

# The Effects of Nest Location and Beach Environment on Hatching Success for Leatherback (*Dermochelys Coriacea*) and Green (*Chelonia Mydas*) Sea Turtles on Bioko Island, Equatorial Guinea

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## Research Article

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# Abstract

Hatching success in sea turtles is hindered by a variety of biotic and abiotic factors. This study of the nesting ecology of leatherback (*Dermochelys coriacea*) and green (*Chelonia mydas*) sea turtles, investigated how several environmental factors and beach characteristics on Bioko Island, Equatorial Guinea influence sea turtle reproductive success. Average clutch hatching success was 40.4% for green turtles and 41.73% for leatherback turtles. For leatherback turtles, clutch elevation relative to the high tide line (HTL) was found to be the most influential factor in determining hatching success, highlighting the sensitivity of this species to sea level rise (SLR). Multiple linear regression analysis demonstrated that nest distance to vegetation and sand conductivity also played significant roles in leatherback clutch hatching success. For leatherback clutches, 33% percent of experimental nests were affected by inundation and 17% by predation. An optimum clutch elevation range for leatherback turtles was identified, where a distinct increase in hatching success was observed between -0.286 m to -0.0528 m above the HTL. For green sea turtles, 64% of experimental nests were affected by predation, confounding conclusions about the roles of environmental characteristics in green turtle hatching success. We propose further investigation into influential characteristics in green turtle nests and confirmation of the observed optimum elevation range on Bioko Island and other nesting grounds. Identified sensitivities of each species to SLR and beach characteristics will be used to encourage the government of Equatorial Guinea to consider the vulnerability of their resident turtle populations when planning for future coastal development.

# Introduction

Embryogenesis in reptiles is affected by the environmental conditions of the nest site (Vitt and Pianka 2014; Packard 1991). Incubation conditions can specifically influence sex determination, hatching success, development, hatchling traits, and incubation period in many egg-laying reptiles (Piña et al. 2003; Hare et al. 2002; Ji and Du et al. 2001; Spotila et al. 1994). Due to the life history characteristics of marine turtles, their reproductive success is specifically vulnerable to sea level rise and alterations to the complex microclimate of their nest environment (Eckert and Eckert 1990; Matsuzawa et al. 2002; Wallace et al. 2004; Foley et al. 2006).

Sea turtle embryonic development is a intricate process where factors that decrease hatching success are many and include egg predation (Fowler 1979), nest inundation (Limpus et al. 2006; Hamann et al. 2007), human interference (Tomillo et al. 2008; Salleh and Sah 2015; James and Melero 2015), microbial infection (Phillott and Parmenter 2001; Wallace et al. 2004; Honarvar et al. 2011; Bezy et al. 2015; Rosado-Rodríguez and Maldonado-Ramírez 2016), and environmental characteristics within the nest chamber (Eckert and Eckert 1990). Important environmental qualities include sand moisture content (McGehee 1990; Foley et al. 2006), conductivity (Foley et al. 2006), temperature (Packard and Packard 1988; Matsuzawa et al. 2002; Segura and Cajade 2010), and gas permeability, which can influence respiration (Ackerman 1975; Ackerman 1977; Packard and Packard 1988).

Successful incubation is likely hindered by beaches with poorly-sorted substrates, high silt, saline, or clay-like composition (Mortimer 1990). Poorly-sorted substrates can affect por space, which can result in increased salinity within the nest environment, decreased water availability, and egg desiccation. Silt and clay-sand composition can result in increased water retention within the nest, decreasing the potential for necessary gas exchange (Mortimer 1990). These sand characteristics decrease the likelihood that the highest proportion of individuals will develop successfully into hatchlings and can reduce clutch hatching success (Mortimer 1990).

Regarding general nest placement, nesting too close to the water will cause nest inundation and embryo mortality, whereas nesting too far from the water's edge can cause egg desiccation, increased predation risk of the eggs as well as the hatchlings at time of emergence. There is also an increased likelihood for hatchlings to encounter crawling obstructions or experience orientation difficulty the further away the nest is placed relative to the high tide line (HTL) (Wood and Bjorndal 2000). An increased freshwater table and inundation of nests and body pits attributable to sea level rise has been observed on Raine Island, Australia (Limpus et al. 2006; Hamann et al. 2007) and at the Ten Thousand Island in Florida, where it has resulted in decreased hatching success (Foley et al. 2006). Nesting too seaward also poses threats based on sensitivities to moisture content, chloride, and conductivity (Bustard and Greenham 1968; McGehee 1990; Foley et al. 2006). Increased inundation can cause embryo suffocation and metabolism disruption from high salinity exposure to developing embryos (Whitmore and Dutton 1985). In Suriname, the observed decreased leatherback hatchling success (46%) in comparison to green sea turtle hatchling success (80%) was attributed to the leatherback sea turtles' tendency to nest closer to the extreme HTL (Whitmore and Dutton 1985).

It has been found on a hawksbill sea turtle nesting beach on Shidvar Island, Iran that turtles preferred a specific elevation range, where hatchling emergence was the highest and emergences decreased at both higher and lower elevations (Zare et al. 2012). In Iran and another beach in Barbados, hatching success increased when nests were closer to the average nest elevation and decreased with deviation from the mean (Horrocks and Scott 1991; Zare et al. 2012). This specific elevation range where hatching success was at its highest could be located in an area that represents a balance between both seaward and inland threats (Zare et al. 2012).

The 19 km of beaches on the southern side of Bioko Island, Equatorial Guinea are considered home to the second largest nesting aggregation of leatherback (*Dermochelys coriacea*) as well as green (*Chelonia mydas*) sea turtles in West Africa (Tomás et al. 2010; Honarvar et al. 2016). In the present study we investigate how nest location, environmental factors, and beach characteristics affect clutch hatching success in leatherback and green sea turtles on two of Bioko's nesting beaches.

## **Materials And Methods**

### ***Study Site and Experimental Design***

Within the Grand Caldera and Southern Highlands Scientific Reserve on the south side of Bioko Island are five sea turtle nesting beaches (Beaches A-E), totaling 10.75 km, of four marine turtle species: leatherback sea turtle (*Dermochelys coriacea*), green sea turtle (*Chelonia mydas*), olive ridley sea turtle (*Lepidochelys olivacea*), and hawksbill sea turtle (*Eretmochelys imbricata*) (Honarvar et al., 2016). Although control nests were studied on all five nesting beaches, experimental nests were concentrated on Beach C (2.9 km) and Beach D (2.5 km), most commonly characterized by a mixture of green turtle and leatherback turtle nesting activity (Honarvar et al. 2016; Veelenturf et al. 2020) (Fig. 1).

This study was conducted from November 2016 through February 2017. An ONSET weather station (Part # RX3000, Advanced model, ONSET Computer Corporation, Bourne, MA) was deployed to record temperature, rainfall, relative humidity, solar radiation, wind speed, and wind direction every 15 minutes. During nightly beach patrols on beaches C and D, turtles were tagged using the Passive Integrated Transponder (PIT) tagging method (AVID Identification Systems Inc., Norco, CA) (Dutton and McDonald 1994), and nest locations were recorded using GPS (Garmin GPSMap 64) for 10 green and 10 leatherback clutches on Beach C and 1 green and 14 leatherback clutches on Beach D. Turtles were tagged to ensure that more than one nest from the same turtle were not included in the study. These clutches are referred to as “experimental nests”. Additionally, “control nests” were dug to the average observed depth of experimental nests for both leatherback ( $78.5 \text{ cm} \pm 11.28$ ,  $n = 24$ ) and green ( $66 \text{ cm} \pm 6.02$ ,  $n = 11$ ) nests but contained no eggs. The center of the control nest chamber was calculated using the average distance from the bottom of the nest to the top of nest chamber in leatherback ( $27.19 \text{ cm} \pm 9.52$ ,  $n = 24$ ) and green ( $19.33 \text{ cm} \pm 6.02$ ,  $n = 11$ ) experimental nests. Control nest locations were randomly selected within the observed beach zones where each species nested. Zones were defined in relation to the vegetation and high tide lines, where Zone 1 was below the HTL, Zone 2 was between the HTL and vegetation line, and Zone 3 was behind the vegetation line. Green turtle control nests were dug in Zone 3 and leatherback turtle control nests in Zone 2. There were 3 leatherback and 3 green control nests on Beach C and Beach D.

## ***Nest Site Characteristics***

Nest characteristics were measured twice, during the nesting event and during nest excavations for both experimental and control nests. Nest characteristics measured in the field included: pH, conductivity, sand temperature, distance to the HTL, distance to the vegetation line, and depth of the top, middle, and bottom of the nest chamber. A Bluelab Combo Meter (BLU2300E, Bluelab, Tauranga, NZ) was used to measure pH, conductivity, and temperature of the surface sand and sand at the top, middle, and bottom of the nest chamber. Readings in the nest chamber were taken directly from the cavity walls, and surface samples were taken from an undisturbed patch of sand closest to the cloaca of the nesting female. The presence or absence and type of vegetation at each nest site was recorded. The presence or absence of roots within each nest was also recorded. To record temperature during the nest incubation period, Onset HOBO Pendant Temperature Data Loggers (UA-002-08, ONSET Computer Corporation, Bourne, MA) were deployed at time of nesting and set to log nest temperature every 30 minutes. Loggers were gently placed

in the nest after 30-40 eggs were laid to ensure that the logged temperatures were an indication of the temperature in the center of the clutch. Distance from the nest to the high tide line, distance to the vegetation, depth of the top and bottom of the nest chamber from the surface, and depth of the data logger were also recorded.

The measured beach characteristics at nest sites on Beaches A-E included slope, elevation, general morphology (presence or absence of flats or berms), sand moisture content, sand grain size, and the presence of vegetation, rivers and lagoons. A distinct morphological category was designated “flats”, to describe areas of the beach with adjoining rivers, and lagoons that separated the vegetation from the beach, as well as having significantly lower slopes and elevations than beaches without these water features. Beach profiling data was used to create a triangulated irregular network model (TIN) (Veelenturf et al. 2020). The experimental nest GPS points were overlain with the TIN model layer in ArcMAP (Esri version 10.4), the nest elevation on the surface of the model was determined. The distances from the surface of the sand to the top of the nest chamber and the bottom of the nest chamber were used to calculate the precise elevation in meters of the center of the clutch. Slope at each nest site was extracted from the TIN model (Veelenturf et al. 2020).

In order to determine the sand moisture content in each nest chamber, an average of 450 g per nest of sand samples were collected in Nalgene screw top containers from the surface as well as the top, middle, and bottom of the nest chamber wall. The percent moisture content of the sand was calculated from wet and dry weights of the sand samples (Bustard and Greenham 1968; McGehee 1979; Lutz and Dunbar-Cooper 1984). Dry weight was measured by oven heating sand samples (of at least 50 g in volume) for all experimental and control nests until each sample maintained a constant weight for at least 4 hours. A field oven was created using four wooden planks, a small grill, and a kerosene stove. Percent moisture was calculated using the formula:

$$\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100.$$

In order to determine the sand grain size distribution at each nest site, sand samples from the surface sand and top, middle, and bottom of the nest chamber were passed through six sieves with mesh sizes of 2, 1, 0.5, 0.25, 0.125, and 0.0625 mm, which correspond to the values of -1 (very coarse), 0 (coarse), 1 (medium), 2 (fine), 3 (very fine), and 4 (silt), on the Phi scale of particle size classification. Samples were shaken in the sieves using a mechanical shaker for 5 minutes, and each tray’s contents were weighed and recorded as a percentage of the total sample (Roe et al. 2013).

