2009, there was a temperature gradient along Laganas beach where the west (26.4 ± 1.6°C) was cooler than the center (27.9 ± 2.2°C) and east (29.4 ± 1.4°C). While the beach profile of the nesting area was not visually different, the center and east part of the beach, much like Kalamaki beach, had dunes or clay cliffs behind the beach adds reflected solar radiation to those areas of the beach. The temperature difference could also be due to differences in thermal conductivity of the sand, or due to the temperature and depth of the water table below the beach. It is possible that the water between Marathonissi and west Laganas beach was cooler than the rest of Laganas Bay, contributing to the lower temperatures on those beaches.

The sea surface temperature of Laganas Bay is distributed with warmer water downwind (Schofield et al. 2009). Due to the topography behind Sekania beach, wind direction data showed a lot of variability and were not reported here, however the weather station at Zakynthos airport, located 1 km inland from Laganas Bay, reported wind direction for the summer of 2009 and 2010 to be predominantly from the north, but variable from all directions except south-west (Weather Underground Inc.). With the warm surface water moving away from the beaches, the cooler water from below moves to the surface. In shallower areas of the bay, especially around nesting beaches with a gradual near shore slope, the deeper water is still warm. Laganas Bay is mostly shallow and has clear water with sea grass (*Posidonia oceanica*) beds covering much of the floor from 5-15 m depth (Tsirika et al. 2007). For beaches with steeper near shore slopes, the water can be more than 2°C cooler at >10m depth than <5m depth (Schofield et al. 2009). It was possible that this interaction contributed to the lower temperatures on Marathonissi beach, and to a lesser extent, Laganas beach.

The loggerhead populations that use Kyparissia and Zakynthos as rookeries are among the largest in the Mediterranean (Margaritoulis 2003) and are major sources of genetic variation in the eastern basin (Carreras et al. 2007). The variation in temperature among beaches provides many suitable incubation options for the eggs, and can provide the population with a buffer to climate change. These beaches provide a unique system for studying natural variance of thermal conductance among sands that deserves more study, given the importance of temperature to the turtle population that uses this ecosystem for egg incubation.

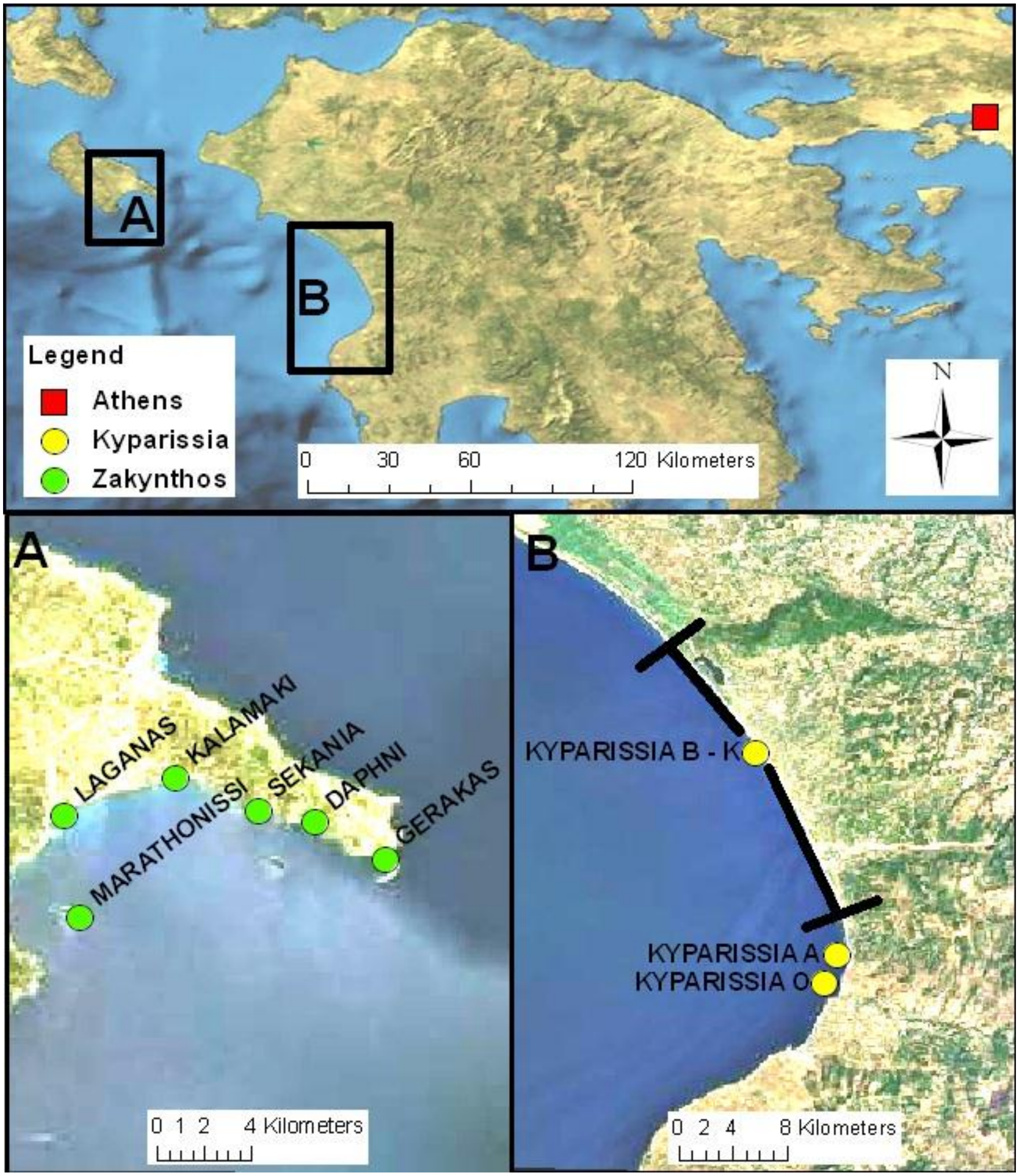

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Figure 2-1. The loggerhead turtle nesting beaches of Zakynthos Island (A) and Kyparissia Bay (B), Greece.

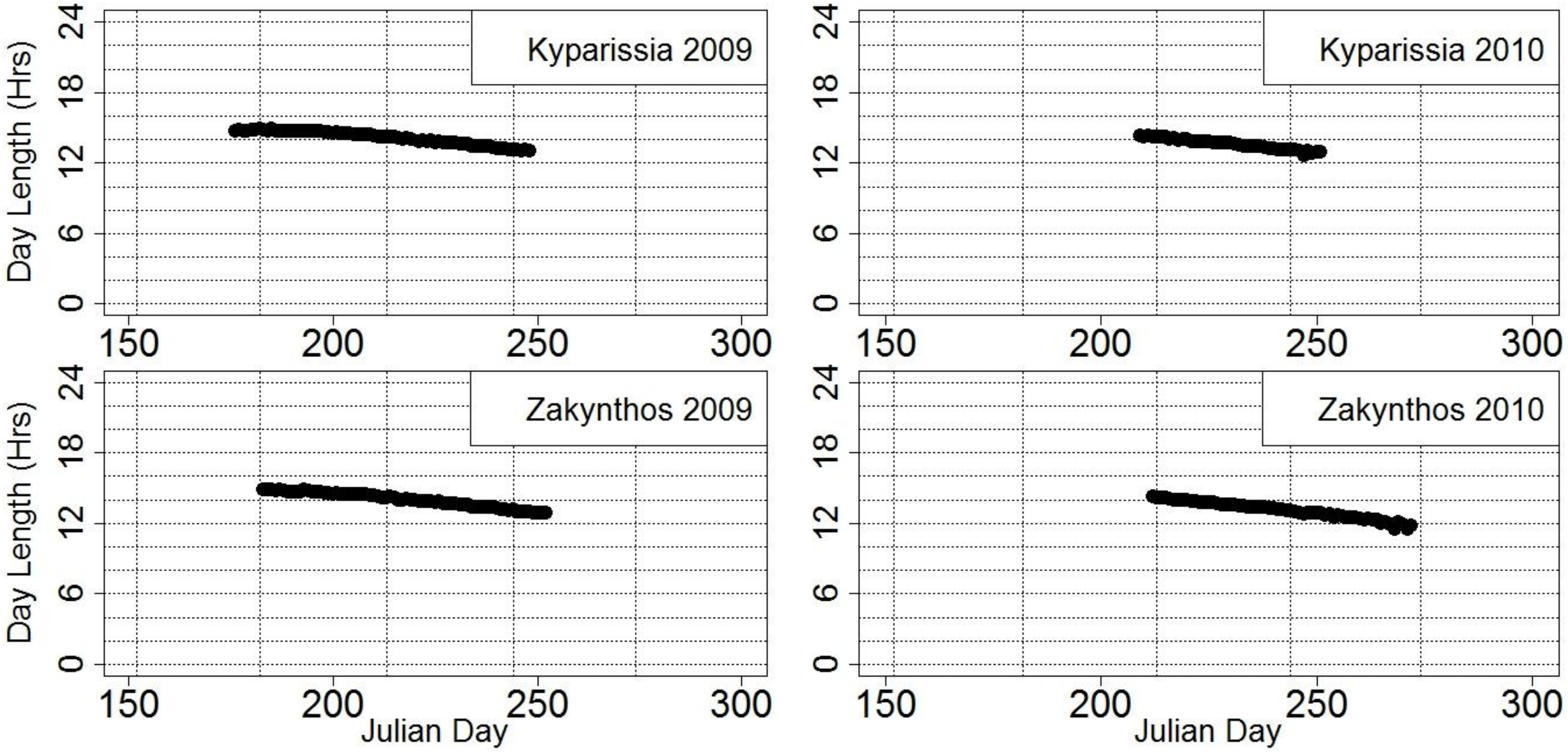

Figure 2-2. During the nesting season for loggerhead turtles in Greece, the day length decreases, reducing the amount of time that the beach is exposed to solar radiation. The vertical grid lines correspond to the first day of the calendar months of June through October.

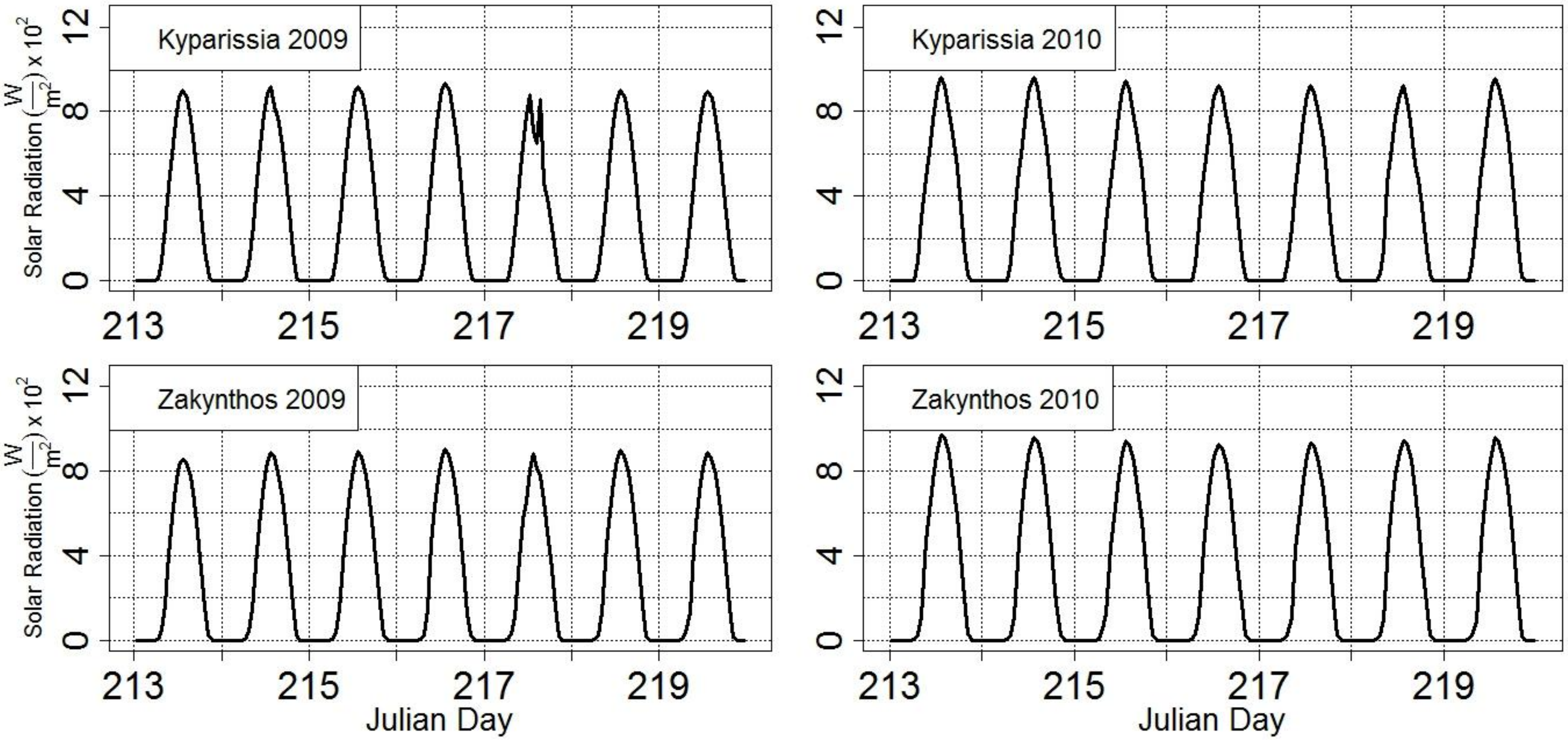

Figure 2-3. Hourly mean solar radiation had a strong diurnal pattern during the week of 01-07 August 2009 and 2010, with higher values during the middle of the day when there is direct sunlight. Some clouds at Kyparissia on Julian day 217, 2009 resulted in the abnormal pattern on that day. All other days describe skies. Vertical grid lines are at 00:00 hrs.

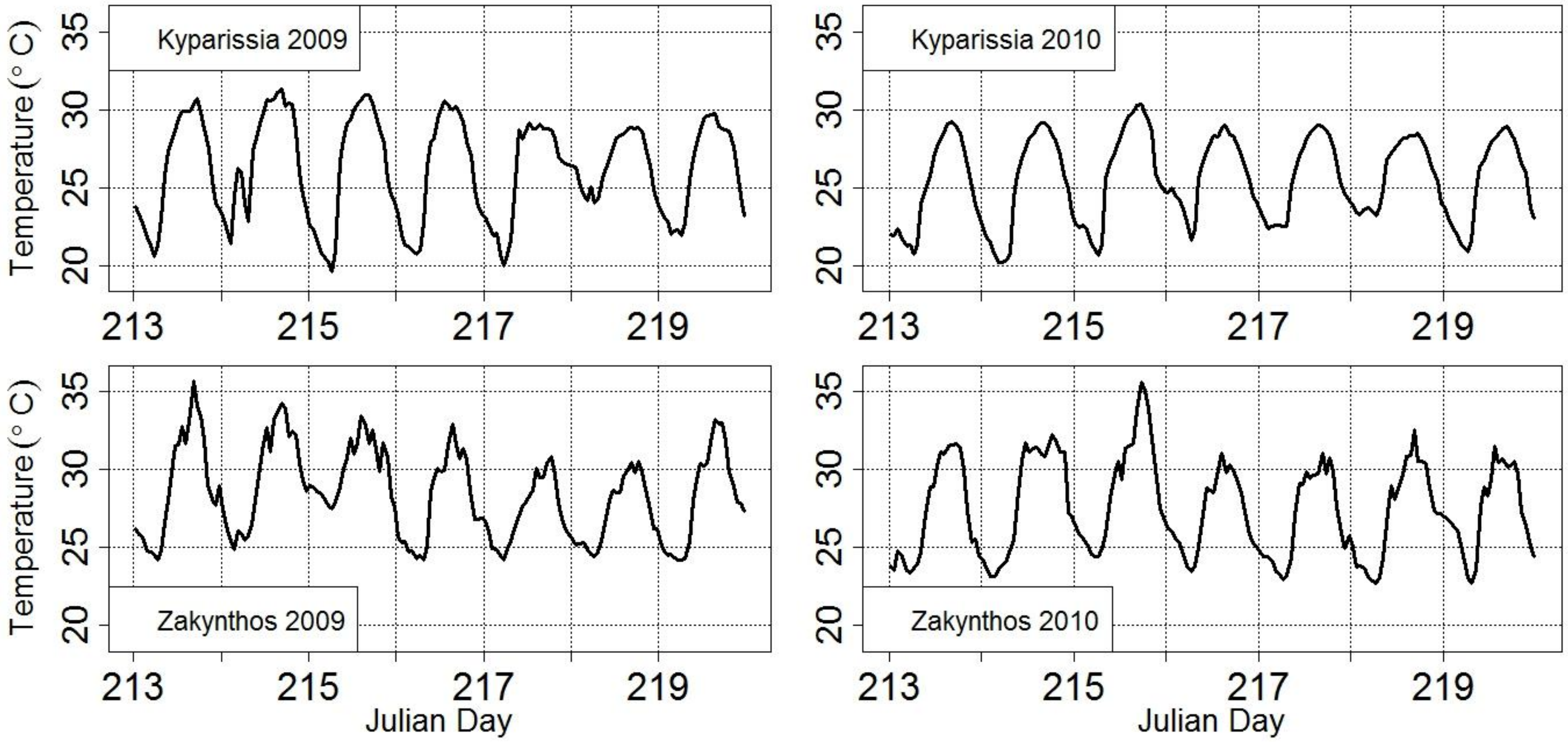

Figure 2-4. Hourly mean temperature had a strong diurnal pattern during the week of 01-07 August 2009 and 2010 with higher values during the day. Temperatures were higher at Zakynthos than at Kyparissia. Vertical grid lines are at 00:00 hrs.

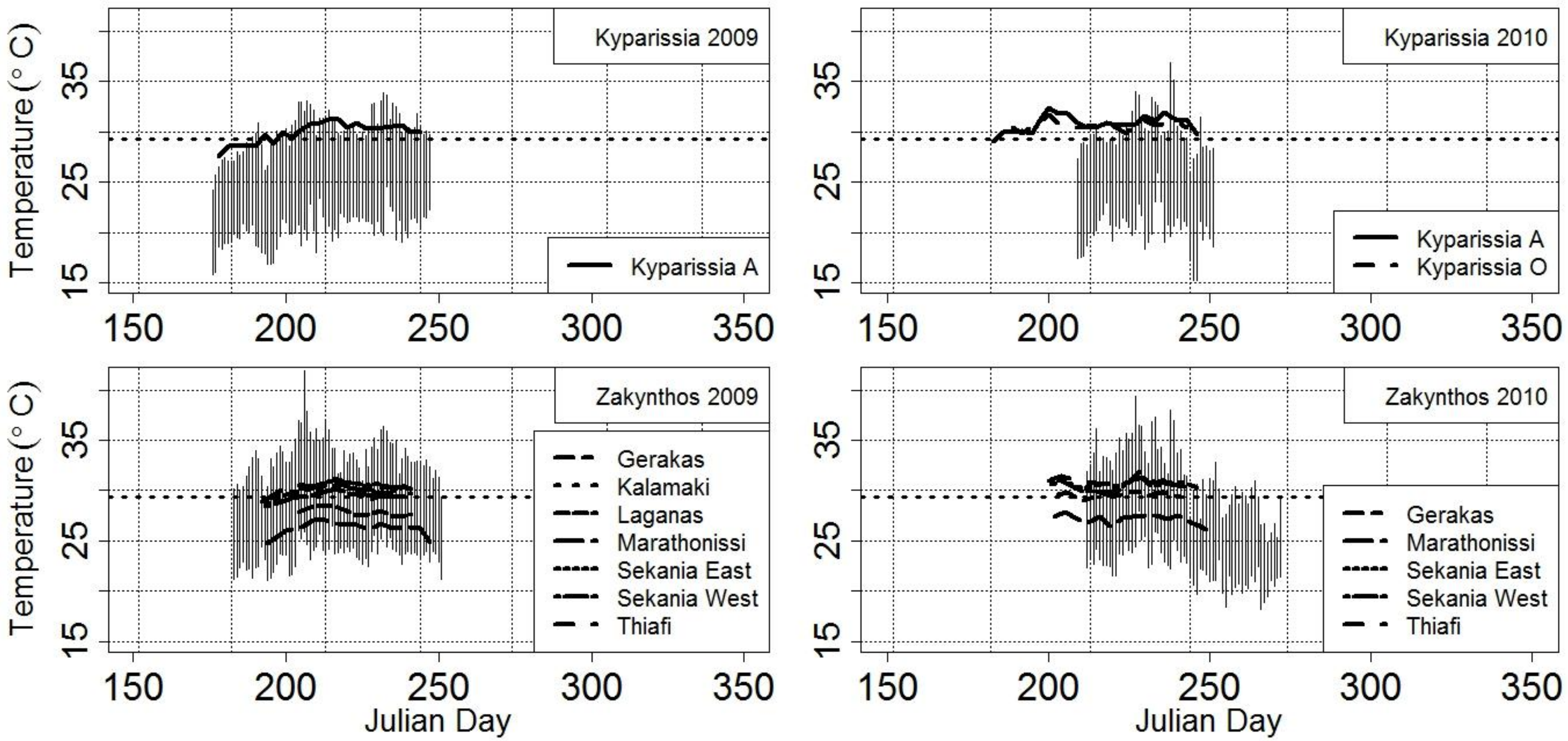

Figure 2-5. Sand temperatures at nest depth during the nesting seasons correspond to the range of daily temperatures (solid vertical lines) differently at different beaches. Most sand temperatures are close to the pivotal temperature for sex determination for loggerheads (29.3 °C, dotted line) except Marathonissi (both years) and Laganas (2009). Sand temperatures for Kyparissia beach sectors were often higher than observed air temperatures in both years. Vertical grid lines correspond to the first day of the calendar months of June through December.

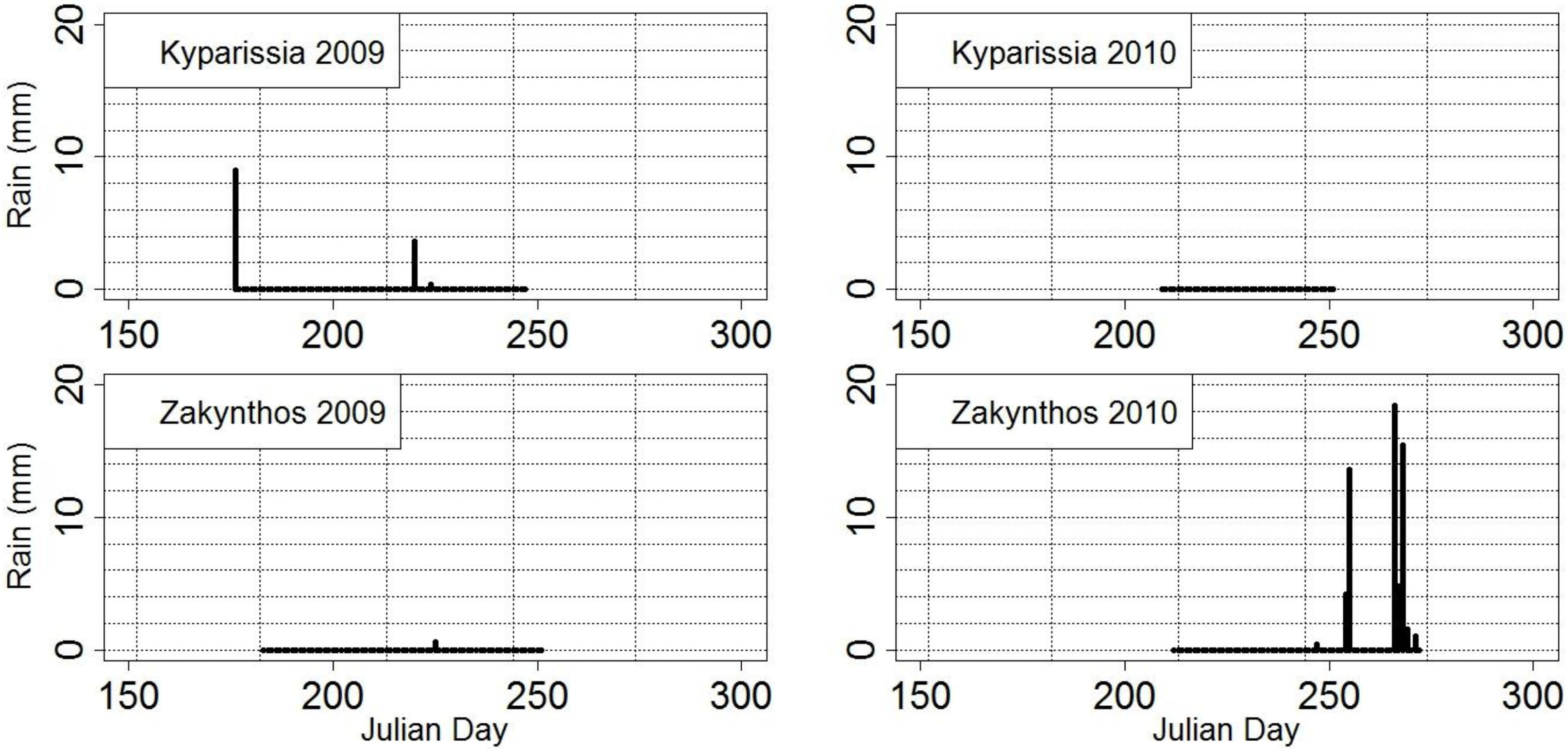

Figure 2-6. Total rainfall at Kyparissia and Zakynthos during the loggerhead turtle nesting seasons in 2009 and 2010. The vertical grid lines correspond to the first day of the calendar months of June through October**.**

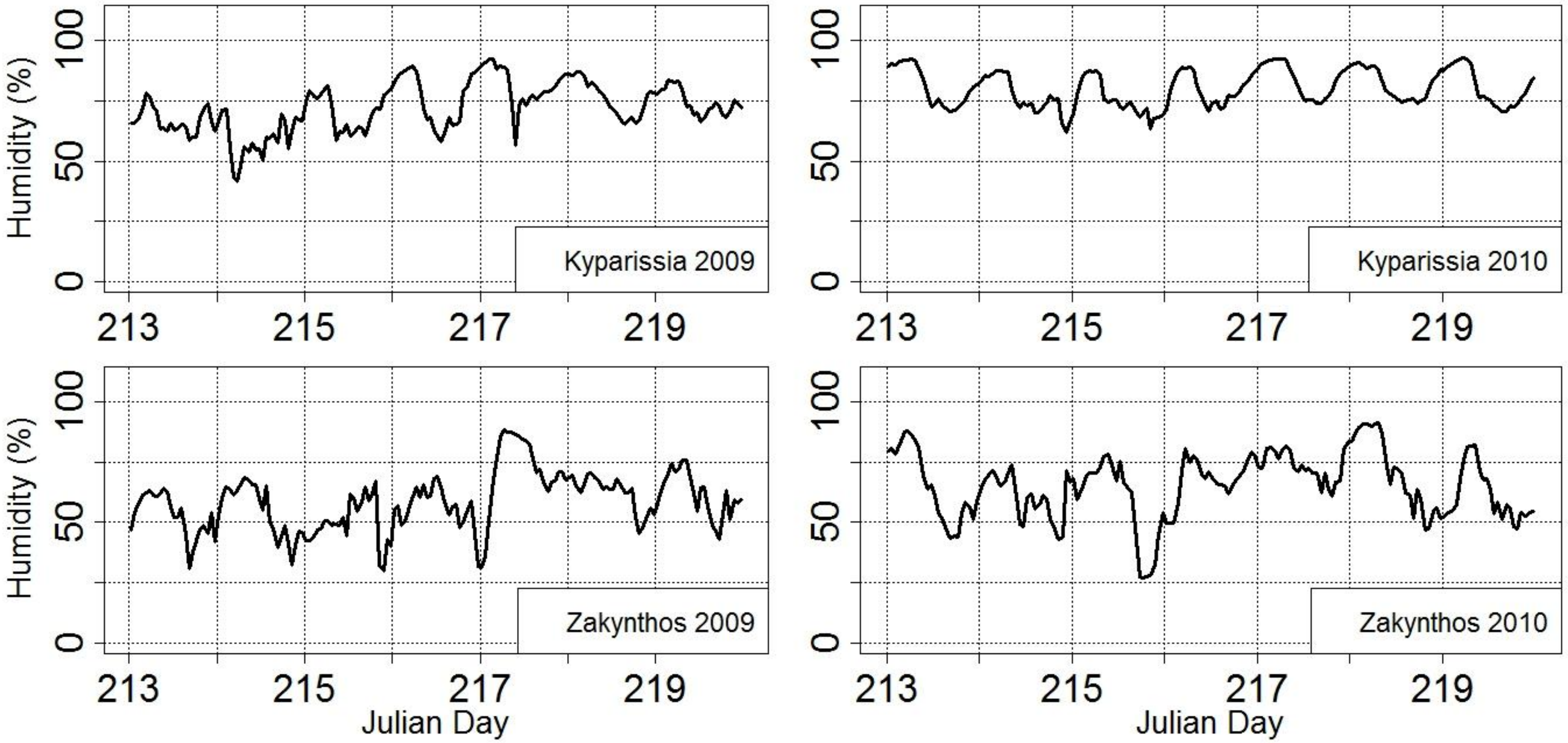

Figure 2-7. Hourly mean relative humidity showed a cyclical diurnal pattern that was higher in the night and early morning during the week of 01-07 August 2009 and 2010. Humidity is higher and the diurnal pattern is clearer for Kyparissia than Zakynthos for both years. Vertical grid lines are at 00:00 hrs.

