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Variations in leatherback turtle nest environments: consequences for hatching success

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ABSTRACT: Physical and biological conditions of nests in which sea turtle embryos develop can vary among and within nesting beaches. Monitoring these conditions and their effects on embryonic development should be considered when assessing conservation efforts to increase sea turtle hatchling production. Sandy Point National Wildlife Refuge (SPNWR), St. Croix, US Virgin Islands, hosts a leatherback turtle *Dermochelys coriacea* nesting colony that has increased exponentially in the past 2 decades, due in part to an ongoing egg relocation program. We characterized the influence of nest environment conditions (e.g. partial pressures of oxygen, pO_2, and carbon dioxide, pCO_2, and temperature) on hatching success of relocated eggs at 3 different sites at SPNWR to evaluate potential intra-beach variation in nest environment conditions and hatching success. Although nest conditions varied significantly among sites, hatching success did not vary significantly among relocation sites. Among all clutches and sites, hatching success varied significantly with minimum pO_2, maximum pCO_2, and maximum temperatures measured in leatherback nests. Thus, leatherback embryos collectively affected their nest environment (i.e. decreased pO_2, increased pCO_2, and temperature), and appeared to show developmental sensitivity to low pO_2 and high levels of pCO_2 and temperature in nests. Our study shows the importance of considering sea turtle nest environment conditions when designing and executing beach-based conservation strategies such as egg relocation programs.

KEY WORDS: Leatherback turtle · Nest environment · Hatching success · Egg relocation

INTRODUCTION

Nest environmental conditions that affect sea turtle embryonic development can vary within and among nesting beaches according to geographic location, weather, human interactions, and sand characteristics (Mortimer 1990, Ackerman 1997, Wallace et al. 2004). In addition, biological factors, such as number of eggs (and thus developing embryos) per clutch and rates of embryonic oxygen consumption and carbon dioxide (CO_2) production can vary within and among sea turtle egg clutches (Ackerman 1997, Miller 1997, Ralph et al. 2005). Abiotic factors in sea turtle nests, such as respiratory gas concentrations and temperature, can influence embryonic development and hatching success (for review, see Ackerman 1997).

Gas exchange in sea turtle nests occurs primarily by diffusion through the surrounding sand to reach incubating eggs (Ackerman 1972) and can influence embryonic development (Ackerman 1980). Due to the low gas conductance of sand, diffusion of oxygen into and CO_2 out of sea turtle nests occurs more slowly than these gases are consumed and produced, respectively. Thus, as oxygen consumption and CO_2 production by developing sea turtle embryos increase rapidly during the second half of incubation, the partial pressures of

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oxygen (pO_2) and CO_2 (pCO_2) decrease and increase, respectively, in nests (Ackerman 1981). This trend has been reported in nests of several sea turtle species across several study sites (e.g. Ackerman 1977, Maloney et al. 1990, Wallace et al. 2004, Ralph et al. 2005).

Temperature within nests influences incubation duration and determines sex of hatchlings (Mrosovsky 1978, Yntema & Mrosovsky 1980, 1982, Mrosovsky & Yntema 1997, Broderick et al. 2000). Both the proportion of male hatchlings and incubation duration typically decrease with increased nest temperature in sea turtle nests (Godfrey et al. 1996, Ackerman 1997). Additionally, hatching success in sea turtles and other turtle species can be reduced due to incubation at extremely high temperatures (Packard & Packard 1987, Spotila et al. 1994, Santidrián Tomillo et al. 2009).

Average hatching success of leatherback turtle *Dermochelys coriacea* egg clutches (~40 to 60%) is the lowest among all sea turtle species (Miller 1997, Eckert et al. in press), but the causes of this phenomenon remain unclear. Bell et al. (2004) reported that low hatching success was not due to infertility in leatherback clutches at Playa Grande, Costa Rica, thus implicating other intrinsic factors (e.g. maternal identity, developmental factors, genetics), or extrinsic factors, such as nest environment conditions. However, hatching success was not related to decreased oxygen levels in leatherback nests at Playa Grande, likely due to ventilation of nests by tidally induced fluctuations in the water table (Wallace et al. 2004). Whether nest conditions contribute to low average leatherback hatching success at other sites is unknown.