## ***Clutch Hatching Success***

The number of eggshells greater than 50% in size, unhatched eggs, dead and alive hatchlings discovered within the nest chamber and the column of sand above the nest, and the number of predated eggs were recorded during excavations. In the present study, eggs that were recorded as predated were those that had obvious signs of disturbance such as bore holes from ants and/or insect larva inside. Clutch hatching success, or hatching success, was calculated for experimental nests by counting the number of eggshells greater than 50% in size as hatched individuals and dividing this number by the total number of eggs laid. Un-hatched eggs were visually classified at various stages of development, zero through three, using the field-staging protocol of Leslie et al. (1996). While excavating, any nest that had pooling water in the bottom of the nest chamber or was completely flooded was considered inundated. Potential bacteria and fungus presence or absence was evaluated visually by identification of distinct discoloration along the inside of the eggshells and covering the embryonic material (Sarmiento-Ramírez et al. 2014).

## ***Analysis***

All statistical analyses were conducted in R Studio (Version 1.0.136).

Environmental characteristics in the nests were compared between species using ANOVA. Pearson correlations were conducted on abiotic characteristics of the nest environment and their spatial distribution in terms of distance to the HTL, distance to the vegetation line, and elevation relative to the HTL. Pearson correlations were also conducted between leatherback and green clutch hatching success including and excluding nests affected by predation. One-way ANOVA tests were used to compare differences between abiotic nest environmental characteristics in groups of nests per species that had a clutch hatching success below 21.24% and above 38.24%. The natural break in hatching success data between 21.24% and 38.24% was used to separate the nests into these two groups to see how the environmental characteristics varied (Table 1). Tukey HSD post hoc tests were conducted to further distinguish significant difference between groups. A regression analysis was conducted to investigate the potential of a cause and effect relationship between possible bacteria and fungus presence with clutch hatching success.

Due to the close relationship between clutch elevation and distance to vegetation, a single coordinate, a “beach coordinate”, was calculated and used to describe the location of the nests on the beach. To determine the beach coordinate, ordinary least squares were used to fit the scatter plot of the distance to vegetation versus clutch elevation with a quadratic expression. To map the nest locations onto this plot, the point on a large array of points on the parabola was found that is closest to the nest location. This is an orthogonal projection of the nest location onto a curved line. The relationship between beach coordinate and hatching success was plotted. To determine the exponents of the beta distribution, the minimize the negative log likelihood

$-\log(L(a,b,c|data)) = -\sum a + b \log(bc[i]) + c \log(1-bc[i])$  was used with respect to the parameters  $(a,b,c)$ , so the distribution is proportional to  $\text{beta}(b+1,c+1)$ .

The beach coordinate can be used to partition the beach into 10 decile bins, each 0.1 beach coordinate units wide and centered at 0.05, 0.15,..., 0.95. Data are summarized for each decile by adding the number of hatching eggs and number of failed eggs for all nests in each bin. For two deciles bins centered on 0.35 and 0.85, no leatherback nests were observed. In those cases, the weakly informative prior of 40% (2/5) hatching success proportion and a standard deviation of approximately 20% were used. The posterior distributions are beta distributions with parameters  $a = \text{count of surviving eggs} + 2$  and  $b = \text{count of failing eggs} + 3$ .

The leatherback clutch optimum elevation range was determined by isolating the continuous high clutch hatching success rates occurring at a clutch elevation of -0.286 m to -0.0528 m, which is a corresponding surface elevation of 0.319 m to 0.438 m relative to the HTL. Nests were placed into hypothetical bins based on their elevation ranges. One-way ANOVA tests were conducted between characteristics of nests in each bin, and Tukey HSD post hoc tests were conducted to characterize significant differences.

A multiple linear regression (MLR) analysis was conducted to determine the nest characteristics that affect hatching success in leatherback turtles, including and excluding nests that were affected by predation. Data available for the model included: percent of eggs showing potential signs of bacteria and fungus presence, distance to vegetation, mean distance to HTL, mean pH, mean conductivity, moisture content at time of nesting, mean moisture content, nest elevation, mean temperature, maximum temperature, and temperature data points over 35 °C. An all-subsets regression was performed using the leaps package (Lumley 2017) to select a subset combination of independent variables that would produce the most representative reduced model with the best fit.

The posterior distribution of the mean clutch hatching success proportion parameter was obtained by Gibbs sampling, using the clutch hatching success of leatherback turtles and green turtles, as priors for each other's mean and variance hyperparameters (Fig. 2). The decision to use each as a prior for the other is justified by the fact that local conditions on Bioko Island such as illegal egg take, beach erosion, ant and crab predation, and high-water tables were experienced by both groups. These factors are at least as relevant as worldwide data on species-specific hatching success rates. Additional hyperparameters used in the normal model were set to the weakly informative level ( $N_0=1$  and  $K_0=1$ ). The Gibbs sampling used 10000 random events.

## Results

This study was conducted from November 2016 through February 2017, coinciding with the leatherback and green sea turtle nesting season on Bioko Island (Fig. 1). During the incubation period for the experimental nests (November 2, 2016 – February 15, 2017) the mean air temperature was  $26.12 \text{ }^\circ\text{C} \pm 2.68 \text{ SD}$ ,  $n = 2755$ . The maximum temperature reached was  $32.38 \text{ }^\circ\text{C}$ , and the minimum was  $20.89 \text{ }^\circ\text{C}$ . There were 435.14 mm of rain, and mean relative humidity was  $92.74\% \pm 7.77 \text{ SD}$ ,  $n = 2755$  during the incubation period. Mean wind speed was  $0.50 \text{ m/s} \pm 0.93 \text{ SD}$ ,  $n = 2755$ , mean gust speed was  $1.12 \text{ m/s} \pm$

1.65 SD, n = 2755, and mean wind direction was  $238.79 \text{ }^\circ \pm 88.84 \text{ SD}$ , n = 2755. The mean solar radiation during the incubation period of our experimental nests was  $144.53 \text{ W/m}^2 \pm 224.37 \text{ SD}$ , n = 2755.

Out of the 35 experimental nests, there were only 17 unaffected by either predation or inundation. Nests that were affected by inundation and/or predation were unable to be used in the analysis of the influence of environmental characteristics on hatching success, as other factors generated the observed clutch hatching success in these cases. Nests were placed into groups to isolate those where the clutch hatching success data was unaffected by inundation or predation. The groups are: total experimental nests (11 green, 24 leatherback), those not affected by predation (4 green, 20 leatherback), those not affected by inundation (11 green, 16 leatherback), those not affected by predation or inundation (4 green, 12 leatherback).

Clutch hatching success between the two species was not significantly different (ANOVA,  $F(1,31) = 0.01$ ,  $P = 0.913$ ). Average hatching successes for green and leatherback sea turtles were  $40.04 \% \pm 40.60 \text{ SD}$ , n=9, and  $41.73 \% \pm 38.25 \text{ SD}$ , n=24, respectively (Fig. 2). There was not a significant difference between average leatherback hatching success on Beach C (39.22%, n=10) and Beach D (35.52%, p=14) (ANOVA,  $F(1,22) = 0.07$ ,  $P = 0.793$ ). Out of the 35 experimental nests, a total of 11, or 31.43%, were affected by predation. Crabs, species *Ocyropodecursor* and *Ocyropode africana*, and ants, potentially of the genus *Dorylus*, were the major nest predators on Bioko Island. For the leatherback turtle clutches affected by predation, 7.32% to 43.53% (min to max) of the eggs were affected, and for green sea turtles 35.42% to 100% were affected. As nests were located closer to the vegetation, they were significantly more likely to be predated (ANOVA,  $F(1,32) = 5.37$ ,  $P = 0.027$ ). When data for the nests affected by predation were omitted from the analysis, the difference between the mean hatching success of leatherback ( $44.16\%$ ,  $\pm 38.40 \text{ SD}$ , n = 21) and green sea turtles ( $82.63\%$ ,  $\pm 12.95 \text{ SD}$ , n = 4) was not statistically significant ( $t_{15} = -3.63$ ,  $P = 0.001$ ). There were 8 nests affected by predation on Beach C, and only 3 affected by predation on Beach D. When omitting all nests affected by predation and inundation (n=17), hatching success was not significantly different between leatherback (70.6%) and green turtle nests (82.63%) (ANOVA,  $F(1,15) = 1.07$ ,  $P = 0.317$ ).

By the end of the incubation period, 8 leatherback and zero green turtle nests were partially or fully inundated with a mean clutch hatching success of 22.86%. Inundated leatherback nests had a significantly lower hatching success than those that were not inundated (ANOVA,  $F(1,32) = 5.00$ ,  $P = 0.033$ ). Inundated nests were at a significantly lower clutch elevation (ANOVA,  $F(1, 33) = 7.04$ ,  $P = 0.012$ ) and surface elevation (ANOVA,  $F(1,33) = 8.60$ ,  $P = 0.006$ ) than non-inundated nests. There was a significantly higher conductivity in nests affected by inundation than those that were not (ANOVA,  $F(1,33) = 32.50$ ,  $P < 0.001$ ).

All experimental nests were placed into two categories, those with low (0-22%) and high (38-100%) hatching success. The natural break in hatching success data between 21.24% and 38.24% was used to separate the nests into these two groups (Table 1). As the global average leatherback turtle clutch hatching success is 40-60% (Miller 1997), the group with low hatching success was selected to be for

nests considerably below this global average. Leatherback nests including and excluding predation were considered, but only green turtle nests including predation were considered, as there were no green turtle nests that had a hatching success below 22% when nests affected by predation were excluded. For green turtles there were 4 nests with low and 5 with high hatching success, and for leatherbacks there were 11 with low and 13 with high hatching success. For all leatherback nests the distance to the vegetation, mean temperature during the entire and second half of the incubation period, moisture content, percent of eggs with signs of bacteria and fungus present, and maximum temperature were significantly different between the two groups (Table 1). For leatherback turtle clutches, excluding those affected by predation, the mean temperature within nests of hatching success less than 22% and greater than 38% was not significantly different (ANOVA,  $F(1,16) = 3.00$ ,  $P = 0.103$ ), but the maximum temp was significantly higher in nests with a hatching success greater than 38% ( $34.13\text{ }^{\circ}\text{C} \pm 1.91\text{ SD}$ ,  $n = 13$ ) than those below 22% ( $31.64\text{ }^{\circ}\text{C} \pm 1.11\text{ SD}$ ,  $n = 8$ ) (ANOVA,  $F(1,16) = 11.41$ ,  $P = 0.004$ ). All leatherback nests that had a hatching success less than 22% (0-21.24%) had a significantly higher percent of eggs with signs of fungus and bacteria present than those nests that had a hatching success greater than 38% (38.24-100%) (ANOVA,  $F(1,20) = 8.08$ ,  $P = 0.010$ ) (Table 1). For leatherback turtle nests it was found that distance to vegetation, moisture content, average conductivity, and percent of eggs with signs of bacteria and fungus present were all negatively and significantly correlated with hatching success (Table 2).

For both species there were no significant differences between the mean temperatures in the control ( $28.66\text{ }^{\circ}\text{C} \pm 1.97\text{ SD}$ ,  $n=11$ ) and experimental nests ( $29.81\text{ }^{\circ}\text{C} \pm 1.545\text{ SD}$ ,  $n=31$ ) (ANOVA,  $F(1,40) = 3.84$ ,  $P = 0.057$ ). The average temperature throughout the extent of the incubation period was not significantly different between leatherback experimental nests ( $30.14\text{ }^{\circ}\text{C} \pm 1.60\text{ SD}$ ,  $n = 24$ ) and leatherback controls ( $29.65\text{ }^{\circ}\text{C} \pm 1.88\text{ SD}$ ,  $n = 3$ ) (ANOVA,  $F(1,25) = 0.41$ ,  $P = 0.530$ ), but during the last third of the incubation period, this difference was significant (ANOVA,  $F(1,760) = 3.85$ ,  $P < 0.001$ ). Green experimental ( $29.117 \pm 1.22\text{ SD}$ ,  $n = 11$ ) and control ( $27.485 \pm 1.45\text{ SD}$ ,  $n = 3$ ) nests did experience a significant difference in mean temperature (ANOVA,  $F(1,13) = 5.30$ ,  $P = 0.039$ ) throughout the incubation period.