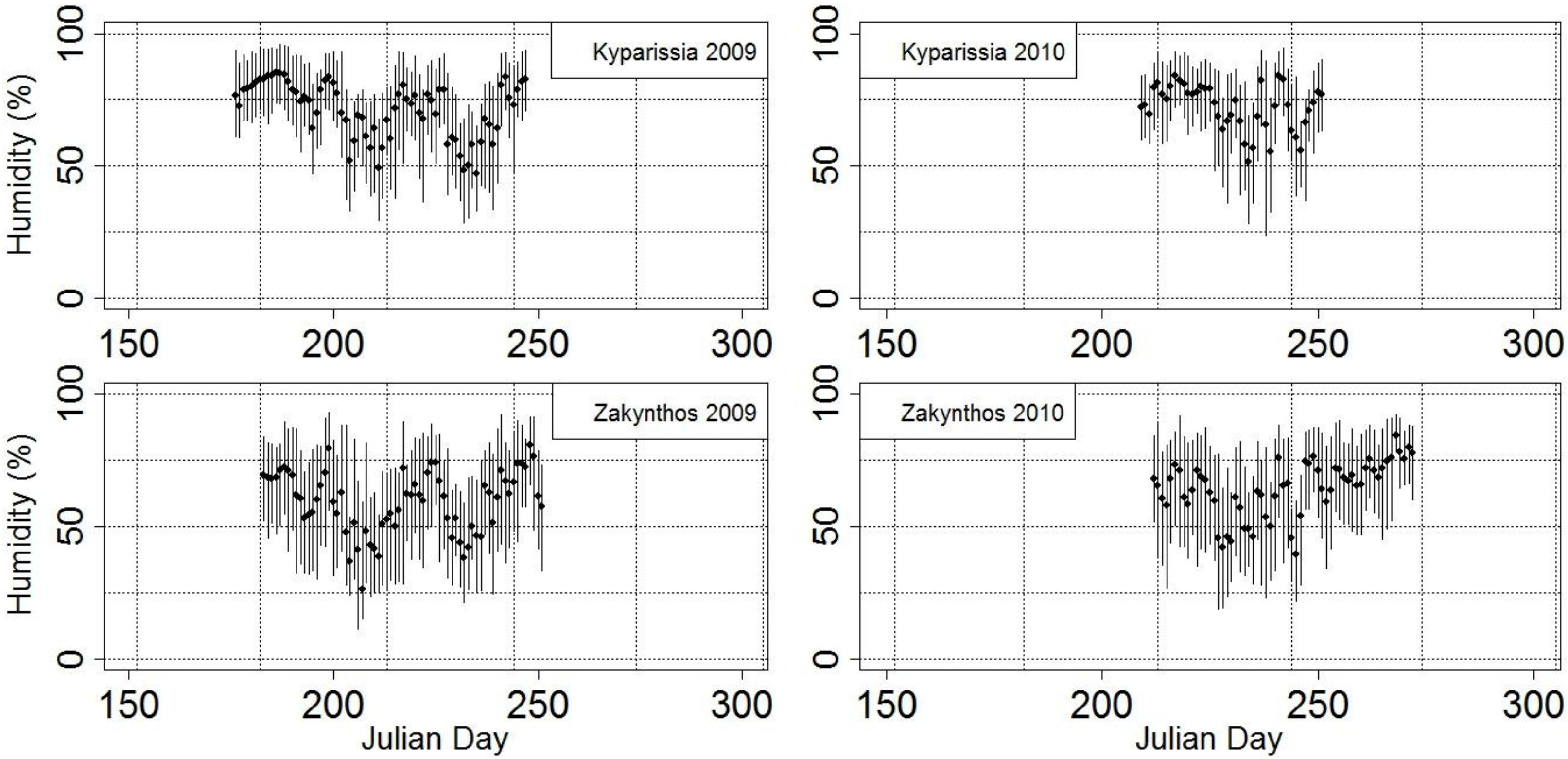

Figure 2-8. Daily mean relative humidity was higher at Kyparissia than Zakynthos throughout the nesting season. Error bars designate the daily range of humidity values. The vertical grid lines correspond to the first day of the calendar months of June through October.

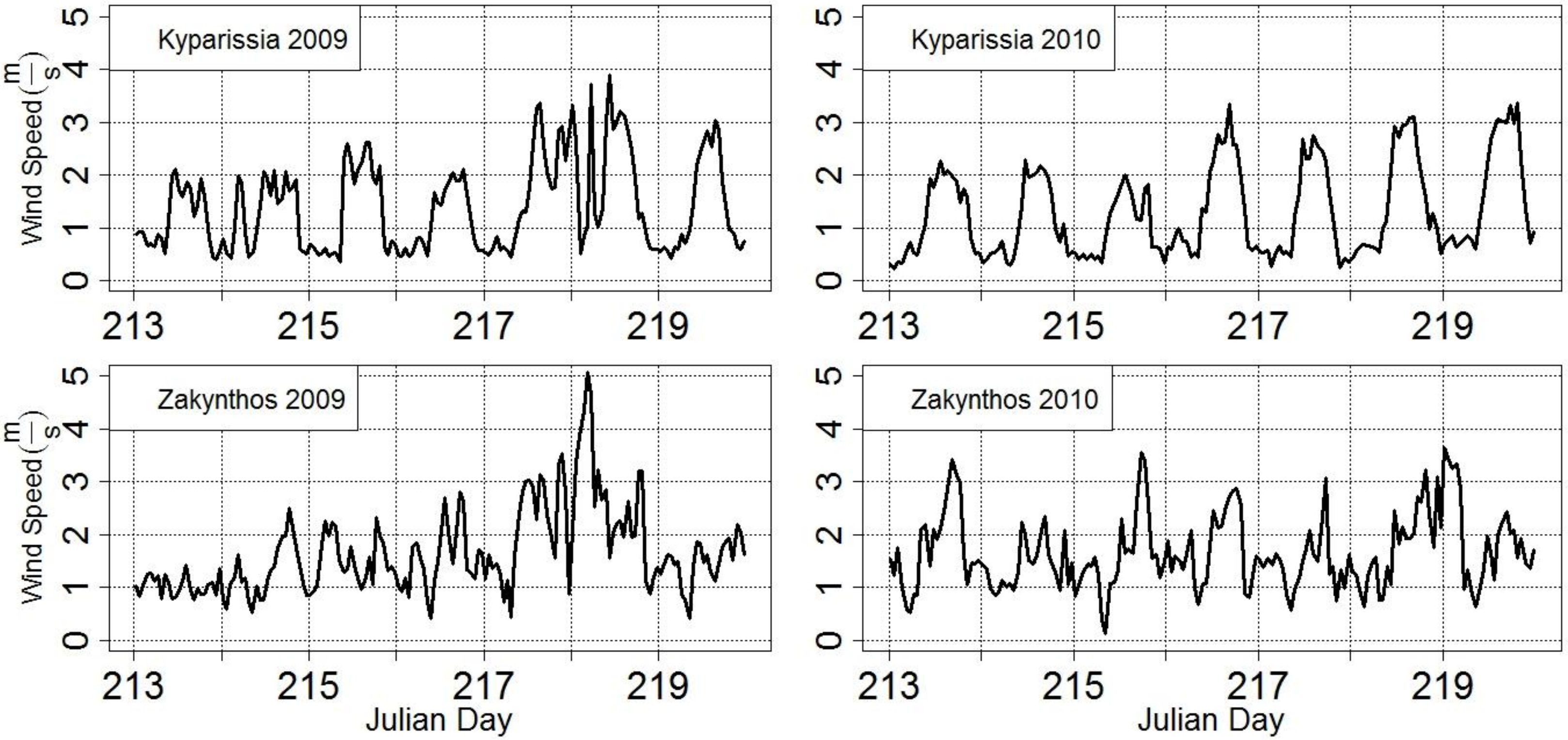

Figure 2-9. Mean hourly wind speed showed a diurnal pattern of windy days and calm nights that was clearer at Kyparissia during the week of 01-07 August 2009 and 2010. Vertical grid lines are at 00:00 hrs.

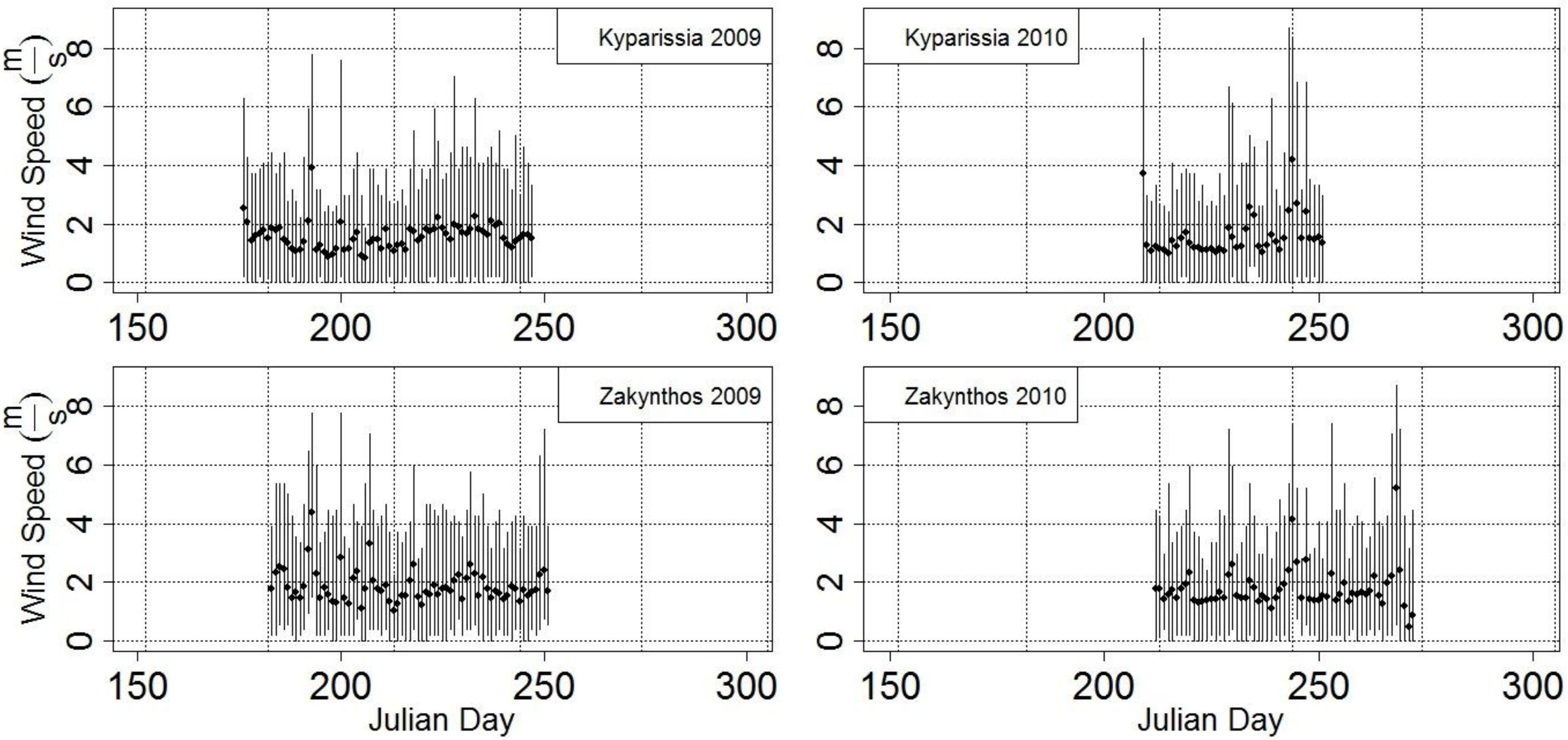

Figure 2-10. Mean daily wind speed at Kyparissia and Zakynthos, Greece during the loggerhead turtle nesting seasons in 2009 and 2010. Vertical grid lines correspond to the first day of the calendar months of June through October.

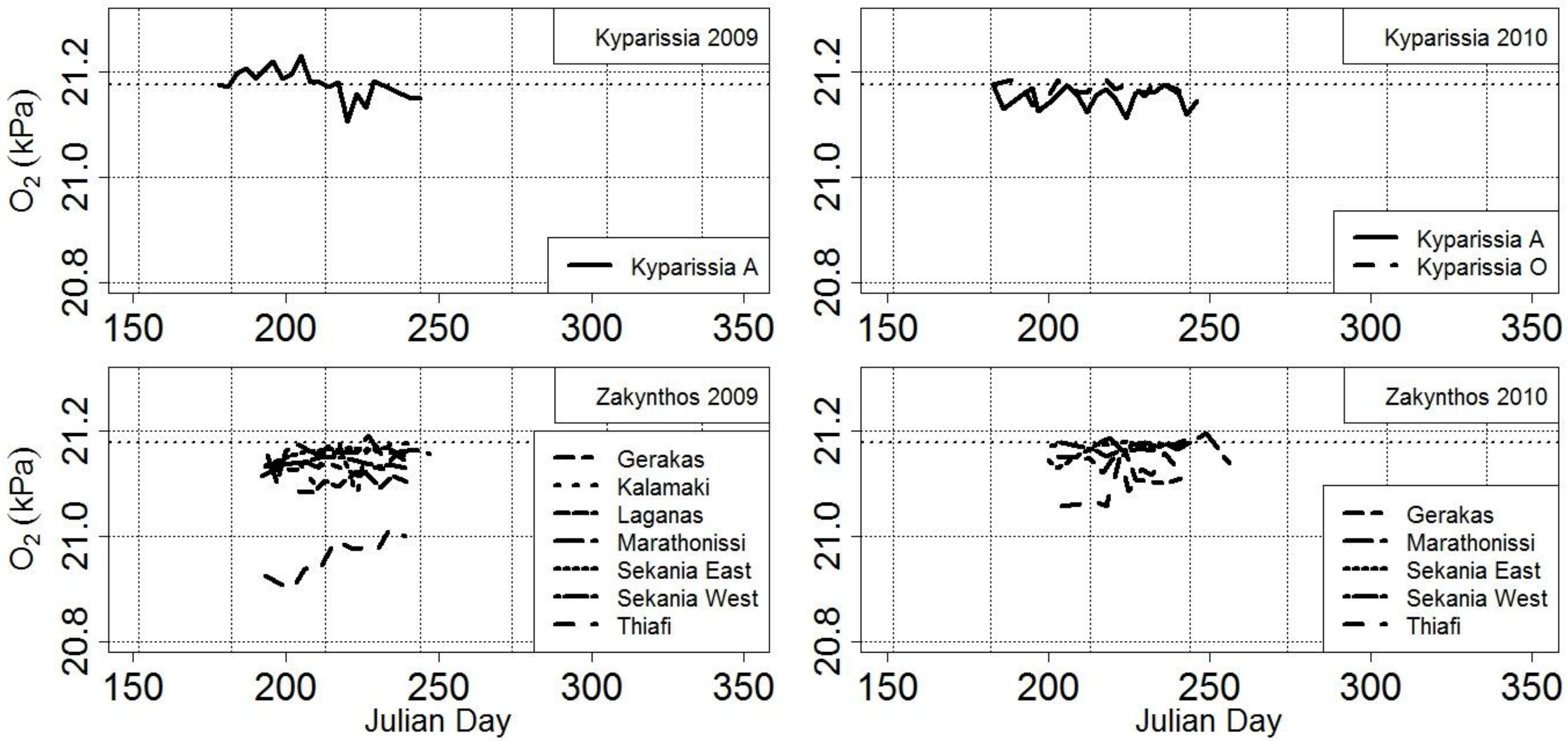

Figure 2-11. Oxygen concentrations at nest depth were generally below the atmospheric oxygen concentration (21.18 kPa, dotted line). Thiafi sand had lower oxygen in 2009. Vertical grid lines correspond to the first day of the calendar months of June through December.

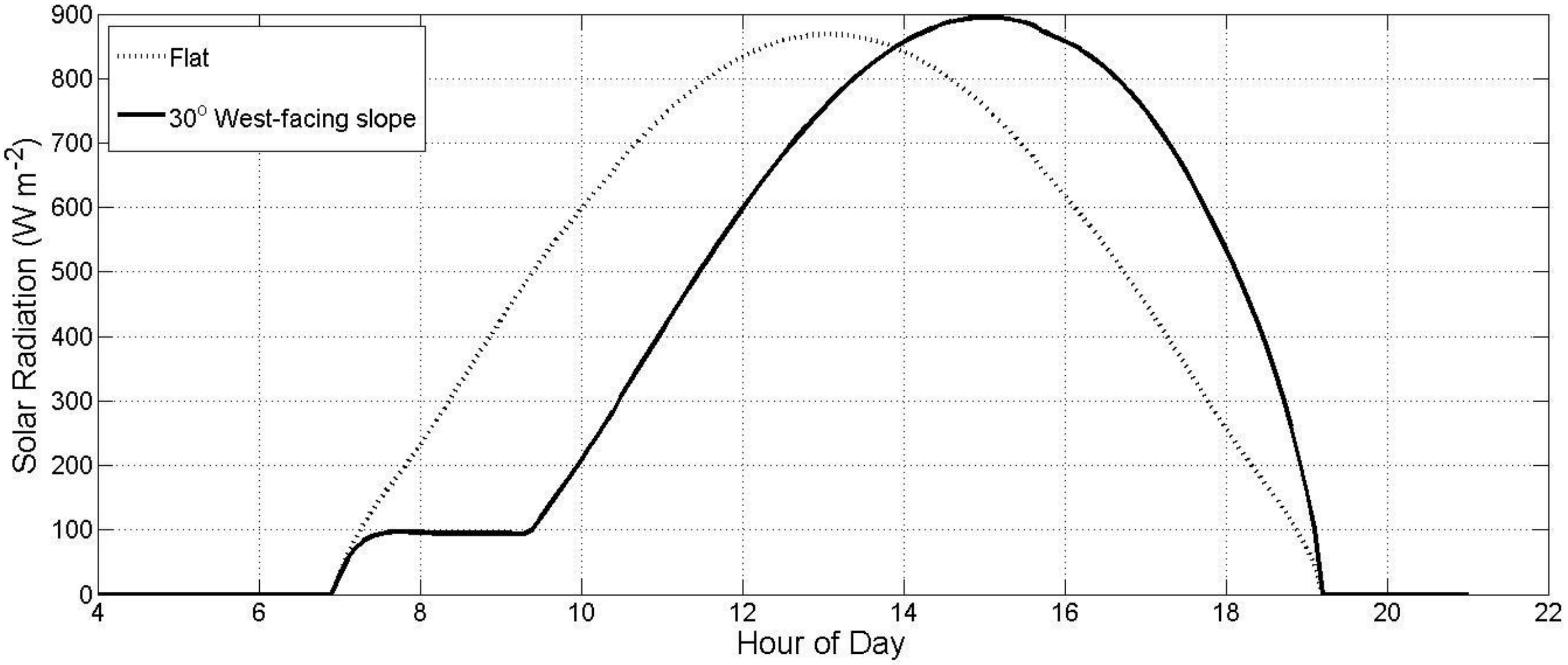

Figure 2-12. Daily profile for solar radiation on a flat beach and a beach with a 30° west-facing slope at 40°N latitude on the equinox (22 March or 22 September). The sand on the west-facing slope is exposed to a higher peak solar radiation two hours later than on the flat beach. Between 07:00 and 09:00, the west-facing slope is only exposed to diffuse solar radiation**.**

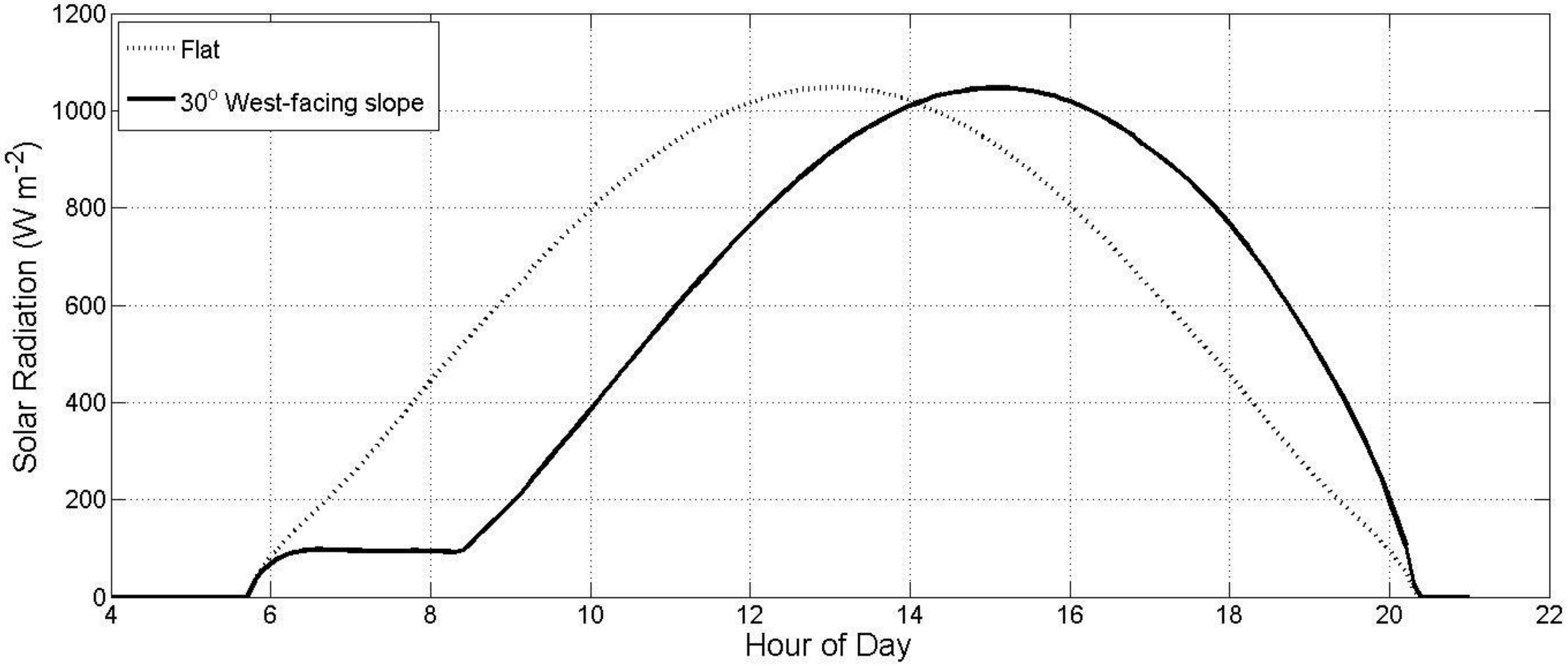

Figure 2-13. Daily profile for solar radiation on a flat beach and a beach with a 30° west-facing slope at 40°N latitude on the summer solstice (22 June). The sand is exposed to the most solar radiation two hours later on the west-facing slope than the flat beach. Between 05:45 and 08:30, the west-facing slope is only exposed to diffuse solar radiation.

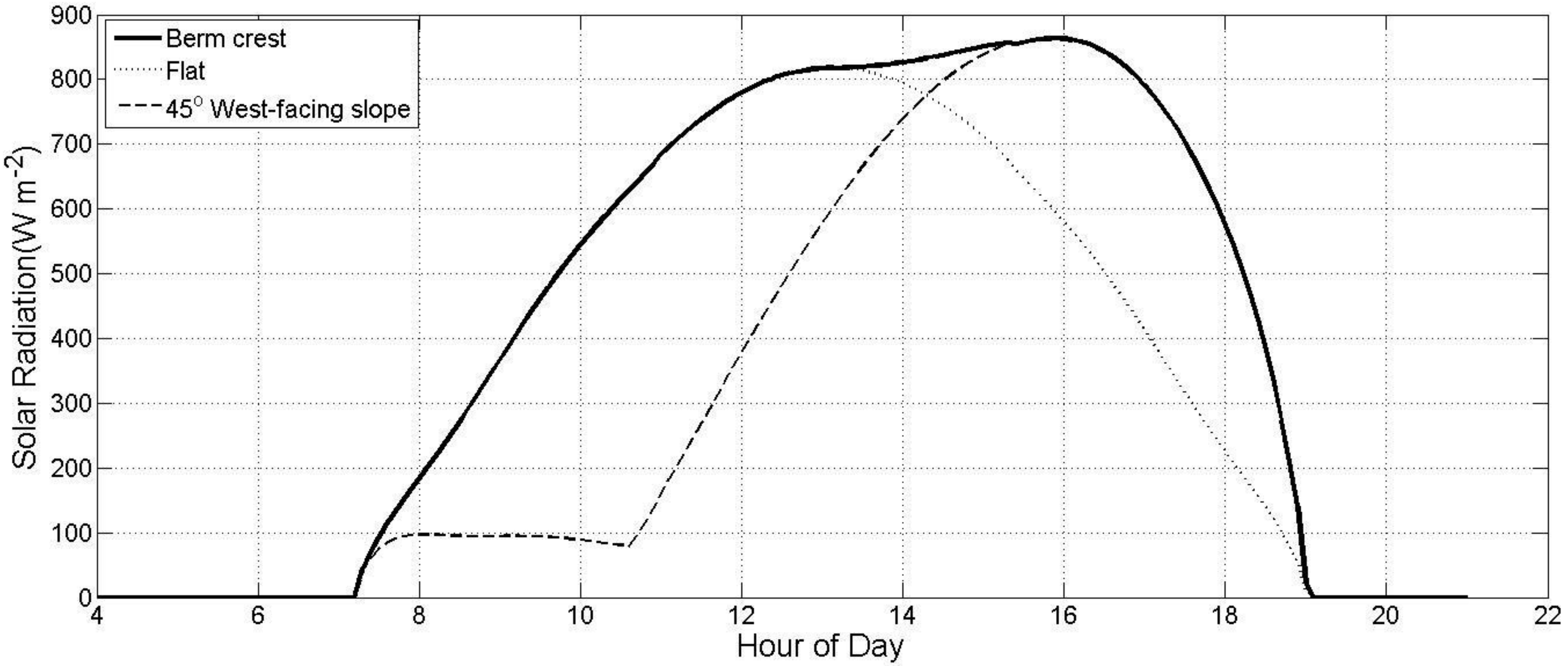

Figure 2-14. Daily profile for solar radiation on the crest of a beach berm consisting of a flat top and a 45° west-facing slope at 40°N latitude on the equinox (22 March or 22 September). The sand at the curve of crest is exposed to peak solar radiation from 13:00 until 16:00 and more total solar radiation than either the flat beach or west-facing slope alone.

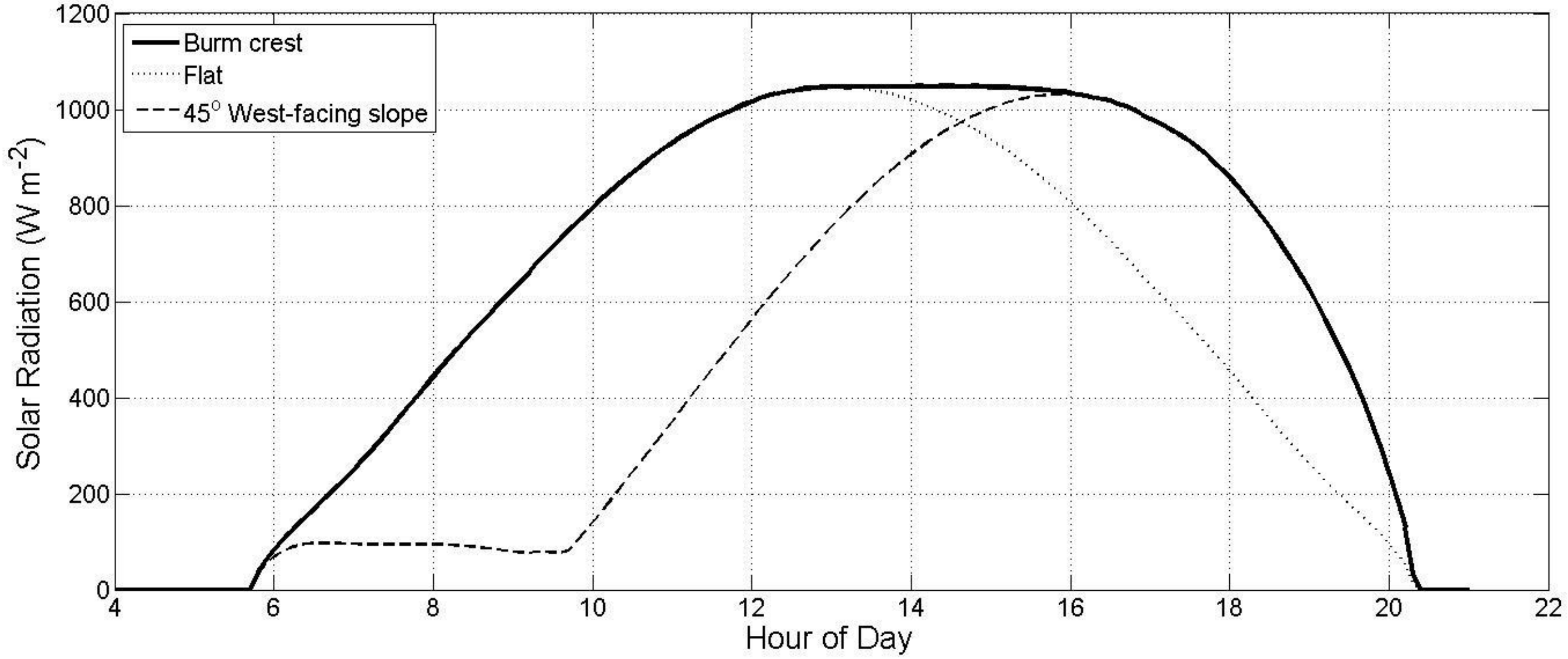

Figure 2-15. Daily profile for solar radiation on the crest of a beach berm consisting of a flat top and a 45° west-facing slope at 40°N latitude on the summer solstice (22 June). The sand at the curve of crest is exposed to peak solar radiation from 13:00 until 16:00 and more total solar radiation than either the flat beach or west-facing slope alone.

CHAPTER 3: WATER RELATIONS OF SEA TURTLE EGGS AND NESTING BEACH SAND

Introduction

Female loggerhead turtles bury clutches of eggs on beaches worldwide. Each egg contains resources necessary for growth and development and exchanges water, gases and heat with the environment (Ackerman et al. 1985). Water is an important factor for embryos developing in buried eggs and for the formation of new tissue (Packard 1999). The embryo can obtain water either directly from the yolk and albumin or through exchange with the environment (Ackerman 1997). The magnitude and direction of the water exchange between the environment and the egg contents are driven by a water potential gradient.

The water potential of the sea turtle egg has been reported as -750 to -800 kPa (Booth 2005). Pure water has a water potential of 0 kPa. Water movement accounts for changes in mass of the egg and its components (Ackerman and Lott 2005) as it moves down a water potential gradient such that if the surrounding sand has a water potential of -1000 kPa, the egg will lose water mass to the incubation environment according to:

$$\frac{dM}{dt} = K_e * A(\psi_e - \psi_s) \qquad (1)$$

where $dM\ dt^{-1}$ is the change in mass (mg) over time, K_e (mg cm^{-2} kPa) is the hydraulic permeability of the eggshell, and ψ_e (kPa) and ψ_s (kPa) are the water potentials of the interior of the egg and of the incubation environment (sand), respectively (Ackerman and

Lott 2005). Water is contained in a given environment dependent on the matric and osmotic potential of the material and the amount of water already present. Water potential increases as water content increases, thus increasing the potential for water to move out of the environment.

In this chapter, I generated soil water characteristic curves from sand samples of loggerhead turtle nesting beaches in Greece and compared to the water content of those beaches throughout the nesting seasons. I considered the eggs to be in hydric stress when the water potential of the surrounding sand is below -750 kPa and hypothesize that water potential will be lower in larger grained sands at similar water contents.