In some cases, conservation efforts on nesting beaches directed at increasing early stage (i.e. eggs and hatchlings) survivorship can contribute to population increases (Dutton et al. 2005, Chaloupka et al. 2008). Relocation of sea turtle eggs (hereafter referred to as egg relocation) from areas that are in danger of erosion, poaching, or predation is a widespread management practice in sea turtle conservation. Eggs are collected during or immediately after oviposition and are relocated to a natural area (e.g. Boulon 1999) or to a hatchery (e.g. Mortimer 1999) on the same beach. However, several potential problems associated with egg relocation, such as reduced hatching success, sex ratio biases, manipulation of natural nest site selection patterns by nesting females, and inappropriate use of conservation resources (i.e. funding, personnel), must be considered to optimize the conservation benefits of this practice (Yntema & Mrosovsky 1980, Grand & Beissinger 1997, Lutcavage et al. 1997, Rees & Margaritoulis 2004, Mrosovsky 2006, Pintus et al. 2009). Considering that many of the potential effects of egg relocation mentioned above are influenced by incubation conditions in the nest, abiotic and biotic effects on the nest environment must be taken into account when designing and executing an egg relocation program.

The leatherback nesting colony at Sandy Point National Wildlife Refuge (SPNWR) in St. Croix, US Virgin Islands, has been the subject of a long-term conservation and research program (Dutton et al. 2005). SPNWR is a high-energy beach characterized by seasonal erosion that can result in annual loss of 45 to 60% of all clutches laid (Eckert et al. 1982, Eckert & Eckert 1983). Due to this high likelihood of clutch loss, researchers relocate egg clutches in danger of erosion to locations on the beach that ensure safe incubation. In fact, increased hatchling production resulting from relocation of eggs from erosion-prone areas appears to have contributed to the exponential increase in nesting adult leatherbacks documented over the past 2 decades at SPNWR (Dutton et al. 2005). Relocated egg clutches had reduced hatching success compared to *in situ* egg clutches at St. Croix (Eckert & Eckert 1990), but conditions of nest environments were not reported.

We characterized the influence of nest environmental conditions on hatching success of relocated leatherback eggs during the 2007 nesting season at SPNWR. Specifically, our goals were to determine the degree of variation in the hatching success of relocated leatherback clutches and to examine relationships between hatching success and nest conditions affecting clutches relocated to different sites at SPNWR.

MATERIALS AND METHODS

Study site. We conducted this study during the 2007 leatherback nesting season (April to August) at SPNWR (Fig. 1). Patrols occurred within the entire 3.0 km long nesting area for leatherbacks within the SPNWR. All experimental clutches were collected from leatherbacks that were nesting at Site B (see below for site descriptions), in areas at imminent risk of consistent tidal inundation and erosion. Clutches were collected in a new plastic bag during oviposition, and entire clutches (including shelled albumen gobs; Sotherland et al. 2003) were then buried within 2 h of deposition at 3 suitable areas. Artificial nests were constructed according to specific shape and dimensions: 30 to 35 cm wide at the neck of the nest and 70 to 75 cm deep in a boot-like shape (Dutton et al. 1992).

About half of the patrolled beach is on the leeward (north-facing) side of SPNWR and features a wide sandy beach known as the 'sandy side.' The windward side of the beach (south-east facing), termed the 'grassy side,' exhibits heavy wave action and relatively large amounts of debris. Two of the 3 study sites (i.e. A and B) were located on the sandy side. Site A was located in the accretion zone of the sandy side, where