Mean temperature during the second half of the incubation period ( $p < 0.001$ ) and maximum temperature ( $p < 0.0001$ ) were positively correlated with hatching success for leatherback turtles (Table 2). When the nests affected by predation and inundation were omitted from the analysis, both of these correlations were no longer significant (Table 2). No significant difference was found between the mean temperature in leatherback ( $30.14\text{ }^{\circ}\text{C} \pm 1.600\text{ SD}$ ,  $n = 24$ ) and green ( $29.11\text{ }^{\circ}\text{C} \pm 1.218\text{ SD}$ ,  $n = 11$ ) experimental nests (ANOVA,  $F(1,29) = 3.16$ ,  $P = 0.086$ ). There was, however, a significant difference between the average maximum temperature reached in leatherback ( $32.85\text{ }^{\circ}\text{C} \pm 2.00548\text{ SD}$ ,  $n = 24$ ) versus green experimental nests ( $31.21\text{ }^{\circ}\text{C} \pm 1.944\text{ SD}$ ,  $n = 11$ ) (ANOVA,  $F(1,29) = 4.61$ ,  $P = 0.040$ ).

Large rainfall events appeared to influence temperature within experimental nests (Fig. 3). Inundated nests experienced significantly lower maximum temperatures, excluding nests affected by predation (ANOVA,  $F(1,16) = 8.76$ ,  $P = 0.009$ ). Including predation, the average temperature in inundated nests was not significantly different (ANOVA,  $F(1,19) = 4.96$ ,  $P = 0.304$ ).

Measured nesting habitat characteristics were correlated with leatherback and green hatching success, clutch elevation, distance to the HTL, and distance to the vegetation line to provide insight on the spatial distribution of these biotic characteristics. For leatherback sea turtles there was not a difference between the nest characteristics in control versus experimental nests (mean conductivity: ANOVA,  $F(1,28) = 0.00$ ,  $P = 0.965$ ; mean pH: ANOVA,  $F(1,27) = 1.02$ ,  $P = 0.321$ ; mean temperature: ANOVA,  $F(1,25) = 0.41$ ,  $P = 0.530$ ; mean nest moisture content: ANOVA,  $F(1,28) = 0.75$ ,  $P = 0.395$ ). For green sea turtles there were no significant differences between characteristics of control versus experimental nests except for the mean temperature (mean conductivity: ANOVA,  $F(1,14) = 0.44$ ,  $P = 0.519$ ; mean pH: ANOVA,  $F(1,11) = 0.85$ ,  $P = 0.378$ ; mean temperature: ANOVA,  $F(1,13) = 5.30$ ,  $P = 0.039$ ; and mean moisture content: ANOVA,  $F(1,14) = 0.84$ ,  $P = 0.374$ ). Mean conductivity was significantly higher in green control nests on Beaches A (ANOVA,  $F(1,70) = 4.94$ ,  $P = 0.029$ ) and B (ANOVA,  $F(1,70) = 4.12$ ,  $P = 0.046$ ) than on Beach D (Fig. 4), but conductivity in leatherback control nests on Beaches A (ANOVA,  $F(1,82) = 1.76$ ,  $P = 0.189$ ) and B (ANOVA,  $F(1,82) = 2.14$ ,  $P = 0.147$ ) was not significantly different than on the conductivity in leatherback control nests on Beach D. There was no significant correlation between pH and average moisture content in control nests (Pearson's correlation,  $r = 0.29$ ,  $p = 0.096$ ,  $n = 6$ ). The distribution of sand grain sizes across the five sea turtle nesting beaches and in green and leatherback experimental nests located on Beaches C and D are shown in Fig. 5. There was an observed significant effect on sand grain size distribution based upon beach origin (Beaches A-E) (MANOVA, Wilks'  $\lambda = 0.238$ ,  $F_{24,1034} = 21.945$ ,  $P < 0.0001$ ; Fig. 5). Leatherback turtle nest site sand was characterized by significantly higher proportions of sand particles in the very fine and silt size classes compared to sand from green turtle nest sites (MANOVA, Wilks'  $\lambda = 0.716$ ,  $F_{6,137} = 9.051$ ,  $p < 0.0001$ ; Fig. 5). The sand grain size distribution of clutches with varying clutch hatching success is plotted in Fig. 6. Sand grain size did not significantly influence hatching success leatherback turtle experimental nests (Pearson's correlation,  $r = 0.047$ ,  $p = 0.83$ ,  $n = 24$ ).

Flats were defined by lagoons dividing the beach area from the vegetation, significantly lower elevations (ANOVA,  $F(1,22) = 11.51$ ,  $P = 0.003$ ), and significantly lower slopes ( $F(1,22) = 4.51$ ,  $P = 0.045$ ) in comparison to the rest of the beach. Three flats were identified on Beach D and four flats were identified on Beach C. The flats are approximately 600 m long on Beach D (22.64% of Beach D's length) and 700 m on Beach C (20.69% of Beach C's length). Approximately 29.79% of all 2017 leatherback nests where a waypoint was taken ( $N=47$ ,  $n=14$ ), were located in the flat on Beach D

In the section of Beach C patrolled, there are only three flats. Out of the leatherback experimental nests, 33.33% ( $N=24$ ,  $n=8$ ) were located within the flats. Approximately 29.79% ( $n=14$ ) of all 2017 geolocated leatherback nests were located in the flat on Beach D. The average leatherback clutch hatching success in and outside of flats was significantly different (ANOVA,  $F(1,22) = 9.81$ ,  $P = 0.005$ ). Excluding experimental nests affected by predation, the average leatherback clutch hatching success within flats ( $12.32\% \pm 26.63\%$  SD,  $n = 8$ ) on Beach C and Beach D is significantly less than that of the clutch hatching success outside of these flats ( $54.37\% \pm 34.89\%$  SD,  $n = 16$ ,  $F(1,19) = 17.42$ ,  $p < 0.001$ ). Of the experimental nests, none of the leatherback nests affected by predation were located in the flats on either Beach C or D. There was not a significant difference between the mean sand conductivity (ANOVA,  $F$

(1,19) = 3.45,  $P = 0.080$ ), pH (ANOVA,  $F(1,19) = 0.10$ ,  $P = 0.758$ ), mean temperature (ANOVA,  $F(1,16) = 0.00$ ,  $P = 0.949$ ), maximum temperature (ANOVA,  $F(1,16) = 1.22$ ,  $P = 0.285$ ), and moisture content (ANOVA,  $F(1,19) = 0.00$ ,  $P = 0.993$ ) in leatherback nests located inside and outside of the flats. Leatherback nests, not including those affected by predation, within the flats ( $n = 8$ ) were at significantly lower clutch elevations than those outside of the flats ( $n = 16$ ) (ANOVA,  $F(1,19) = 18.99$ ,  $P < 0.001$ ).

The average percent of eggs with potential bacteria and fungus presence within a clutch for green sea turtles was  $5.18\% \pm 4.74$  SD,  $n = 9$ , and that of leatherback sea turtles was  $24.21\% \pm 29.7$  SD,  $n = 22$  (ANOVA,  $F(1,29) = 3.59$ ,  $P = 0.068$ ). The potential presence of bacteria or fungus was observed in at least 24% (24.76-100%) of egg clutches affected by inundation. Inundated nests contained a significantly higher percentage of eggs with potential presence of bacteria and fungus than those that were not partially or fully inundated with water at time of excavation (ANOVA,  $F(1,18) = 24.44$ ,  $P < 0.001$ ). Regression showed a significant negative relationship between the percent of total eggs within a nest containing evidence of the potential presence of bacteria and fungus and nest hatching success ( $r^2 = 0.22$ ,  $F_{1,29} = 8.30$ ,  $p = 0.007$ ). The observed Pearson correlation between average distance to the HTL and potential percent bacteria presence was not significant (Pearson's correlation,  $r = -0.39$ ,  $P = 0.103$ ,  $n = 31$ ). Regression showed a significant positive relationship between nest distance from the vegetation line and potential percent of eggs with bacteria and fungus present ( $r^2 = 0.24$ ,  $F_{1,28} = 8.70$ ,  $p = 0.006$ ). The potential percent of eggs with bacteria and fungus present in a nest was not correlated with clutch elevation (Pearson's correlation,  $r = -0.15$ ,  $p = 0.540$ ,  $n = 31$ ), but was significantly correlated with moisture content (Pearson's correlation,  $r = 0.50$ ,  $p = 0.029$ ,  $n = 31$ ). Leatherback nests, not including those affected by predation, within the flats ( $n = 8$ ) had a significantly higher percent of eggs containing signs of potential fungus and bacteria presence (ANOVA,  $F(1,17) = 6.34$ ,  $P = 0.022$ ).

The beach coordinate, a variable described by a combination of proportional clutch elevation (CE) and proportional distance to vegetation (DV), was defined by the quadratic equation  $DV = c_0 + c_1 CE + c_2 CE^2$ , where  $(c_0, c_1, c_2) = (0.96, -2.37, 1.59)$ . The beach coordinate is used to comprehensively describe the location on beach relative to DV and CE and is the proportional arc length measured from the upper left (low clutch elevation, high distance to vegetation) to the lower right (high clutch elevation, low distance to vegetation) of the curve. The function of the relationship between beach coordinate and hatching success was plotted in Fig. 7. The posterior distribution of the mean clutch hatching success proportion by decile of beach coordinate is shown in Fig. 8.

A leatherback optimum clutch elevation range of  $-0.286$  to  $-0.0528$  m and corresponding surface elevation of  $0.319$  to  $0.438$  m, relative to the HTL at  $0$  m was found to be optimum for clutch hatching success. Within this range hatching success was  $81.64\% \pm 8.17\%$  SD,  $n=9$ , and significantly higher than the hatching success outside of this range,  $17.78\% \pm 26.82$  SD,  $n=15$  (ANOVA,  $F(1,22) = 31.92$ ,  $P < 0.001$ ) (Fig. 9). This optimum clutch elevation range was evident even when leatherback and green experimental nests affected by predation and inundation were omitted. The 24 experimental nests were hypothetically placed into 3 bins based upon their elevations (Fig. 9). In Bin 1, 0 nests were affected by predation; in Bin 2, 2 nests were affected by predation; and in Bin 3, 2 nests were affected by predation. In Bin 1, 7 nests

were affected by inundation; in Bin 2, 0 nests were affected by inundation; and in Bin 3, 1 nest was affected by inundation (Fig. 9). Out of the 2016/2017 nesting events where a GPS point was taken, only 15.38% of the nests were located in this elevation range, 41.03% below and 43.59% above.

Results from an all subsets regression analysis and multiple linear regression analysis can be found in Table 3.

## Discussion/conclusion

Insights into the major abiotic and biotic influences on hatching success have been gained through this study for both leatherback and green turtles in this critically important nesting habitat of the Southeast Atlantic. This is the first study to report leatherback clutch hatching success and the first scientific journal publication to report green turtle clutch hatching success on Bioko Island.