Methods

Water Content

In 2010, I collected sand samples from non-nest locations on nesting beaches bi-weekly during the nesting season. I determined gravimetric water content by weighing samples to the nearest 0.01 g on an OHAUS balance, drying for 24 hours at 100°C and reweighing; I considered water mass to be the difference in mass. Water content was the water mass per dry sand mass. I did not include any samples in the analysis that had longer than 1 week between collection and weighing.

Sand grain size characteristics

I manually shook dried sand for 15 minutes through standard soil sieves of sizes 2, 1.4, 1, 0.5, 0.355, 0.25, 0.15, 0.09 and 0.063 mm and weighed the contents of each bin. The bin sizes were converted to the Φ scale ($-\log_2$(mm)) to account for lognormal distribution during sedimentation (Folk 1980). Beaches were differentiated by the median grain size (Φ_{50}) and sorting (σ_Φ) for analyses according to Folk (1966) where

$$\sigma_\Phi = \left(\frac{\Phi_{84} - \Phi_{16}}{4}\right) + \left(\frac{\Phi_{95} - \Phi_5}{6.6}\right) \tag{2}$$

I interpolated values for Φ at the 5th, 16th, 50th, 84th and 95th percentiles from the slope of the line between the points on either side of each percentile.

Water Potential

I generated soil water characteristic curves from four samples from nest depth (30, 40, or 50 cm) from every beach using a WP4 Water Potentiameter (Decagon) calibrated with KCl. Laboratory water contents between 0.2% and 10% were prepared gravimetrically by adding water to dry sand and mixing thoroughly. I ran each water content preparation in triplicate. A water potential curve consisted of no less than four water content preparations for each sample.

I calculated the water content of the sand at osmotic equilibrium (ψ_e is equal to ψ_s in Equation 1) from the slope of the straight line between points on either side of -0.75 MPa. I calculated high and low estimates of the osmotic equilibrium sand water content. This is the water potential of a turtle egg, so this value represents the minimum water content at which an egg will not desiccate.

Data Analysis

I performed all statistical analyses using R. I used an ANOVA to test differences in water content at osmotic equilibrium between beaches. I used an ANCOVA to test for differences between beaches of water content during the incubation period. Since grain sizes are lognormally distributed, I present all sand grain data as $\Phi = -\log_2$ (mm), such that finer grains have larger Φ. I performed least squares regressions with Φ_{50} and σ_Φ as independent. For all statistical tests I accepted a significance level of $\alpha = 0.05$. Means are reported with two standard errors.

Results

Water Content and Water Potential

The soil water characteristic curve showed a similar pattern among all beaches (Figure 3-1). There were no differences between the water content of all beaches in 2010 (ANCOVA: $F(13,176) = 1.7$, $p > 0.05$). There were no differences for the water content at egg water potential (-0.75 MPa) between beaches (ANOVA: $F(6,21) = 1.9$, $p > 0.05$).

Sand Grain Size

Median grain size (Φ_{50}) did not affect the water content at osmotic equilibrium (Least Squares Regression: $F(1,26) = 2.1$, $p > 0.05$). Sand grain sorting (σ_Φ) had a significant effect on the water content at osmotic equilibrium (Least Squares Regression: $F(1,26) = 4.9$, $p = 0.04$, $R^2 = 0.16$).

Discussion

The water potential for all beaches at field water content (Table 3-1) was greater than -0.75 MPa, the water potential of a sea turtle egg (Figure 3-1). The loggerhead turtle eggs incubating on these beaches are not under hydric stress and should not strongly influence hatching success. The field water content of the beaches were all greater than the high water content values (Table 3-1) so there would be no net water loss from the eggs. Eggs incubated in cool and moist environments have higher hatching success than those incubated in hot and dry environments (Packard 1999) and as such there may be a hatching success gradient among these beaches. More likely, some beaches, such as on Kyparissia and Thiafi, which have right-shifted water potential curves (Figure 3-1) and relatively low field water content (Table 3-1) may have lower quality hatchlings by limiting the amount yolk converted to tissue (Booth and Yu 2009).

The sand grain size distribution characteristics did not explain the variations between beaches. Median grain size (Φ_{50}) did not affect the osmotic equilibrium sand water content and sorting (σ_{Φ}), while statistically significant, only explains 16% of the variation. It should be noted that water vapor moves through the environment mainly through a water vapor pressure (P) gradient similar to the water potential (ψ) gradient in equation 1, but there are five orders of magnitude less vapor permeability than liquid permeability at the same driving pressure (Ackerman and Lott 2005). Water vapor moves through a porous environment through the air-filled pore space while liquid water uses the water-filled pore space and displaces the air (Bear 1972). The significant effect of sorting on water content at osmotic equilibrium was most likely due to its correlation with air-filled porosity (Beard 1973) and the similarities between liquid and water vapor movement.

It is important that physiological studies take care to describe the water in the environment appropriately. Liquid and vapor water move through an environment through a water potential gradient determined by the osmotic, matric and gravitational force, but water vapor is also strongly influenced by temperature and air-filled porosity (Ackerman et al. 1985). Parchment-shelled eggs of reptiles are able to exchange water with the environment through both vapor (Packard et al. 1979) and liquid processes (Tracy et al. 1978). The most appropriate measurements to be reported are water potential to describe water movement, volumetric water content to describe gas or water vapor movement and gravimetric water content to describe the amount of water consumed. Since eggs are not consuming the substrate, gravimetric water content should not be reported as an explanatory variable in physiological studies.

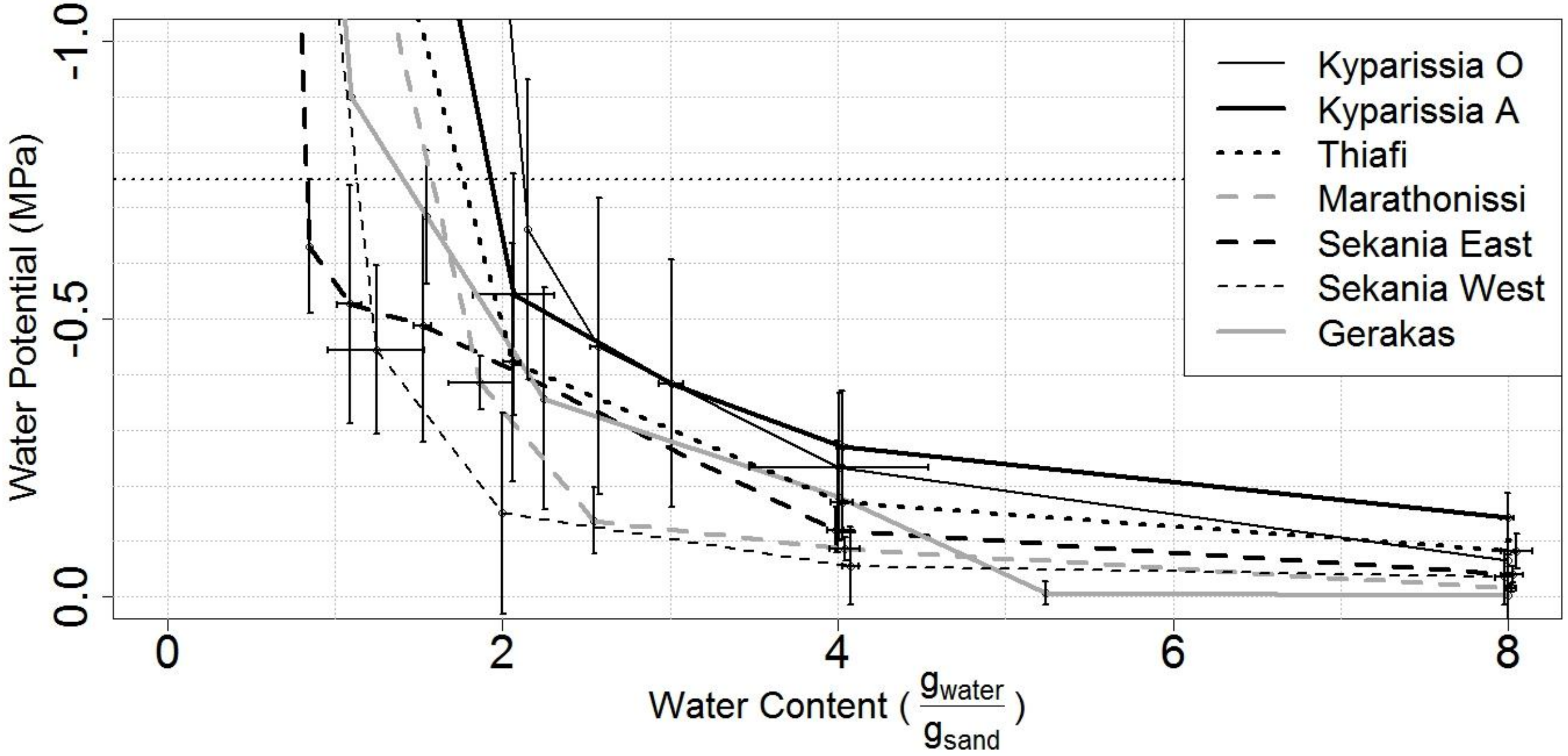

Figure 3-1. The water potential curve for loggerhead turtle nesting beaches at Zakynthos and Kyparissia, Greece demonstrates that when the water potential of the sand has a higher value (less negative) than the water potential of the sea turtle egg shell (-0.75 MPa, horizontal dotted line) the eggs are not under hydric stress. Error bars are two standard errors of the mean**.**

Table 3-1. Sand water content values (g g^{-1}) at osmotic equilibrium were calculated from the slope of the line in Figure 3-1. High and low water contents were calculated from the slope of the line among positive error points and negative error points, respectively and represent the range of water content at the hydric stress boundary. Field water content was the average value of the 2010 nesting season. Median grain size (Φ_{50}) and sorting (σ_{Φ}) are Φ ($-\log_2$(mm)) for the samples used to generate the water potential curves. Errors are two standard errors of the mean**.**

Beach	**Sand Water Content at ψ_{Egg}**	**Low Water**	**High Water**	**Field Water Content**	**Φ_{50}**	**σ_{Φ}**
Kyparissia O	2.13	1.97	2.55	3.85 (±0.46)	-0.66 (±0.33)	0.89 (±0.14)
Kyparissia A	1.93	1.41	2.36	3.52 (±0.15)	0.03 (±0.34)	0.67 (±0.15)
Thiafi	1.76	1.53	2.00	2.97 (±0.39)	1.15 (±0.14)	1.32 (±0.21)
Marathonissi	1.58	1.28	1.83	4.38 (±0.41)	1.23 (±0.23)	0.78 (±0.13)
Sekania East	0.83	0.77	0.85	2.61 (±0.21)	1.97 (±0.10)	0.90 (±0.19)
Sekania West	1.13	0.85	1.47	3.26 (±0.26)	1.83 (±0.19)	0.71 (±0.25)
Gerakas	1.41	0.97	1.70	3.21 (±0.25)	2.21 (±0.21)	0.52 (±0.05)

CHAPTER 4: PHYSICAL CHARACTERISTICS OF SAND FROM SEA TURTLE NESTING BEACHES AFFECT GAS MOVEMENT

Introduction

Gas movement within soils is an important process for the organisms that reside there, from plants to micro- and macrofauna. Historically, there has been much interest in gas flux through soil in agriculture as it relates to oxygen supply to plant roots (Bakker and Hidding 1970, Troeh et al. 1982), in addition to the use of pesticides, herbicides and fertilizers in agriculture (Bruckler et al. 1989), and more recently in assessing greenhouse gas emissions (Mancinelli 1995, Lange et al. 2009) and carbon cycling (Pataki et al. 2011). The structure of soil consists of solid, liquid and gas phases, and as such, a material moving through soils will follow the path of least resistance. Respiratory gases move through sand mainly through the air-filled pore space, although oxygen diffusion through water is also possible, but occurs at a much slower rate than in the air.

For organisms living within the soil matrix soil diffusivity may constrain gas exchange for aeration of their metabolic output. Sea turtles bury egg masses in the sand where they are left to incubate until hatching. Oxygen concentration decreases throughout incubation reaching minimum O_2 and maximum CO_2 just before hatching (Ackerman 1977, Maloney et al. 1990, Miller 2008). The low-oxygen environment is due to increased oxygen consumption during embryonic development (Ackerman 1981, Reid et al. 2009), as well as the resistance to gas exchange of the nest environment (Ackerman 1977). The nests of other organisms that bury eggs, such as megapode birds (Seymour et al. 1986) and crocodilians (Booth 2000) have similar oxygen profiles during development.

Generally, the minimum oxygen concentration in the nest corresponds to the number of developing embryos (Wallace et al. 2004), but if the nest becomes too hypoxic, hatching success can decrease (Honarvar et al. 2008, Garrett 2010). Sand characteristics explain differences in hatching success of green turtle nests (Maloney et al. 1990), but have not explained variations in gas concentrations (Chen et al. 2010). Estimates of sand diffusivity have been made through modeling (Ackerman 1977), but diffusivity alone may not be adequate to explain nest gas concentrations, especially on beaches with large tidal fluctuations (Wallace et al. 2004).

The sea turtle nest acts as an oxygen sink and a CO_2 source, and offers an excellent model for gas movement. Oxygen concentrations in the nest are lower than in the surrounding sand resulting in a net diffusion of oxygen into the nest, at the same time CO_2 diffuses out (Ackerman 1977). Bulk flow of gases into and out of the nest can occur through tidal movement, as the water table rises and falls with the tide, it acts as a piston under the beach producing high and low pressures, which can ventilate the nest (O'Connor et al. 2011). Bulk flow of gases can also be generated through the gas sink environment in the nest. The sea turtle nest produces a respiratory quotient (RQ) of 0.7 (Ackerman 1977), meaning that for every 10 mL of oxygen consumed, 7 mL of CO_2 is produced, leaving 3 mL that needs to be replaced. As the clutch of eggs metabolizes at this RQ, the nest containing the eggs becomes an area of low pressure and the net convective flux of gas will be into the nest (O'Connor et al. 2011).

Diffusion is the dominant force of gas exchange in the soil (Buckingham 1904), where the gas flux (μg s^{-1}) is governed by Fick's first law:

$$F = AD\frac{\Delta C}{\ell} \tag{1}$$

where A is the cross-sectional area (cm^2), D is the diffusivity of the material ($cm^2\ s^{-1}$), ΔC is the concentration difference (μg cm^{-3}) through the material over a distance ℓ (cm). Diffusive fluxes are therefore driven by the partial pressure differences, or differences in concentration, of a gas. The differences in diffusive gas exchange through a variety of porous media can be described through the D of the gas in each medium.

Many attempts have been made to clarify the relationship between the gas diffusion constant (D_s) and soil structure (van Bavel 1952, Freijer 1994). Generally, there is a positive relationship between diffusivity and air-filled porosity (ε_{air}) (Buckingham 1904, Penman 1940, Marshall 1959) which can be described as

$$\frac{D_S}{D_0} = \alpha\varepsilon^{x} + \beta \tag{2}$$

where D_s is the diffusion coefficient in the soil ($cm^2\ s^{-1}$), D_0 is the diffusion coefficient in air ($cm^2\ s^{-1}$), ε is the air-filled porosity ($cm^3\ cm^{-3}$) and α,β, and x are characteristics of the gas and medium. These parameters are affected by tortuosity (Troeh et al. 1982, Ullman and Aller 1982, Freijer 1994), constrictivity (Freijer 1994), pore continuity (Bruckler et al. 1989), compaction (Currie 1984a, Fujikawa 2005), morphology (Bruckler et al. 1989), water content (Bakker and Hidding 1970) and water potential (Currie 1984b, Pereira and Cruciani 2001, Thorbjorn et al. 2008). All of these complexity factors define the active pore space (Moldrup 2005). Predictive models of gas diffusivity in soils have demonstrated that the relationship differs depending on disturbance and repacking (Moldrup 2000). Additionally, diffusion in soils may have a larger vertical component than horizontal (Kühne et al. 2012). While no single equation can adequately describe diffusion in all materials (Currie 1960), the general relationship that diffusivity depends on porosity has passed scientific scrutiny for more than a century.

Gas can also move through a soil via bulk flow driven by a total pressure gradient. This movement is driven by Darcy's Law:

$$Q = \frac{kA\Delta P}{\mu\ell} \tag{3}$$

where Q is the convective flux ($m^3\ s^{-1}$), k is the intrinsic permeability (m^2), A is the cross-sectional area for flow (m^2), ΔP is the difference in total pressure (Pa), μ is the gas viscosity (Pa s) and ℓ is the flow path length (m). In this chapter, I report conductivity (c, $m^3\ s\ kg^{-1}$) as the k of the sample over the μ_{air} at 30ºC and 90% humidity (Tracy et al. 1980). Convective fluxes are driven by total pressure differences across a boundary. The differences in convective gas flow through a variety of porous media may be described through the c of gas in the medium.

Conductivity through soil is usually reported as hydraulic conductivity, which includes a gravity component not applicable to gas flows in our system. Hydraulic conductivity through soil is affected by the pore radius usually estimated through moisture retention (Campbell 1974). Intrinsic permeability varies as a function of air-filled porosity (Olson et al. 2001). Different porosities, median grain size, and sorting factors affect the pore radius (Bezrukov et al. 2001).

Air-filled porosity consistently affects gas movement through soils via both diffusive and convective fluxes. Porosity varies with sorting, a standard estimate of the variance in the grain distribution, but is independent of median grain size (Beard 1973, Bezrukov et al. 2001). Grain size distributions on natural beaches are mixtures of log-normally distributed populations of grain sizes produced by different sedimentation processes (Spencer 1963), with sorting, skewness and kurtosis being descriptors of the sum of the influence of each of these processes on the beach (Folk and Ward 1957). Median grain size and morphology describe the distance traveled and the maturity of the source population (Folk 1954, Pettijohn 1957).

In this chapter, I report on the factors that affect gas movement through the sand on sea turtle nesting beaches. In order to generate better models of sea turtle nest gas exchange, I measured the diffusivity and conductivity of sands from sea turtle nesting beaches around the world with visibly different characteristics.

Methods

Sand Collection

I generated a library of sand from sea turtle nesting beaches in Greece (Kyparissia, Thiafi, Marathonissi, Sekania, and Gerakas), Costa Rica (Ostional, Naranjo, and Tortuguero), Bioko Island and Equatorial Guinea (Playa Moaba), in addition to commercially available “Play Sand.” These beaches were used by loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), green (*Chelonia mydas*), East Pacifc green (*Chelonia agassizi*) and hawksbill (*Eretrmochelys imbricata*) sea turtles.

I collected sand in a core of 50 cm length and 5 cm diameter from control sites on loggerhead turtle nesting beaches in Greece. A preliminary depth profile sieve analysis found no significant difference in sand structure in the first 50 cm; hence, after visual confirmation that there was no stratification, I dried and pooled each core. On Bioko Island, sand was collected in 50 mL screw-top Falcon tubes from the sides of leatherback turtle nests, up to 1 m depth. A preliminary depth profile sieve analysis found no significant difference in sand structure in the first meter, so I pooled those dry samples by nest site. Sand collections on the Costa Rica beaches occurred prior to this study and I re-dried them before analysis. I dried the sand at 100ºC for 24 hrs prior to international transportation per USDA regulations. For all experiments, the sand tested was a sub-sample of the pooled sand.

Sand Grain Size

I manually shook dried sand for 15 minutes through standard soil sieves of sizes 2, 1.4, 1, 0.5, 0.355, 0.25, 0.15, 0.09 and 0.063 mm, and weighed the contents of each bin. The bin sizes were converted to the Φ scale ($-\log_2(\text{mm})$) to account for lognormal distribution during sedimentation (Folk 1980). Beaches were differentiated by the median grain size (Φ_{50}) and sorting (σ_Φ) for analyses according to Folk (1966) where:

$$\sigma_\Phi = \left(\frac{\Phi_{84} - \Phi_{16}}{4}\right) + \left(\frac{\Phi_{95} - \Phi_5}{6.6}\right) \tag{4}$$

I interpolated values for Φ at the 5th, 16th, 50th, 84th and 95th percentiles from the slope of the line between the points on either side of each percentile.

Porosity

I measured porosity in five random samples from each site. I packed dry sand by vibration into a 60 mL graduated syringe and capped the syringe with a rubber stopper. I recorded mass (+/- 0.005 g) and volume (+/- 0.5 ml). The syringe connected to a low-flow rotameter via tygon tubing and a vapor trap. I introduced a known volume of water by suction at 20 mL/min against gravity and under light vibration to minimize trapped air bubbles. When water coming out of the syringe appeared as an uninterrupted stream, I recorded the amount of water in the syringe as the void volume. Total porosity (ε_T) was equal to the void volume per total volume and included both gas and liquid phases.

Since gas movement occurs through the gas phase of the soil matrix, I calculated air-filled porosity (ε_{air}) following experiments using the equation:

$$\varepsilon_{air} = \varepsilon_T + \left(\frac{\left(V_T - \frac{M_S}{\rho_S} \right) - V_W}{V_T} \right) \qquad (5)$$

where V_T was total volume occupied by sand (cm^3), V_W was volume of water in sand (cm^3), M_S was mass of dry sand (g), and ρ_S was density of dry sand in the experiment. The first phrase in the numerator was a volume correction for the packing of wet sand. I performed a separate sieve analysis on the individual sand samples to determine the relationship between porosity and grain size distribution parameters (Φ_{50} and σ_Φ).

Sand Preparation for diffusivity and conductivity experiments

I added water to dry sand for the desired water content (% of dry sand mass) and thoroughly mixed it. I packed wet sand into the chamber with ~3 kg force and vibrated it to obtain maximal compaction. The chamber was sealed and capped before being inserted into the apparatus assembly. Sealed chambers were checked regularly for leaks by a pressurized soap bubble test.

Conductivity

I built a test chamber following the methods of (Olson et al. 2001). I calculated the specific volume of test sand as ρ_S^{-1} from the volume of the conductivity chamber (cm^3) over the mass of the dried sand (g). Specific volume was a proxy for the ability to pack sand into the chamber. I flowed N_2 at 90% humidity through the chamber (verified with a Qubit Q-S161 RH/Temperature Analyzer) and used that humidity to calculate viscosity from Tracy et al. (1980).

Following Figure 4-1, I increased the flow using a rotameter (Brooks) and recorded the difference in pressure with an HH800 differential manometer (OMEGA). The intrinsic permeability (K, m^2) was calculated according to Olson et al. (2001):

$$K = \frac{\mu V \ell}{A \Delta P} \tag{6}$$

where μ = viscosity (Pa s), V = flow rate ($m^3\ s^{-1}$), ℓ = column length (m), A = cross-sectional area (m^2), and ΔP = differential pressure (Pa). In this chapter, I report gas conductivity (c, $m^3\ s\ kg^{-1}$) as K/μ_{air} where $\mu_{air} = 1.08\text{x}10^{-5}$ (Pa s) for air at 30ºC and 90% humidity.

Diffusivity

I modified the two flow system from (Batterman et al. 1996) to determine oxygen diffusivity through sand (Figure 4-2). I established humidified gas flow rates of ~15 ml/min of pure O_2 and pure N_2 using a rotameter (Brooks) and monitored differential pressure using a digital manometer (OMEGA) to ensure no directional convective flow. A S100 O_2 Sensor (Qubit) measured O_2 concentration on the outflows and I analyzed it in LoggerPro (Vernier). Diffusivity (D, $cm^2\ s^{-1}$) was calculated according to (Batterman et al. 1996):

$$D = \frac{\ell C_{N_2 out} V_{N_2 out}}{A\left(C_{O_2 out} - C_{N_2 out}\right)} \tag{7}$$

where ℓ = column length (cm), C = concentration of O_2 at the outflow of the chamber (%) where the outflow ports are indicated by the respective inflow gas (O_2 or N_2), V = flow rate (mL s^{-1}), A = cross-sectional area (cm^2), and dP = differential pressure (Pa).

Statistics

I performed all statistical analyses in R and MATLAB. For the sieve analysis, I used a Pearson correlation between Φ_{50} and σ_Φ, and I used a MANCOVA to analyze the clustering differences by beach of the dependent variables. I analyzed the porosity data in least squares linear regression versus the separate sieve analysis of those specific samples. I analyzed diffusivity and conductivity data versus the median grain size (Φ_{50}) and sorting (σ_Φ) for the sand core and ε_{air} as calculated from the water content of the sample and average ε_T of the sand core following equation 5. I used a multiple regression of ε_{air} and Φ_{50} on conductivity, and ANCOVA of ε_{air} and beach as a factor variable on diffusivity. All means are reported ± 2 standard errors of the mean.

Results

Grain Size

Cumulative distribution of sand grain sizes (% larger grain size by mass) was different in different beaches (Figure 4-3). There was a significant difference between beaches on the sand Φ_{50} and σ_Φ (MANOVA: $F(11,117) = 112.29$, $p < 0.0001$; Pillai's Trace = 1.83) (Table 4-1). There was a statistically significant, but small correlation between Φ_{50} and σ_Φ ($R = -0.31$, $p < 0.0001$, Figure 4-4).

Porosity

Total porosity (ε_T) varied as a function of σ_Φ between sands from different beaches (linear regression: $F(1,89) = 180.4$, $p < 0.0001$, $R^2 = 0.67$, Figure 4-5). There was also a statistically significant relationship between ε_T and Φ_{50} (linear regression: $F(1,89) = 77.49$, $p < 0.0001$, $R^2 = 0.47$, Figure 4-6). Since gas conductivity and diffusivity depend upon air-filled porosity (ε_{air}) (Currie 1970b), I calculated ε_{air} using equation 5 from the mean ε_T for each core.

Conductivity, Diffusivity and Specific Volume

During the analysis of gas conductivity and diffusivity versus water content, sand at 0% water content had lower conductivity (Figure 4-7 Top) and diffusivity (Figure 4-7 Middle) than sand at 2% moisture. Specific volume increased between 0 and 2% moisture but plateaued as water was added beyond that (Figure 4-7 Bottom). An increase in specific volume means a decrease in sand density and thus an increase in void space. This is why ε_{air} was not always less than ε_T because repacking wet sand increased the specific volume of the sand.

Gas conductivity through sand (Figure 4-8) varied as a function of the air-filled porosity and grain size (Multiple regression: $F(2,315) = 1294$, $R^2 = 0.89$, $p < 0.0001$). Large sand grain sizes and large air-filled porosities increased gas conductivity.

Gas diffusivity through sand varied as a function of air-filled porosity and beach (ANCOVA: $F(12,198) = 54.9$, $p < 0.0001$, $R^2 = 0.77$, Figure 4-9). Differences among beaches could not be explained from Φ_{50} or σ_Φ ($p > 0.05$), but were related to differences in air filled porosity of those beaches. The diffusion constants ranged from 2-16% of O_2 in air with a mean of 7.7%.

Discussion

In this chapter, I report on the factors that influence gas movement through the sand of sea turtle nesting beaches. I show that the gas diffusion coefficient was linearly determined by the air-filled porosity (ε_{air}) of the sand (Figure 4-8), that gas conductivity was collinearly determined by ε_{air} and the median grain size (Φ_{50}) of the sand (Figure 4-7), and that ε_{air} was linearly related to the sorting (σ_{Φ}) of the sand. I measured these variables on sands from a variety of sea turtle nesting beaches that had different Φ_{50} and σ_{Φ}.

In the grain size distribution analysis, the relationship between Φ_{50} and σ_{Φ} was statistically significant, however that relationship should not be used to predict sand grain size distribution on the beaches (Figure 4-4). The sand distribution was unique to each beach because there were potentially different sources and different distribution forces that produced differences in not just the Φ_{50} and σ_{Φ}, but also factors not measured for this study, such as kurtosis and skewness in addition to non-distribution factors such as grain roundness and smoothness (Spencer 1963, Folk 1980). All of these factors could contribute to the amount of air space in the soil matrix, which in turn affects the gas movement through beach sand. Overall, the beaches in this study vary in terms of texture and are representative of the range of beaches used for sea turtle nesting found previously (Mortimer 1990; Figure 4-4).

In the porosity analysis, I found a significant relationship between ε_T and Φ_{50}; however, when this relationship is visualized (Figure 4-6) and compared to the relationship between Φ_{50} and σ_Φ (Figure 4-4), it is revealed that these are mirror images, which can be explained by the negative slope in Figure 4-5. Therefore, the relationships I found between ε_T and σ_Φ (Figure 4-5) and between ε_T and Φ_{50} (Figure 4-6) are similar to the previous findings that porosity is independent of grain size, but varies with sorting (Beard 1973). In that study, they were able to fill a wider array of sand grain distributions through artificial mixing which can fill in the gaps of this study, however, they point out, many of those distributions are not likely to occur in nature, especially fine sands with poor sorting (Beard 1973).

Conductivity values from these experiments correspond to the range previously suggested for sand of $1*10^{-7}$ to 10^{-4} m^3 s kg^{-1} (Bear 1972). Previous studies have found a relationship between intrinsic permeability and air-filled porosity (Olson et al. 2001), as well as both Φ_{50} and σ_Φ (Beard 1973). While porosity, and as an extension σ_Φ, explains the total amount of pore space available for fluid movement through sand, Φ_{50} can be used to estimate pore diameter from the spherical contact distribution function (Bezrukov et al. 2001). Sand grain size determines pore diameter which is functionally related to the shear resistance to flow (Sperry and Peirce 1995).

The relationship between diffusivity and air-filled porosity I report is comparable to previous studies in that as air-filled porosity increases, so does diffusivity (Batterman et al. 1996, Bartelt-Hunt and Smith 2002). However I found a straight line relationship while others have proposed a curve or exponential relationship (Penman 1940, Millington and Quirk 1959, Currie 1970a), which may be due to the relatively small range of low porosities in sands as compared to soils (Shimamura 1992). The differences between the diffusivity of beaches could not be attributed to Φ_{50} or σ_{Φ}, and may be related to grain shape or tortuosity.

Sand Repacking and the Cluster Concept

In agriculture, plowing of the soil increases the porosity, diffusivity and permeability (Ball 1981); similarly, as sea turtles dig the nest, they disturb the soil and increase the porosity. I have demonstrated that repacking wet sand in a tube has a greater pore volume than with dry sand. The difference in conductivity (and to a lesser extent, diffusivity) between dry (0% water g g^{-1}) sand and lightly wetted sand (2.5% water g g^{-1}) is due to the specific volume because less wet sand (g) than dry sand (g) is required to fill the same volume. This phenomenonis consistent with the cluster concept of soil structure (Barden and Pavlakis 1971). The effect of increased specific volume between dry and lightly wetted sand was more pronounced in the conductivity column (Figure 4-7 Top) than the diffusivity column (Figure 4-7 Middle) and could be due to a number of factors: I used nearly twice the mass of sand for the conductivity experiment than for diffusivity, there was greater length to width ratio of the conductivity column, and there was a greater amount of surface area of the sand contacted by the flexible screen in the diffusivity column. The increase in specific volume was because ε_{air} was greater for wet sand than for dry sand. Although it was counterintuitive that adding liquid to a solid could increase the amount of gas in a column, a greater mass of dry sand than wet sand could be packed into a conductivity column, leaving less air space. It is important that future studies examine the relative gas exchange through the egg chamber versus the undisturbed sand surrounding the nest.