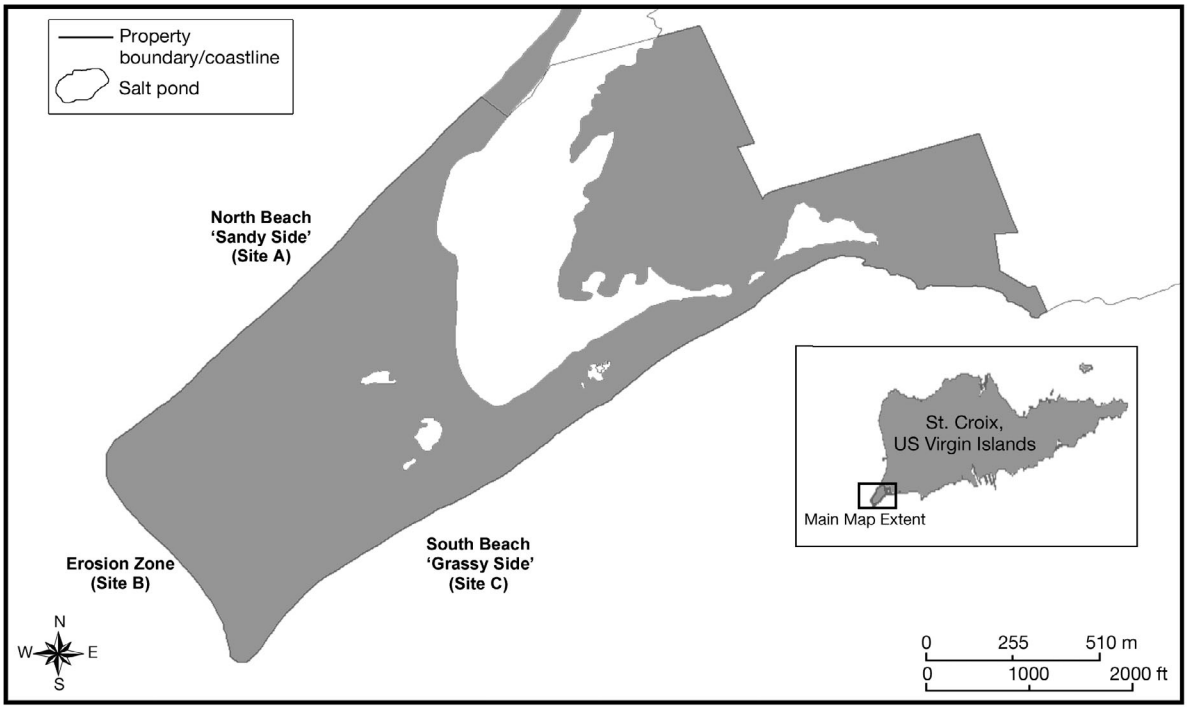

Fig. 1. Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Labeled sites correspond to sites to which experimental clutches were relocated, as described in 'Materials and methods'. Site A: 'Sandy side' or North Beach; Site B: 'Erosion zone'; Site C: 'Grassy side' or South Beach. Numbers (e.g. 157) indicate stake numbers placed at 20 m intervals

a large amount of sand is deposited as the nesting season progresses. It has little vegetative cover and is the area to which most clutches are relocated during the nesting season (Garner & Garner 2007). Site B was located in the erosion zone on the sandy side, which is characterized by sand erosion as the summer months progress, due to prevailing longshore current dynamics. In addition, this is the site where the majority of nesting activity occurs (Garner & Garner 2007). Site B is characterized by heavy vegetative cover and reduced open sand areas, and most clutches are relocated from this area during the nesting season. Site C was located on the grassy side, which is characterized by higher amounts of silt and organic materials than the other sites. This site typically hosts a very small number of nesting leatherbacks; e.g. 2.4% of *in situ* clutches in 2007.