Compared to the global average of leatherback clutch hatching success, 40-60% (Miller 1997), the leatherback sea turtles on Bioko fared at the lower end of this range, mostly due to nest inundation. Globally green sea turtle hatching success is approximately 60-90% (Broderick and Godley 1996; Antworth et al. 2006; Cheng et al. 2009), which is at least 20% higher than the green hatching success reported here. Without including nests affected by predation or inundation, the hatching success of leatherback sea turtles was higher than the global average and green hatching success was in the global average range. An unexpected 31.45% of the 35 experimental nests were affected by predation, specifically by ants and crabs. A larger proportion of green turtle experimental nests (7/11) were affected than that of leatherback turtle experimental nests (4/24), and overall more green nests were affected than leatherback nests. This is likely caused by green sea turtles nesting closer to or past the vegetation line, whereas leatherback sea turtles normally nest in the zone between the high tide line and the vegetation line. On the two beaches studied, as nests were located closer to the vegetation line, they were significantly more likely to be predated. This is in line with the findings of other studies (Crossland 2003, Leighton et al. 2011, Da Silva et al. 2016). Increased hawksbill nest infestation by invertebrates in locations closer to the vegetation was observed on Pip beach in Brazil (Da Silva et al. 2016). In Suriname, fire ants were associated with nests in areas with increased vegetation cover (Crossland 2003), and in Barbados predation risk was calculated to be highest in and near vegetation (Leighton et al. 2011). Studies have noted increased mortality in hatchlings stung by fire ants (Moulis 1997; Krahe 2005), but in predated nests on Bioko, ants and ant larvae were found feeding on egg material inside the unhatched eggs. Egg infestation by ants has been observed in a South African hatchery, where almost complete egg mortality occurred (Hughes 1970). Not including nests that were predated, green sea turtles had a much higher hatching success than leatherback nests (Fig. 2), indicating that predation affected green sea turtles more than leatherback sea turtles, likely due to their placement in or near the vegetation.

Of the experimental nests, 8 leatherback nests and 0 green nests were affected by inundation, a total of 22.86%. In French Guiana, about one third (30.34%) of *Dermochelys coriacea* nests were washed by the tides at least once during the incubation period, and on average hatching success was significantly lower

in overwashed versus non-overwashed nests (Caut et al. 2010). Our results show that any nest below -0.311 m in clutch elevation was either partially or completely inundated with water at time of excavation. Based on these results, inundation more frequently threatens leatherback nests, and predation affects both species. Often times leatherback sea turtles have a lower hatching success than other species nesting on the same beach, and it is thought that as a species, they are specifically sensitive to certain nest environmental characteristics (Miller 1997). Unexpectedly, there was no significant difference between the clutch hatching success of green and leatherback sea turtles, or even a difference greater than 2%. Omitting data for nests affected by predation and inundation did result in a significant difference between the clutch hatching success of both species, which supports the idea of species-specific sensitivities to the nest environment on Beaches C and D.

The successful thermal range of sea turtle egg incubation is thought to be between 25-33 °C (Yntema and Mrosovsky 1982). In this study, incubating clutches were not at a great risk of consistently reaching potentially lethally high temperatures, but rather many were at a greater inundation risk, subsequently experiencing correspondingly lower temperatures. These results show that mean temperature was significantly and positively correlated with clutch hatching success in leatherback turtles, including predated and inundated nests. The average temperature during the second half of the incubation period was significantly and positively correlated with clutch hatching success for leatherback sea turtles including and excluding predation. Other studies on leatherback sea turtles have found the opposite, that increasing temperatures are negatively correlated with hatching success (Tomillo et al. 2009; Garrett et al. 2010). When omitting data for predated and inundated nests in the present study, these correlations were no longer significant. The loss of the significant correlation suggests that the original observed relationship can be attributed to the significantly lower maximum temperatures of inundated nests, which had very low hatching success, versus non-inundated nests. The result that average temperature in inundated nests was not significantly different than non-inundated nests when predated nests were included in the analysis indicates that predated nests, or nests closer to the vegetation line, also experience lower temperatures than nests located farther from the vegetation line. This difference is likely due to increased shading closer to the vegetation and decreased exposure to metabolic heat attributable to early mortality.

Mean temperatures are correlated less strongly than maximum temperatures with clutch hatching success for both species. Green turtle nests are significantly correlated with maximum temperatures reached in the nests but not mean temperatures. On average, leatherback nests reached significantly higher maximum temperatures than green nests, which is likely due to the increased consistency of direct sunlight in the beach zone between the HTL and vegetation. For green sea turtles there was a positive correlation only between maximum temperature in the nest and hatching success. Maximum temperature increased with distance from the vegetation, indicating, again, that vegetation likely shaded nests and decreased temperature. Nests closer to the vegetation were significantly more likely to be affected by predation, indicating that the correlation between hatching success and maximum temperature is possibly due to decreasing predation with increasing temperature reached. When nests affected by

predation were omitted from the analysis, this correlation is not significant. Shading from vegetation may be a more effective constant cooling agent than fluxing tidal proximity or heavy rainfall.

The maximum temperature reached in the nest, percent of eggs exhibiting a potential presence of bacteria and fungus, average nest conductivity, average moisture content, and distance to the vegetation line were all significantly and consistently correlated with leatherback clutch hatching success, excluding nests affected by predation and excluding those affected by both predation and inundation. When the nests were separated into two groups, nests with a hatching success below 21.24% and above 38.24%, the only nest characteristic that was significantly different for leatherback turtle nests, green turtle nests, and leatherback turtle nests not including nests affected by predation was the maximum temperature reached inside each clutch. For leatherback nests, excluding those affected by predation, the moisture content, percentage of eggs with bacteria and fungus present, maximum temperature, mean temperature during the second half of the incubation period, and distance to vegetation were all significantly different within these two groups. This result suggests that these environmental characteristics likely played a role in determining hatching success. Excluding nests affected by both predation and inundation, there were no nests with a hatching success less than 21.24%, indicating that the most detrimental threat to leatherback nests on Bioko is nest placement too close to the HTL. For the green sea turtle, there were no nests with a hatching success less than 22% that were not affected by predation, indicating that the largest threat affecting green sea turtle nests is their placement and corresponding probability of predation.

The fact that the mean conductivity was significantly higher in those nests that were affected by inundation attributes the observed increase in moisture content to tidal inundation and not random rainfall events or a high freshwater table due to nearby rivers. The significant, positive correlation between moisture content and percent of eggs with potential presence of bacteria and fungus indicates that frequent tidal inundation does not decrease bacterial growth in this area, but the moisture content may actually foster bacterial growth. It is important to note that the observed potential fungal and bacterial growth in experimental nests could have begun before or after embryo mortality. Fungal pathogenic species *Fusarium falciforme* and *Fusarium keratoplasticum* have been associated with decreased sea turtle hatching success globally in six sea turtle species (Sarmiento-Ramírez et al. 2014). *Fusarium* thrives at the same temperature ranges that are optimum for sea turtle egg incubation and therefore makes these endangered reptiles particularly susceptible to infection (Sarmiento-Ramírez et al. 2014). Nests located in beach zones most prone to tidal inundation typically exhibit increased susceptibility to fungal infection and rates of mortality attributable to *Fusarium* invasion (Sarmiento-Ramírez et al. 2014). For example, loggerhead nests experiencing tidal inundation and high proportions of clay/silt sediment in Cape Verde showed significantly higher occurrence of fungal infestation than drier nests with greater gas diffusion potential (Sarmiento-Ramírez et al. 2014). *Fusarium* has been isolated in *Caretta caretta* eggs on Boa Vista Island and Ascension Island off the coast of West Africa (Sarmiento-Ramírez et al. 2014), suggesting that this genus could be the same one potentially propagating on Bioko. Future studies are needed to identify observed potential microbial biomasses found in both green and leatherback eggs on Bioko Island.

Leatherback clutch elevation is not significantly correlated linearly with leatherback hatching success, but a distinct increase in hatching success is evident in a specific elevation range from -0.285541 to -0.0528 m. The corresponding surface elevation range is 0.319 to 0.438 m, relative to the high tide line at a surface elevation of 0 m, and peaking at a beach coordinate of 0.58. Here, the elevation range of Bin 2 has been termed the optimum clutch elevation range for leatherback sea turtles on Beaches C and D on Bioko Island. Below the optimum elevation range, 7 experimental nests were affected by inundation, but in the optimum elevation range, none of the nests were affected by inundation. Above the optimum elevation range, one of the experimental nests was inundated, but this leatherback nest inundated in Bin 3 was in a section of Beach D where the freshwater table is visibly higher than other parts of the beach. A waterfall flows in the forest behind the vegetation line in this location, and at times, the sand appears visibly wetter than neighboring sections of this beach, even at low tide (Paladino and Veelenturf, *personal observations*). Regardless of its elevation, this nest was likely to be inundated. In the optimum elevation range, 2 nests were affected by predation, and the 2 others affected by predation were in Bin 3. Even though there were two nests affected by predation in the optimum elevation range, these nests were not completely predated, whereas those above the optimum elevation range had hatching successes of 0 and 3.25%, largely due to predation. This suggests that even though there are two predated nests in Bins 2 and 3 each, the observed predation risk was still greater in Bin 3. Farther inland the threat from ant and crab predation increases, and farther seaward the threat from inundation and bacteria and fungus infestation increases. These pressures, which have less to do with specific nest conditions for successful incubation and more to do with nest location, are likely driving optimum elevation towards the observed range. When omitting nests affected by predation and inundation, an increase in hatching success was still observed in this range, indicating that there are likely other characteristics of this range that increase nest success. Fig. 8 supports this idea by exhibiting that nests with central beach coordinates had higher hatching success. Further studies with a larger sample size are needed to additionally assess these nest characteristics in leatherback turtles. The percentage of nests during the 2016/2017 season located in the optimum elevation range for leatherback sea turtles was only about 15.38%, which suggests that leatherback sea turtles are not showing a pattern of selecting for the range that, based on the data, seems to have the greatest reproductive output. It will be important to test this trend in the following years to investigate the extent of the optimum elevation range and if hatching success continues to follow similar trends.

The all subsets regression analysis indicated that the strongest and most highly correlated predictors of hatching success for any leatherback nest on Bioko Island's beaches are the distance to the vegetation line, nest elevation, mean temperature during the second half of the incubation period, sand moisture content, and sand conductivity. From the MLR analysis, mean conductivity, distance to vegetation, and clutch elevation showed significant influence, and moisture content and mean temperature during the second half of the incubation period did not show significant influences. Although moisture content and temperature during the second half of the incubation period do not have a significant influence on hatching success according to the model, there was a strong biological justification for their inclusion in the model. Nest groups with hatching successes above 38% and below 22% had a significantly different

temperature and moisture content. The mean temperature during the second half of the incubation period and moisture content were significantly correlated with hatching success in leatherback turtles. Inundated nests had a significantly lower hatching success than non-inundated nests, providing further evidence that these two variables needed to be included in the model. This interpretation was justified by other studies finding that temperature (Packard and Packard 1988; Matsuzawa et al. 2002; Segura and Cajade 2010) and moisture content (McGehee 1990; Foley et al. 2006) influenced hatching success in sea turtles. Despite collinearity between variables used in this regression analysis and violation of the assumption of independence, their inclusion was important for attaining a full understanding of the complex nest environment. The amount of variance of these nest characteristics in the presence of each other provides further insight regarding their influence on hatching success. Other interrelated biological factors associated with each variable that could be affecting hatching success include nest sand porosity (Mortimer 1990), nest shading (Janzen 1994), and sand organic material (Ditmer and Stapleton 2012). Without quantifying these additional variables, it is important to complete system understanding not to omit characteristics solely due to collinearity. Sand grain size did not significantly influence leatherback turtle hatching success, as has been seen at other nesting sites for leatherback (Garrett et al. 2010) and green turtles (Stewart et al. 2020). Observed significant differences between nest sand grain size amongst species and beaches (which vary in nesting species composition) could suggest that this characteristic could play a role in nest site selection on Bioko Island, as has been observed in other areas and species (Karavas et al. 2005; Zare et al. 2012; Roe et al. 2013)

The results from the MLR analysis and comparisons between bins suggest that perhaps on Bioko, the most important predictor of hatching success is clutch elevation and distance to the vegetation line. Measurements of distance to HTL and vegetation line were less normally distributed and had more variance than clutch elevation in leatherback turtles. This finding may indicate a greater sensitivity to elevation during the nest-site selection process than distance to HTL or vegetation line, as was also observed on a hawksbill nesting beach in Iran (Zare et al. 2012). In terms of environmental characteristics, apart from nest placement at a specific elevation or near the vegetation, the data suggest that conductivity has the greatest influence on hatching success. When data for nests affected by inundation were omitted from the analysis, environmental characteristics were not significantly different between nests with high clutch hatching success and nests with low clutch hatching success, and clutch hatching success was not significantly correlated with environmental nest characteristics. These results may be an indication of an absence of severe sensitivities to environmental characteristics in the nest environment, when a nest was not simply laid too close to the water. Leatherback turtles have shown the capability to be adaptable to changing beach conditions. It has previously been documented on Bioko Island that about 89% (n=26) of the time that a leatherback was found digging her nest below the HTL and the nest filled with water, she aborted that nest to choose a drier location closer to the vegetation (Veelenturf et al. 2020).