The difference specific volume between repacked wet and dry sand is consistent with the cluster concept (Barden and Pavlakis 1971). When dried soil is mixed with water, particles will cluster, held together by soil suction, and commonly referred to as aggregates or peds. Water forms a membrane around each particle, with water suction promoting particle aggregation. Water suction is greater in the intra-cluster micropores than the inter-cluster macropores and, upon compaction, water gathers around the tight junctions, rather than in the void space (Barden and Pavlakis 1971). The wet aggregates essentially act as larger particles that have larger void space for air to flow through both inside the aggregate and between aggregates.

The difference between the intra-cluster micropore and the inter-cluster macropore affects functional pore space (Fish and Koppi 1994). Since bulk flow was affected by pore size, conductivity showed a large response due to clustering. Diffusivity was affected by porosity, or the ratio of void space, and increasing the effective size of particles through aggregation did not drastically increase the ratio of inter-cluster macropore volume to aggregate volume. The beach sands that displayed an increase in diffusivity between dry and lightly wetted sand had functional intra-cluster micropores that can allow for diffusion through an aggregate. This underscores the importance of reporting volume relationships, rather than mass relationships, of water to soil when discussing gas exchange.

Gas exchange of underground nests

Measurements of diffusivity on the substrate of megapode nests (0.08-0.093 cm^2 s^{-1}) were much higher than found in this study (Seymour et al. 1986). This is most likely due to high organic content of the megapode mounds. Large amounts of clay and organic material in soil can increase the porosity and affect tortuosity (Troeh et al. 1982) allowing for more diffusion. All of the measurements in this study were on sandy beaches with <1% fines (diameter of less than 63 µm) and very little organic material; increasing the amount of fines in soils affects diffusivity relationship with water and compaction (Shimamura 1992).

My data supported previously reported *D* for O_2 in sea turtle nesting beach sand of 0.015 and 0.025 cm^2 s^{-1} (Ackerman 1977). The previous estimates of diffusivity on sea turtle nesting beaches did not account for bulk flow. Ackerman (1977) estimated a diffusivity of 0.015 cm^2 s^{-1} for Tortuguero, Costa Rica and 0.025 cm^2 s^{-1} for Hutchinson Island, Florida. My measurements of diffusivity for most beaches correspond well to Ackerman’s (1977) Tortuguero estimate; indeed, all beaches with water contents between 5-7.5% could have a diffusivity of 0.015 cm^2 s^{-1}. Interestingly, the only sand that does not fall within that range were from Tortuguero, but there could be variation in sand along the beach, sand could have changed between Ackerman’s work and this study, or Tortuguero may have had a high water content for Ackerman’s (1977) estimate.

In this study, less than 5% of diffusivity values were greater than 0.025 cm^2 s^{-1}, the estimate for Florida sand (Ackerman 1977). This overestimation may be due to bulk flow in the system not considered in the model. The tidal amplitude at Limon, Costa Rica (15 km from Tortuguero, http://www.surf-forecast.com/breaks/Tortuguero/tides/latest) is usually below 0.5 m; however, Fort Pierce Inlet, Florida (20 km from Hutchinson Island, http://saltwatertides.com/cgi-local/floridaatlantic.cgi) has regular tidal amplitudes of over 1 m. Assuming that the water table fluctuations below nests are attenuated to 20% of the tide (O'Connor et al. 2009), sea turtle nests would experience vertical bulk air movement of 10 cm (Tortugero) and 20 cm (Hutchinson Island). Ackerman's (1977) measurements of effective nest volumes suggest that the minimum diameter of a sea turtle nest of 100 eggs is 21.6 cm (*C. caretta*, Florida) and 23 cm (*C. mydas*, Tortuguero). Bulk air movement would affect less than half of the nest at Tortuguero, but almost the entire nest air volume would be displaced at Hutchinson Island each day.

Wallace et al. (2004) found that a prediction from the values of diffusivity from Ackerman (1977) overestimated the oxygen consumption in the nests of *D. coriacea* at Playa Grande, Costa Rica. I used the calculations from Wallace et al. (2004) to compute diffusivity values necessary to match their observed oxygen concentration in the nest. The authors assumed that the oxygen concentration in the nest (P_{O2}, kPa) was proportional to the nest oxygen consumption (V_{O2}, $cm^3\ s^{-1}$) over the gas conductance (G_{O2}, $cm^3\ s^{-1}\ kPa^{-1}$) and calculated the conductance from their data. Gas conductance can also be calculated from Wangensteen et al. (1970) by:

$$G_{O_2} = \frac{ADT_0}{T\ell P_{atm}} \tag{8}$$

where T is the temperature (ºK), T_0 is freezing temperature (273.15 ºK), A is the area (cm^2), ℓ is the distance (cm) and P_{atm} is the atmospheric pressure at T_0 (101.325 kPa).

Assuming that the temperature is 30ºC, the *D. coriacea* nest has a radius of 15 cm at a depth of 40 cm (Wallace et al. 2004), then the D needed to increase G_{O2} sufficiently for the data is 0.05 $cm^2 s^{-1}$, which is two to five times higher than my measurements suggest would be reasonable for beach sand. Tamarindo, Costa Rica (5 km from Playa Grande, http://tamarindotides.com/Months/August2012.html) has regular tidal fluctuations of 2-3 m. Under the previous assumption of water table attenuation to 20% of tidal value, the vertical bulk air flow could be 40-60 cm, which is of sufficient amplitude to flush the nest with reoxygenated air daily.

In Florida, some sea turtle nesting beaches are being renourished after erosion by hurricanes and can affect hatching success and gas concentrations in the nest (Mota 2009). The sand that is transported to the beaches often comes from inland sand mines or offshore dredges, which can include higher percentages of silts and clays than is normally found on the beach. These sands are not generated from the same processes that produced the beaches; hence, this difference can affect the mean grain size, sorting, skewness, and kurtosis of the beach sand population (Spencer 1963). I have demonstrated that various properties of sand affect gas movement; therefore, it is important that beach renourishment programs consider the gas environment for organisms of the in-beach ecosystem.

Climate change models project heating for sea turtle nesting beaches (Saba et al. 2012) that can affect hatchling mortality (Santidrian Tomillo et al. 2012). In addition, heating would create a deeper dry sand layer that is less permeable to gases than the repacked wet sand. This would restrict the tidal flow of gases, which contributed to the higher than expected oxygen concentrations for *D. coriacea* nests at Playa Grande

(Wallace et al. 2004). Furthermore, introduction of dry sand into nests through relocation could decrease gas exchange with the sea turtle nest, and may be a contributing factor to lower hatching success in relocated nests (Sieg 2011).

Sea turtles are important environmental engineers of beaches, with the construction of nests being hypothesized to maximize gas exchange (Ackerman 1975). Recent modeling attempts on the gas exchange of sea turtle nests have assumed that the sand surrounding the nest is uniform (O'Connor et al. 2009, O'Connor et al. 2011). The nest shaft is filled with repacked sand by the female turtle as she finishes laying, and so would have ε_{air} greater than that of the undisturbed sand to the side of the nest, leading to less resistance to gas movement above the nest than to the sides. Although densely nested beaches have lower gas concentrations in neighboring nests, this effect was only seen at densities of 9 nests per meter (O'Connor et al. 2009). It is therefore imperative to measure the air-filled porosities of undisturbed sand to determine the difference between gas movement within the nest versus the rest of the beach. As beaches continue to be developed, eroded or renourished, the undisturbed state of the interstitial environment must be understood in order to properly mitigate and restore specific beach ecosystems.

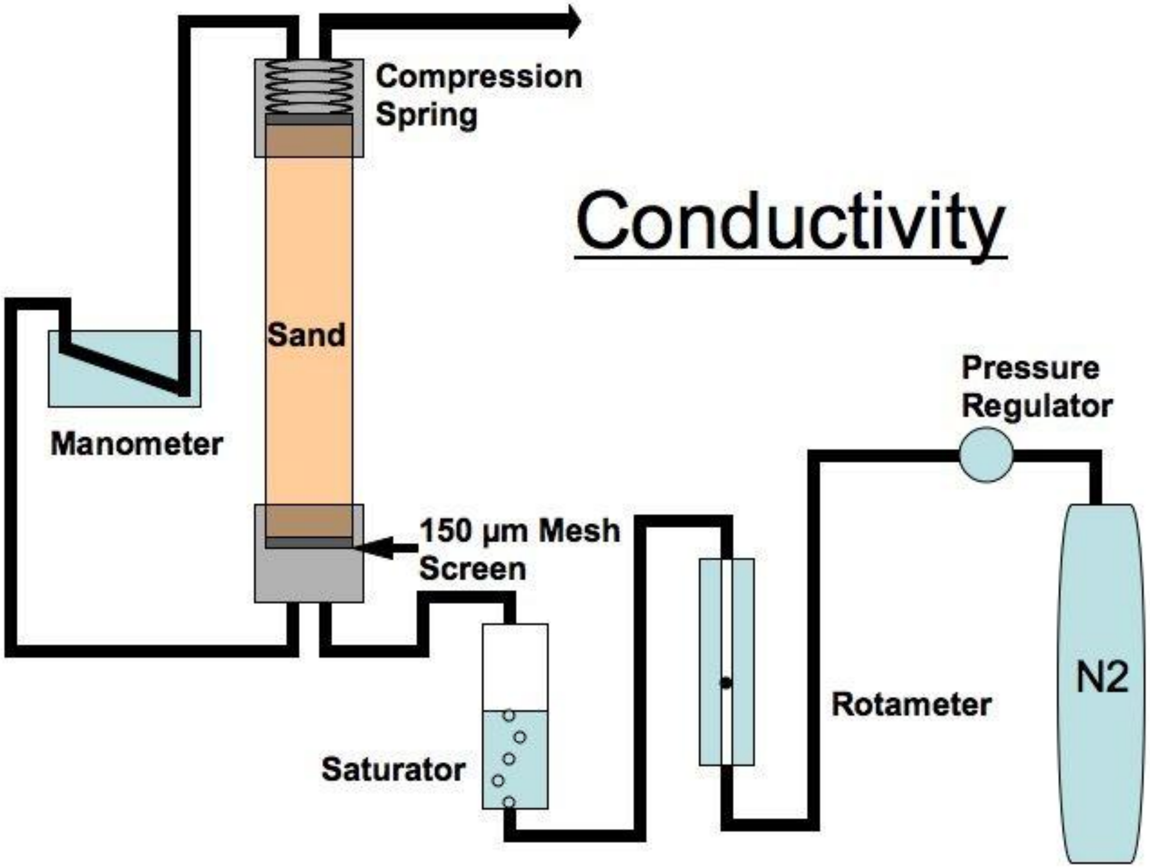

Figure 4-1. Diagram of the laboratory set-up for testing sand conductivity. I measured differential pressure (manometer) across a column of sand using unidirectional flow-controlled (rotameter) water-saturated nitrogen.

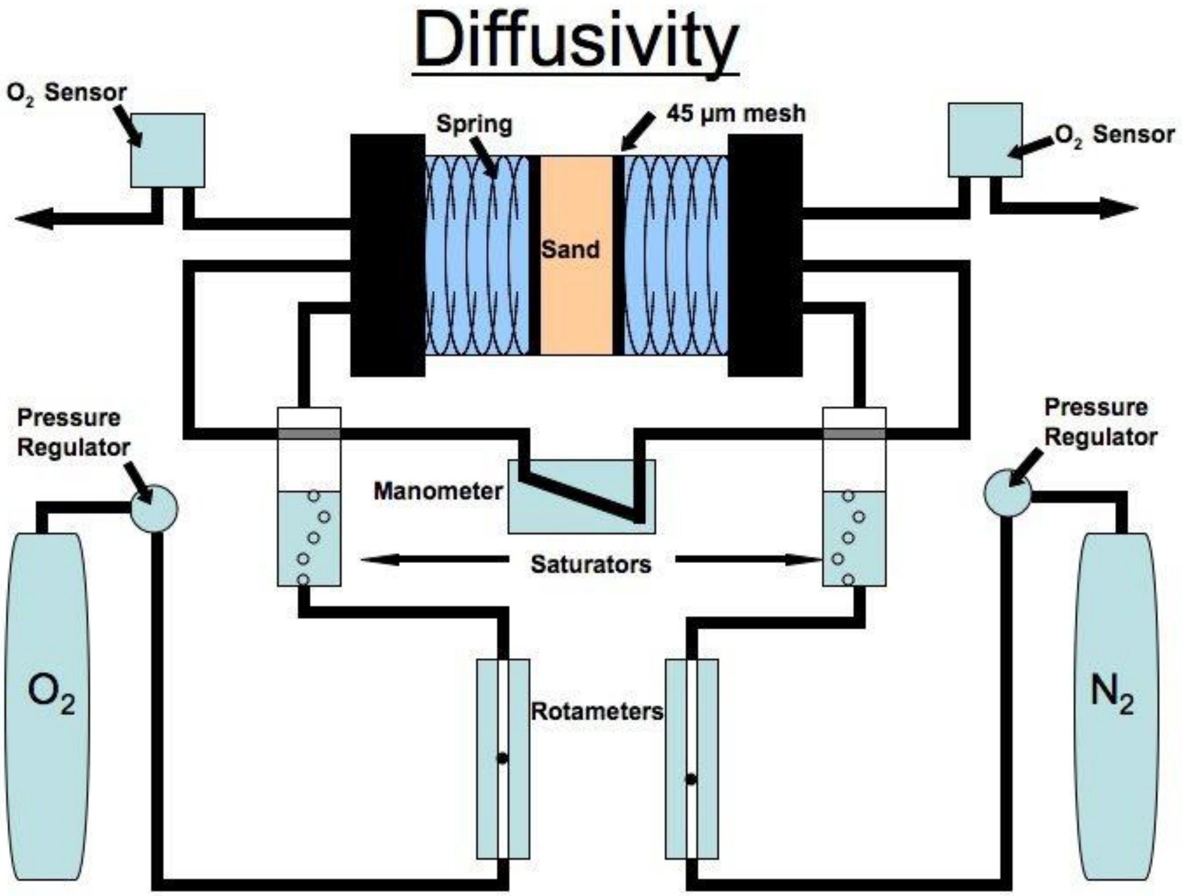

Figure 4-2. Diagram of the laboratory set-up for testing sand diffusivity. I measured [O_2] on either end of column of sand using bi-directional flow-controlled (rotameter) water-saturated nitrogen and oxygen gases. I measured the differential pressure to ensure no unidirectional flow**.**

Table 4-1. Median grain size and sorting. The values represent the mean ± 2 SE.

Beach	**Country**	$\mathbf{\Phi_{50}}$	$\boldsymbol{\sigma_{\Phi}}$
Kyparissia O	Greece	-0.43 (±0.180)	0.86 (±0.083)
Kyparissia A	Greece	-0.45 (±0.076)	0.77 (±0.034)
Thiafi	Greece	1.14 (±0.062)	1.16 (±0.035)
Marathonissi	Greece	1.37 (±0.123)	0.77 (±0.032)
Playa Ostional	Costa Rica	1.56 (±0.363)	1.17 (±0.037)
Sekania East	Greece	1.68 (±0.047)	0.44 (±0.011)
Play Sand	Commercial	1.75 (±0.029)	0.71 (±0.015)
Playa Naranjo	Costa Rica	1.76 (±0.035)	0.76 (±0.004)
Sekania West	Greece	1.79 (±0.041)	0.48 (±0.034)
Gerakas	Greece	2.14 (±0.091)	0.49 (±0.006)
Tortuguero	Costa Rica	2.24 (±0.139)	0.61 (±0.014)
Playa Moaba, Bioko Island	Ecuatorial Guinea	2.41 (±0.047)	0.53 (±0.039)

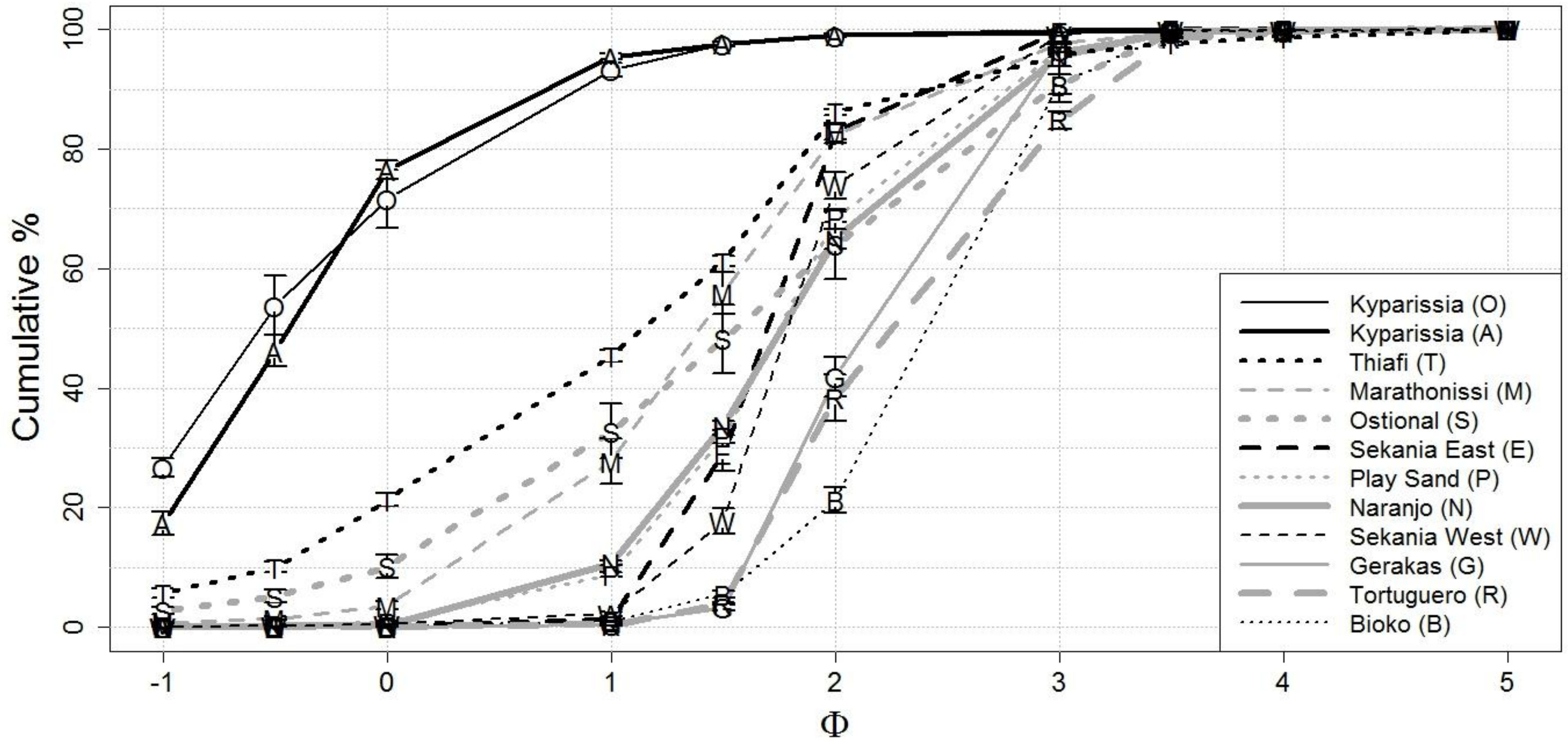

Figure 4-3. Cumulative grain size for all experimental beaches. Each point represents the cumulative percentage of sand mass retained in each sieve and all coarser screened sieves. The coarser sands have lower Φ. Median grain size (Φ_{50}) is the Φ at which the cumulative distribution is equal to 50%. Sorting (σ_{Φ}) can be visualized on this graph through the slope: poorly sorted sands have less steep slopes.

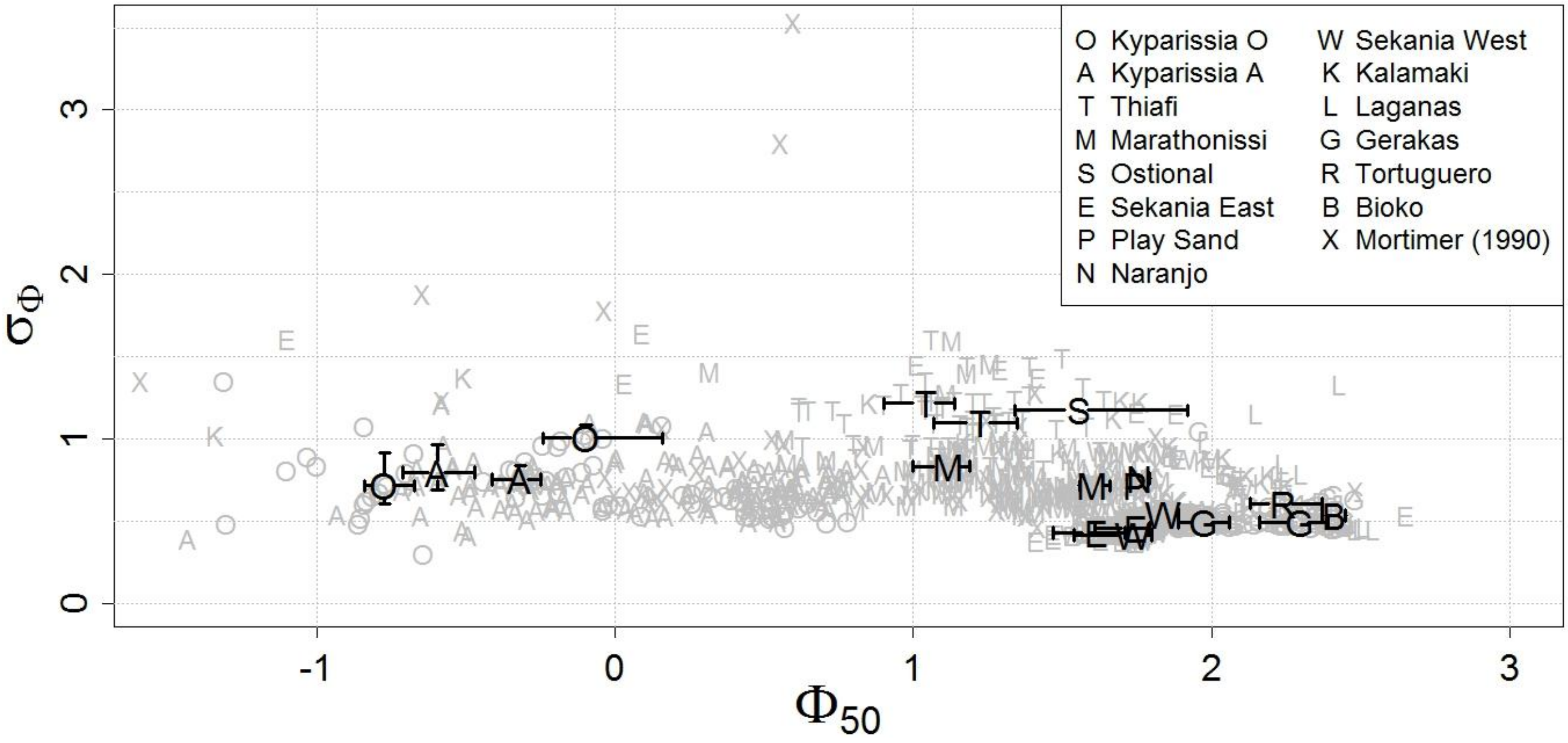

Figure 4-4. Distributions of pooled samples vary according to median grain size and sorting. The beach samples for this study (black letters) overlap with distributions from Mortimer (1990) (gray X) and samples from turtle nests on the experimental beaches (all other gray letters). Error bars represent the range of the pooled samples that could be expected from subsampling (N=7 for each); bars which did not extend beyond the limit of the symbol are not shown. Low values of Φ_{50} are coarse-grained sand. Low values of σ_{Φ} are well sorted and high values are poorly sorted.

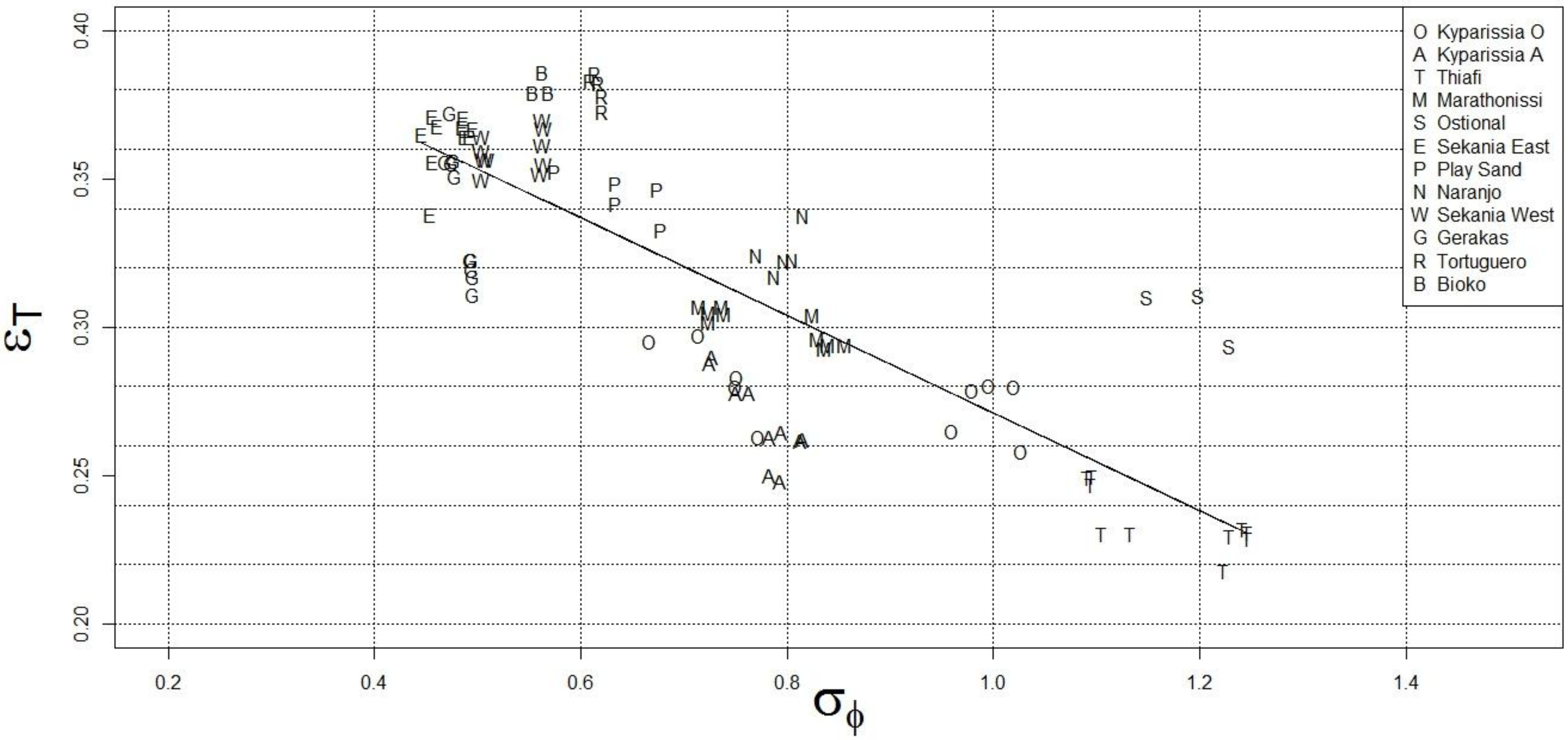

Figure 4-5. Porosity (ε_T) varied as a function of sorting (σ_Φ) on the beaches. The well sorted beaches had higher porosities. The equation for the regression is $\varepsilon_T = -0.16 * \sigma_\Phi + 0.44$ ($R^2 = 0.67$).

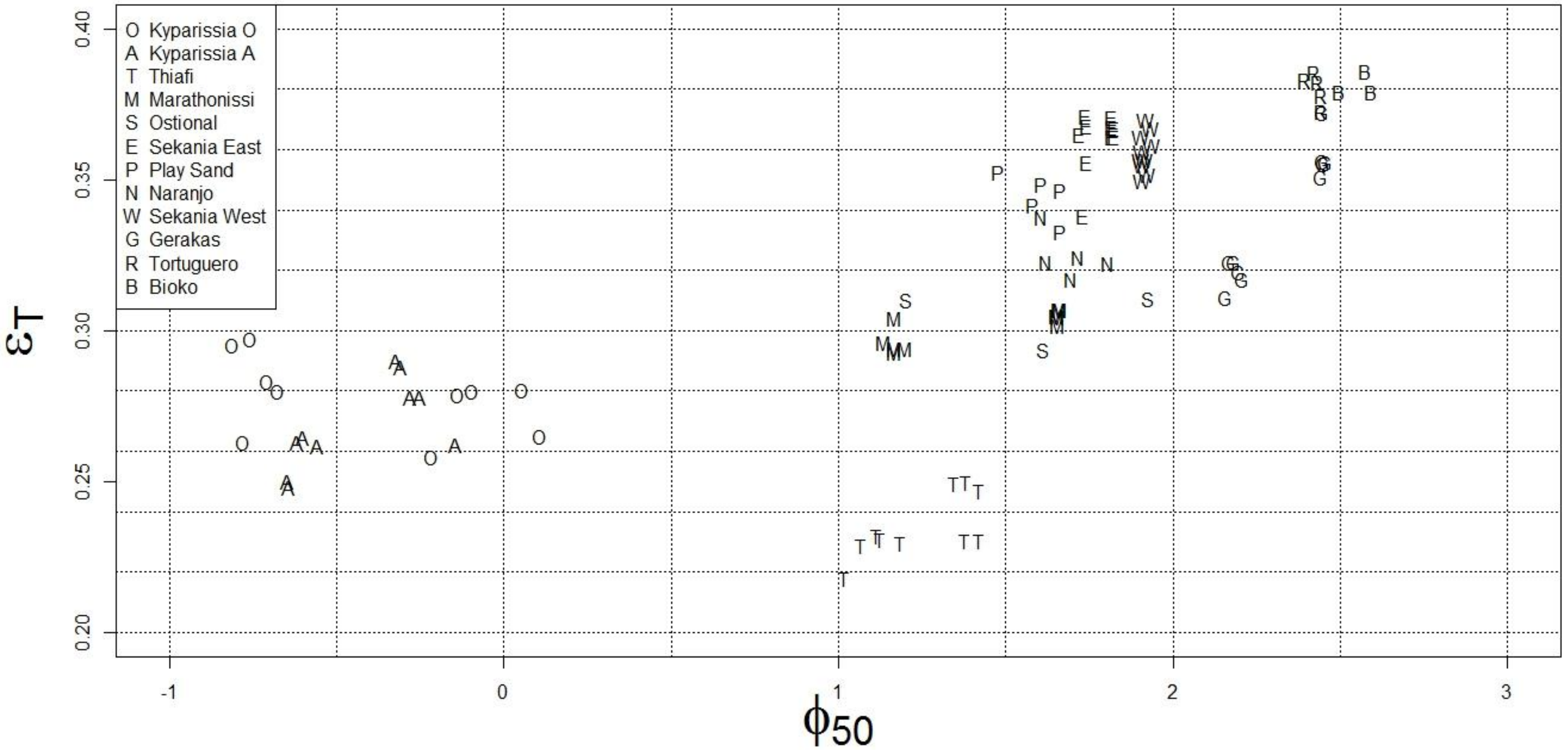

Figure 4-6. Beaches cluster according to their porosity and median grain size. The linear regression line is not shown.

