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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Abstract

Hatching success of sea turtle eggs can be reduced 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 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, temperature, sand grain size, location on the beach, and sand conductivity also played significant roles in leatherback clutch hatching success. For leatherback clutches, 33% percent of 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 and −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.

Keywords Sea turtles · Hatching success · Bioko Island · Climate change · Predation · Sea-level rise

Introduction

Embyrogenesis in reptiles is affected by the environmental conditions of the nest site (Vitt and Pianka 2014; Packard and Packard 1988). Incubation conditions can specifically influence sex determination, hatching success, development, hatching traits, and incubation period in many egg-laying reptiles (Piña et al. 2003; Hare et al. 2002; Ji and Du 2001; Spotila et al. 1994). Due to the life history characteristics of marine turtles and their use of coastal beaches to lay their eggs, 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 an intricate process where factors that decrease hatching success are many and include egg predation (Fowler 1979), nest inundation (Limpus 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 (Ackerman 1975, 1977; Packard and Packard 1988).

Successful incubation is likely hindered by beaches with poorly sorted substrates, high silt, saline, or clay-like composition. Poorly sorted substrates can affect pore 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 and successful hatchling development (Mortimer 1990).

Regarding general nest placement, nesting too close to the water may result in nest inundation and embryo mortality, whereas nesting too far from the water’s edge can cause an increased risk of egg and hatchling predation. Nesting too seaward also poses threats based on sensitivities to moisture content, chloride, and conductivity (Bustard and Greenham 1968; McGeehe 1990; Foley et al. 2006). Increased inundation can cause metabolism disruption in developing embryos due to high salinity exposure and embryo suffocation due to decreased air-filled pore space within the surrounding sand (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 high tide line (HTL) (Whitmore and Dutton 1985). A rising freshwater table and inundation of nests and body pits attributable to sea-level rise has been observed on Raine Island, Australia (Limpus 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).

It has been found on a hawksbill sea turtle nesting beach on Shidvar Island, Iran that turtles preferred a specific elevation range. Emergences decreased at elevations both higher and lower than the optimal range (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). Increased development in the Gran Caldera and Southern Highlands Scientific Reserve has made local conservation efforts on Bioko Island even more important than ever (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 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). Bioko’s nesting beaches are comprised primarily of black volcanic sand with interspersed outcroppings of lava and basalt (Rader et al. 2006).

This study was conducted from November 2016 to February 2017, coinciding with the leatherback and green sea turtle nesting season on Bioko Island (Fig. 1). 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 min. 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 greater than one nest from the same turtle were not included in the study. These clutches are referred to as “experimental nests”. Experimental nests were chosen at random, opportunistically during night patrols. From the beginning of the monitoring period, any nesting female encountered while making a body pit or digging a nest, the beginning stages of the nesting process, was a turtle who’s nest was an experimental nest. Artificial “control” sites were established by digging to the average observed depth of experimental nests for both leatherback (78.5 cm ± 11.28 SD, n = 24) and green (66 cm ± 6.02 SD, n = 11) nests but contained no eggs. The average depth of the centre of the nest chamber per species was calculated using the average distance from the bottom of the nest to the top of nest chamber in leatherback (27.19 cm ± 9.52 SD, n = 24) and green (19.33 cm ± 6.02 SD, n = 11) experimental nests. These depths were used to determine where to obtain nest site measurements from (detailed below). Control sites 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 sites were dug in Zone 3 and leatherback turtle control sites in Zone 2. There were 3 leatherback and 3 green control sites on Beach C and Beach D. Control nests were monitored for the duration of the nesting season, overlapping with the

Fig. 1 Nesting beaches on Bioko Island, Equatorial Guinea (Honarvar et al. 2016). The five nesting beaches are labelled with letters A–E.
incubation period of the experimental nests on each beach (November 2, 2016–February 13, 2017). Although control sites 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 2017; Veelenturf et al. 2020) (Fig. 1).