Green sea turtle clutch hatching success was 82.63%, not including nests affected by predation, which may indicate that green sea turtles are not particularly susceptible to environmental characteristics on Bioko Island, and that predation is the primary threat to clutch hatching success. Correlations were less

significant between hatching success and nest characteristics for green sea turtles than for leatherback sea turtles, but this should be further investigated on Bioko with a larger sample size. More data should be collected for increased assessment of effects on hatching success in green sea turtles. The loss of 7 out of the 11 green experimental nests due to predation, either by ants or crabs, hindered a more robust analysis for this species. Green turtle control nests on Beaches A and B, did have a significantly higher nest sand conductivity than those on Beaches C, D, and E, indicating higher levels of sand inundation on these beaches, which are those characterized by relatively higher concentrations of green turtle nesting activity (Honarvar et al. 2016). It has been documented that on Beaches A and B, relatively advanced levels of beach erosion and the resulting beach berms are preventing green turtles from nesting in Zone 3, or behind the vegetation line, subsequently placing these nests at a lower elevation and an increased risk to tidal overwashing (Veelenturf et al. 2020). At Raine Island, Australia, it has been shown that saltwater inundation directly lowers the viability of green turtle eggs at all embryonic developmental stages. After 1 or 3 hours egg viability was reduced by less than 10%, and 6 hours of inundation reduced viability by approximately 30% (Pike et al. 2015). Nesting in front of the vegetation line to avoid surmounting vegetation berms and increased nest conductivity are already quantifiable changes within green nesting habitat and nest site selection that require further investigation to determine their effects on hatching success and hatchling production. At this time, we can only predict that increased inundation risk due to sea level rise and beach erosion will result in increased nest mortality, and increased sand conductivity will be a significant negative influence on hatching success.

Studies regarding bacteria optimum growth conditions, spatial distribution, and specific species diversity will be useful going forward in better understanding this specific threat to sea turtle nests on Bioko Island. Crabs, ants, bacteria, and fungus likely all have optimum ranges that could be identified and used to determine the best location for a potential future hatchery on Bioko Island. Identification of the primary ant species heavily preying nests on Bioko will be useful in future management. A hatchery could significantly decrease the effects of predation on hatching success. Cages, which have been shown to significantly reduce predation in Florida (Engeman et al. 2016), were used during the 2016-2017 season on Bioko but did not appear to deter crab predation. Nests that were seemingly undisturbed on the surface were heavily predated 50-80 cm deep. By intervening in natural selection processes, it is possible that hatchery work could result in the propagation of genes that do not evolutionarily lead to a species' ability to choose a proper nest site. Hatcheries that are not designed properly can undercut the best of intentions (Pritchard 1980). Due to the significantly lower hatching success observed in flats, relocating nests found in these parts of the beach to other sections with demonstrated higher hatching success could be effective. Another factor to consider is that nests grouped closely together in a hatchery could make an easy target if and when discovered by predators, as was seen in the case of a South African hatchery devastated by predaceous ants (Hughes 1970). If the predation threat can be mediated, moving leatherback nests closer to the vegetation line and eliminating inundation risk altogether will likely increase hatching success significantly.

Based upon the results presented here, climate change will specifically affect reproduction not only by decreasing available nesting habitat, but also by decreasing the beach zone that is optimal for increased

hatching success and thus increasing risks due to the two largest overarching threats to leatherback and green turtle nests: inundation and predation. As erosion increases beach slope and diminishes the distance between the high tide line and vegetation line on Bioko's nesting beaches (Veelenturf et al. 2020), it is likely that the optimum elevation range identified previously will entirely disappear. Before disappearing, the optimum elevation range will shift toward the vegetation, and the probability of nest predation could increase. With decreasing distance between the high tide line and vegetation line, all nests will also be closer to the high tide line, likely increasing potential risk due to inundation and decreased hatching success due to increased conductivity. The three factors that the MLR analysis indicates affect hatching success to the greatest extent, conductivity, distance to the vegetation, and clutch elevation, are all subject to change greatly with increasing sea level rise.

In conclusion, the data presented here suggest that clutch elevation or beach coordinate (the quadratic expression relating clutch elevation and distance to vegetation) appears to be the environmental characteristic that has the greatest influence on clutch hatching success in leatherback turtles. On beaches where predation is less rampant, the observed optimum elevation range will likely be shifted or less prominent. This study provides the basis for characterizing intricacies of the optimum elevation range and its specific qualities that illicit high hatching success in the leatherback sea turtle. Further hatching success studies and profiling on Bioko Island are needed to determine the plasticity of this range and further identify its extent. The greatest threat facing developing green turtle nests is egg predation, but more research with a larger sample size is required to isolate specific influential environmental characteristics.

## Declarations

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**Conflicts of interest/Competing interests** The authors declare that they have no conflict of interest.

**Availability of data and material** The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

**Code availability** The code generated during the current study is available from the corresponding author upon reasonable request.

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**Consent to participate** This work was conducted under appropriate permits form Universidad Nacional de Guinea Ecuatorial (permit #289/2016).

**Consent for publication** All authors of this manuscript have agreed to be listed, approve the submitted version of the manuscript.

### **Compliance with Ethical Standards**

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical standards: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee at Purdue University (IACUC), IACUC protocol #1410001142.

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## Tables

**Table 1.** One-way ANOVA tests between different nest environmental characteristics in nests that had a hatching success less than 22% and greater than 38%. This analysis was conducted within each species, including and excluding data for nests affected by predation. Significant ( $p < 0.05$ ) differences between

the two groups are denoted by an asterisk (\*). There were no green sea turtles with a hatching success less than 22% that were not affected by predation. Without nests affected by predation or inundation, there is only one leatherback nest with a hatching success less than 22% (21.24%)

	Green HS <22 n=4 and >38 n=5 with predation	F (df)	Leatherback HS <22 n=11 and >38 n=13 with predation	F (df)	Leatherback HS <22 n=11 and >38 n=13 without predation	F (df)
Elevation (m)	0.835	0.05 (1,7)	0.491	0.49 (1,22)	0.085	3.47 (1,19)
Distance to HTL (m)	0.155	2.55 (1,7)	0.175	1.96 (1,22)	0.140	2.38 (1,19)
Distance to Vegetation (m)	0.756	0.105 (1,7)	*0.006	9.17 (1,21)	*<0.001	16.34 (1,18)
Moisture Content (%)	0.338	1.0574 (1,7)	*0.031	5.33 (1,22)	*0.015	7.17 (1,19)
Mean Conductivity (EC)	0.407	0.778 (1,7)	*0.013	7.24 (1,22)	*0.006	9.74 (1,19)
Bacteria and Fungus (%)	0.336	1.0694 (1,7)	*0.010	8.08 (1,20)	*0.003	11.83 (1,17)
Mean °C	0.266	1.507 (1,6)	*0.040	4.86 (1,19)	0.103	3.00 (1,16)
Maximum °C	*0.007	16.149 (1,6)	*<0.001	15.54 (1,19)	*0.004	11.41 (1,16)
Mean °C 2 <sup>nd</sup> half of incubation	0.053	5.795 (1,6)	*<0.001	18.60 (1,19)	*<0.001	20.05 (1,16)
Slope	0.804	0.0663 (1,7)	0.856	0.034 (1,22)	0.227	1.56 (1,19)

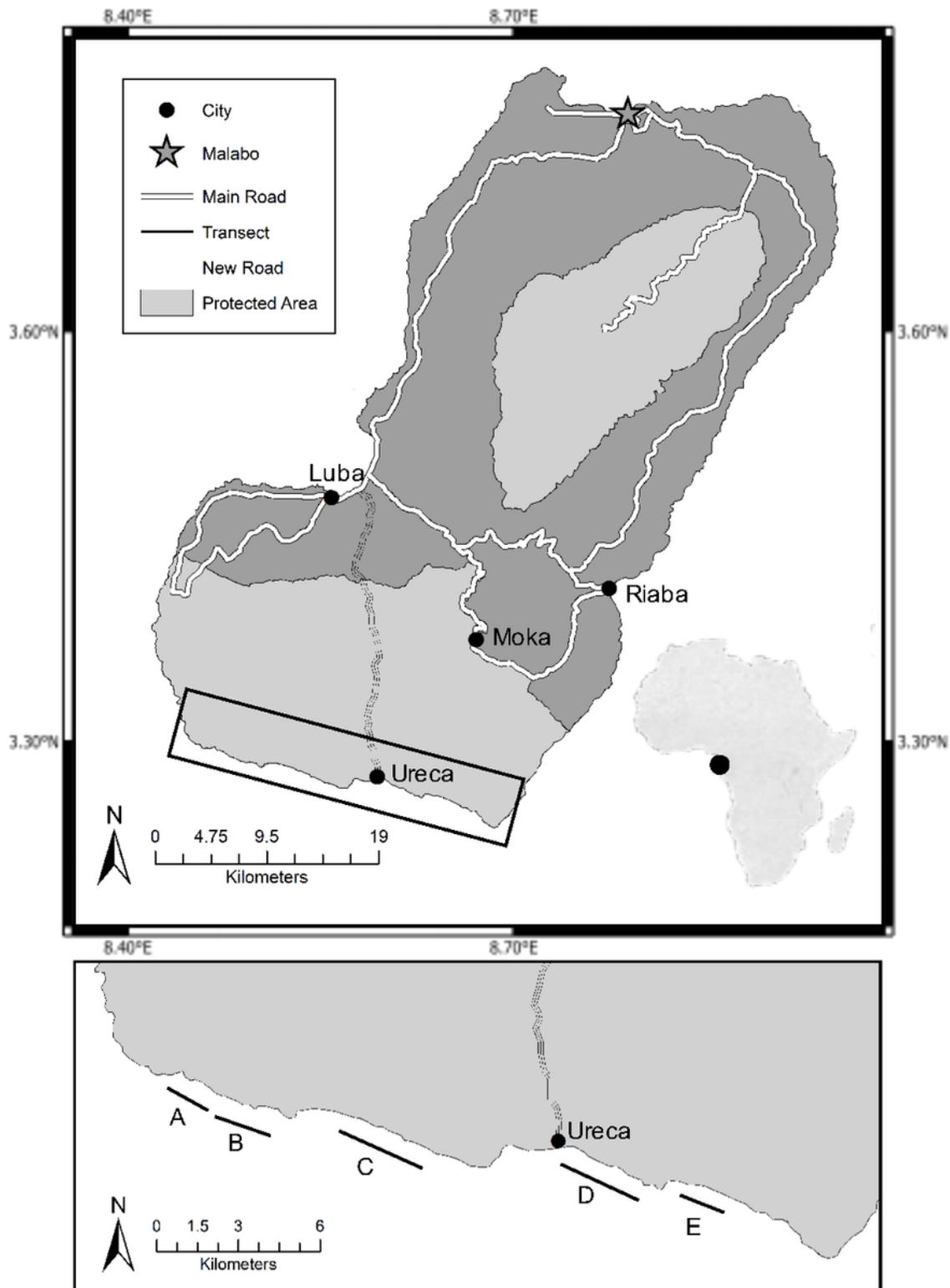
**Table 2.** Pearson correlations (r(p)) between leatherback and green hatching success (HS) including and excluding data for nests that were affected by predation and/or inundation. Significant (p<0.05) Pearson correlation values denoted with an asterisk (\*). All green nests not including predation had a mean conductivity of 0, making correlation calculation inapplicable