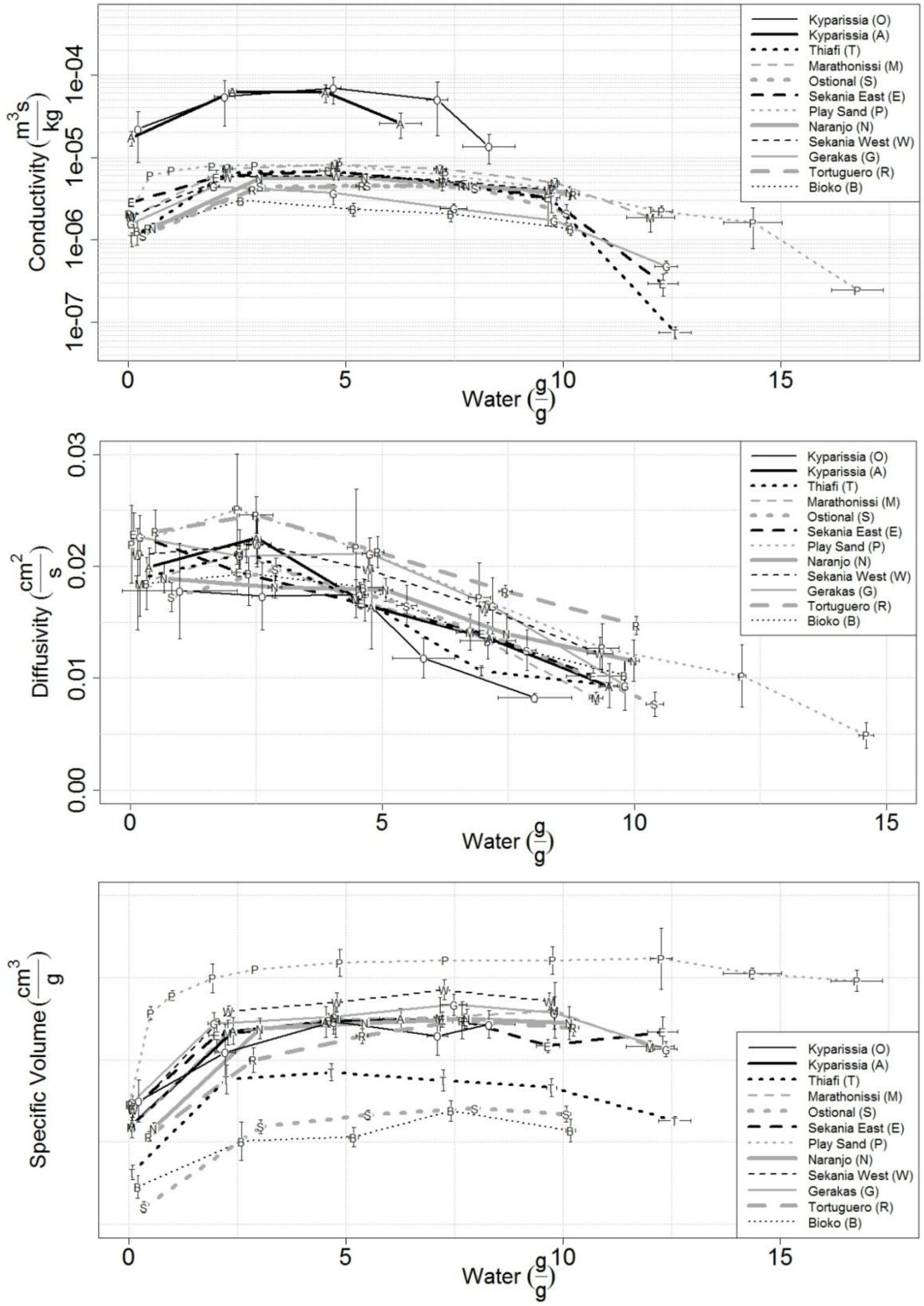

Figure 4-7. Dry sand (0% moisture by mass) had lower conductivity (Top) and diffusivity (Middle) than lightly wetted sand (2% moisture by mass). Specific volume (bottom) increases between 0 and 2% moisture, but plateaus as water is added beyond that.

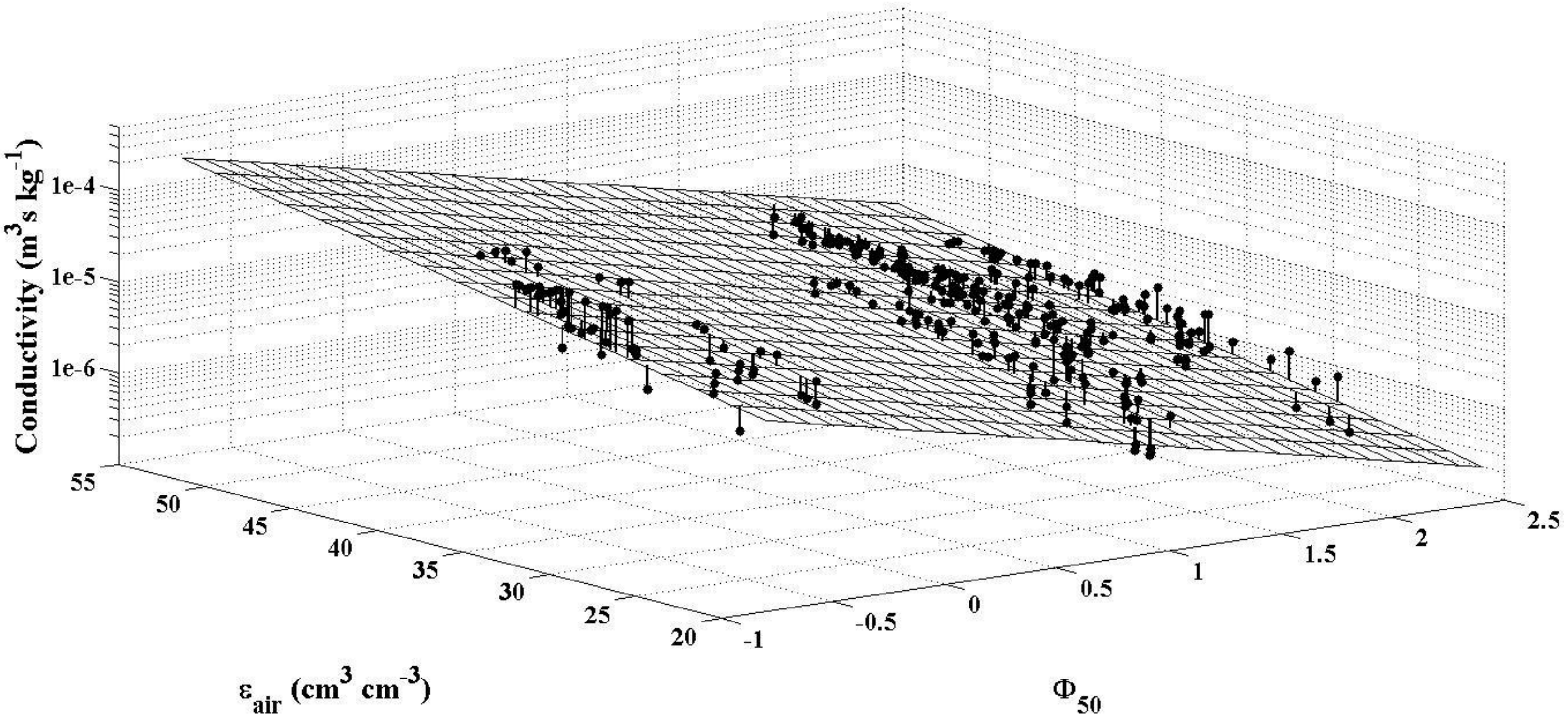

Figure 4-8. Conductivity varies with median grain size and air-filled porosity. The median grain size can be used to estimate pore size (Bezrukov et al. 2001).

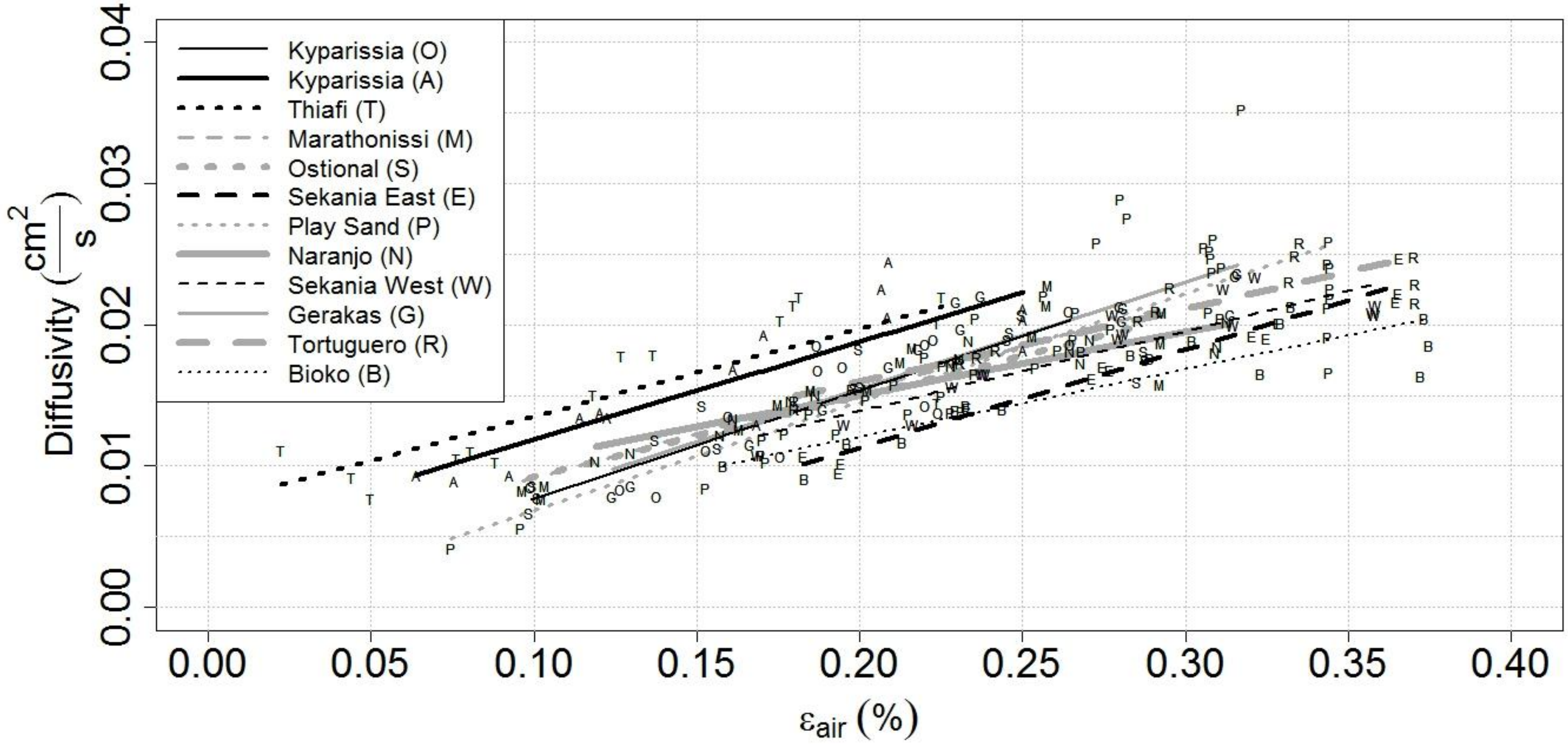

Figure 4-9. Diffusivity of O_2 in sand varies by beach and air-filled porosity. Values shown here range from 2-16% of the diffusivity for O_2 in air (0.230 $cm^2\,s^{-1}$).

CHAPTER 5: ABIOTIC AND BIOTIC EFFECTS ON THE GAS EXCHANGE ENVIRONMENT OF LOGGERHEAD TURTLE NESTS IN GREECE

Introduction

Sea turtle eggs are an immobile life stage exposed to the environment for 40-80 days without parental care. In all sea turtle species, a clutch of eggs is buried deep in the sand of a beach where the highly porous eggs are adapted to the high humidity and low oxygen in the underground environment (Seymour 1980). To survive this life stage, eggs respond to stressors physiologically. Proper development requires food, water, oxygen and temperature in a range suitable for development. While the egg comes preloaded with food in the form of yolk, the incubation medium affects the thermal, hydric and gaseous environment (Ackerman 1977, Seymour 1980, Seymour et al. 1986).

If the incubation medium does not allow for adequate gas exchange, the sea turtle nest may become chronically hypoxic resulting in lower hatching success (Ackerman 1980, Garrett 2010), or growth and metabolism of the eggs in the clutch may be depressed (Kam 1993). Embryos exposed to hypoxia do show some physiological plasticity in ability to transport oxygen (Kam 1993, Crossley et al. 2003), smaller size with larger hearts (Crossley and Altimiras 2005, van Bergen 2005), and vasodilation (Crossley and Altimiras 2005) that may reduce the physiological response to future hypoxia (Eme et al. 2011b) but may also result in hypertension and low heart rate (Eme et al. 2011a). The reptilian sympathetic and parasympathetic reflex loops may play important roles in the response of embryos to naturally occurring hypoxic nest conditions (Eme et al. 2011b).

The sea turtle egg shell is flexible and parchment-like (Packard et al. 1979) with gas permeability across the sea turtle eggshell being twice that of the chicken egg (Wangensteen et al. 1970) due to a higher shell porosity (Ackerman and Prange 1972) that allows for a greater flux of gas and water across the shell (Tracy et al. 1978). In avian eggs, the resistance to gas exchange is through the hard shell that minimizes water loss in the air. In contrast, in the turtle egg, the major resistance to gas exchange is in the sand around the nest rather than in the porous shell (Ackerman and Prange 1972). Ackerman (1977) hypothesized that the sand surrounding the nest acts as an extension of the turtle eggshell, thereby decreasing the effective gas permeability to that of a hard-shelled avian egg. Female sea turtles influence the gas exchange of their clutch of eggs by constructing a nest and by ovipositing an appropriate metabolic mass in the nest (Ackerman 1977, Warner and Andrews 2002). Gas exchange is ultimately limited by the physical characteristics of the nest and beach (Ackerman 1975) and affects survivorship of eggs (Ackerman 1980, Wallace et al. 2004, Honarvar et al. 2008, Garrett 2010). Embryos at the center of the clutch may suffer greater mortality, grow more slowly and hatch later than the eggs at the periphery of the nest (Ackerman 1980, Wallace et al. 2004).

Within the amniote egg, yolk serves as a source of food for the developing embryo; it consists of proteins, fats and sugars that form the building blocks for structural development, and acts as an energy sounce. Yolk composition depends upon the condition of the mother, and is representative of the condition of the foraging site. The predominant hypothesis for differences in remigration intervals among sea turtle populations is that foraging in low productivity areas increases the time between nesting bouts (Saba et al. 2007, Wallace et al. 2007, Reina et al. 2009). Other maternal effects, linked with foraging site and female condition, include egg size and clutch size. In this study, I measured the clutch characteristics as a proxy for maternal effects on hatching success.

Generally, the oxygen consumption of a clutch exhibits a peaked pattern, low at first, with exponential increase that may either flatten or decrease just before hatching (Prange and Ackerman 1974, Ackerman 1981, Thompson 1989), which would allow for improved gas exchange in the final days of development (Kraemer and Richardson 1979). Increased embryonic growth rates are associated with increased oxygen consumption (Ackerman 1980).

Many studies have examined the effects of sand grain size on sea turtle nesting. Sand grain size in Brazil appeared to affect incubation duration, but the authors did not measure nest temperature as an explanatory variable (Ferreira Júnior 2008). Mortimer (1990) found that sands with larger air-filled porosities had lower hatching success in *Chelonia mydas* nests, which was most likely due to lower water exchange. Bustard (1968) found that *C. mydas* nesting success on Heron Island, Australia, was highest when there were many roots and high moisture. *Chelonia mydas* nest oxygen concentration was influenced by numbers of eggs and temperature, but not sand grain size on Wan-an Island, Taiwan (Chen et al. 2010). Sand oxygen concentrations of *Lepidochelys olivacea* in Costa Rica may be affected by nest density (Honarvar et al. 2008) and organic material in the sand (Clusella Trullas 2007). Hatching success of *Caretta caretta* nests in Florida is affected by salinity and water content from inundations (Foley et al. 2006), as well as compaction and calcium carbonate content (Mota 2009). Sand characteristics did not affect hatching success of *Dermochelys coriacea* in Costa Rica (Clune 2005).

In this study, I investigated the physical properties of a series of beaches in Greece and the hatching success of loggerhead turtle eggs laid in those beaches. Zakynthos Island and the mainland site of Kyparissia, Greece, host the two largest loggerhead rookeries in the Mediterranean accounting for 857-2018 (16 years) and 286-927 (15 years) nests per year, respectively (Rees 2005).

Occasional records of interchange have been reported between these rookeries from external flipper tags of nesting females (Margaritoulis 1998). From 2006-2008, out of 13 tracked females from Laganas Bay, there were four forays to neighboring rookeries at Kyparissia, other neighboring Peleponesse bays and Kefalonia Island (Schofield et al. 2010). Given the differences between sand characteristics of these beaches, it is not clear if turtle nesting behaviors are selected for laying their eggs on a particular beach. Loggerheads in Greece use sand characteristics for oviposition sites displaying preferences for sand grain size distributions that maximize aeration and water drainage and provide structural support during egg chamber construction (Karavas 2005, Mazaris et al. 2006). Since adaptive behaviors are related to survival and reproductive success (Williams and Nichols 1984), the same sand characteristics that influence nest placement should influence the survival of the eggs within the nest (Hays and Speakman 1993). Sands on the beaches of Zakynthos and Kyparissia differ visually in grain size and color (personal observation). These differences in sand characteristics may affect development of turtle eggs. I hypothesized that the beaches with larger sand grains would produce clutches with higher oxygen concentrations due to increased nest ventilation and that those clutches would have higher hatching success than clutches on beaches with smaller grain sizes.

Methods

Locations

I conducted this study on the Greek island of Zakynthos (Figure 5-1A) in the Ionian Sea and on the beach at Kyparissia Bay (Figure 5-1B) on the Peleponesse Peninsula of mainland Greece. Laganas Bay is located at the southern end of Zakynthos Island, and has 6 nesting beaches: Marathonissi, Laganas, Kalamaki, Sekania (East, West and Thiafi), Daphni, and Gerakas. Laganas Bay is a NATURA 2000 site protected under the National Marine Park of Zakynthos (NMPZ) and the beaches have limited public access and strict regulations. I monitored nests on Marathonissi, Sekania, and Gerakas. Daphni beach experienced an increased number of cobbles and pebbles prior to the 2009 season due to winter storms, and turtles had difficulty nesting there. Laganas and Kalamaki beaches had grain size distributions between those found at Sekania East and Gerakas. For these reasons, I decided not to include those beaches in this study.

Marathonissi is a spit on a beach consisting of white rocky and biogenic sand of moderate coarseness. The beach is on the northeastern side of the island of Marathonissi within Laganas Bay and is separated from the mainland by a channel to the north and west and shallow sea grass beds to the east that constitute most of the bay floor. Between 2008 and 2009, sand was deposited on the east side of the spit from winter storms, increasing the size of the beach (G. Schofield, unpublished data). Marathonissi has cool nest temperatures and long incubation times and is an important male-producing beach (Zbinden 2007). Visitors to the beach are restricted to the area adjacent to the water to preserve nesting habitat and nests are caged by NMPZ wardens and ARCHELON volunteers to protect from human trampling.

Sekania consists of three crescent beaches (East, West and Thiafi) connected via rocky outcroppings. For this study, I differentiated between the three beaches of Sekania because of differences in sand grain size distribution and nest density. All three beaches have limited access via a steep walking path only accessible to personnel and volunteers from ARCHELON, The Sea Turtle Protection Society of Greece, NMPZ and World Wildlife Foundation; the marine area falls within Zone A of the NMPZ, with all water craft being prohibited to protect sea turtles and fisheries resources. Sekania East and West had similar moderately fine sand but Sekania East also had occasional pebbles, cobbles and hard packed clay, especially in the river bed discharge area (Karavas 2005). During the 2009 and 2010 nesting seasons, the river beds were dry and had relatively few successful nesting attempts. Both beaches are less than 200 m in length, but Sekania West has a spit with steeper slope and decreased beach width on the west side. There are three coves of calm water separated by a rocky outcrop between East and West and the spit on Sekania West.

Thiafi is located west of Sekania West and has a small nesting area about 10 m wide nestled between a rocky outcrop and a cliff. The beach consists of moderate and poorly sorted rocky sand and contained between 10 and 20 nests in 2009 and 2010; as such, Thiafi had high nesting density. The beach of Thiafi continued less than 100 m west to a tall cliff, but there was no suitable nesting area and very few attempts. Turtles usually accessed the nesting beach on Thiafi from the cove to the west, but occasionally would access it from the cove between Sekania West and Thiafi.

Kyparissia Bay is on the western coast of Greece and the beach, dunes and coastal forest are a NATURA 2000 site (Rees 2005). The nesting beach extends the entire 44 km length of the bay, but the majority of nesting occurs in the southern 9.5 km (Figure 5-1B). For this study, only sectors A and O were used. Kyparissia A consists of the first 2500 meters of the beach north of the village of Kalo Nero to the hillock, Vounaki, and has coarse sand grains with very little clay. I monitored nests on the first 1500 meters of sector A. Kyparissia O consisted of the beaches directly in front of Kalo Nero and had coarse grained sand with some clay that often made it slightly muddy. Kyparissia O had rocky outcrops that separated nesting areas on the beach. There was greater wave action in Kyparissia Bay than Laganas Bay that can account for a one-meter berm next to the water and large amounts of debris from winter storms.

For definitions and description of the Kyparissia Bay sectors see Margaritoulis and Rees (2001). For descriptions of all Zakynthos nesting beaches, see Margaritoulis (1982).

Field Studies of Nests

In 2009, I monitored nine nests on Kyparissia and 21 nests on Zakanthos Island. In 2010, I monitored 15 nests on Kyparissia and 41 nests on Zakanthos Island (Table 5-1). Turtles laid the clutches in this study between 23 June and 30 July 2009 and 29 June and 23 July 2010. I collected gas samples from nests via a sampling port constructed from a perforated film canister (volume ≈ 75-cm^3). I placed a sampling port in the center of each clutch as the female turtle was laying her eggs, and I held it in place as she covered the nest naturally. A 70-mm length of Tygon tubing (2-mm internal diameter) extended to the surface through the neck of the nest chamber to allow for gas samples to pass from the nest to the gas analyzing equipment (Wallace et al. 2004, Honarvar et al. 2008, Garrett 2010).

I also collected gas samples from 2-4 control sites on each beach. I excavated a nest similar to a turtle nest in a location that would be suitable for a turtle nest. I determined suitable nesting sites by selecting locations that were surrounded by visible body pits or previously marked nests within 15 meters, and at an intermediate distance to the sea. Each control site contained the same gas sampling port as the nests, placed at nest depth of 40-cm (Margaritoulis 2005). I covered the hole and packed it with sand to approximate the conditions of a natural nest, but without eggs.

I collected gas samples twice per week from the nests and controls on individual beaches in the morning or evening. For real-time measurements I used a Qubit Systems RP2LP-FCM High Ranges Respiration Package. I used a gas pump calibrated to 50 mL min^{-1} and drew air from the nest for three minutes for dependable gas measurements. I compared O_2 readings within the nest to atmospheric readings immediately prior to sampling and to samples from control sites. Due to unreliable measurements, I do not report CO_2 concentrations.

Each sampling port also contained a Cu/Cn thermocouple to record nest temperature concurrent with gas sampling throughout incubation. The control locations also had thermocouples at depths of 0, 10, 25, 40, and 50 cm for a thermal profile of the beach. I used OMEGA HH200A thermocouple readers (±0.1) for real-time temperature measurements of the nests and beach thermal conditions. I calibrated thermocouples to ±0.05 °C. I calculated metabolic heating from temperature measurements in the nests compared to the grand mean temperatures of the beach from control thermocouples at 25, 40, and 50 cm depth.

Nests within close proximity of each other affect gas tensions and, at high densities, decrease hatching success (Honarvar et al. 2008). In 2009, I counted and measured the distance to all hatched nests within 10 m of a monitored nest or control site. On many beaches, the distance from the back of the beach to the sea was less than 30 m. Hence, when inter-nest distances were large, this technique underestimated the density of nests on beaches because the circular quadrat would overlap with areas that were not suitable for nesting, such as vegetation behind the beach, rocky outcrops, and the sea. In addition, some beaches had many areas where a monitored nest or control site had no nests within the quadrat. Due to this underestimation, nest densities from 2009 are not reported here.

In 2010, I measured the distance from the center of each nest or control site to the center of the nearest five hatched nests. For each nest I determined the mean distance to the nearest five nests. Distance is reported as the grand mean for nests on each beach.

Egg and hatchling characteristics

In 2010, I collected up to twenty (20) eggs from each monitored clutch as they were laid, individually weighed them in a plastic bag attached to a 60 g PESOLA spring scale, before carefully placing them back in the egg chamber prior to covering. Eggs were not manipulated in any way during handling, and I recorded the mass of the empty bag before each measurement as sand and cloacal fluid accumulated. This usually totaled less than 10 g. I attempted to return as much sand as possible to the nest. I measured masses to the nearest 0.5 g.

In 2010, I collected up to 20 hatchlings after they emerged from each nest through a boxing method (Hays and Speakman 1993). This method was already being used by ARCHELON monitoring program at Kyparissia. At sunset I placed a box on each of the monitored nests that was expected to hatch and checked it every 1.5 hours throughout the night. I removed the boxes at dawn. The first 20 hatchlings were weighed, measured (straight carapace length and width) with calipers to the nearest mm and released together. No hatchling was held captive for longer than 3 hours and most for less than 30 minutes. These measurements were taken as a proxy of maternal condition. If I did not already have 20 measurements, I measured hatchlings found in the nest during excavations but did not weigh them because they were not representative of a healthy hatchling.

Hatching Success

I excavated nests by hand 10-days (Kyparissia) and 17-days (Zakynthos) post-emergence. I carefully removed eggs and egg shells from the nest and sorted them into hatched eggs, unhatched eggs and hatchlings (dead or alive). I determined the number of hatched eggs by counting egg shells greater than 50% of a whole egg shell. I calculated clutch size as the sum of hatched and unhatched eggs, and hatching success as the percentage of hatched eggs relative to clutch size. I opened all unhatched eggs and recorded the embryonic stage at death according to ARCHELON monitoring protocols as: no visible embryo, embryo contains an eyespot, embryo has visible carapace with coloration but smaller than the yolk sac (stage 1), embryo size is approximately equal to the size of the yolk sac (stage 2), or embryo is larger than the yolk sac (stage 3). For this study, I considered embryos in stages 2 and 3 to have contributed to the gas concentrations in the final 10 days of incubation. During excavations, I also measured the distance from the surface to the top and bottom of the egg chamber and used these measurements to calculate the depth of the middle of the nest.

Sand Collection

In 2009, I only collected sand from beaches before clutches were laid and after they hatched, so water content was not considered for that year because the samples did not reflect hydric conditions during the incubation period. Those samples were used only for grain size analyses. In 2010, I collected sand in 50 mL screw top Falcon tubes from the side of the egg chamber during laying, from control sites every 2-3 weeks, and from nests at excavation in order to measure water content throughout the season. When sand was sampled at control locations, the depth of the dry sand layer was measured as the distance from the surface to the wet, hard-packed layer. This measurement was only done on the Zakynthos beaches during 2010.

Water Content

I determined sample water mass by weighing samples to the nearest 0.01 g, drying for 24 hours at 100 °C and reweighing using an OHAUS balance calibrated at 200 g. The mass difference between the wet and dry sand was water mass. I calculated volume of sand and water (mL) by converting from mass through preliminary measurements of specific volume (mL g^{-1}) on sand from the beaches and water density at 25 °C (0.99704 g mL^{-1}). Volumetric water content was equal to the volume of water per volume of dry sand. I did not include any samples in the analysis that had longer than one week between collection and weighing. A preliminary analysis showed that properly sealed screw top Falcon tubes did not lose substantial moisture (>1%) until almost two weeks. When discussing gas exchange issues in sea turtle nests, volumetric water content of the sand is the most appropriate measurement because it can be used to calculate air-filled porosity.

Sand grain size characteristics

I manually shook dried sand through a stack of 2, 1.4, 1, 0.5, 0.355, 0.25, 0.15, 0.09, and 0.063 mm sieves (-1, -0.5, 0, 1, 1.5, 2, 3, 3.5, and 4 Φ, respectively) and recorded the mass of the contents of each sieve to nearest 0.01g. I recorded cumulative grain size for each beach as the percent mass greater than each sieve size. Statistical analyses were performed on the median sand grain size (Φ_{50}), calculated for each sample using the equation:

$$\Phi_{50} = \Phi_{Low} + \frac{(50 - C_{Low}) * (\Phi_{Hi} - \Phi_{Low})}{(C_{Hi} - C_{Low})} \qquad (1)$$

where Φ_{Low} was the size of the sieve that accounted for the largest cumulative mass less than 50%, Φ_{Hi} was the size of the sieve that accounted for the smallest cumulative mass greater than 50%, C_{Low} was cumulative mass in the lower sieve and C_{Hi} was cumulative mass in the lower sieve. This assumed a straight-line distribution of grain sizes between the two sieves.

I also calculated the sorting factor (σ_Φ) for the beaches according to Folk (1966):

$$\sigma_\Phi = \left(\frac{\Phi_{84} - \Phi_{16}}{4} \right) + \left(\frac{\Phi_{95} - \Phi_{5}}{6.6} \right) \qquad (2)$$

where Φ values for each cumulative percentile (5, 16, 84 and 95) were calculated according to equation 1, with the desired percentile substituted in for 50. Sorting describes the spread of the grain sizes and can be considered analogous to standard deviation (Folk 1966). There is also a strong correlation between σ_Φ and air-filled porosity (Beard 1973), an important component for gas movement through sand.