Measurements of nest conditions. At each of the 3 beach relocation sites, nest environmental conditions were monitored in 8 relocated clutches and 1 'control' nest, which had the same dimensions as other nests, but contained hollow plastic spheres instead of eggs. Each nest was spaced at least 1 m from adjacent nests (distance measured between centers of nests) to eliminate inter-nest influence on nest conditions during incubation (Wallace et al. 2004). Measurements of pO_2 and pCO_2 in experimental nests followed the protocol of Wallace et al. (2004) and Ralph et al. (2005). Briefly, before the nest was filled with sand, we placed a sampling port consisting of a perforated hollow plastic film container into the center of each clutch. The sampling ports were connected to plastic tubing running up the shaft of the nest to the sand surface to allow gas sampling without physically disturbing the egg clutches. Each sampling port also contained a 24-gauge Cu/Cn thermocouple (Omega Engineering) to record nest temperatures along with gas concentrations throughout incubation. An oxygen sensor and a CO_2 analyzer were used to measure partial pressures of both gases in samples of nest air drawn from the sampling port using a DC pump system (Qubit Systems). Air was circulated through the sampling apparatus in the following order: (1) from the nest through a Drierite column to remove water vapor, (2) through the CO_2 sensor, (3) through a soda lime column to remove CO_2, (4) to the oxygen analyzer, and (5) back into the nest. Raw data were analyzed using Logger Pro Software 2.2.1 (Qubit Systems). The sampling duration was approximately 3 min $nest^{-1}$ with at least 1 min periods of sampling ambient air to allow the system to recalibrate between nests. The partial pressures of respiratory gases and temperatures were measured no more frequently than once every other day to minimize sampling effects on nest environmental conditions (e.g. bulk flow effects;

Wallace et al. 2004). At the same time nest gases were sampled (typically in the late afternoon), we recorded nest temperatures using a BAT-12 thermocouple meter (Physiotemp), or, in some nests, temperatures were recorded using HOBO H20-001 and U22-001 dataloggers (Onset Computer Corporation). Thus, the temperature data that we present here are individual daily (late afternoon) measurements of nest temperatures, made by either instrument. However, due to instrument failures, we were unable to obtain temperature measurements for 4 nests (1 at Site A, 2 at Site B, and 1 at Site C). Thermocouples and dataloggers were calibrated in ice baths and at room temperature prior to being placed in nests to ensure comparable temperature measurements. We also measured moisture content and sand particle size in experimental clutches, but neither factor varied significantly across sites or was significantly related to hatching success (data not shown).

We excavated nests within 2 d of hatchling emergence to determine hatching success of the clutches. We counted hatched eggs and opened unhatched eggs to assess embryonic development according to staging procedures determined by Whitmore & Dutton (1985). Hatching success was determined using the following formula: hatched shells/total yolked eggs laid × 100 (Eckert & Eckert 1990, Garner et al. 2005).

To remove the confounding effects of female identity and clutch sizes on response variables (e.g. minimum pO_2, maximum pCO_2, maximum temperature measured in each nest during incubation), we used residuals of linear intercept models, treating maternal identity and clutch size as explanatory variables in the analyses, unless otherwise noted. One-way analysis of variance (ANOVA) and Tukey's post hoc comparison test were used to determine any significant differences in hatching success and environmental factors across sites. We used linear and non-linear regression analyses to characterize relationships between environmental factors and hatching success among leatherback clutches. Although we used residuals in the analyses, figures present observed data. All means are reported ± SD.

RESULTS

Hatching success

Hatching success of all relocated clutches, including experimental clutches, (39.7 ± 21.3%, n = 83) was significantly lower than that of *in situ* clutches (46.7 ± 22.2%, n = 257; p = 0.01). However, hatching success of experimental clutches considered separately (59.5 ± 20.5%, n = 24) was higher than hatching success of *in situ* clutches (p = 0.009), as well as hatching success of all other relocated clutches (36.2 ± 21.5%, n = 59; p < 0.0001).

Hatching success of experimental clutches was extremely variable across sites (Table 1); coefficients of variation (CVs) ranged from 26.5 to 56.6%. However, there were no significant differences in hatching success of experimental clutches among the 3 research sites ($F_{2,22}$ = 0.722, n = 24 clutches, p = 0.497).