	Leatherback HS (n=24)	Green HS (n=11)	Leatherback HS without predation (n=21)	Green HS without predation (n=4)	Leatherback HS without predation and inundation (n=12)
Mean Distance to HTL (m)	0.283 (0.180)	-0.508 (0.162)	0.328 (0.147)	0.0245 (0.976)	0.110 (0.721)
Distance to Veg (m)	*-0.490 (0.0176)	-0.00596 (0.988)	*-0.620 (0.00357)	-0.534 (0.466)	-0.391 (0.187)
Clutch Elevation (m)	-0.000138 (0.999)	-0.105 (0.788)	0.204 (0.376)	*-0.991 (0.00855)	-0.125 (0.683)
Moisture Content (%)	*-0.421 (0.0407)	-0.426 (0.253)	*-0.472 (0.0306)	-0.676 (0.3239)	0.214 (0.483)
Average pH	-0.286 (0.175)	0.409 (0.275)	-0.389 (0.0810)	0.754 (0.2456)	-0.334 (0.265)
Mean Conductivity (EC)	*-0.471 (0.0201)	-0.361 (0.340)	*-0.552 (0.00947)	—————	0.0170 (0.956)
Bacteria and Fungus (%)	*-0.614 (0.00238)	-0.353 (0.351)	*-0.731 (0.000373)	-0.552 (0.4485)	-0.355 (0.234)
Mean °C /Nest	*0.469 (0.0319)	0.361 (0.380)	0.417 (0.0855)	-0.701 (0.5058)	0.109 (0.7487)
Maximum °C/Nest	*0.708 (0.000331)	*0.889 (0.00317)	*0.684 (0.00175)	0.905 (0.280)	0.481 (0.134)
Mean °C 2 <sup>nd</sup> half incubation	*0.671 (0.000867)	0.645 (0.0844)	*0.691 (0.001482)	-0.481 (0.680)	0.291 (0.3848)
Slope	0.00147 (0.995)	0.0262 (0.947)	0.226 (0.324)	-0.404 (0.596)	0.0499 (0.871)

**Table 3.** Results from the multiple linear regression analysis for environmental variables influencing leatherback sea turtle hatching success (n=20), excluding the 4 nests that were affected by predation. Asterisks (\*) indicates a significant p-value resulting from the model ( $p < 0.05$ )

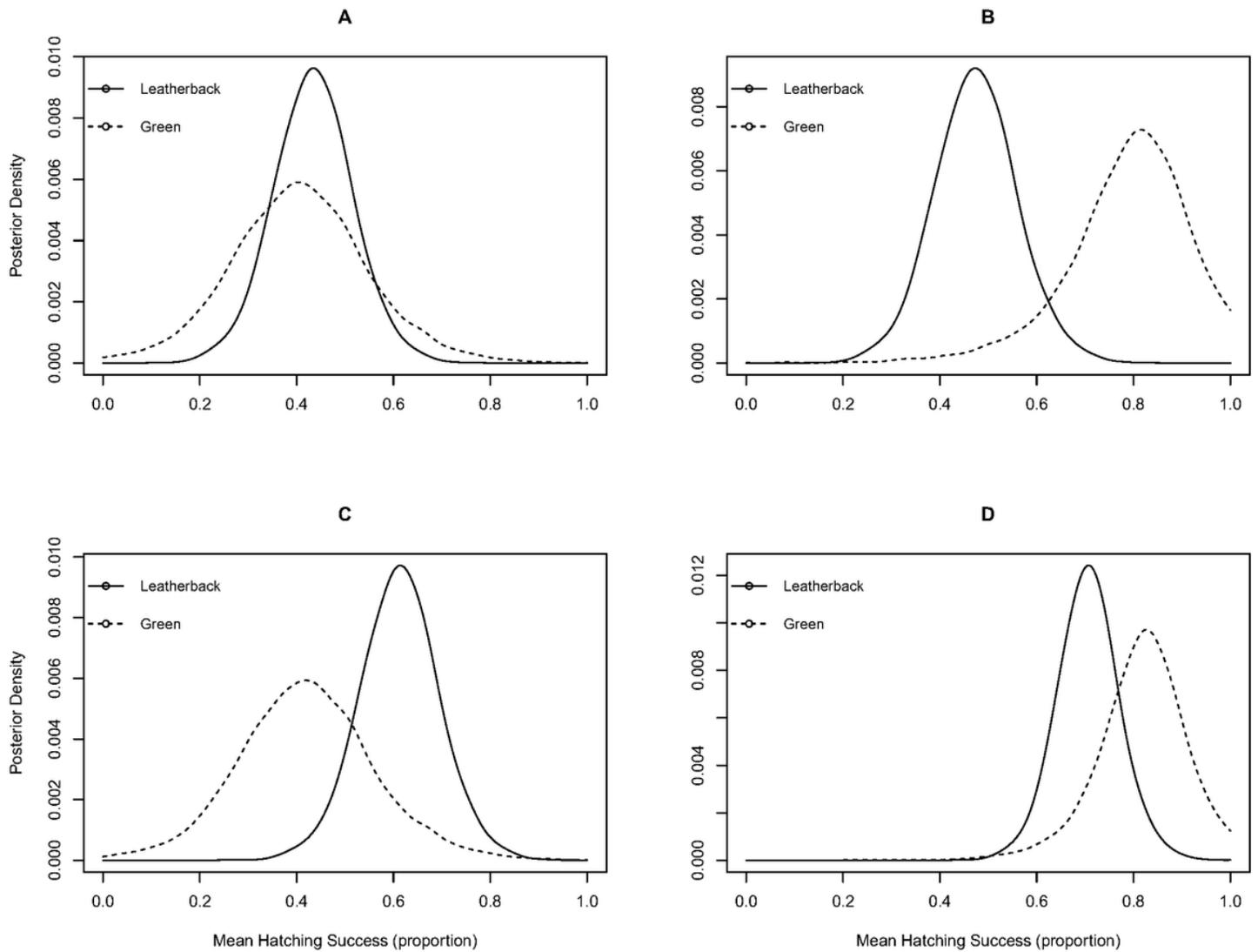
Multiple $r^2 = 0.8687$ , F (5,11) = 14.55, p-value < *0.001	
Variables	P-values
Moisture Content (%)	0.090
Mean Conductivity (EC)	*0.021
Mean °C 2 <sup>nd</sup> half incubation	0.499
Distance to Vegetation (m)	*0.001
Clutch Elevation (m)	*0.014

## Figures



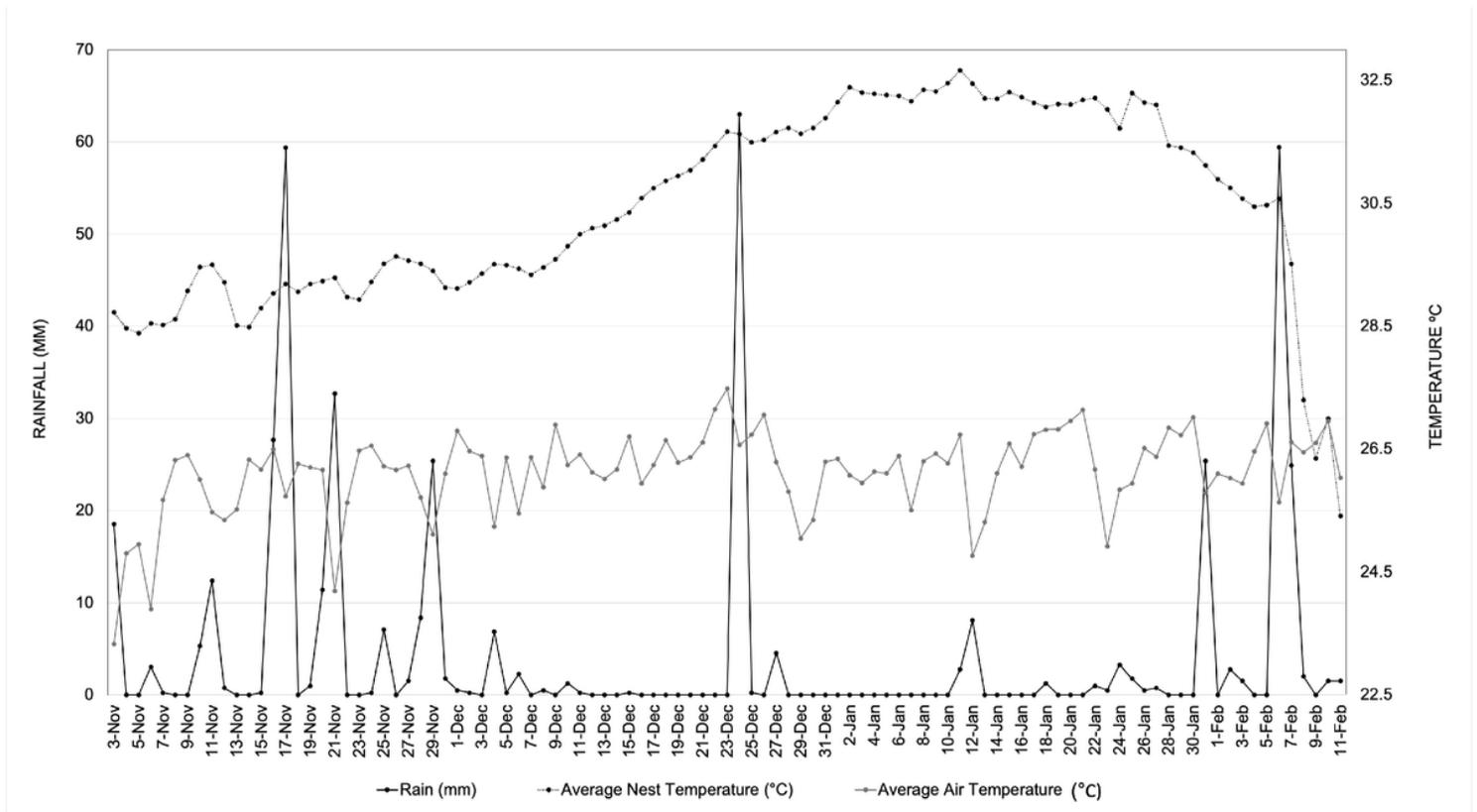
**Figure 1**

Nesting beaches on Bioko Island, Equatorial Guinea (Honarvar et al. 2016). The five nesting beaches are labelled with letters A–E Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