Nest site characteristics

Nest characteristics were measured twice, during the nesting event and during nest excavations for both experimental nests and control sites. 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 in every nest and set to log nest temperature every 30 min. 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 centre 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 transects were conducted on all five of Bioko’s nesting beaches (A–E). Beach characterization methods were consistent with a similar SLR prediction model for 13 beaches on the island of Bonaire, Dutch Antilles (Fish et al. 2005). The profile of each beach was recorded at 50 m intervals along the beaches using a 60 m measuring tape. The transects on each beach were 50 m apart, perpendicular to the water line, and spanned the distance from the vegetation line to the drop off during lowest tide. A metre tape, compass and Abney level, a surveying instrument consisting of a sighting tube, movable spirit level and protractor scale, were used to create profiles of beach topography and dimensions at each change in slope along the transect (Fish et al. 2008). Accuracy to ground truth was relative to the stake GPS point (Garmin GPSMap 64) at the start of each transect. To ensure maximum accuracy, up to 6 different waypoints for the same stake on each beach were averaged to generate an average stake reference point to be used in the following spatial analysis. During the process of beach profiling, the location of the high tide line was indicated. Three times throughout the nesting season, the high tide line of all beaches was walked to create a GPS track that could then be used in conjunction with the HTL identified during profiling to standardize the average location of the high tide line. The circular error probable for each stake was calculated. A program was written in Python to generate a waypoint and elevation at each change in slope on the transects and at the present elevation of the high tide line. In ArcMap (Esri version 10.4), GPS points with their respective elevation values were entered as x, y, z data and then projected as shapefiles. All elevations were relative to the HTL, which for the purposes of this project is at an elevation of 0 m. The weighted average elevation of each beach was determined by utilizing the average elevation and length of all transect segments. The vertical error of each profile segment was determined using the generally accepted measurement error of the Abney level (4.31%) (Pariyar and Mandal 2019). The total error of each profile was determined by adding the sequence of vertical errors along the profile in quadrature. The average vertical error of all profiles was determined to be 0.074 m ± 0.027 (SD) (Veelenturf et al. 2020). 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 metres of the centre of the clutch. Slope at each nest site was extracted from the TIN model (Veelenturf et al. 2020).

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 immediately before oviposition. The percent moisture content of the sand was calculated from wet and dry weights of the sand samples (Bustard and Greenham 1968; McGeehee 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 nests and control sites until each sample maintained a constant weight for at least 4 h.
A field oven was created using four wooden planks, a small grill, and a kerosene stove. Percent moisture was calculated using the formula:

\[
\text{percent moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100
\]

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 min, 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. Excavations occurred three days after hatchling emergence. 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. We did not need to account for clutch destruction by other nesting females. 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 in the clutch. 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 and was below the most recent high tide line was considered inundated. Potential microbial presence or absence was evaluated visually by identification of distinct discoloration along the exterior of the egg and inside the eggshell membrane (Sarmiento-Ramirez 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. ANOVA analyses were robust against mild to moderate heteroskedastic and unequal variances. Pearson correlations were calculated 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 calculated between leatherback and green clutch hatching success including and excluding nests affected by predation. Due to the close relationship between clutch elevation and distance to vegetation, a single coordinate (i.e., 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 nests were projected onto the parabola to minimize the total distance of the projection. The beach coordinate was determined to be the nearest point on the parabola to the nest. This is an orthogonal projection of the next 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

\[
- \log(L(a,b,c|data)) = - \Sigma a + b \log(bc[i]) + c \log(1 - bc[i])
\]

was used with respect to the parameters \((a,b,c)\), so the distribution is proportional to \(\beta(a + 1, c + 1)\).

The beach coordinate was 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 were 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 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 \(DV = c0 + c1 \times CE + c2 \times CE^2\), where \((c0,c1,c2) = (0.96, -2.37, 1.59)\). The beach coordinate was 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. 2. The leatherback clutch optimum elevation range was determined by isolating the continuous high clutch hatching success rates occurring at a clutch elevation from −0.286 to −0.0528 m, which is a corresponding surface elevation from 0.319 to 0.438 m relative to the HTL.