Nest gas concentrations

pO_2 decreased and pCO_2 increased during incubation for all metabolizing nests (Fig. 2A). Minimum pO_2 in leatherback nests during incubation ranged from 11.3 to 19.5 kPa, and was lower than control nests by 2.4 (Site A) to 4.5 kPa (Site B; Table 1). Minimum pO_2 varied significantly ($F_{2,22}$ = 8.445; p = 0.002) across sites, with significant differences between Sites A and C (p = 0.039) and Sites B and C (p = 0.001), but not

Table 1. Nest environment parameters measured in leatherback turtle nests at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Data are means ± 1 SD (range), for 8 experimental nests and 1 control nest, respectively, at each site (24 total for partial pressures of oxygen, pO_2, and carbon dioxide, pCO_2, 20 total for temperature: n = 7 at Sites A and C, n = 6 at Site B). See 'Materials and methods' for details on individual parameters and methodology

Site	Hatching success (%)	Min. pO_2 (kPa)	Min. pO_2 (kPa) control	Max. pCO_2 (kPa)	Max. pCO_2 (kPa) control	Max. temp. (°C)	Max. temp. (°C) control
A	64.8 ± 17.2 (28.8–84.3)	16.2 ± 1.4 (13.8–18.1)	18.6	4.3 ± 0.8 (3.2–5.7)	2.3	34.2 ± 1.6 (30.9–36.4)	32.5
B	61.7 ± 11.3 (45.2–76.6)	13.7 ± 1.8 (11.3–16.7)	18.2	6.4 ± 1.2 (4.3–8.0)	2.4	34.6 ± 0.6 (32.3–35.3)	32.4
C	51.5 ± 29.2 (15.2–91.3)	17.1 ± 1.5 (14.8–19.5)	20.2	4.1 ± 1.2 (2.1–5.5)	1.5	34.0 ± 1.3 (32.5–35.7)	32.3
Overall	59.1 ± 20.8 (15.2–91.3)	15.7 ± 1.8 (11.3–19.5)	19.0	4.9 ± 1.3 (2.1–8.0)	2.1	34.5 ± 1.1 (30.9–36.4)	32.4

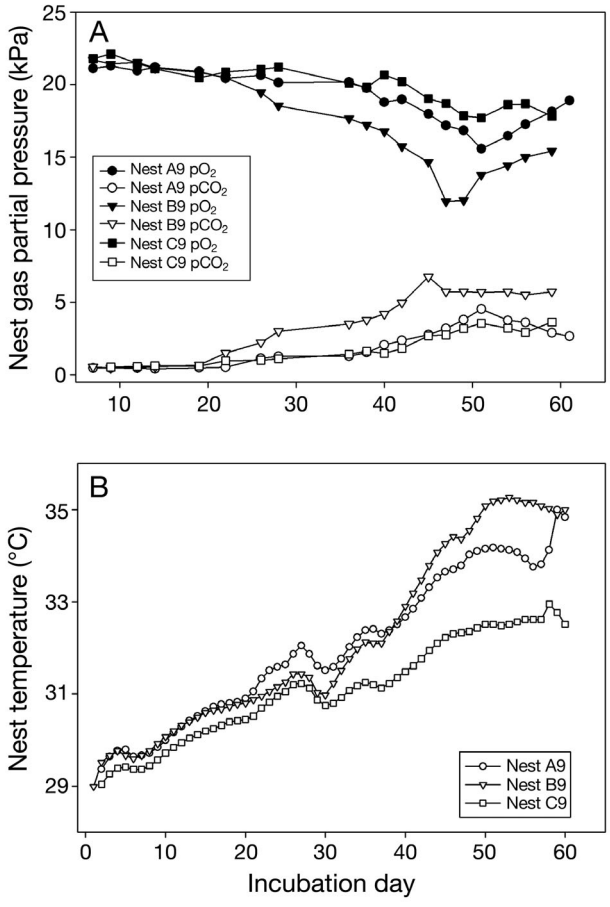

Fig. 2. *Dermochelys coriacea*. (A) Partial pressures of oxygen (pO_2; filled symbols) decreased, and carbon dioxide (pCO_2; open symbols) and (B) nest temperatures increased as incubation progressed in leatherback turtle nests. One nest from each site is shown: Nest A9 from Site A (○; hatching success = 46.1%), Nest B9 from Site B (△; hatching success = 60.2%), and Nest C9 from Site C (□; hatching success = 42.9%) at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Hatchling emergence occurred on the last day shown for each nest (incubation day 60 for A9 and C9, day 62 for B9)

between Sites A and B ($p > 0.05$; Table 1). We found a significant linear negative correlation between hatching success and minimum pO_2 among experimental nests ($r^2 = 0.378$; $p = 0.001$), but a quadratic curvilinear equation resulted in a better fit ($r^2 = 0.481$; Fig. 3A).