Data Analysis

I performed all statistical analyses using R. I tested differences among nests on the minimum PO_2 and maximum temperature experienced during the 3-10 days prior to emergence. Since grain sizes are naturally log-normally distributed, I present all sand grain data as $\Phi = -\log_2$ (mm), such that finer grains have larger Φ. I arcsine transformed the hatching success data, all other data were normally distributed. I used ANOVAs for all tests, except for an ANCOVA of water content during the incubation period and accepted a significance level of $\alpha = 0.05$. Post-hoc tests from ANOVAs were performed using a Tukey-Kramer test. Means are reported with two standard errors. I used the physical characteristics of nests to predict minimum oxygen concentration, and I compared these predicted values to the observed minimum oxygen concentration using a paired t-test.

Results

Hatching Success

Hatching success ranged from a mean of 70% on Kyparissia A to 92% on Thiafi. Kyparissia A had lower hatching success than all other beaches, except Sekania East and West (ANOVA: $F(6,78) = 2.81$, $p = 0.016$; Figure 5-2). One nest on Sekania East was inundated by sea water during high winds on 22-23August 2010, but may have been affected by the water table prior to that date due to its close proximity (5.37 m) to the water. That nest was removed from all analyses because of unreliable gas measurements and my inability to excavate underwater to collect accurate clutch size or development stage data.

During excavations, I noticed that some nests had many unhatched eggs that were already broken, suggesting mechanical crushing of the eggs. When I removed nests with more than 20% of eggs broken from the analysis, Kyparissia A had significantly lower hatching success than all other beaches, including Sekania East and West (ANOVA: $F(6,59) = 4.26$, $p = 0.001$). There were seven, six, five and one nests from Kyparissia A, Sekania East, Sekania West, and Gerakas, respectively removed from that analysis.

Nest Oxygen

Loggerhead turtle nest PO_2 decreased throughout incubation (Figure 5-3). Oxygen concentration in all nests followed a peaked pattern, with an initial period of slow decrease followed by exponential decrease until concentrations steady or even increase before hatching. Maximum PO_2 deficit differed between Marathonissi and Gerakas (ANOVA: $F(6,74) = 3.17$, $p = 0.008$; Tukey-Kramer: $M < G$, $p = 0.04$), but did not differ significantly between any other beaches (Figure 5-4). Minimum oxygen partial pressure in nests varied by beach as a function of the number of developing embryos (ANCOVA: $F(7,73) = 6.10$, $p < 0.0001$, $R^2 = 0.37$); however, median grain size did not contribute to the multiple regression as an explanatory variable of PO_2 ($F(1,72) = 0.29$, $p > 0.05$). Thiafi and Kyparissia O had low sample sizes, which may have contributed to the low R^2 value. The number of developing embryos (of at least stage 2) was a better explanatory variable for oxygen levels by beach than the number of hatched and pipped eggs (ANCOVA: F (7,73) = 5.41, $p < 0.0001$, $R^2 = 0.34$), although both were significant. Since the minimum oxygen was usually between 3 and 14 days before emergence, the number of developing embryos at a late stage of development more accurately described the metabolic mass at that time.

Nest Temperature

Maximum nest temperature (Figure 5-5) was lower on Marathonissi than other beaches, but did not differ among any other beaches (ANOVA: F (6,64) = 24.26, $p < 0.001$). Metabolic heating did not differ between the beaches (ANOVA: F (6,64) = 0.59, $p > 0.05$). Maximum nest temperature also explained some of the variation in nest oxygen concentration along with beach and number of developing embryos (ANCOVA: F(8,60) = 5.83, $p < 0.0001$, $R^2 = 0.44$).

Physical factors on the beaches and nest density

Beaches differed in median particle size of sand grains (ANOVA: F(6,404) = 271.9, $p < 0.001$). Kyparissia sectors A and O had medium-coarse sand, Marathonissi and Thiafi had medium sand, Sekania East and Sekania West had medium-fine sand, and Gerakas had fine sand (Table 5-2).

Thiafi, Sekania West and Sekania East had the shortest distance between nests and Marathonissi, Kyprissia A, Gerakas, and Kyparissia O had the longest distance between nests (ANOVA: F (6,368) = 15.45, $p < 0.001$; Table 5-2). There were no differences between the water content of all beaches and there was no significant change in water content of the beaches during the incubation period in 2010 (ANCOVA: F

(13,176) = 1.72, p = 0.061; Figure 5-7). The nesting season in Greece is characterized by a seasonally arid summer. Because of the lack of rain, the surface sand dried out to a depth of 7-35 cm on the beaches of Zakynthos. Within the first two days after a rain event on 12 Sept 2010, the dry sand depth was between two and six cm, but after a week of dry weather, the dry sand depth was back to 10-26 cm before another rain event on 23 September. Dry front was not measured during sand collection on Kyparissia. The dry sand layer did not penetrate to the depth of the loggerhead turtle nests. Rain storms from 23-30 September 2010 totaled 41 mm and increased surface sand water content on Marathonissi so there was no dry sand layer, but did not affect the water content at nest depth. No other beaches were sampled after that time for water content since all monitored nests had hatched.

Egg and hatchling characteristics

There were no significant differences (p >0.05) among the beaches for egg and hatchling characteristics of egg mass, hatchling mass, hatchling width, hatchling length (Figure 5-6), or clutch size (not shown). Loggerhead egg mass was 33.0 ± 0.2 g from a total of 791 eggs from 43 clutches (N = 10 (Sekania East, Sekania West, Gerakas and Marathonissi), N = 2 (Kyparissia A), N = 1 (Thiafi)). Hatchling mass was 15.6 ± 0.1 g from a total of 706 hatchlings from 44 clutches (N = 10 (Gerakas), N = 9 (Sekania East, Kyparissia A), N = 8 (Sekania West), N = 4 (Kyparissia O), N = 3 (Marathonissi), N = 1 (Thiafi)). Hatchling length was 41.9 ± 0.1 mm and hatchling width was 32.4 ± 0.1 mm from 770 hatchlings from 50 clutches (N = 10 (Gerakas), N = 9 (Sekania East, Kyparissia A, Marathonissi), N = 8 (Sekania West), N = 4 (Kyparissia O), N = 1 (Thiafi)). Clutch size was 103.3 ± 4.3 eggs from 85 clutches over both seasons (N = 19 (Kyparissia A), N

= 15 (Gerakas, Marathonissi, Sekania West), N = 14 (Sekania East), N = 5 (Kyparissia O), N = 2 (Thiafi)).

Discussion

In this chapter, I have reported the oxygen concentration in developing loggerhead turtle nests on a series of beaches in Greece. The nesting beaches of Laganas Bay, Zakynthos, and Kyparissia Bay, Peleponessus, have different sand grain size characteristics but this did not affect hatching success or nest oxygen concentration.

Oxygen concentration in all nests followed a peaked pattern, with an initial period of slow decrease followed by exponential decrease until concentrations steady or increase before hatching (Figure 5-3). This trend is similar to those found in previous studies of sea turtle nests (Ackerman 1977, Maloney et al. 1990, Wallace et al. 2004, Honarvar et al. 2008, Chen et al. 2010, Garrett 2010). Although early embryonic oxygen consumption may be underestimated in most studies that use void-volume sampling (Miller 2008), the increased cost of using a fiber-optic method for gas sampling was not feasible for this study. Nest density has been shown to affect the oxygen concentration in neighboring nests (Honarvar et al. 2008), while microbial activity in decomposing nests may be responsible for lower sand oxygen concentration (Clusella Trullas 2007); however, these effects were not seen in this study. Volumetric water content was not different among beaches or throughout the incubation period, and did not affect the gas exchange environment for the nests (Figure 5-7). Although low oxygen concentration can be an important stressor for turtle clutches (Ackerman 1977, Kam 1993), it did not threaten sea turtle clutch development in Greece.

The values of egg mass from this study were higher than those reported previously for Kyparissia of 30.3 g (Reid et al. 2009) and is probably due to the inability to clean eggs of cloacal fluid and sand. Margaritoulis (2005) reports the typical clutch characteristics for *Caretta caretta* in Greece to be 120 eggs of 32g. Previous reports of hatchling mass, carapace length and carapace width were similar to this study (Reid et al. 2009).

Hatching success in this study was among the highest for loggerheads. The hatching success for all of the beaches of Laganas Bay, Zakynthos has previously been reported to be 73.6% (N=4017) from 2003-2009 (Margaritoulis et al. 2011). Clutches on Zakynthos and Kyparissia have much higher hatching success than on other nesting beaches. For example, loggerhead nests in Turkey have a hatching success of 10.7% in a good month, and most of the mortality is due to predation (Erk'akan 1993). Loggerhead nesting beaches in Florida have hatching success of 68% (Antworth 2006), but can vary between 30-80% and differences may be due to sand characteristics (T. Tucker, personal communication).

Hatching success was similar on most beaches of Zakanthos Island and Kyparissia Bay. However, hatching success on Kyparissia A was the lowest and was significantly lower than on all the beaches except Sekania East and West (Figure 5-2). There were no data to support the hypothesis that physical factors of the beaches measured were responsible for this difference. While grain size parameters differed between the beaches it did not appear to affect hatching success or oxygen concentration.

While Kyparissia O had among the largest median grain size with Kyparissia A (Table 5-2), that beach did not have the lower hatching success of its counterpart. There were farms behind the dunes at Kyparissia A, but there have been no investigations into what if any effects those farms had on the conditions on the beach. Since the physical factors in the sand did not appear to be affected, it may be that some form of contamination was reaching the beach from the agricultural activities. Future studies should investigate the possibility of contamination of the beach sand by pesticides, heavy metals and other agricultural byproducts. The grain size analysis for Sekania East and West are similar to previous studies (Karavas 2005) and suggests that the beach has been under a stable sedimentation dynamic for the last decade.

Temperature was lower on Marathonissi than the other beaches, but this did not affect hatching success or metabolic heating. It did affect the oxygen concentration in the nest most likely by decreasing the oxygen consumption. Beach temperatures were similar to those found previously (Zbinden 2007). The lower temperature slowed developmental rates on Marathonissi, but did not affect hatchling size. Nests on Marathonissi had the lowest oxygen deficit as well, but it did not affect hatching success. Due to the beach temperature being below the pivotal sex-determining temperature, Marathonissi appears to be an important male-producing beach for this rookery (Zbinden 2007), although early season nesting tends to produce males on all beaches (Katselidis et al. 2102).

Sekania East and West had slightly higher hatching success than Kyparissia A, but not significantly different from either group. During excavations, I noticed that the nests with lower hatching success had many of their eggs broken (personal observation). Because of the high density of nests (Margaritoulis 2005), access to these beaches was restricted to Marine Park, World Wildlife Fund and ARCHELON personnel; however, nests were not marked, and while care was taken to minimize stepping on nests, it was likely that it occurred (G. Schofield, unpublished data). When these nests were removed from the analysis, the hatching success of Sekania East and West was 92.4 (±2.1)% and 90.8 (±2.3)%, accounting for 10-15% fewer hatchlings produced. Increasing the hatching success on Sekania beaches can have a greater positive effect on the population than any other nesting beach conservation effort. Indeed, modeling results have estimated that a 15% hatching success increase on Sekania would result in 10,000 more hatchlings produced (Mazaris et al. 2009).

The high hatching success for loggerhead clutches on all beaches in the Zakynthos and Kyparissia rookeries suggest that the physical conditions on the beach are very supportive for egg incubation. For instance, temperatures are moderate, sand grain size varies but allows for sufficient gas exchange, water content is within normal range for maintenance of water balance in the eggs. Minimum oxygen percentages were related to the number of developing late term embryos in the nests. This relationship also occurs in leatherback turtle nests (Wallace et al. 2004) and green turtle nests (Chen et al. 2010). Clutch size is the strongest determinant of the concentration of oxygen in the nest. Conductance of the sand is limiting in that oxygen content does drop as metabolism of the clutch increases. However, in general these beaches are excellent incubators for loggerhead turtle eggs.

Modeling abiotic and biotic factors affecting clutch metabolism

In order to determine the relationship of the biotic and abiotic factors that shaped nest metabolism, I modeled gas exchange between clutches in their nests and the sand in the beach beyond the nest. I calculated a predicted nest oxygen value inside the nest using Fick's Law (Wangensteen and Rahn 1970, Wallace et al. 2004) and the beach characteristics and nest variables that I measured such that:

$$PO_{Nest} = PO_{Sand} - \frac{VO_2(x)}{GO_2} \tag{3}$$

where PO_{Nest} was the partial pressure of oxygen in the nest (kPa), PO_{Sand} was the partial pressure of oxygen in the sand without a nest (kPa), VO_2 was the oxygen consumption (cm^3 s^{-1}) per embryo, x was the number of developing embryos and GO_2 was the gas conductance of the sand (cm^3 s^{-1} kPa^{-1}).

Since there were no trends in PO_{Sand} measurements from the control sites for any beach in either year (ANCOVA: F(13,128)=1.62, p>0.05), I used mean sand oxygen level for beach and year as PO_{Sand} in this model.

The maximum VO_2 for *C. caretta* hatchlings is 1.13*10^{-3}, 1.06*10^{-3} and 8.66*10^{-4} (cm^3 s^{-1} egg^{-1}) at 27.6, 30, and 31.8 °C, respectively (Reid et al. 2009). I used the maximum nest temperature (T) with the curve generated from those values to estimate the VO_2 for each egg:

$$VO_2 = -1.045*10^{-5}*T^2 + 6.843*10^{-4}*T - 1.006*10^{-2}. \tag{4}$$

I then multiplied by the number of developing eggs to get the numerator in equation 3.

I calculated GO_2 for each nest from equation (8) of Wangensteen et al. (1970):

$$GO_2 = \frac{AD_{Sand}T_0}{\ell T P_0} \tag{5}$$

where A was the surface area of the nest (cm^2), D_{Sand} was the diffusivity of the sand (cm^2 s^{-1}), T_0 was the standard temperature (273.15 °K), P_0 was the pressure at sea level (101.325 kPa), ℓ was the depth of the middle of the nest from the surface of the sand (cm), and T was the maximum nest temperature (°K).

Diffusivity of the sand (D_{Sand}) was calculated as:

$$D_{Sand} = a\varepsilon_{Air} - b \tag{6}$$

where a and b were the slope and intercept unique to the beach in Table 5-3 and ε_{Air} was the air-filled porosity (% volume) for sand from each beach. I estimated ε_{Air} of the beaches as

$$\varepsilon_{Air} = \varepsilon_{Total} - W_{Sand} \tag{7}$$

where ε_{Total} was the total porosity of packed dry sand and W_{Sand} was the mean volumetric water content for the beach over the season. I calculated ε_{Total} from preliminary relationship measurements from the equation of the least squares regression,

$$\varepsilon_{Total} = 0.44 - 0.16 * \sigma_{\Phi} \tag{8}$$

where σ_Φ is the sorting of the sand grain sizes in Φ units.

I assumed that the eggs were arranged in a tetrahedral lattice in a spherical nest and that the minimum air-filled space was 34.01% (Hilbert and Cohn-Vossen 1999). Therefore I calculated the surface area (*A*) of the nest where the volume of the nest was equal to the sum of the volume of the total number of eggs laid plus 34.01% of that volume. I used 2.1 cm as the radius of *C. caretta* eggs from Ackerman (1977). The average volume (5371 cm^3) and surface area (1477 cm^2) for 86 nests with an average clutch size of 103 eggs were similar to values calculated by Ackerman (1977) of 5242 cm^3 and 1465 cm^2 for a nest 100 eggs.

I input variables that I measured specific to each nest or beach into Equation 3 to obtain predicted values for PO_{Nest}. I compared these values to measured minimum oxygen in each nest to test the accuracy of the model.

The PO_{Nest} predictions from this model underestimated the oxygen deficit observed in the nests by 0.9 to 1.2 kPa (Paired T (80) = -12.55, $p < 0.0001$; Figure 5-8). Although significantly different from the observed values, this predictive model improved upon previous models by providing insights into the process of gas exchange between the nest and the beach. Previous models assumed that GO_2 and VO_2 were constants and that PO_{Nest} was a function of clutch size (Wallace et al. 2004).

From equation 1, there are three ways to decrease the PO_{Nest}. First, a lower PO_{Sand} decreases the maximum oxygen levels that could be found in the nest. Second, a higher VO_2 means that more oxygen is consumed by the eggs and that would decrease the oxygen levels in the nest. Finally, a smaller GO_2 would allow less gas exchange so that less of the low oxygen gas in the nest would be replaced by the higher oxygen in the surrounding sand. I address each of these possibilities as I discuss the assumptions of this model.

The first assumption was that the beach was uniform in water content, grain size, temperature, and gas concentration. I considered water content of the nest to be equal to the average of the water content measured at the control sites throughout the season. Although there was no significant temporal difference of water content at nest depth, seasonal summer dry weather and winter rainfall common to Mediterranean climates may account for the visual trend of higher water content at the beginning and end of incubation (Figure 5-7). Control location sampling does not account for cloacal fluid that could penetrate the sand immediately adjacent to the nest during oviposotion, further decreasing the air-filled porosity of a thin layer of sand around the nest. Another factor to consider in beach heterogeneity is the location of old nests. Microbial and fungal decomposers are abundant on densely nested beaches (Clusella Trullas 2007), indeed old nests were found during excavations, attempts at control locations, and during nesting events, especially on Sekania (personal observation). While all of these are possible, the measurements of water content and sand grain size were not different between control sites and nests, temperature at nest depth and oxygen concentration did not differ among control sites on the same beach, and there was no difference in nest density among nests and controls from the same beach. I have no reason to suspect that beach measurements used in these calculations affected the PO_{Sand}.

The depth to the surface is assumed to be the minimum distance of the diffusive path length, although this may not always be the case. Another nest may have been (and in some cases was) closer to the nest in question than was the surface. This situation would also change the assumption that the PO_{Sand} was equal to the control location for the beach. Nests in close proximity can affect the PO_{Nest} and the hatching success of that nest (Honarvar et al. 2008). Mathematical models suggest that sea turtle nests can affect the PO_{Sand} up to 1 m distance (O'Connor et al. 2009, O'Connor et al. 2011). The estimates of PO_{Sand} that I used for the model were from measurements of the control nests and there was no difference in distance to the nearest 5 nests between controls and nests among beaches. Again, control location sampling was sufficient to account for any variation in PO_{Sand} due to nest placement.

I also assumed that the relationship of temperature to maximum VO_2 was a constant curve and, as such, many of these values were extrapolations, especially for the higher temperatures. I used the maximum values of VO_2 reported in (Reid et al. 2009), but they only measured temperatures at 27.6, 30, and 31.8 °C, while I measured temperatures up to 35 °C. This extrapolation may have underestimated the VO_2 at high temperatures and could reasonably contribute to an underestimation of oxygen deficit in the nest. The VO_2 also assumed that all of the oxygen consumed was due to the sea turtle eggs, yet many bacterial and fungal species exhibit metabolic activity in the nest (Clusella Trullas 2007) that may not be contributing equally to the metabolic heating; however, data on microbial metabolism in the sand would have been seen in the estimates of PO_{Sand} from the control sites.

I further assumed that there was no influence of convection in these nests. For some systems, a tidal pressure gradient can drive bulk air flow though the beach (Wallace et al. 2004, O'Connor et al. 2011). Greece has very small tides and thus total pressure gradients on the beach would be driven stochastically by wind and weather events. For most of the incubation duration, the weather is dominated by a high pressure system, typical for a Mediterranean summer climate, and changes in weather are rare. Additionally, gas sink convection due to the lower CO_2 production than oxygen consumption at a respiratory quotient (RQ) of 0.7 (Ackerman and Prange 1972, Reid et al. 2009) can drive bulk flow of gases into the nest. An addition of bulk flow would increase the estimate of GO_2, but this is unlikely to occur for sea turtle nests in Greece.

The most reasonable explanation for the underestimation of oxygen deficit by the model is that sand diffusivities (D_{Sand}) were higher in the model than they may have been in nature. From equation (5), smaller D_{Sand} decreases GO_2. I estimated D_{Sand} to be in the two-standard-deviation range of 0.015 to 0.026 cm^2 s^{-1} based on the air-filled porosity (ε_{Air}) from equations (6-8). These values are reasonable estimates and correspond to the two-standard-deviation (2SD) range from my laboratory experiments (0.007 to 0.027 cm^2 s^{-1}; Chapter 4) and values from previous models (0.015 and 0.025 cm^2 s^{-1}; Ackerman (1977)). However, by removing the inactive pore space (ε_{in}) from equation (6) such that:

$$D_{Sand} = a * (\varepsilon_{Air} - \varepsilon_{in}) - b \qquad (9)$$

where ε_{in} was 40% of ε_{Air}, or a mean of 0.11 cm^3 cm^{-3}, the 2SD range of D_{Sand} became 0.09 to 0.017 cm^2 s^{-1} and there was no difference between the observed and predicted values (Paired T (80) = -0.30, $p > 0.05$, Figure 5-9). It is common to overestimate the soil diffusion coefficient when transferring from laboratory models to field measurements, especially in moist soils, and the adjustment has been to subtract the inactive pore space

(0.07 to 0.13 $cm^3\ cm^{-3}$ for Yolo County, CA loam, for example) from ε_{Air} (Moldrup 2005). In moist soils, water accumulates in the tight junctions and reduces the effective pore space by increasing the constrictivity (Freijer 1994) and decreasing the pore continuity (Bruckler et al. 1989).

The number of eggs explained half of the variation in predicted oxygen concentration from the adjusted model (Least Squares Regression F (1,79) = 73.5, $p < 0.0001$, $R^2 = 0.48$), the rest of the variation was generated in the model by other biotic and abiotic factors unique to each nest. Biotic factors include the nest size, depth, metabolic heating, and oxygen concentration in the sand affected by other nearby nests and microorgainisms; abiotic factors include ambient beach temperature and sand physical characteristics such as grain size, sorting and water content as it relates to porosity. Overall, the model shows the importance factors that can affect PO_{Sand}, VO_2, and GO_2. Each nest has individual factors, both biotic and abiotic, that affect the oxygen concentration so we cannot assume that GO_2 is constant among nests, even if they are on the same beach.

The model demonstrates that the physical attributes of the beach are important components for sea turtle egg incubation. The adjustment for inactive pore space in the model underscores the importance of ground-truthing for models and understanding the limitations of laboratory experiments. It also demonstrates that changes in beach conditions to lower porosity conditions could decrease the oxygen levels in the nests. Under the beach conditions at Zakynthos and Kyparissia, Greece in 2009 and 2010, the nests did not experience hypoxic conditions that negatively affected hatching success. In the absence of tidal ventilation, the nests rely on diffusion for aeration, and lower porosities due to increased water content or increased grain sorting would be detrimental. It is important to continue to monitor the beach activities in Greece so that the conditions stay in the range that continues to provide recruits to the Mediterranean loggerhead turtle population.

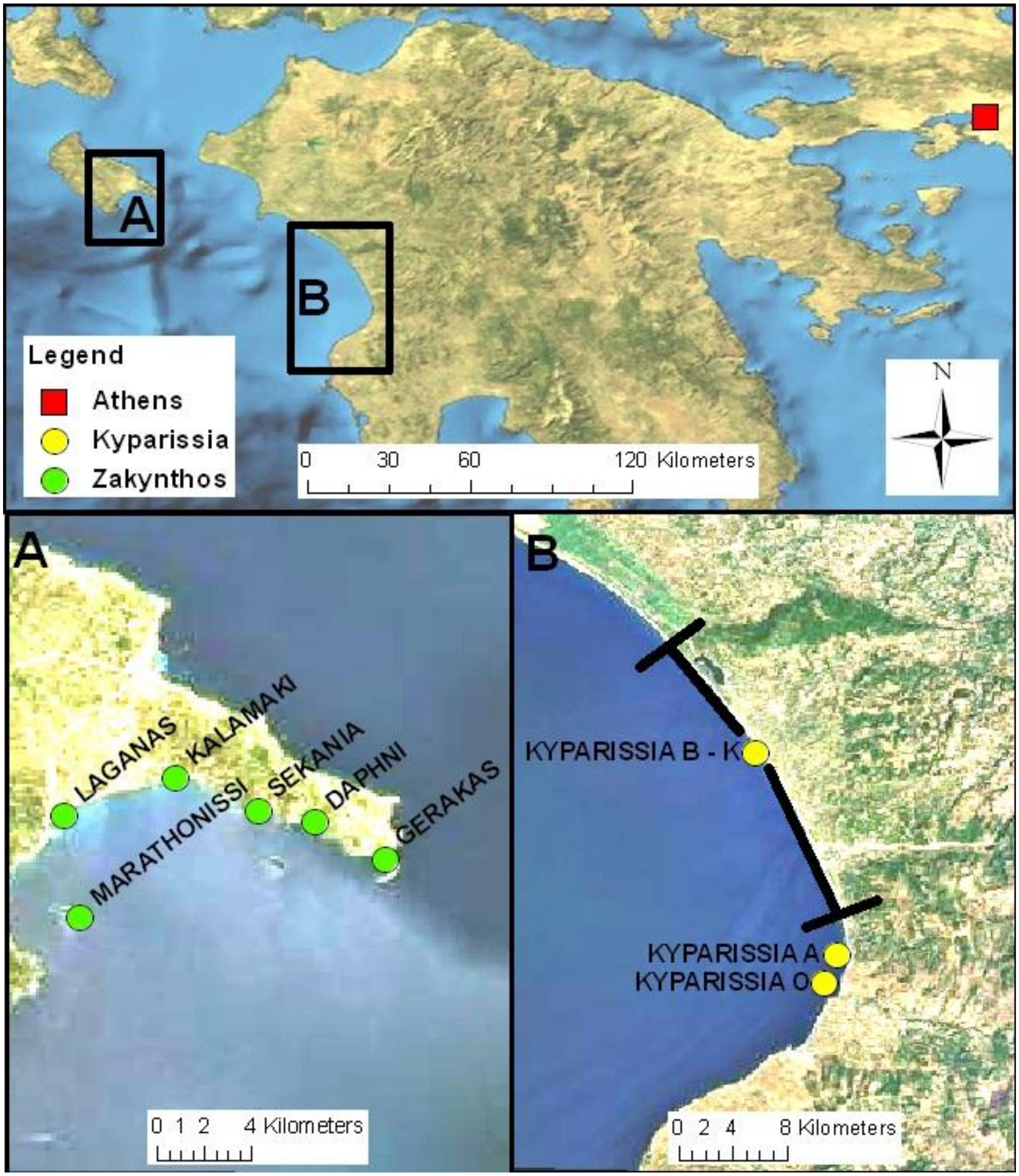

Figure 5-1. The loggerhead nesting beaches of Zakynthos Island (A) and Kyparissia Bay (B), on the Peleponesse peninsula of Greece.

Table 5-1. Number of loggerhead turtle nests and controls on each beach on Zakanthos Island and Kyparissia Bay in Greece in 2009 and 2010. The code is used in the graphs.

Beach	**Code**	**Nests**		**Controls**	
		2009	**2010**	**2009**	**2010**
Kyparissia O	O	0	5	0	3
Kyparissia A	A	9	10	4	3
Thiafi	T	1	1	2	2
Marathonissi	M	5	10	3	3
Sekania East	E	5	10	3	3
Sekania West	W	5	10	3	3
Gerakas	G	5	10	3	3

Table 5-2. Median sand grain size, sand grain sorting and nest density for loggerhead nesting beaches on Zakynthos Island and Kyparissia Bay in Greece.

Beach	**Sand**		**Density**
	Φ_{50} (±2 SEM)	σ_{Φ} (±2 SEM)	Distance to nearest 5 nests (m) (±2 SEM)
Kyparissia O	-0.09 (±0.19)	0.66 (±0.03)	17.90 (±5.15)
Kyparissia A	0.17 (±0.10)	0.69 (±0.02)	12.33 (±2.12)
Thiafi	1.26 (±0.10)	1.20 (±0.06)	2.09 (±0.70)
Marathonissi	1.29 (±0.07)	0.77 (±0.04)	11.48 (±1.84)
Sekania East	1.64 (±0.12)	0.57 (±0.05)	6.91 (±0.92)
Sekania West	1.78 (±0.03)	0.51 (±0.01)	3.94 (±0.77)
Gerakas	2.00 (±0.06)	0.54 (±0.01)	13.46 (±2.87)

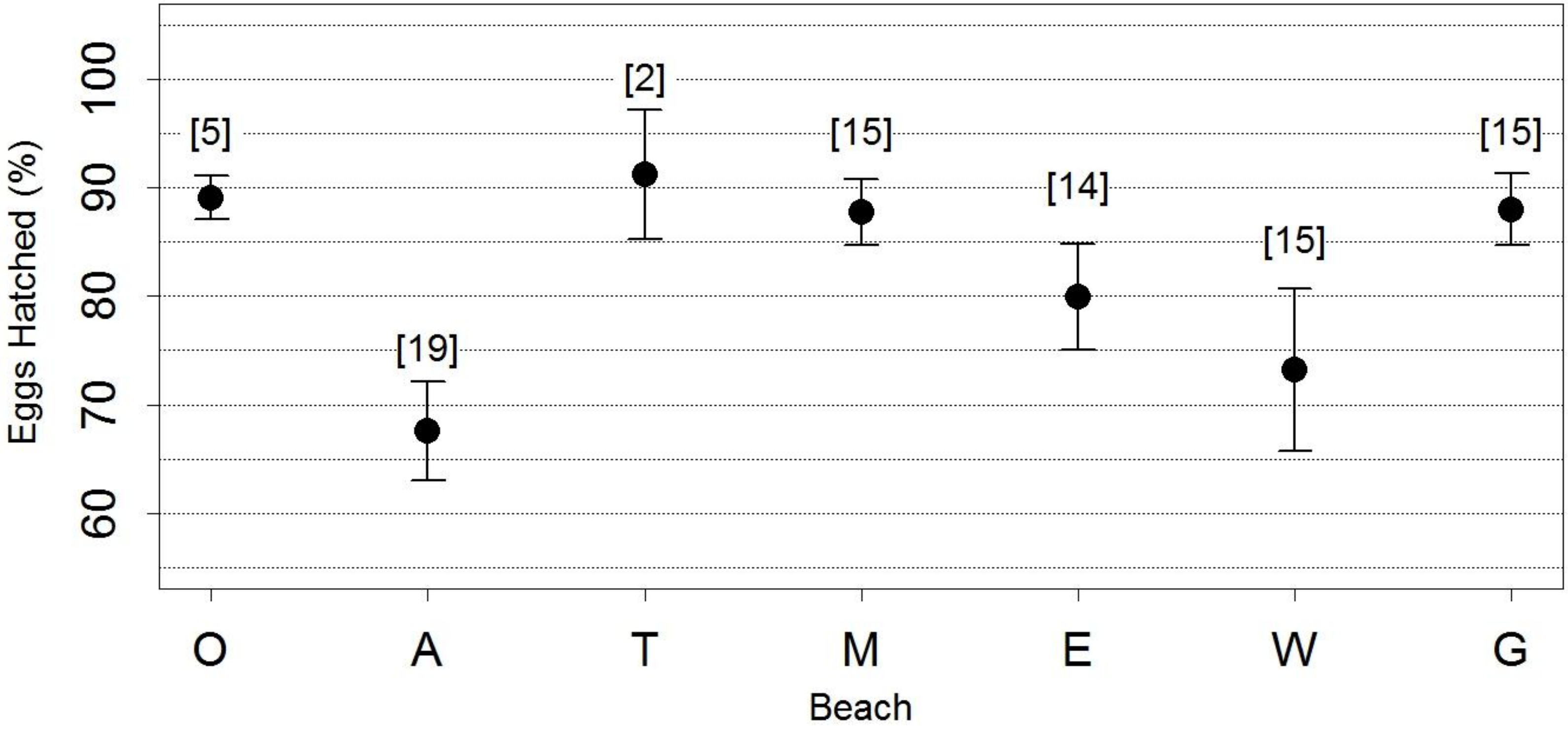

Figure 5-2. Hatching success for loggerhead turtle clutches on Kyparissia A was lower than on all other beaches except Sekania East and West. Error bars are 2 standard errors of the mean; numbers in brackets are the sample size.