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 inundation. A MLR analysis was not conducted for green turtle data due to
a low sample size ($n = 11$). Data available for the model included: distance to vegetation, mean distance to HTL, mean pH, mean conductivity, mean moisture content, clutch elevation, mean temperature, mean temperature during the second half of the incubation period, nest location (inside or outside of flats), maximum temperature, and mean sand grain size. Model selection using stepwise analysis was conducted to minimize the Bayesian Information Criterion and 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. 3). 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 10,000 random events.

![Figure 2](image)

**Fig. 2** The expected hatching success rate for leatherback turtles is an increasing-then-decreasing function of beach coordinate. The functional form is $\beta(8.9, 6.6) \sim bc^7.9 (1-bc)^5.6$ 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 $(0.05, 0.15, ..., 0.95)$ 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.

### Results

During the monitoring period for the experimental nests (November 2, 2016–February 15, 2017) the mean air temperature was $26.12 \pm 2.68$ SD, $n = 2755$. The maximum temperature reached was $32.38$ °C, and the minimum was $20.89$ °C. There were 435.14 mm of rain, and mean relative humidity was $92.74\% \pm 7.77$ SD, $n = 2755$. Mean wind speed was $0.50$ m/s $\pm 0.93$ SD, $n = 2755$, mean gust speed was $1.12$ m/s $\pm 1.65$ SD, $n = 2755$, and mean wind direction was $238.79 \text{ø} \pm 88.84$ SD, $n = 2755$. The mean solar radiation during the incubation period of our experimental nests was $144.53$ W/m² $\pm 224.37$ SD, $n = 2755$. The average circular error probable for the beach profiling reference points was $2.43$ m $\pm 1.44$ SD, indicating that $2.43$ m is the radius of a circle centered around the mean position of each reference stake that contains 50% of the reference stake GPS points. Similarly, the circular error probable was $3.85$ m $\pm 2.51$ SD for 98% of the reference stake GPS points (Veelenturf 2017; Veelenturf et al. 2020).

Out of the 35 experimental nests, there were only 17 unaffected by either predation or inundation. Nests that were affected by predation were not included 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 grouped 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 of all nests 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% ± 40.60 SD, $n=9$, and 41.73% ± 38.25 SD, $n=24$, respectively (Fig. 3).

Two of the green turtle nests were so heavily depredated that we were unable to determine a hatching success. When data for the nests affected by predation were omitted, mean hatching success was 44.16%, ± 38.40 SD, $n=21$ for leatherback turtles and 82.63%, ± 12.95 SD, $n=4$ for green turtles. When omitting all nests affected by predation and inundation, hatching success was not significantly different between leatherback (70.6%) and green turtle nests (82.63%) (ANOVA, $F(1,15)=1.07, P=0.317$). 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 *Ocypode cursor* and *Ocypode africana*, and ants, potentially of the genus *Dorylus*, were the major nest predators on Bioko Island. For the leatherback turtle clutches affected by predation, from 7.32 to 43.53% (min to max) of the eggs were affected, and for green sea turtles 35.42–100% were affected. As nests of both species were located closer to the vegetation, they were significantly more likely to be predated (ANOVA, $F(1,32)=5.37, P=0.027$).

By the end of the incubation period, 8 leatherback and zero green turtle nests were partially or fully inundated.

![Posterior distributions of mean hatching success (percent of hatched eggs out of total number of eggs laid) for leatherback ($n=24$) and green turtle nests ($n=11$)](image)
with a mean clutch hatching success of the leatherback nests 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, \( F (1,33) = 32.50, P < 0.001 \)).

Pearson correlations between leatherback and green turtle hatching success and environmental characteristics are shown in Table 1.

For both species there were no significant differences between the mean temperatures in the control sites (28.66 °C ± 1.97 SD, \( n = 11 \)) and experimental nests (29.81 °C ± 1.54 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 °C ± 1.60 SD, \( n = 24 \)) and leatherback control sites (29.65 °C ± 1.88 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 nests (29.117 ± 1.22 SD, \( n = 11 \)) and control (27.485 ± 1.45 SD, \( n = 3 \)) sites did experience a significant difference in mean temperature (ANOVA, \( F (1,13) = 5.30, P = 0.039 \)) throughout the incubation period.