Maximum pCO_2 in nests during incubation ranged from 2.1 to 8.0 kPa, was higher than control nests by 2.0 to 3.9 kPa (Table 1), and varied significantly among sites ($F_{2,22} = 7.685$; $p = 0.003$), with significant differences between Sites A and B ($p = 0.007$) and Sites B and C ($p = 0.007$), but not between A and C ($p > 0.05$; Table 1). There was a positive linear correlation between maximum pCO_2 and hatching success across all sites ($r^2 = 0.355$, $p = 0.002$), but the fit was better described by a quadratic curvilinear equation ($r^2 = 0.509$; Fig. 3B).

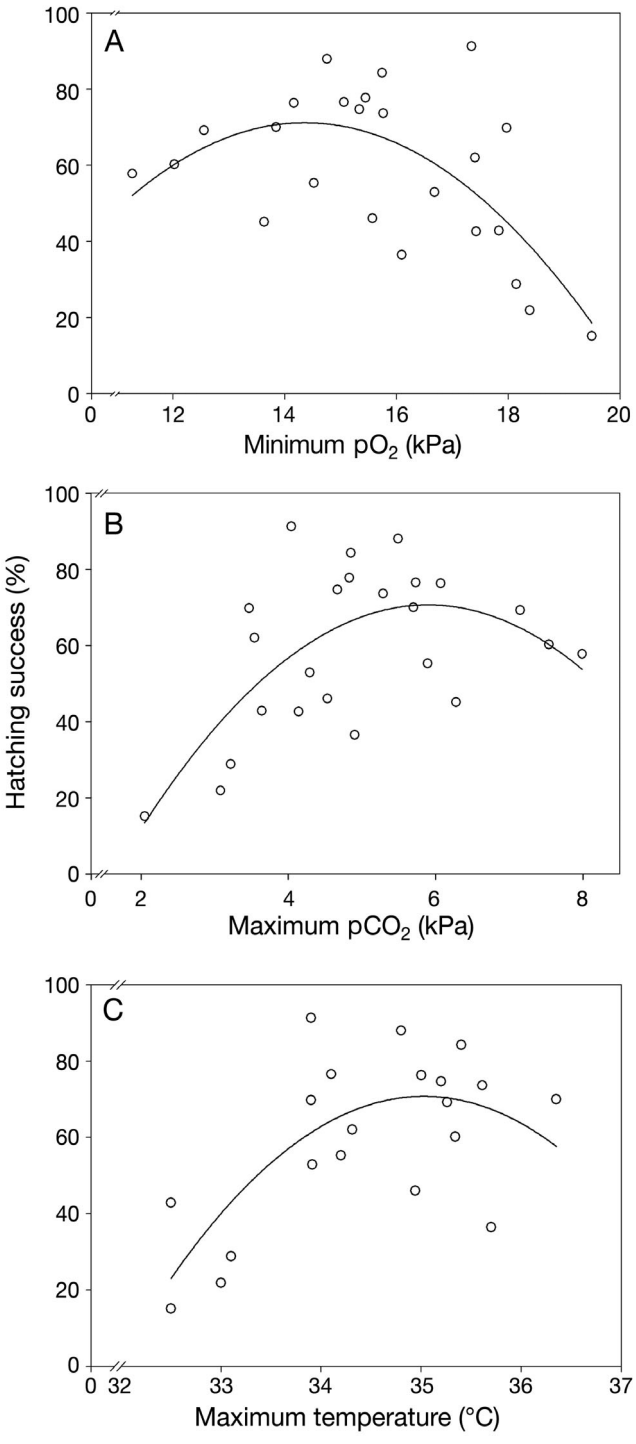

Fig. 3. *Dermochelys coriacea*. Hatching success of leatherback turtle clutches related to (A) minimum partial pressure of oxygen (pO_2; $r^2 = 0.481$, n = 24 nests), (B) maximum partial pressure of carbon dioxide (pCO_2; $r^2 = 0.509$, n = 24 nests), and (C) maximum temperature ($r^2 = 0.589$; n = 20 nests) values measured in leatherback nests at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Data shown are observed values, but analyses were performed on residual values, following fit of intercept models (female and clutch size effects removed). Each data point represents a single nest

Nest temperatures

Temperature in leatherback nests increased throughout incubation and peaked shortly before emergence (Fig. 2B). Maximum nest temperatures during incubation ranged from 30.9 to 36.4°C, were higher than control nests by 1.7 (Sites A and C) to 2.2°C (Site B; Table 1), and did not vary significantly among sites ($F_{2,19}$ = 0.305, p = 0.741; Table 1). Maximum temperature was inversely related to hatching success at Sites A (r^2 = 0.86; p = 0.012), and B (r^2 = 0.93; p = 0.006), but not at Site C (r^2 = 0.50; p = 0.258). There was a negative linear correlation between maximum temperatures and hatching success (r^2 = 0.417; p = 0.002) when considering all clutches across sites, but a quadratic curvilinear equation resulted in a better fit (r^2 = 0.589; Fig. 3C).

DISCUSSION