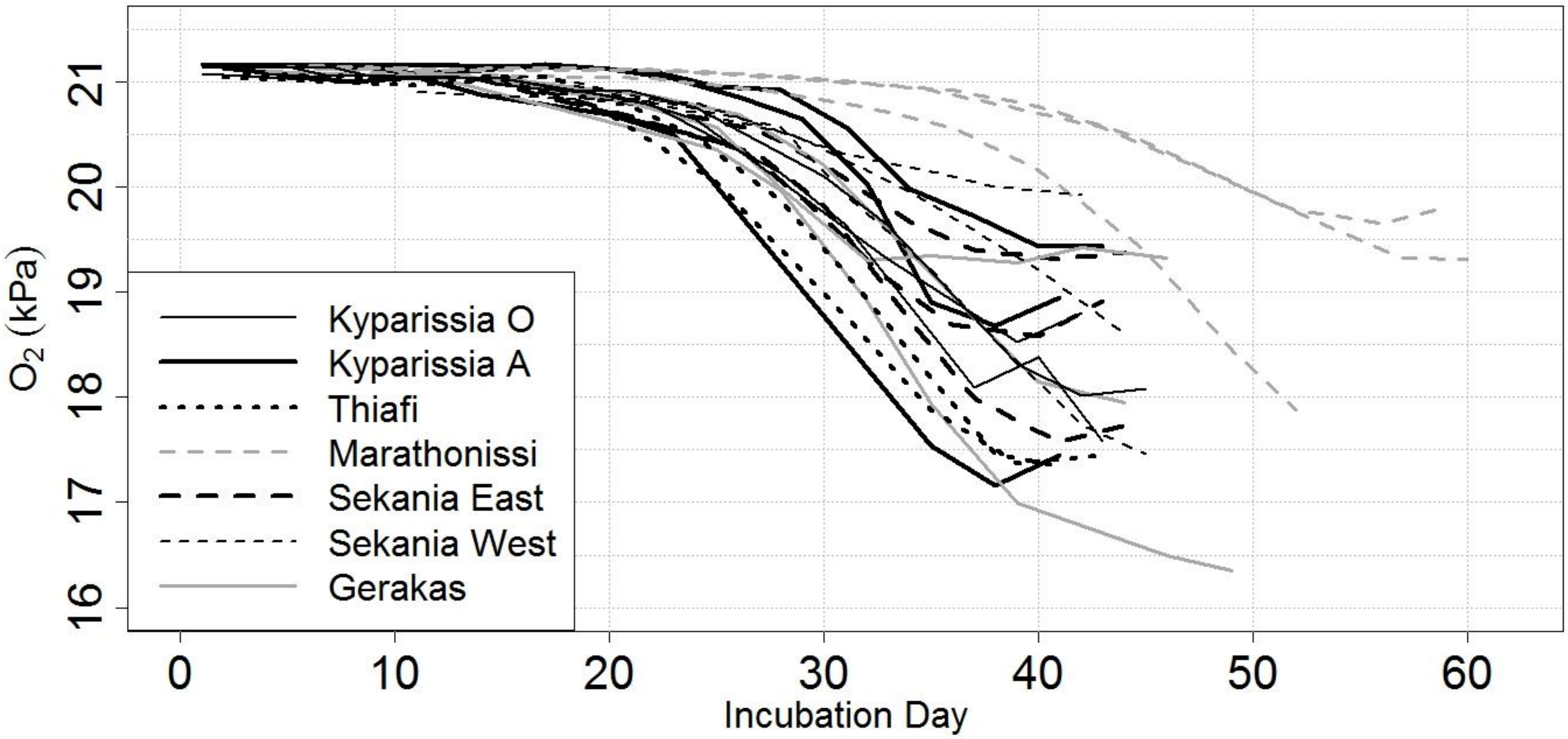

Figure 5-3. Loggerhead turtle nest PO_2 decreased throughout incubation. Each beach is represented with the profiles for the three nests that had the median and penultimate high and low minimum PO_2.

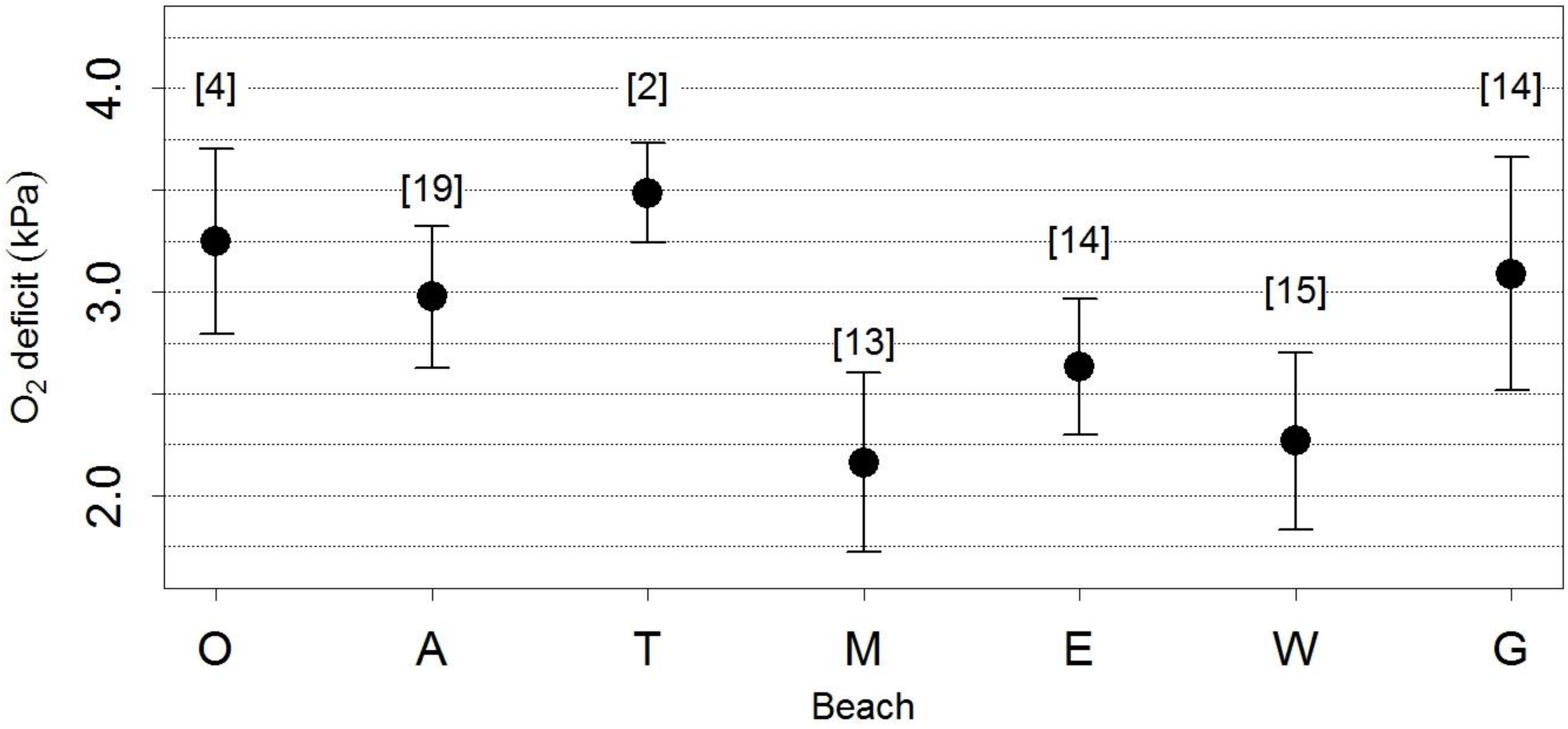

Figure 5-4. Maximum PO_2 deficit in loggerhead turtle nests differed between Marathonissi and Gerakas on Zakynthos. Error bars are 2 standard errors of the mean; numbers in brackets are the sample size.

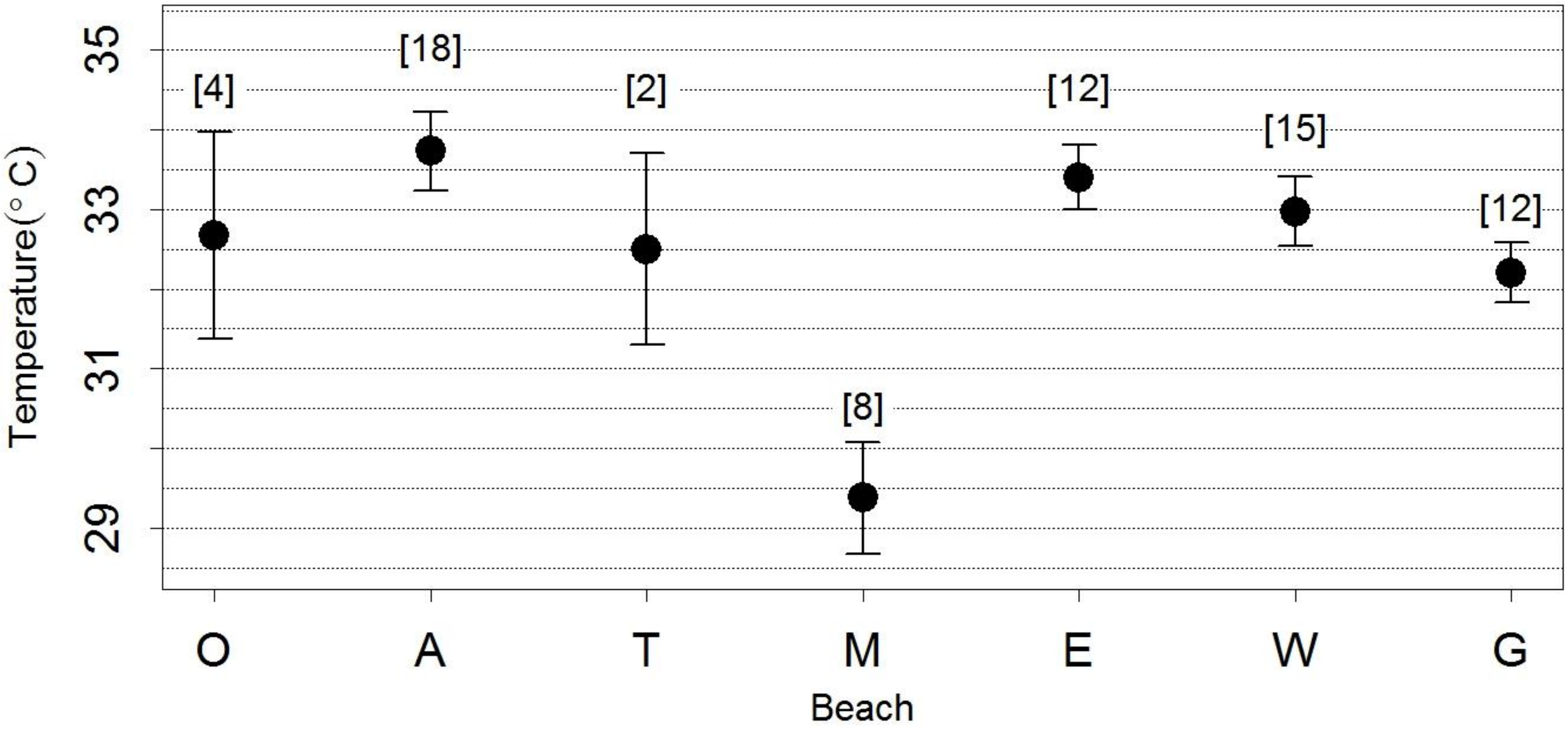

Figure 5-5. Maximum nest temperature was lower on Marathonissi than on all other beaches. Error bars are 2 standard errors of the mean; numbers in brackets are the sample size.

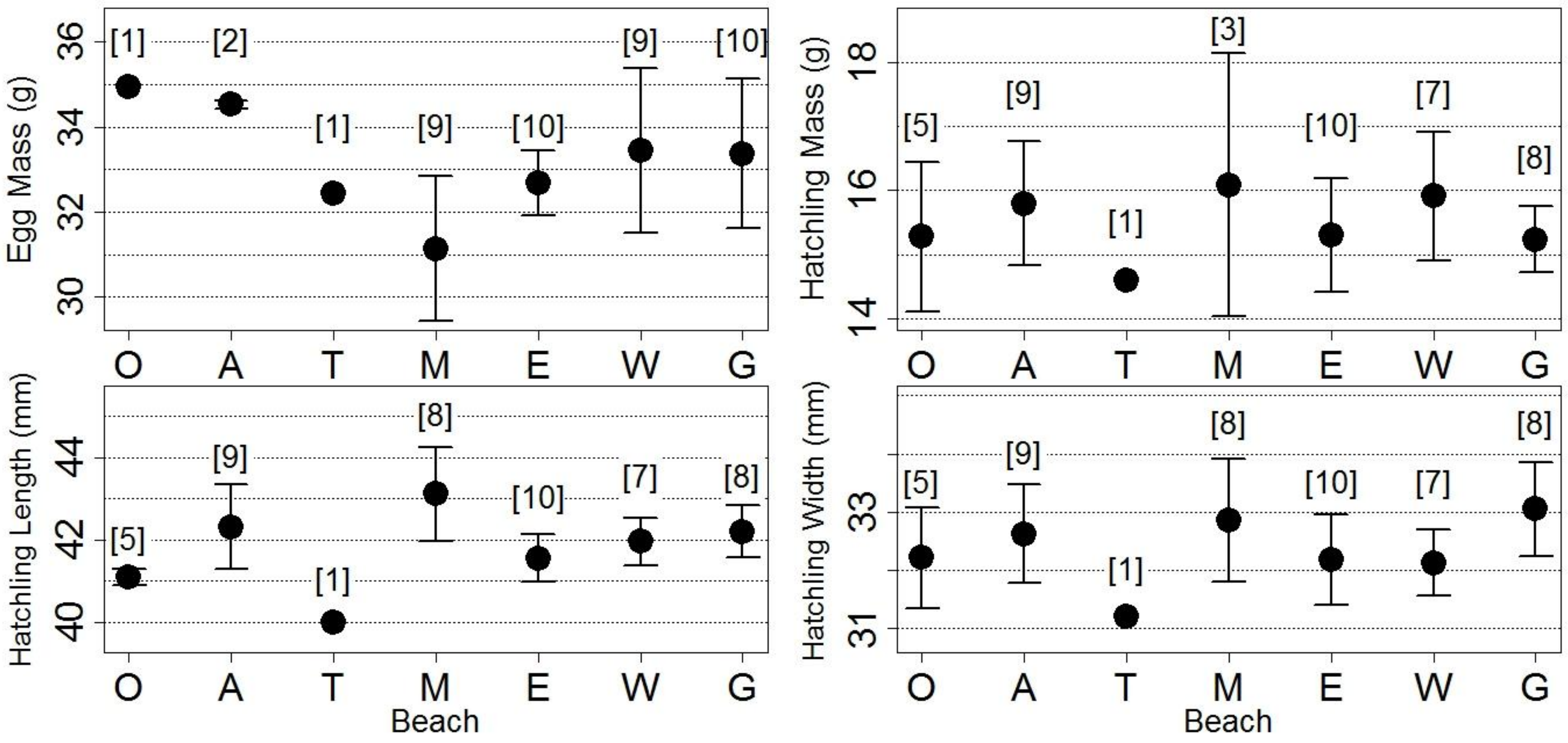

Figure 5-6. Egg mass (top left), hatchling mass (top right), hatchling length (bottom left) and hatchling width (bottom right) did not differ among hatchlings of loggerhead turtles from different beaches on Zakynthos Island and Kyparissia Bay suggesting that there was no difference in maternal quality of loggerheads nesting on the different beaches. Error bars are 2 standard errors of the mean, numbers in brackets are the number of clutches. The mean value represents the grand mean for the beach of the clutch mean values.

Table 5-3. Slope and intercept from equations used to predict diffusivity from air-filled porosity in sand on loggerhead turtle nesting beaches of Zakynthos and Kyparissia, Greece.

Beach	**Intercept**	**Slope**
Kyparissia O	1.90×10^{-5}	7.68×10^{-2}
Kyparissia A	4.93×10^{-3}	6.93×10^{-2}
Thiafi	7.26×10^{-3}	6.24×10^{-2}
Marathonissi	3.00×10^{-3}	6.19×10^{-2}
Sekania East	2.54×10^{-3}	6.93×10^{-2}
Sekania West	2.48×10^{-3}	5.70×10^{-2}
Gerakas	4.88×10^{-4}	7.50×10^{-2}

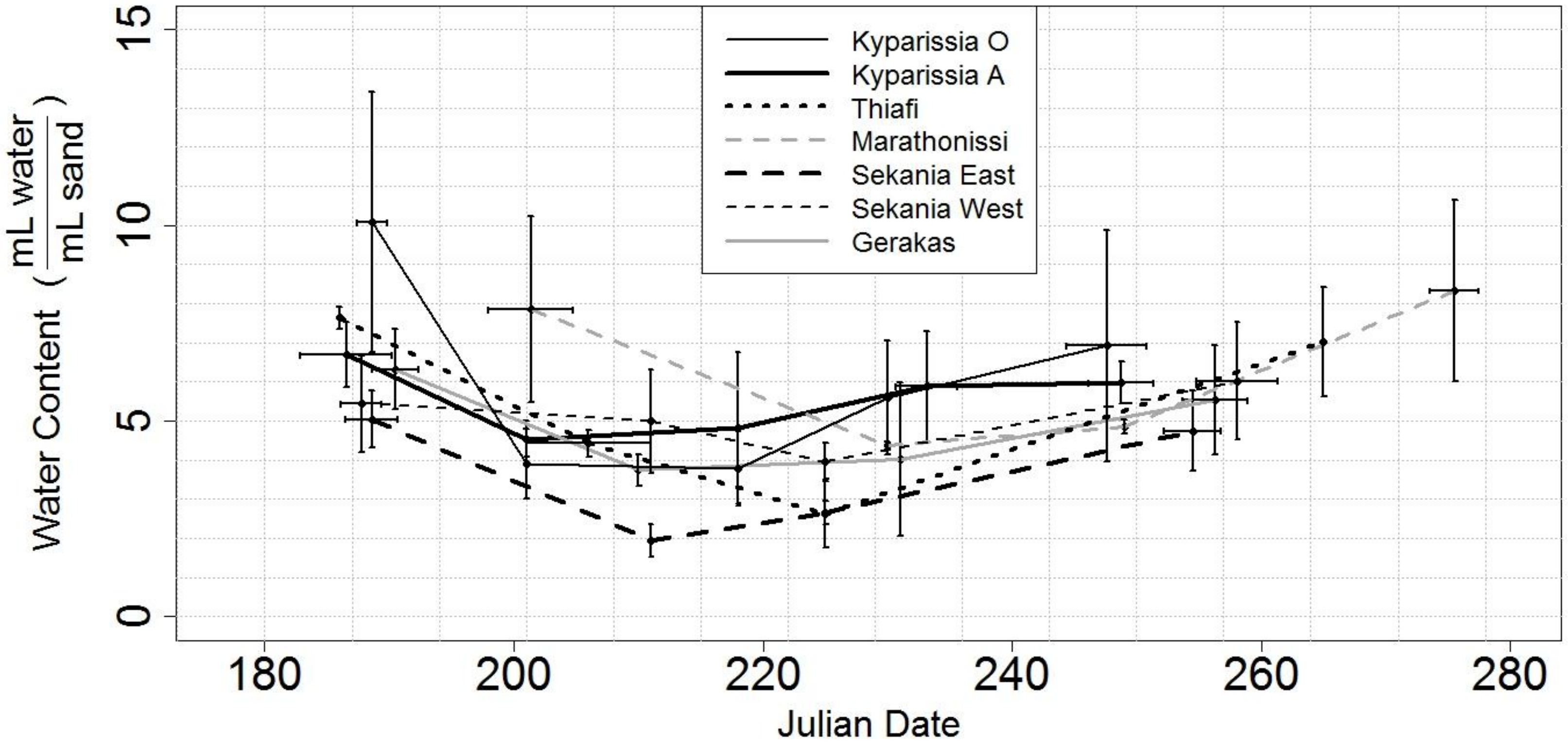

Figure 5-7. The volumetric water content of the beaches on Zakynthos Island and Kyparissia Bay did not differ statistically between the beaches or during the incubation period in 2010. Rain occurred at the beginning of the nesting season in May and June, and in September after most nests had hatched, except for the nests on Marathonissi Island. Error bars are 2 standard errors of the mean.

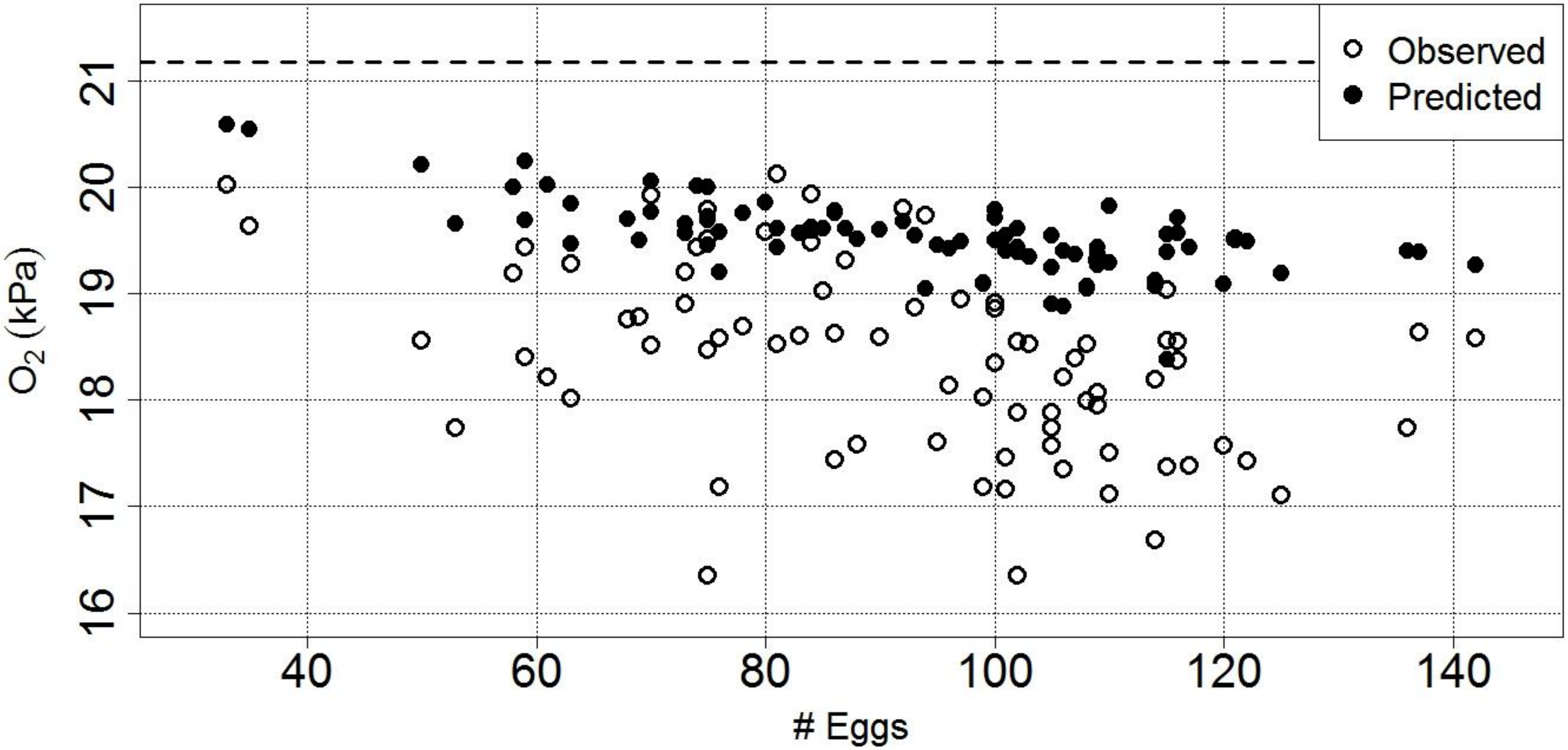

Figure 5-8. Minimum oxygen concentration in the sea turtle nest as a function of the number of eggs where the embryo developed to at least the size of the yolk. The open circles (○) are the observed minimum oxygen concentration from loggerhead turtle nests at Zakynthos and Kyparissia, Greece. The closed circles (●) are oxygen concentrations predicted from equation (3). The dashed line indicates atmospheric oxygen concentration.

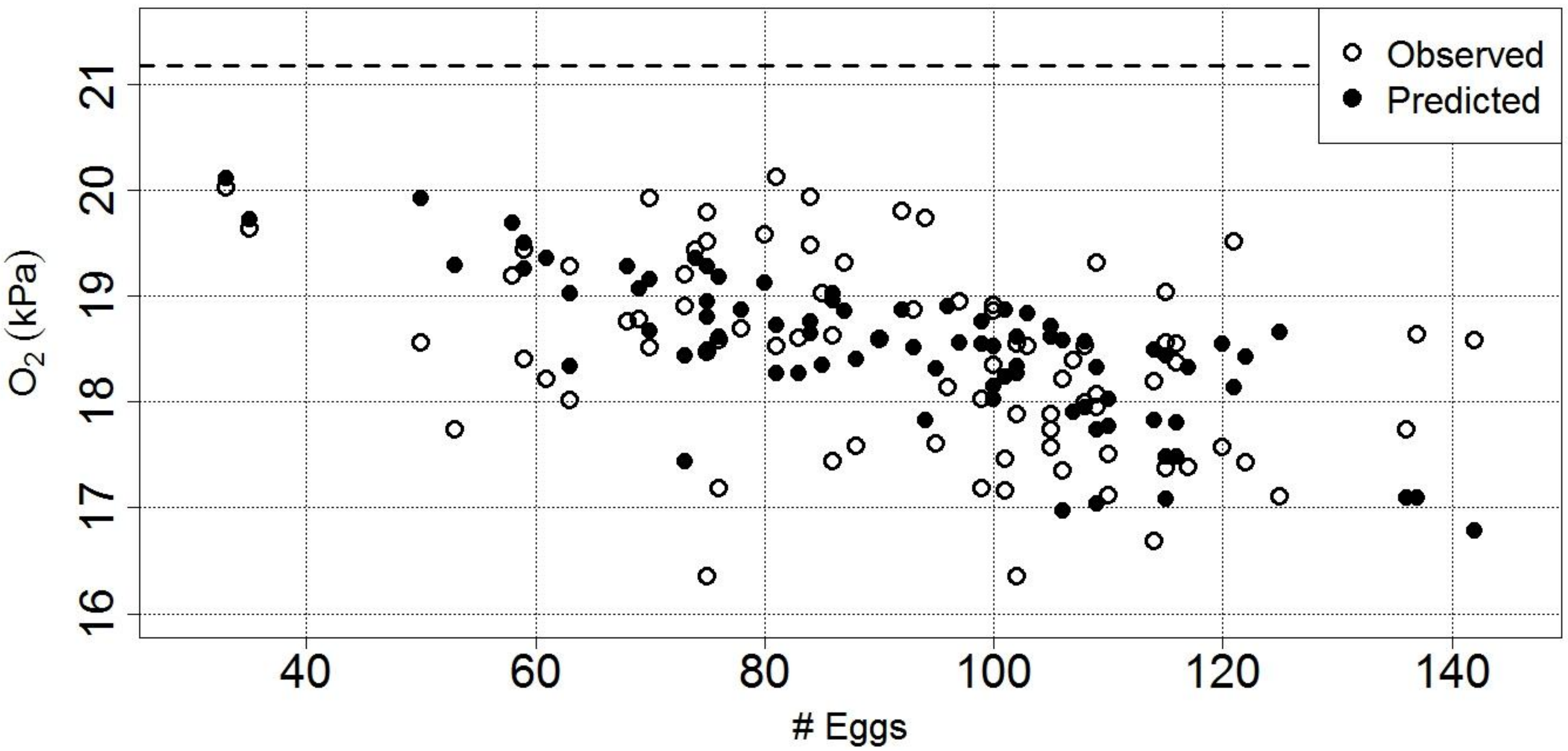

Figure 5-9. Minimum oxygen concentration in the sea turtle nest as a function of the number of eggs where the embryo developed to at least the size of the yolk. The open circles (○) are the observed minimum oxygen concentration from loggerhead turtle nests at Zakynthos and Kyparissia, Greece. The closed circles (●) are the adjusted predictions of oxygen concentrations from equation (3) when equation (9) is used to predict sand diffusivity instead of equation (6). The dashed line indicates atmospheric oxygen concentration.

CHAPTER 6: CONCLUSION AND MANAGEMENT SUGGESTIONS

Many reptiles, including testudines, iguanids, megapodes and some snakes, bury clutches of eggs underground where they are incubated by the soil environment. These eggs contain the embryo and the yolk resources needed for growth and development, but must exchange heat, water and gases with the environment. The rates of these exchanges are limited by egg shell and soil permeability. Soil is a matrix of solid, liquid and gas phases, and the movement of materials through the matrix depends on the relative proportions of each of these phases. The herpetological literature provides relatively few examples about how the incubation medium affects the physiology of egg clutches, but there is increasing awareness that climate can influence soil properties.

Sea turtles bury large masses of eggs on many different types of beaches worldwide. These beaches may vary in water availability, temperature, texture and tidal amplitude, but they are all favorable for egg development. Burying eggs deeply in sand offers protection from predation and provides a humid environment with fairly constant temperature, but also constrains the diffusion of gases. Other underground nesters, such as megapodes, crocodilians, freshwater turtles, snakes and iguanids bury the nests less deeply, provide burrows of free air for the incubating eggs, or bury in soils with greater air-filled porosity (Seymour 1980, Seymour et al. 1986, Booth 2000). Gas exchange is such a problem for mudskipper nests that the adult males gulp air to transport to the incubating eggs (Ishimatsu et al. 1998).