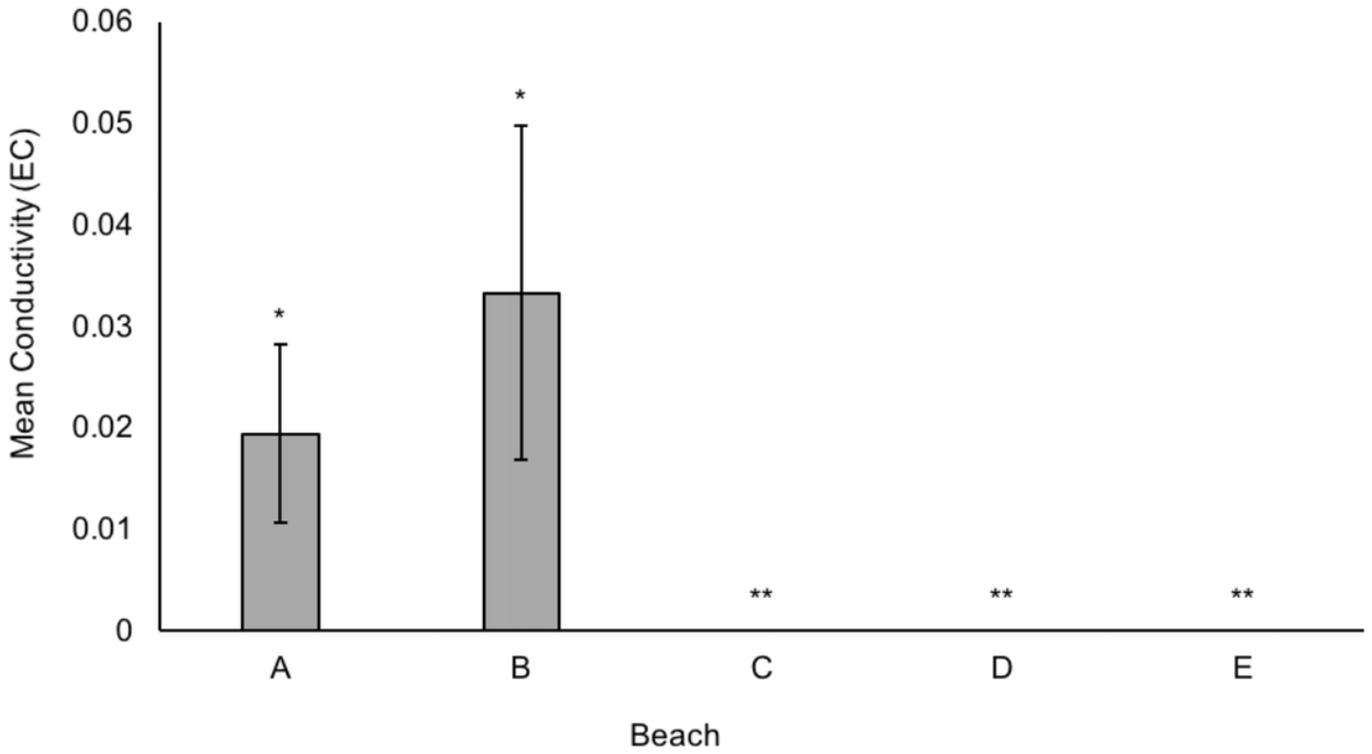
**Figure 2**

Posterior distributions of mean hatching success (percent of hatched eggs out of total number of eggs laid) for leatherback (n=24) and green turtles (n=11) a) for all cases, b) green (n=4) and leatherback (n=21) nests excluding predation, c) green (n=11) and leatherback (n=16) excluding inundation, and d) green (n=4) and leatherback (n=12) nests excluding inundation and predation



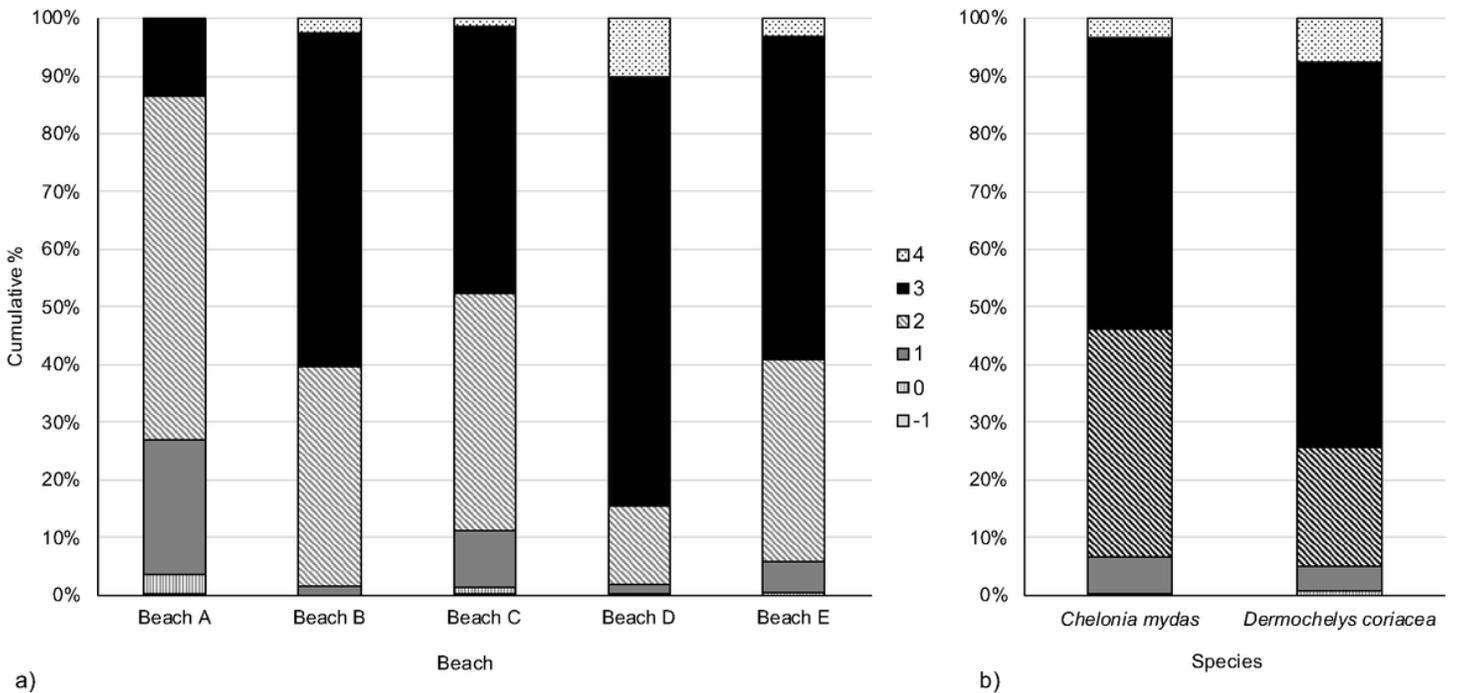
**Figure 3**

Total rainfall per day (mm) shown in blue, average temperature per day (°C) inside the center of leatherback clutches on Beach D shown in red, and average air temperature (°C) shown in grey. Black arrows highlight consistent decreases in temperature across all nests located on Beach D after spikes in rainfall



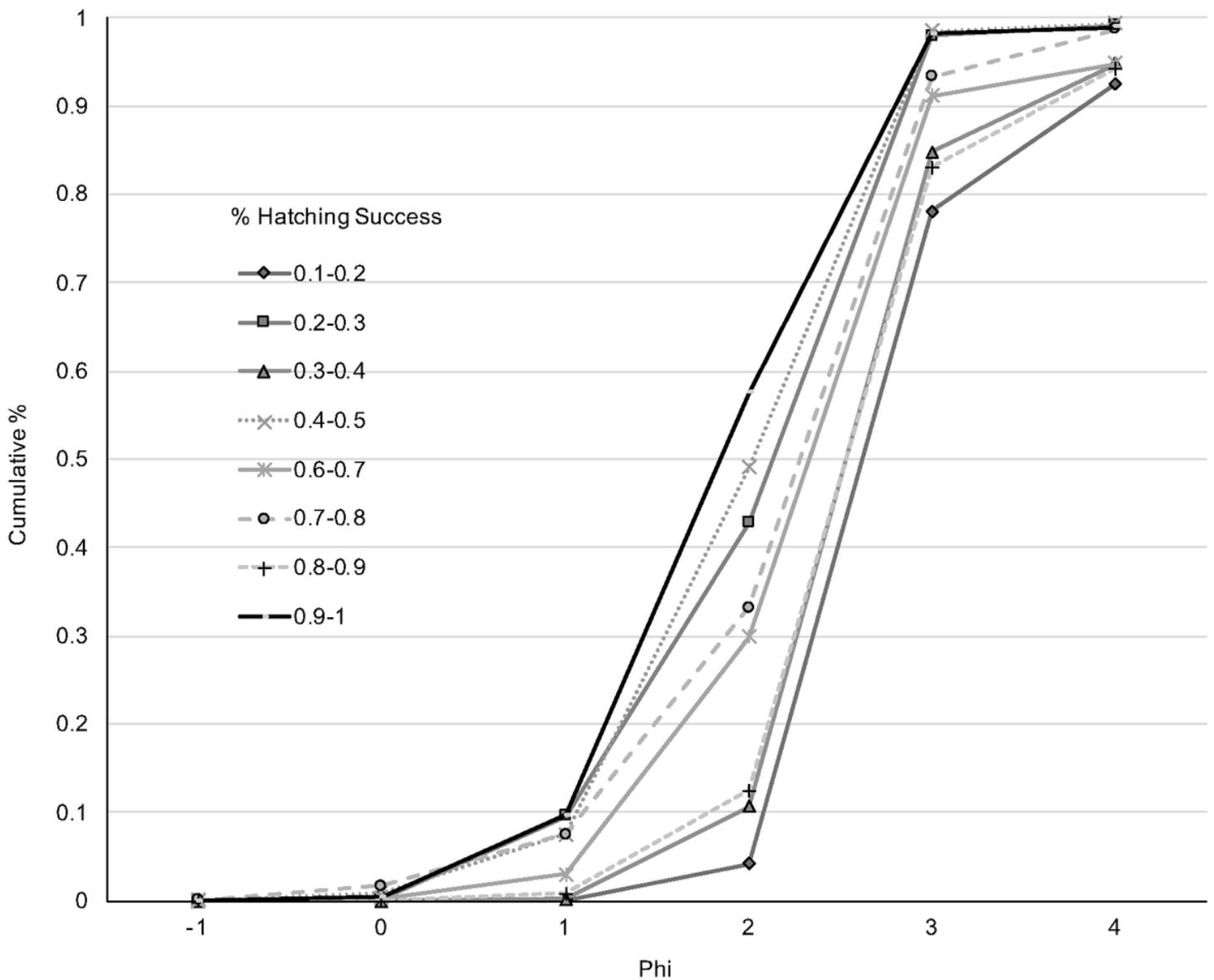
**Figure 4**

Mean conductivity (EC) readings in green turtle control nests between beaches. Error bars are standard error from the mean. Stars indicate significant differences between groups. The conductivity of beaches C, D, and E registered below the detection limit of our equipment (0.0001 mHO/m)