Careful monitoring of environmental conditions in nests can provide insights that could lead to improved conservation practices to maximize hatchling production while avoiding detrimental effects on natural embryonic development (e.g. Pintus et al. 2009). In our study, clutches left *in situ* had significantly higher hatching success than relocated clutches, and inter-clutch variation in hatching success among females was high (Table 1), corroborating previous reports for the SPNWR leatherback rookery and for leatherbacks generally (Eckert & Eckert 1990, Dutton et al. 1992, Garner & Garner 2007, Wallace et al. 2007, Eckert et al. in press). Similar to results from previous studies (Wallace et al. 2004, Ralph et al. 2005), developing embryos collectively affected their nest environments, as nest gas concentrations and temperatures varied significantly with embryonic development (Figs. 2 & 3). Overall, our results suggest that conditions of nest environments for different relocation sites at SPNWR appear to be relatively moderate and within the range of tolerance for developing leatherback embryos. Given that clutch loss due to natural erosion would exceed 50% in the absence of egg relocation, and the lack of significant variation in hatching success across study sites, we conclude that the egg relocation program at SPNWR continues to be an effective tactic to produce hatchlings for this leatherback rookery.

As previous studies have reported, relocated clutches (including experimental clutches) had lower hatching success than did *in situ* clutches (Eckert & Eckert 1990, Pintus et al. 2009, Eckert et al. in press). The *in situ* hatching success value included clutches affected by tidal erosion and inundation, as well as other factors (Garner & Garner 2007), which might have contributed to this result. However, hatching success of experimental clutches was significantly higher than that of other relocated clutches and *in situ* clutches. Although there is no clear explanation for this finding, it might suggest differences in handling and artificial nest construction of experimental clutches when compared to other relocated clutches, or some potential effect of our sampling protocol. Average hatching success of all clutches was similar to that reported in previous studies at SPNWR (Eckert & Eckert 1990, Alexander et al. 2002) and beaches in other parts of the world (Santidrián Tomillo et al. 2009, Eckert et al. in press). Low hatching success in leatherback clutches is not due to infertility (Bell et al. 2004), which implicates other intrinsic factors, such as maternal identity, developmental genetics, or extrinsic factors, such as nest environment.