No significant difference was found between the mean temperature in leatherback (30.14 °C ± 1.60 SD, \( n = 24 \)) and green (29.11 °C ± 1.218 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 °C ± 2.00548 SD, \( n = 24 \)) versus green experimental nests (31.21 °C ± 1.944 SD, \( n = 11 \)) (ANOVA, \( F (1,12) = 4.61, P = 0.040 \)). Inundated nests experienced significantly lower maximum temperatures, excluding nests affected by predation (ANOVA, \( F (1,16) = 8.76, P = 0.009 \)).

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 sites 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 sites 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 sites 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 Beaches C, D and E, but conductivity in leatherback control sites 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 sites on Beaches C, D and E. 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. 4. 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. 4). 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’ \( \lambda = 0.238, F_{24,1034} = 21.945, P < 0.0001 \); Fig. 4).

### Table 1 Pearson correlations \((r_p)\) between leatherback and green hatching success \((HS)\) and environmental characteristics, excluding data for nests that were affected by predation and excluding those affected by inundation in column 3

| Characteristic                      | Leatherback HS \((n = 21)\) | Green HS \((n = 4)\) | Leatherback HS without inundation \((n = 12)\) |
|------------------------------------|------------------------------|----------------------|-----------------------------------------------|
| Mean distance to HTL (m)           | 0.328 (0.147)                | 0.0245 (0.976)       | 0.110 (0.721)                                 |
| Distance to veg (m)                | * -0.620 (0.004)             | -0.534 (0.466)       | -0.391 (0.187)                                |
| Clutch elevation (m)               | 0.204 (0.376)                | -0.991 (0.009)       | -0.125 (0.683)                                |
| Moisture content (%)               | * -0.472 (0.031)             | -0.676 (0.324)       | 0.214 (0.483)                                 |
| Average pH                         | -0.389 (0.081)               | 0.754 (0.246)        | -0.334 (0.265)                                |
| Mean conductivity (EC)             | * -0.552 (0.010)             | -0.017 (0.956)       |                                              |
| Mean °C /Nest                      | 0.417 (0.086)                | -0.701 (0.506)       | 0.109 (0.749)                                 |
| Maximum °C /Nest                   | 0.684 (0.002)                | 0.905 (0.280)        | 0.481 (0.134)                                 |
| Mean °C 2nd half incubation        | 0.691 (0.002)                | -0.481 (0.680)       | 0.291 (0.385)                                 |
| Slope                              | 0.226 (0.324)                | -0.404 (0.596)       | 0.050 (0.871)                                 |

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.
Wilks’ $\lambda = 0.716$, $F_{6,137} = 9.051$, $P < 0.0001$; Fig. 4). The sand grain size distribution of clutches with varying clutch hatching success is plotted in Fig. 5.

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. Flats were also characterized by significantly different sand grain size distributions than other parts of the beach (MANOVA, Wilks’ $\lambda = 0.0168$, $F_{12,290} = 162$, $P < 0.0001$; Fig. 4). 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 way-point 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. Excluding experimental nests affected by predation, the average leatherback clutch hatching success within flats (12.32% ± 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% ± 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. ANOVA analyses between the characteristics of leatherback experimental nest not affected by predation located in ($n = 8$) and outside ($n = 16$) of flats shown in Table 2.

The average percent of eggs with potential microbial presence within a clutch for green sea turtles was 5.18% ± 4.74 SD, $n = 9$, and that of leatherback sea turtles was 24.21% ± 29.7 SD, $n = 22$ (ANOVA, $F(1,29) = 3.59$, $P = 0.068$). The potential presence of microbes 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 microbial presence than those that were not partially or fully inundated with water at time of excavation (ANOVA, $F(1,18) = 24.44$, $P < 0.001$). Leatherback nests, not including those affected by predation, within the flats ($n = 8$) had a significantly higher percent of eggs containing signs of potential microbial presence (ANOVA, $F(1,17) = 6.34$, $P = 0.022$).