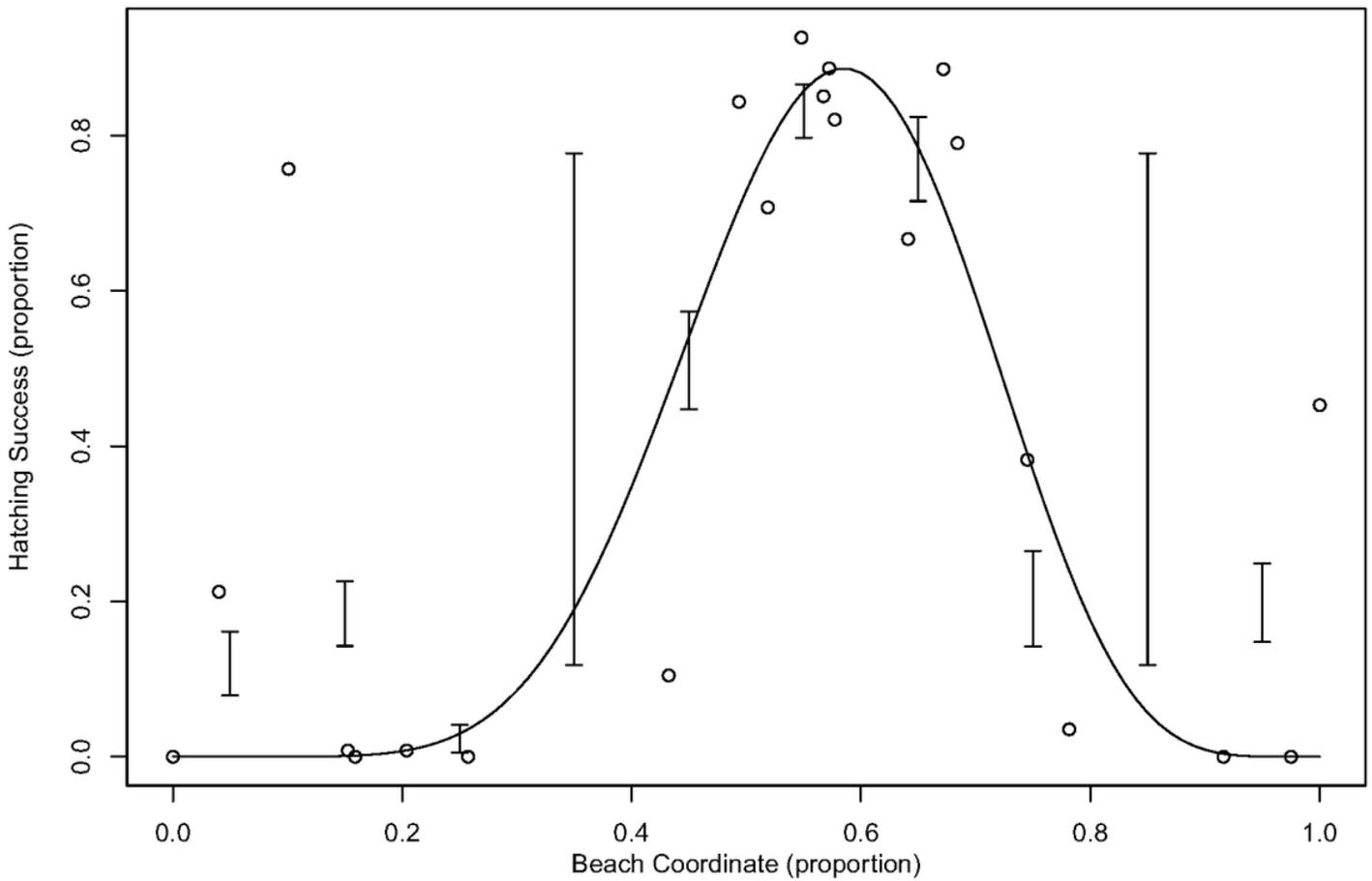
**Figure 5**

Percentage composition of sand particles in 6 size classes on sea turtle nesting Beaches A-E and in green turtle (*Chelonia mydas*) and leatherback turtle (*Dermochelys coriacea*) experimental nests. Particle classes correspond to the Phi scale ranging from very coarse (Phi = -1, 2 mm diameter) to silt (Phi = 4, 0.0625 mm diameter)



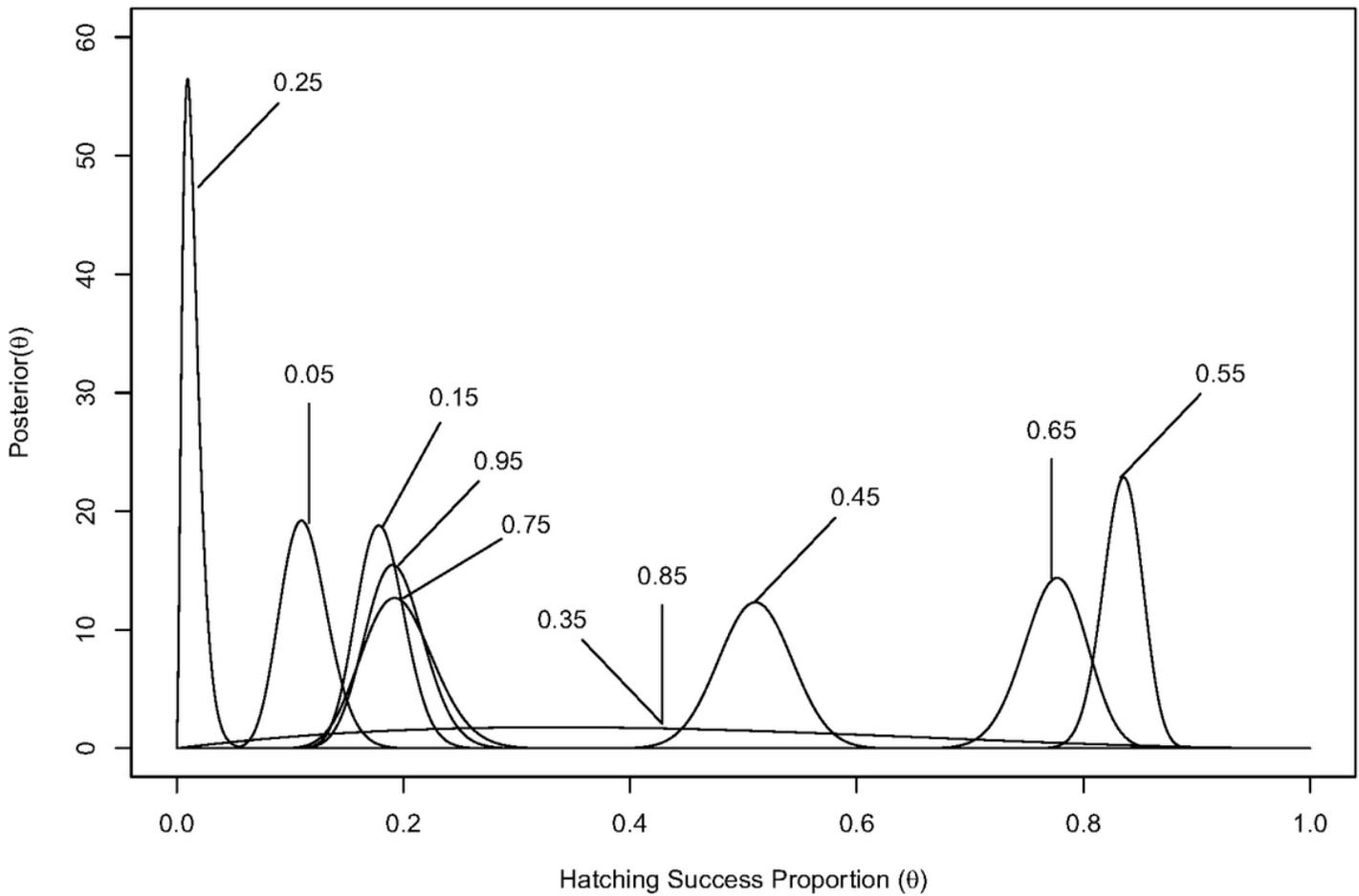
**Figure 6**

Comparison of average cumulative nest sand particle size distributions among clutch hatching success of 10-20%, 20-30%, 30-40%, 40-50%, 60-70%, 70-80%, 80-90% and 90-100% in leatherback turtle experimental nests on Bioko Island, Equatorial Guinea. Particle classes correspond to the Phi scale ranging from very coarse (Phi = -1, 2 mm diameter) to silt (Phi = 4, 0.0625 mm diameter)



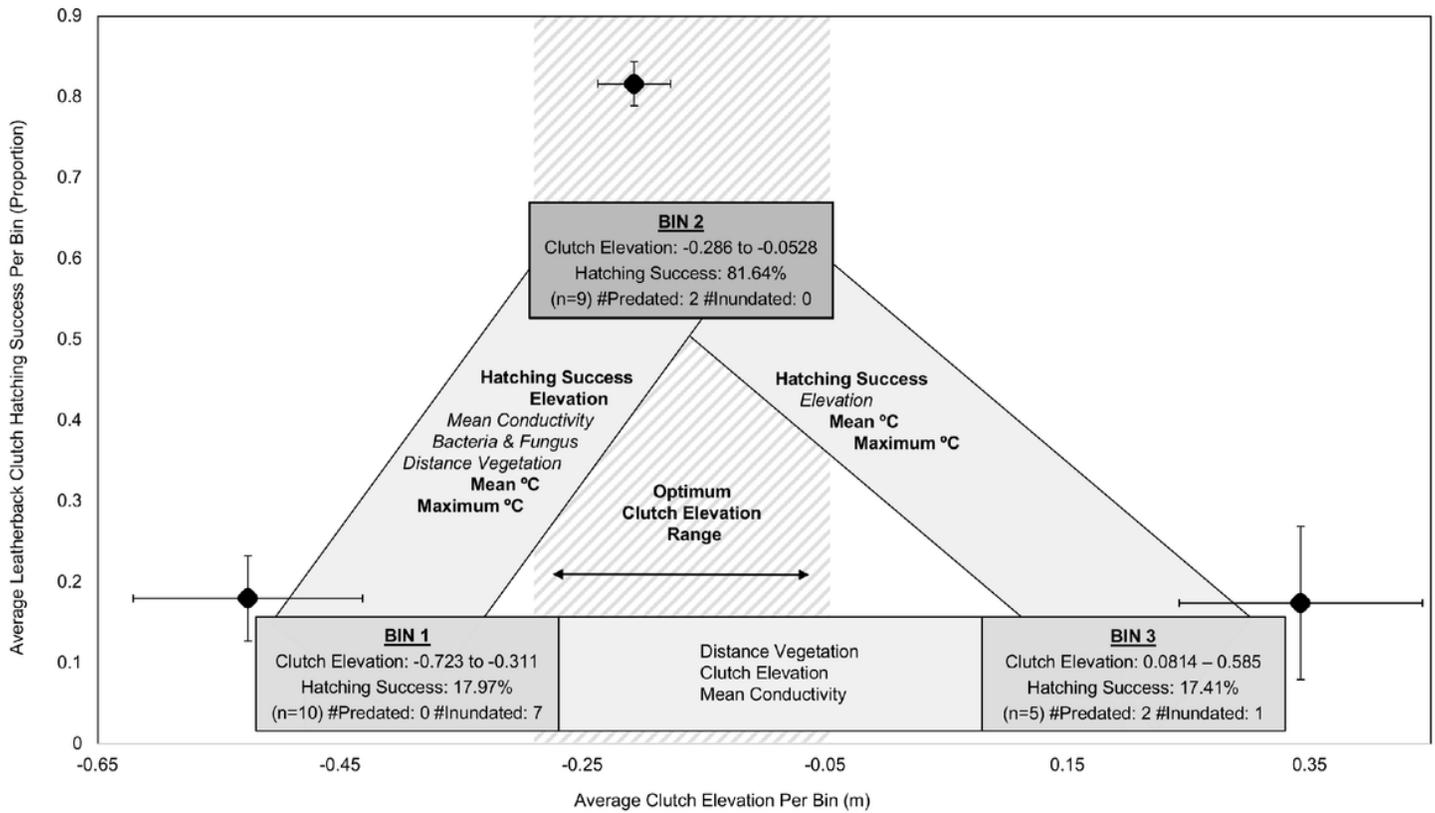
**Figure 7**

The expected hatching success rate for leatherback turtles is an increasing-then-decreasing function of beach coordinate. The functional form is  $\text{beta}(8.9, 6.6) \sim bc^{7.9} (1-bc)^{5.6}$  and has a maximum at about 0.58. The beach coordinate is the normalized (proportional) arc length of the curve defined by the quadratic equation  $DV = c_0 + c_1 CE + c_2 CE^2$ , where  $(c_0, c_1, c_2) = (0.96, -2.37, 1.59)$ . Open circles indicate the leatherback nests. Error bars are given for hatching success proportion based division of nests into deciles by beach coordinate. Values of  $(0, 0.05, 0.15, \dots, 0.95)$  are used to collect and summarize data. Two deciles (0.35 and 0.85) were not observed and their error bars reflect the weakly informative Bayesian prior



**Figure 8**

The posterior distribution of the mean hatching success proportion by decile of beach coordinate. The lowest deciles and the highest deciles show low mean hatching success proportion while the middle deciles show the highest success proportions. Two deciles (0.35 and 0.85) were not directly observed so their points are described by the broad and weakly informative Bayesian prior distributions



**Figure 9**

Nests were placed in bins based upon their elevation. The average elevation and hatching success for each bin are marked. Nest characteristics were compared between bins, and significantly different characteristic between bins are displayed. Italicized characteristics were significantly higher in that bin than in that of the adjoining bin, and bolded characteristics were significantly lower than that of the adjoining bin. Only significantly different characteristics between Bins 1 and 3 are shown. The optimum clutch elevation range observed for the 24 leatherback nests studied is indicated by the patterned horizontal lined rectangle. Nests in the optimum clutch elevation range had a significantly higher hatching success than those outside this range (ANOVA,  $F(1,22) = 31.92, p < 0.001$ )