Oxygen concentration in loggerhead turtle (*Caretta caretta*) nests in Greece reduced throughout incubation and approached, but never reached a physiological threshold affecting hatching success. The threshold for oxygen levels in leatherback turtle (*Dermochelys coriacea*) nests was 14 to 16 kPa (Garrett 2010), but oxygen deficits that do not reach this point are functions of the number of developing embryos (Wallace et al. 2004). This relationship describes a limit to the clutch size that should approach, but not exceed the point that the total metabolic output would suffocate itself.

I have described a relationship that can be useful in determining the limits of reproduction parameters for sea turtles (see Chapter 5). Accordingly, lower oxygen concentrations in the ambient sand could occur through the metabolic output of high nest density (Honarvar et al. 2008) and microbial and fungal decomposers (Clusella Trullas 2007). In addition to increasing the number of eggs, higher nest temperature also increases the oxygen consumption (Reid et al. 2009) and beach topography can play an important role in sand heating. Wet sand has among the lowest air-filled porosity of all unsaturated soils (Fredlund and Rahardjo 1993), and therefore the lowest diffusivity. Indeed, the diffusion coefficient of oxygen in sand was 2-16% of oxygen in free air but 40% for the diffusion coefficient of oxygen through the mound soil of megapode nests (Seymour et al. 1986).

The surface area of the nest is limited by the number of eggs and the shape of the nest where the smallest surface area to volume is a sphere. Curiously, sea turtle nests are roughly spherical which allows for the lowest possible gas conductance, especially for the eggs in the center of the clutch (Ackerman 1977). To this point, it would appear that either there is no strong selection against incubation under depressed oxygen conditions or that there is a selective advantage to these conditions. Gas exchange may limit clutch size, and may be an important factor in the evolution of multiple nesting events in sea turtles.

Embryos exposed to hypoxia show physiological plasticity by an increased ability to transport oxygen (Kam 1993, Crossley et al. 2003), smaller body size with larger hearts (Crossley and Altimiras 2005, van Bergen 2005), and vasodilation (Crossley and Altimiras 2005) that may reduce the response to future hypoxia exposure (Eme et al. 2011b) but also result in hypertension and low heart rate (Eme et al. 2011a). The reptilian sympathetic and parasympathetic reflex loops may play important roles in the response of embryos to naturally occurring hypoxic nest conditions (Eme et al. 2011b). Among the underground nesters, sea turtles, freshwater turtles, crocodilians and mudskippers spend portions of their lives underwater where gas exchange is limited, so exposure to depressed but non-lethal oxygen concentration during incubation could provide an advantage for diving or brumation.

Depth is another important component that can affect the gas conductance in the nest such that deeper nests have a smaller conductance and a greater oxygen deficit per egg. The importance of depth is two-fold however, because the nest must not be exposed to the dry sand layer or risk desiccation due to the low water potential of dry sand. Dry sand also has lower specific volume than the repacked wet sand in the egg chamber. This may have important consequences for gas exchange, especially on beaches with large tidal excursion where bulk flow of gases can be a large component of gas exchange (O'Connor et al. 2011).

Management Suggestions

Overall, the loggerhead turtle nesting beaches of Zakynthos and Kyparissia, Greece, are capable of sustaining a healthy population if the hatching success is kept above 70%. These rookeries are the largest for loggerheads in the Mediterranean (Margaritoulis 2003); therefore, improving the hatching success of nests on these beaches can have a greater positive effect on the population than any other nesting beach conservation effort. Indeed, modeling results have estimated that increasing hatching success by 15% on Sekania alone would result in 10,000 more hatchlings produced (Mazaris et al. 2009). Public usage and population monitoring techniques need to be constantly scrutinized for minimizing damages to nests and eggs.

The Greek loggerhead turtle sub-population is a vital source of genetic variation for the Mediterranean population (Carreras et al. 2007); hence, protection of these beaches is crucial for maintaining population sustainability. Regulations already in place at Zakynthos through the National Marine Park must continue to be strictly enforced on all beaches. However, at Kyparissia, local species-specific conservation efforts through ARCHELON, The Sea Turtle Protection Society of Greece, remain the most visible face of European Union regulations.

In Florida, some sea turtle nesting beaches are being renourished after erosion by hurricanes and this can affect hatching success and gas concentrations in the nest (Mota 2009). The sand that is transported to the beaches often comes from inland sand mines or offshore dredges which can include higher percentages of silts and clays than is normally found on the beach. These sands are not generated from the same processes that produced the beaches, this difference can affect the mean grain size, sorting, skewness and kurtosis of the beach sand population (Spencer 1963). I have demonstrated that various properties of sand affect gas movement, it is important that the beach renourishment consider the gas environment for organisms of the in-beach ecosystem.

Climate change models project heating for sea turtle nesting beaches (Saba et al. 2012) that can affect hatchling mortality (Santidrian Tomillo et al. 2012). In addition, heating would create a deeper dry sand layer that is less permeable to gases than the repacked wet sand. This would restrict the tidal flow of gases, which can aerate nests (Wallace et al. 2004, O'Connor et al. 2011). Furthermore, introduction of dry sand into nests through relocation could decrease gas exchange with the sea turtle nest and may be a contributing factor to lower hatching success in relocated nests (Sieg 2011).

Sea turtles are important environmental engineers of the beaches, and they are adapted to construct nests conforming to an optimal gas exchange environment. As beaches continue to be developed, eroded or renourished, the undisturbed state of the interstitial environment must be understood in order to properly mitigate and restore the beach ecosystem.

REFERENCES

Ackerman, R. A. 1975. Diffusion and the gas exchange of sea turtle eggs. Dissertation. University of Florida, Gainesville, FL.

Ackerman, R. A. 1977. The respiratory gas exchange of sea turtle nests (Chelonia, Caretta). Respir Physiol **31**:19-38.

Ackerman, R. A. 1980. Physiological and Ecological Aspects of Gas-Exchange by Sea Turtle Eggs. American Zoologist **20**:575-583.

Ackerman, R. A. 1981. Growth and Gas-Exchange of Embryonic Sea Turtles (Chelonia, Caretta). Copeia:757-765.

Ackerman, R. A. 1997. The nest environment and the embryonic development of sea turtles. Pages 83-106 *in* P. L. Lutz, John A Musick, editor. The Biology of Sea Turtles. CRC Press, Boca Raton, Florida, USA.

Ackerman, R. A. and D. B. Lott. 2005. Thermal, hydric and respiratory climate of nests. Pages 15-43 *in* D. C. Deeming, editor. Reptilian Incubation: Environment, Evolution and Behaviour. Nottingham University Press, Nottingham, U. K.

Ackerman, R. A. and H. D. Prange. 1972. Oxygen diffusion across a sea turtle (Chelonia mydas) egg shell. Comparative Biochemistry and Physiology Part A: Physiology **43**:905-909.

Ackerman, R. A., R. C. Seagrave, R. Dmi'el, and A. Ar. 1985. Water and Heat Exchange between Parchment-Shelled Reptile Eggs and Their Surroundings. Copeia **1985**:703-711.

Antworth, R. L., David A. Pike, John C. Stiner. 2006. Nesting ecology, current status, and conservation of sea turtles on an uninhabited beach in Florida, USA. Biological Conservation **130**:10-15.

Bakker, J. W. and A. P. Hidding. 1970. The influence of soil structure and air content on gas diffusion in soils. Netherlands Journal of Agricultural Science **18**:37-48.

Ball, B. C. 1981. Pore characteristics of soils from two cultivation experiments as shown by gas diffusivities and permeabilities as air-filled porosities. Journal of Soil Science **32**:483-498.

Barden, L. and G. Pavlakis. 1971. Air and water permeability of compacted unsaturated cohesive soil Journal of Soil Science **22**:302-318.

Bartelt-Hunt, S. L. and J. A. Smith. 2002. Measurement of effective air diffusion coefficients for trichloroethene in undisturbed soil cores. J Contam Hydrol **56**:193-208.

Batterman, S., I. Padmanabham, and P. Milne. 1996. Effective Gas-Phase Diffusion Coefficients in Soils at Varying Water Content Measured Using a One-Flow Sorbent-Based Technique. Environmental Science & Technology **30**:770-778.

Bear, J. 1972. Dynamics of fluids in porous media. American Elsevier Publishing Company, Inc., New York, NY.

Beard, D. C., P. K. Weyl. 1973. Influence of texture on porosity and permeability of unconsolidated soil. The American Association of Petroleum Geologists bulletin **57**:349-369.

Bezrukov, A., D. Stoyan, and M. Bargiel. 2001. Spacial statistics for simulated packing of spheres. Image Analysis and Stereology **20**:203-206.

Booth, D. T. 2000. The Effect of Hypoxia on Oxygen Consumption of Embryonic Estuarine Crocodiles (Crocodylus porosus). Journal of Herpetology **34**:478-481.

Booth, D. T. 2005. Artificial Incubation. Pages 253-263 *in* D. C. Deeming, editor. Reptilian Incubation: Environment, Evolution and Behaviour. Nottingham University Press, Nottingham, U.K.

Booth, D. T., E. Burgess, J. McCosker, and J. M. Lanyon. 2004. The influence of incubation temperature on post-hatching fitness characteristics of turtles. International Congress Series **1275**:226-233.

Booth, D. T. and C. Y. Yu. 2009. Influence of the Hydric Environment on Water Exchange and Hatchlings of Rigid-Shelled Turtle Eggs. Physiol Biochem Zool **84**:382-387.

Bruckler, L., B. C. Ball, and P. Renault. 1989. Laboratory Estimation of Gas Diffusion Coefficient and Effective Porosity in Soils. Soil Science **147**:1-10.

Buckingham, E. 1904. Contributions to our knowledge of the aeration of soils. US Department of Agriculture Bureau of Soils Bulletin.

Bustard, H. R., Peter Greenham. 1968. Physical and chemical factors affecting hatching in the green sea turtle, Chelonia mydas (L.). Ecology **49**:269-276.

Campbell, G. 1974. A simple method for determining unsaturated conductivity from moisture retention data. Soil Science **117**:311-314.

Carreras, C., M. Pascual, L. Cardona, A. Aguilar, D. Margaritoulis, A. Rees, O. Turkozan, Y. Levy, A. Gasith, M. Aureggi, and M. Khalil. 2007. The genetic structure of the loggerhead sea turtle (Caretta caretta) in the Mediterranean as revealed by nuclear and mitochondrial DNA and its conservation implications. Conservation Genetics **8**:761-775.

Chen, C. L., C. C. Wang, and I. J. Cheng. 2010. Effects of biotic and abiotic factors on the oxygen content of green sea turtle nests during embryogenesis. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **180**:1045-1055.

Clune, P. R. 2005. Natural and human induced variations on beach dynamics and their effects on leatherback sea turtle conservation. Purdue University.

Clusella Trullas, S., F. V. Paladino. 2007. Micro-environment of olive ridley turtle nests deposited during an aggregated nesting event. Journal of Zoology **272**:367-376.

Crossley, D. A. and J. Altimiras. 2005. Cardiovascular development in embryos of the American alligator Alligator mississippiensis: effects of chronic and acute hypoxia. Journal of Experimental Biology **208**:31-39.

Crossley, D. A., W. W. Burggren, and J. Altimiras. 2003. Cardiovascular regulation during hypoxia in embryos of the domestic chicken Gallus gallus. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology **284**:R219-R226.

Currie, J. A. 1960. Gaseous diffusion in porous media Part 2.-Dry granular materials. British Journal of Applied Physics **11**:318-324.

Currie, J. A. 1961. Gaseous diffusion in the aeration of aggregated soils. Soil Science **92**:40-45.

Currie, J. A. 1970a. Movement of gases in soil respiration. S.C.I. Monograph **37**:152-171.

Currie, J. A. 1970b. Sorption and transport processes in soils. SCI Monograph **37**:152-169.

Currie, J. A. 1984a. Gas diffusion through soil crumbs: the effects of compaction and wetting. Journal of Soil Science **35**:1-10.

Currie, J. A. 1984b. Gas diffusion through soil crumbs: the effects of wetting and swelling. Journal of Soil Science **34**:217-232.

Drake, D. L. and J. R. Spotila. 2002. Thermal tolerances and the timing of sea turtle hatchling emergence. Journal of Thermal Biology **27**:71-81.

Eme, J., J. Altimiras, J. W. Hicks, and D. A. Crossley Ii. 2011a. Hypoxic alligator embryos: Chronic hypoxia, catecholamine levels and autonomic responses of in ovo alligators. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology **160**:412-420.

Eme, J., J. Hicks, and D. Crossley. 2011b. Chronic hypoxic incubation blunts a cardiovascular reflex loop in embryonic American alligator (<i>Alligator mississippiensis</i>). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology **181**:981-990.

Erk'akan, F. 1993. Nesting biology of loggerhead turtles Caretta caretta L. on Dalyan Beach, Mugla-Turkey. Biological Conservation **66**:1-4.

Ferreira Júnior, P. D., Maria F. Rosa, Mariângela de Lorenzo, Matheus F. Monteiro, Romildo A. Júnior. 2008. Influência das características geológicas do local de desova na duração da incubação e no sucesso da eclosão dos ovos de Caretta caretta na praia da Guanabara, Anchieta, Espírito Santo. Iheringia. Série Zoologia **98**:447-453.

Fish, A. N. and A. J. Koppi. 1994. The use of a simple field air permeameter as a rapid indicator of functional soil pore space. Geoderma **63**:255-264.

Foley, A. M., S. A. Peck, and G. R. Harman. 2006. Effects of Sand Characteristics and Inundation on the Hatching Success of Loggerhead Sea Turtle (Caretta caretta) Clutches on Low-Relief Mangrove Islands in Southwest Florida. Chelonian Conservation and Biology **5**:32-41.

Folk, R. L. 1954. The Distinction between Grain Size and Mineral Composition in Sedimentary-Rock Nomenclature. The Journal of Geology **62**:344-359.

Folk, R. L. 1966. A review of grain-size parameters. Sedimentology **6**:73-93.

Folk, R. L. 1980. Petrology of Sedimentary Rocks. Hemphill Publishing Company, Austin, Texas.

Folk, R. L. and W. C. Ward. 1957. Brazos River bar [Texas]; a study in the significance of grain size parameters. Journal of Sedimentary Research **27**:3-26.

Fredlund, D. G. and H. Rahardjo. 1993. An Overview of Unsaturated Soil Behaviour. Pages 1-33 *in* S. L. Houston and W. K. Wray, editors. Unsaturated Soils. American Society of Cvil Engineers, New York.

Freijer, J. I. 1994. Calibration of Jointed Tube Model for the Gas Diffusion Coefficient in Soils. Soil Sci. Soc. Am. J. **58**:1067-1076.

Fujikawa, T. 2005. Effects of bulk density and soil type on the gas diffusion coefficient in repacked and undisturbed soils. Soil Science **170**:892-901.

Garrett, K., Bryan P. Wallace, Jeanne Garner, Frank V. Paladino. 2010. Variations in leatherback turtle nest environments: consequences for hatching success. Endangered Species Research **11**:147-155.

Gates, D. M. 1980. Biophysical Ecology. Springer-Verlag, New York.

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

Godley, B. J., A. C. Broderick, J. R. Downie, F. Glen, J. D. Houghton, I. Kirkwood, S. Reece, and G. C. Hays. 2001. Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. Journal of Experimental Marine Biology and Ecology **263**:45-63.

Hays, G. C. and J. R. Speakman. 1993. Nest placement by loggerhead turtles, Caretta caretta. Animal Behaviour **45**:47-53.

Hilbert, D. and S. Cohn-Vossen. 1999. Geometry and the Imagination. Chelsea, New York.

Honarvar, S., M. P. O'Connor, and J. R. Spotila. 2008. Density-dependent effects on hatching success of the olive ridley turtle, Lepidochelys olivacea. Oecologia **157**:221-230.

Ishimatsu, A., Y. Hishida, T. Takita, T. Kanda, S. Oikawa, T. Takeda, and K. H. Khoo. 1998. Mudskippers store air in their burrows. Nature **391**:237-238.

Kam, Y. C. 1993. Physiological effects of hypoxia on metabolism and growth of turtle embryos. Respir Physiol **92**:127-138.

Karavas, N., Kyriacos Georghiou, Margarita Arianoutsou, Dimitris Dimopoulos. 2005. Vegetation and sand characteristics influencing nesting activity of Caretta caretta on Sekania beach. Biological Conservation **121**:177-188.

Katselidis, K. A., G. Schofield, G. Stamou, P. Dimopoulos, and J. D. Pantis. 2102. Females first? Past, present and future variability in offspring sex ratio at a temperate sea turtle breeding area. Animal Conservation **15**:508-518.

Kraemer, J. E. and J. I. Richardson. 1979. Volumetric Reduction in Nest Contents of Loggerhead Sea Turtles (Caretta caretta) (Reptilia, Testudines, Cheloniidae) on the Georgia Coast. Journal of Herpetology **13**:255-260

Kühne, A., H. Schack-Kirchner, and E. E. Hildebrand. 2012. Gas diffusivity in soils compared to ideal isotropic porous media. Journal of Plant Nutrition and Soil Science **175**:34-45.

Lange, S. F., S. E. Allaire, and D. E. Rolston. 2009. Soil-gas diffusivity in large soil monoliths. European Journal of Soil Science **60**:1065-1077.

Maloney, J. E., C. Darian-Smith, Y. Takahashi, and C. J. Limpus. 1990. The Environment for Development of the Embryonic Loggerhead Turtle (Caretta caretta) in Queensland. Copeia **1990**:378-387.

Mancinelli, R. L. 1995. The regulation of methane oxidation in soil. Annual Review of Microbiology **49**:581+.

Margaritoulis, D. 1982. Observations on Loggerhead Sea Turtle Caretta-Caretta Activity during 3 Nesting Seasons (1977-1979) in Zakynthos, Greece. Biological Conservation **24**:193-204.

Margaritoulis, D. 1998. Interchange of nesting loggerheads among Greek beaches. Pages 225-227 *in* S. P. Eppley and J. Braun, editors. Proceedings of the Seventeenth Annual Sea Tutle Symposium. NOAA Technical Memorandum NMPS-SEFC-415, Orlando, Fl.

Margaritoulis, D. 2005. Nesting activity and reproductive output of loggerhead sea turtles, Caretta caretta, over 19 seasons (1984-2002) at Laganas Bay, Zakynthos, Greece: The largest rookery in the Mediterranean. Chelonian Conservation and Biology **4**:916-929.

Margaritoulis, D., R. Argano, I. Baran, F. Bentivegna, M. N. Bradai, J. A. Caminas, P. Casale, G. De Metrio, A. Demetropoulos, G. Gerosa, B. J. Godley, D. A. Haddoud, J. Houghton, L. Laurent, B. Lazar. 2003. Loggerhead turtles in the Mediterranean Sea: Present knowledge and conservation perspectives. Pages 175-198 *in* A. B. Bolten, and B. E. Witherington, editor. Loggerhead Sea Turtles. Smithsonian Books, Washington, D.C.

Margaritoulis, D. and A. F. Rees. 2001. The Loggerhead Turtle, Caretta caretta, population nesting in Kyparissia Bay, Peloponnesus, Greece: Results of beach surveys over seventeen seasons and determination of the core nesting habitat. . Zoology in the Middle East **24**:75-90.

Margaritoulis, D., A. F. Rees, C. J. Dean, and T. Riggall. 2011. Reproductive data of loggerhead turtles in Laganas Bay, Zakynthos Island, Greece, 2003-2009. Marine Turtle Newsletter:2-6.

Marshall, T. J. 1959. The diffusion of gases through porous media. Journal of Soil Science **10**:79-82.

Mazaris, A., S. Kramer-Schadt, J. Tzanopoulos, K. Johst, and G. P. Matsinos, JD. 2009. Assessing the relative importance of conservation measures applied on sea turtles: comparison of measures focusing on nesting success and hatching recruitment success Amphibia-Reptilia **30**:221-231.

Mazaris, A. D., Y. G. Matsinos, and D. Margaritoulis. 2006. Nest site selection of loggerhead sea turtles: The case of the island of Zakynthos, W Greece. Journal of Experimental Marine Biology and Ecology **336**:157-162.

Miller, N. A. 2008. PO2 in loggerhead sea turtle (Caretta caretta) nests measured using fiber-optic oxygen sensors. Copeia **4**:882-888.

Millington, R. J. and J. P. Quirk. 1959. Permeability of porous media. Nature **183**:387-388.

Moldrup, P., T. Olesen, J. Gamst, P. Schjonning, T. Yamaguchi, D. E. Rolston. 2000. Predicting the gas diffusion coefficient in repacked soil: water-induced linear reduction model. Soil Science Society of America Journal **64**:1588-1594.

Moldrup, P., Torben Olesen, Seiko Yoshikawa, Toshiko Komatsu, Ann M. McDonald, Dennis E. Rolston. 2005. Predictive-descriptive models for gas and solute diffusion coefficients in variably saturated porous media coupled to pore-size distribution: III. Inactive pore space interpretations of gas diffusivity. Soil Science **170**:867-879.

Mortimer, J. A. 1990. The Influence of Beach Sand Characteristics on the Nesting Behavior and Clutch Survival of Green Turtles (Chelonia mydas). Copeia **1990**:802-817.

Mota, M. J. 2009. Beach restoration in Florida: Effects on sea turtle nesting and hatchling physiology. University of Florida.

Mrosovsky, N., S. Kamel, A. F. Rees, and D. Margaritoulis. 2002. Pivotal temperature for loggerhead turtles (Caretta caretta) from Kyparissia Bay, Greece. Canadian Journal of Zoology-Revue Canadienne De Zoologie **80**:2118-2124.

O'Connor, M. P., S. Honarvar, P. R. Sotherland, and J. R. Spotila. 2009. Biophysical factors affecting gas exchange in sea turtle nests. Integrative and Comparative Biology **49**:E124-E124.

O'Connor, M. P., J. S. Suss, P. R. Sotherland, and J. R. Spotila. 2011. Diffusive and conductive effects of sand on gas exchange in sea turtle nests. Society for Integrative and Comparitive Biology, Salt Lake City, UT.

Oke, T. R. 1992. Boundary Layer Climates. Routledge, New York.

Olson, M. S., F. D. Tillman, Jr., J. W. Choi, and J. A. Smith. 2001. Comparison of three techniques to measure unsaturated-zone air permeability at Picatinny Arsenal, NJ. J Contam Hydrol **53**:1-19.

Packard, G. C. 1999. Water Relations of Chelonian Eggs and Embryos: Is Wetter Better? American Zoologist **39**:289-303.

Packard, G. C., T. L. Taigen, M. J. Packard, and R. D. Shuman. 1979. Water-vapor conductance of testudinian and crocodilian eggs (class reptilia). Respiration Physiology **38**:1-10.

Pataki, D. E., M. M. Carreiro, J. Cherrier, N. E. Grulke, V. Jennings, S. Pincetl, R. V. Pouyat, T. H. Whitlow, and Z. W. C. 2011. Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions. Frontiers in Ecology and the Environment **9**:27-36.

Penman, H. L. 1940. Gas and vapour movements in the soil: I. The diffusion of vapours through porous solids. The Journal of Agricultural Science **30**:437-462.

Pereira, A. A. A. and D. E. Cruciani. 2001. Estimativa de variações no coeficiente relativo de difusão dos gases no solo com base na análise da curva de retenção da água. Ciencia Rural **31**:245-249.

Pettijohn, F. J. 1957. Sedimentary Rocks, 2nd ed. 2nd edition. Harper and Bros., New York.

Prange, H. D. and R. A. Ackerman. 1974. Oxygen consumption and mechanisms of gas exchange of green turtle (C. mydas) eggs and hatchlings. Copeia:6.

Rees, A. F. 2005. ARCHELON, The Sea Turtle Protection Society of Greece: 21 years studying and protecting sea turtles. Testudo **6**:32-50.

Rees, A. F., Dimitris Margaritoulis. 2004. Beach temperatures, incubation durations and estimated hatchling sex ratio for loggerhead sea turtle nests in southern Kyparissia bay, Greece. Testudo **6**:23-36.

Reid, K. A., D. Margaritoulis, and J. R. Speakman. 2009. Incubation temperature and energy expenditure during development in loggerhead sea turtle embryos. Journal of Experimental Marine Biology and Ecology **378**:62-68.

Reina, R. D., J. R. Spotila, F. V. Paladino, and A. E. Dunham. 2009. Changed reproductive schedule of eastern Pacific leatherback turtles Dermochelys coriacea following the 1997–98 El Nino to La Nina transition. Endangered Species Research **7**:155-161.

Saba, V. S., P. Santidrian-Tomillo, R. D. Reina, J. R. Spotila, J. A. Musick, D. A. Evans, and F. V. Paladino. 2007. The effect of the El Niño Southern Oscillation on the reproductive frequency of eastern Pacific leatherback turtles. Journal of Applied Ecology **44**:395-404.

Saba, V. S., C. A. Stock, J. R. Spotila, F. V. Paladino, and P. S. Tomillo. 2012. Projected response of an endangered marine turtle population to climate change. Nature Clim. Change **advance online publication**.

Santidrian Tomillo, P., V. S. Saba, G. S. Blanco, C. A. Stock, F. V. Paladino, and J. R. Spotila. 2012. Climate Driven Egg and Hatchling Mortality Threatens Survival of Eastern Pacific Leatherback Turtles. PLoS ONE **7**:e37602.

Santidrián Tomillo, P., J. S. Suss, B. P. Wallace, K. D. Magrini, G. Blanco, F. V. Paladino, and J. R. Spotila. 2009. Influence of emergence success on the annual reproductive output of leatherback turtles. Marine Biology **156**:2021-2031.

Schofield, G., C. M. Bishop, K. A. Katselidis, P. Dimopoulos, J. D. Pantis, and G. C. Hays. 2009. Microhabitat selection by sea turtles in a dynamic thermal marine environment. Journal of Animal Ecology **78**:14-21.

Schofield, G., V. J. Hobson, M. K. S. Lilley, K. A. Katselidis, C. M. Bishop, P. Brown, and G. C. Hays. 2010. Inter-annual variability in the home range of breeding turtles: Implications for current and future conservation management. Biological Conservation **143**:722-730.

Seymour, R., D. Vleck, and C. Vleck. 1986. Gas exchange in the incubation mounds of megapode birds. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology **156**:773-782.

Seymour, R. S., Ralph A. Ackerman. 1980. Adaptations to underground nesting in birds and reptiles. American Zoologist **20**:437-447.

Shimamura, K. 1992. Gas Diffusion Through Compacted Sands. Soil Science **153**:274-279.

Sieg, A. E., Christopher A. Binkley, Bryan P. Wallace, Pilar Santidrian Tomillo, Richard D. Reina, Frank V. Paladino, James R. Spotila. 2011. Sex ratios of leatherback turtles: hatchery translocation decreases metabolic heating and female bias. Endangered Species Research **15**:194-204.

Spencer, D. W. 1963. The interpretation of grain size distribution curves of clastic sediments. Journal of Sedimentary Research **33**:180-190.

Sperry, J. M. and J. J. Peirce. 1995. A Model for Estimating the Hydraulic Conductivity of Granular Material Based on Grain Shape, Grain Size, and Porosity. Ground Water **33**:892-898.

Standora, E. A., James R. Spotila. 1985. Temperature dependent sex determination in sea turtles. Copeia **3**:711-722.

Thompson, M. B. 1989. Patterns of metabolism in embryonic reptiles. Respir Physiol **76**:243-255.

Thorbjorn, A., P. Moldrup, H. Blendstrup, T. Komatsu, and D. E. Rolston. 2008. A Gas Diffusivity Model Based on Air-, Solid-, and Water-Phase Resistance in Variably Saturated Soil. Vadose Zone Journal **7**:1276-1286.

Tracy, C. R., G. C. Packard, and M. J. Packard. 1978. Water Relations of Chelonian Eggs. Physiological Zoology **51**:378-387.

Tracy, C. R., W. R. Welch, and W. P. Porter. 1980. Properties of Air. University of Wisconsin-Madison, Madison, WI.

Troeh, F. R., J. D. Jabro, and D. Kirkham. 1982. Gaseous diffusion equations for porous materials. Geoderma **27**:239-253.

Tsirika, A., G. Skoufas, and S. Haritonidis. 2007. Seasonal and bathymetric variations of epiphytic macroflora on Posidonia oceanica (L.) Delile leaves in the National Marine Park of Zakynthos (Greece). Marine Ecology **28**:146-153.

Ullman, W. J. and R. C. Aller. 1982. Diffusion Coefficients in Nearshore Marine Sediments. Limnology and Oceanography **27**:552-556.

van Bavel, C. H. M. 1952. Gaseous Diffusion and Porosity in Porous Media. Soil Science **73**:91-104.

van Bergen, Y. 2005. Mini-Alligators with big hearts. Journal of Experimental Biology **208**:ii.

Wallace, B. P., P. R. Sotherland, P. Santidrian Tomillo, R. D. Reina, J. R. Spotila, and F. V. Paladino. 2007. Maternal investment in reproduction and its consequences in leatherback turtles. Oecologia **152**:37-47.

Wallace, B. P., P. R. Sotherland, J. R. Spotila, R. D. Reina, B. F. Franks, and F. V. Paladino. 2004. Biotic and abiotic factors affect the nest environment of embryonic leatherback turtles, Dermochelys coriacea. Physiol Biochem Zool **77**:423-432.

Wangensteen, O. D. and H. Rahn. 1970. Respiratory gas exchange by the avian embryo. Respiration Physiology **11**:31-45.

Wangensteen, O. D., D. Wilson, and H. Rahn. 1970. Diffusion of gases across the shell of the hen's egg. Respiration Physiology **11**:16-30.

Warner, D. A. and R. M. Andrews. 2002. Nest-site selection in relation to temperature and moisture by the lizard Sceloporus undulatus. Herpetologica **58**:399-407.

Williams, B. K. and J. D. Nichols. 1984. Optimal timing in biological processes. American Naturalist **123**:1-19.

Zbinden, J. A., Christina Davy, Dimitris Margaritoulis, Raphael Arlettaz. 2007. Large spacial variation and female bias in the estimated sex ratio of loggerhead sea turtle hatchlings of a Mediterranean rookery. Endangered Species Research **3**:305-312.

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Santidrian Tomillo, P., F. V. Paladino, **J. S. Suss**, and J. R. Spotila. 2010. Predation of leatherback turtle hatchlings during the crawl to the water. Chelonian Conservation and Biology. 9(1): 18-25.

Suss, J. S., S. Honarvar, A. Panagopoulou, J. R. Spotila, and M. P. O'Connor. (13-16 March 2012). Beach characteristics affect the gas exchange environment for sea turtle nests. International Sea Turtle Symposium, Huatulco, Oaxaca, Mexico.

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