A leatherback optimum clutch elevation range of −0.286 to −0.0528 m and corresponding surface elevation of 0.319–0.438 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% ± 8.17% SD, $n = 9$, and significantly higher than the hatching success outside of this range, 17.78% ± 26.82 SD, $n = 15$ (ANOVA, $F(1,22) = 31.92$, $P < 0.001$). This optimum clutch elevation range was evident even when leatherback and green experimental nests affected by predation and inundation were omitted. 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 multiple linear regression analyses evaluating the strongest predictors of hatching success in leatherback turtles including and excluding nests affected by inundation can be found in Tables 3 and 4, respectively. Collinearity was tested for using variance inflation factor (VIF) on the final model, and the VIF values were less than 10 for all variables. Collinear models are VIF values far in excess of 10.

Fig. 5 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 (excluding those affected by predation and inundation) on Bioko Island, Equatorial Guinea. There were no nests that were unaffected by predation and inundation with a hatching success between 20–30% and 50–60%. Particle classes correspond to the Phi scale ranging from very coarse (Phi = −1, 2 mm diameter) to silt (Phi = 4, 0.0625 mm diameter). Samples were taken from 35 different nest sites on Beaches C and D. At each nest site a total of 4 sand samples were taken from: (1) the surface sand and the (2) top, (3) middle, and (4) bottom of the nest chamber.

Table 2 ANOVA analysis between the characteristics of leatherback experimental nest not affected by predation located in (n=8) and outside (n=16) of flats

| Variables               | F Statistic | p value |
|-------------------------|-------------|---------|
| Clutch elevation (m)    | 18.99 (1.19) | <0.001  |
| Mean conductivity (EC)  | 3.45 (1.19)  | 0.080   |
| Moisture content (%)    | 0.00 (1.19)  | 0.993   |
| Mean pH                 | 0.10 (1.19)  | 0.758   |
| Mean temperature (°C)   | 0.00 (1.16)  | 0.949   |
| Maximum temperature (°C)| 1.22 (1.16)  | 0.285   |
| Hatching success (%)    | 9.81 (1.22)  | *0.005  |

Asterisks (*) indicates a significant p value resulting (p < 0.05)
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

Multiple $r^2 = 0.893$, $F(5,10) = 16.67$, $p$ value $< *0.001$

| Variable                        | $p$ value |
|--------------------------------|-----------|
| Moisture content (%)           | 0.118     |
| Mean conductivity (EC)         | *0.005    |
| Distance to vegetation (m)     | 0.150     |
| Clutch elevation (m)           | *0.001    |
| Beach location (flats/non-flats)| *0.014    |

Asterisks (*) indicates a significant $p$ value resulting from the model ($p<0.05$)

Table 4 Results from the multiple linear regression analysis for environmental variables influencing leatherback sea turtle hatching success (n = 12), excluding the 4 nests that were affected by predation and the 8 nests that were affected by inundation

Multiple $r^2 = 0.960$, $F(4,6) = 16.16$, $p$ value $< *0.009$

| Variable                        | $p$ value |
|--------------------------------|-----------|
| Mean conductivity (EC)         | *0.218    |
| Mean °C 2nd half incubation    | 0.025     |
| Distance to vegetation (m)     | *0.023    |
| Clutch elevation (m)           | *0.002    |
| Mean sand grain size clutch elevation (m) | *0.039 |
| Beach location (flats/non-flats)| *0.049    |

Asterisks (*) indicates a significant $p$ value resulting from the model ($p<0.05$)

**Discussion/conclusion**

Insights into the major abiotic and biotic influences on hatching success have been gained through this study for leatherback turtles in this critically important nesting habitat of the Southeast Atlantic. Hatching success in green sea turtles should be further investigated on Bioko with a larger sample size. 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, as one third of the nests studied were affected by 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. The finding that more green nests were affected by predation than leatherback nests 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. Our observation that as nests were located closer to the vegetation line, they were significantly more likely to be predated is in line with the findings of other studies (Crossland 2003; Leighton et al. 2011; Da Silva et al. 2016). 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).

The observed quantity of inundated nests on Bioko is similar to findings from French Guiana, where about one third (30.34%) of leatherback 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 and 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 and exhibiting a significant and positive correlation between temperature and clutch hatching success in leatherback turtles, including inundated nests. 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). The present observed relationship can likely be attributed to the significantly lower maximum temperatures of inundated...
nests, which had very low hatching success, versus non-inundated nests.

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. It is important to note that the observed potential microbial growth in experimental nests could have begun before or after embryo mortality. The pathogenic fungi species *Fusarium falciforme* and *Fusarium keratoaplasticum* have been associated with decreased sea turtle hatching success globally in six sea turtle species (Sarmiento-Ramírez et al. 2014). 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 0.319–0.438 m, relative to the high tide line at a surface elevation of 0 m, and peaking at a beach coordinate of 0.58. This elevation range 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, nests are more likely to be affected by inundation, and above the optimum elevation range, nests are more likely to be affected by predation by ants and crabs. 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, such as the significant predictors for hatching success from the MLR analysis in Table 4: mean temperature in the second half of the incubation period, sand grain size, and location in flats. Further studies with a larger sample size are needed to additionally assess these nest characteristics in leatherback turtles. The percentage of leatherback nests during the 2016/2017 season located in the optimum elevation range (15.38%) suggests that leatherback 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.

Results from the MLR analysis not including nests affected by predation were similar to other studies finding that temperature (Packard and Packard 1988; Matsuzawa et al. 2002; Segura and Cajade 2010), elevation (Zare et al. 2012), distance to vegetation (Leighton et al. 2011), and sand grain size (Ditmer and Stapleton 2012) influence hatching success in sea turtles. Although moisture content often affects hatching success, it was not included in the final model due to the stepwise model selection process (McGehee 1990; Foley et al. 2006). As all calculated variance inflation factors were less than 10, collinearity in our final models is within acceptable levels. Despite nominal collinearity between variables, their inclusion in the model was determined via stepwise model selection and 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 characteristics when VIF values are within acceptable limits. Including inundated nests, sand grain size did not significantly influence leatherback turtle hatching success, as has been seen at other nesting sites for leatherback turtles (Garret et al. 2010). Hatching success in nests unaffected by inundation were slightly positively influenced by increased sand grain size, as has been observed at other nesting sites (Ditmer and Stapleton 2012; Mortimer 1990). As Bioko Island is an extremely humid environment, the risk of desiccation may play less of a role in the effect of grain size in hatching success than it has in other, more arid nesting environments (Mortimer 1990). Increased air-filled pore space due to coarser grain sizes allow for increased gas exchange, which would benefit the nest environment on beaches characterized by high tides and quantities of rainfall, as seen on Bioko. Observed significant differences between nest sand grain size amongst species and beaches (which vary in nest 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 analyses and Bayesian statistics 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, near the vegetation or in flats, the data suggest that conductivity, grain size and temperature have the greatest influences on hatching success. When data for nests affected by inundation were omitted from the analysis, environmental
characteristics were not significantly correlated with clutch hatching success. 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, but further research is required to better understand the complex ecology of nesting habitat on Bioko. 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 abandoned that nest to choose a drier location closer to the vegetation (Veelenturf et al. 2020).

High green sea turtle clutch hatching success, not including nests affected by predation (82.63%), 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 sites 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 2017; 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 h egg viability was reduced by less than 10%, and 6 h 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 predating nests on Bioko will be useful in future management. A hatchery could significantly decrease the effects of predation on hatching success. Circular cages dug 3 inches into the ground around the nests, 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 towards 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.

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 foster 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.

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Availability of data and materials The datasets generated during and analysed 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.

Declarations

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 guidelines of the Institutional Animal Care and Use Committee at Purdue University (IACUC), IACUC protocol #1410001142.

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