Hatching success of leatherback clutches was variable, though not significantly so, among beach sites (Table 1). Minimum pO_2 was lowest and maximum pCO_2 was highest in clutches incubating at Site B, and high levels of pCO_2, but not low levels of pO_2, were negatively correlated with hatching success at SPNWR. It is important to note that Site B was located in the 'erosion zone' of SPNWR, the area from which most egg clutches are relocated during the nesting season due to high frequency of erosion (Eckert & Eckert 1983). This is also the area with the heaviest vegetative cover, which might have affected nest environments in this area. Therefore, our conclusions support the current relocation program, which involves relocating clutches from Site B – due to high erosion and high incidence of nest loss there – to Site A, which exhibited the highest mean hatching success among sites.

Oxygen levels decreased and CO_2 levels and temperatures increased in nests as incubation progressed (Fig. 1), corroborating previous studies that also demonstrated the ability of developing sea turtle embryos to collectively affect the environmental conditions in the nest (Ackerman 1997, Wallace et al. 2004, Ralph et al. 2005). Changes in respiratory gas levels in leatherback nests as incubation progresses might influence patterns in the timing of embryonic death. Embryonic development and growth are most sensitive to changes in respiratory gas concentration during the second half of development (Ackerman 1981), and embryonic mortality at SPNWR commonly occurs in late stages of development (Eckert & Eckert 1990). This is contrary to the pattern observed in leatherback clutches at Playa Grande, Costa Rica, where most mortality occurs at the early embryonic stages (Bell et al. 2004). Ralph et al. (2005) showed that late-stage embryonic death at Playa Grande was not related to respiratory gas levels in the central region of the nest,

where pO_2 was lowest and pCO_2 was highest. Similarly, in multiple studies, low nest pO_2 was not significantly related to hatching success, despite an inverse relationship between minimum pO_2 and number of metabolizing embryos (Ackerman 1977, Maloney et al. 1990, Wallace et al. 2004).

For leatherback embryos incubating at SPNWR, we found significant curvilinear relationships between hatching success and all 3 nest conditions that we measured (Fig. 3). Thus, pO_2 decreased whereas pCO_2 and temperature increased with the number of metabolizing embryos in a nest until pO_2 fell below 14 to16 kPa, pCO_2 exceeded roughly 5 to 6 kPa, and temperatures exceeded ~35°C, after which point hatching success began to decrease. These results suggest that a depletion of O_2 and a buildup of CO_2 (and increased temperature) in and around nests was due to elevated embryonic development toward the end of incubation by increasing numbers of metabolizing embryos. However, levels of pO_2, pCO_2, and temperatures in nests below (in the case of pO_2) and/or above (pCO_2 and temperature) these putative threshold values could be causing late stage embryonic mortality that drives hatching success patterns at SPNWR. Similarly, Balasingam (1967) reported that dividing leatherback clutches into 2 separate nests resulted in increased hatching success over entire clutches, suggesting that smaller clutches experience higher pO_2 and lower pCO_2 (and temperature) levels, perhaps resulting in increased hatching success.

To explore these patterns further, we regressed late stage embryonic mortality (measured as the number of embryos that survived to late developmental stages but died before hatching, as quantified during nest excavations) against minimum pO_2, maximum pCO_2, and maximum temperature values across all clutches. Late stage embryonic mortality correlated negatively with minimum pO_2 ($r^2 = 0.471$, $p < 0.001$; Fig. 4A) and positively with maximum pCO_2 ($r^2 = 0.385$, $p = 0.001$; Fig. 4B) and maximum temperature ($r^2 = 0.519$, $p < 0.001$; Fig. 4C). Therefore, although nest conditions only explained between ~40 and 50% of variation in late stage embryonic mortality, developing leatherback turtle embryos collectively affect gaseous and thermal environmental conditions within their nests, but appear to be sensitive to decreased pO_2 and/or increased pCO_2 and temperature, which could result in increased late stage embryonic mortality, as reported or suggested in previous studies of these factors (Ackerman 1981, Thompson 1993, Godley et al. 2001, Matsuzawa et al. 2002, Santidrián Tomillo et al. 2009). Whether similar relationships between nest conditions and hatching success exist at other nesting sites for leatherbacks and other sea turtle species merits further investigation.

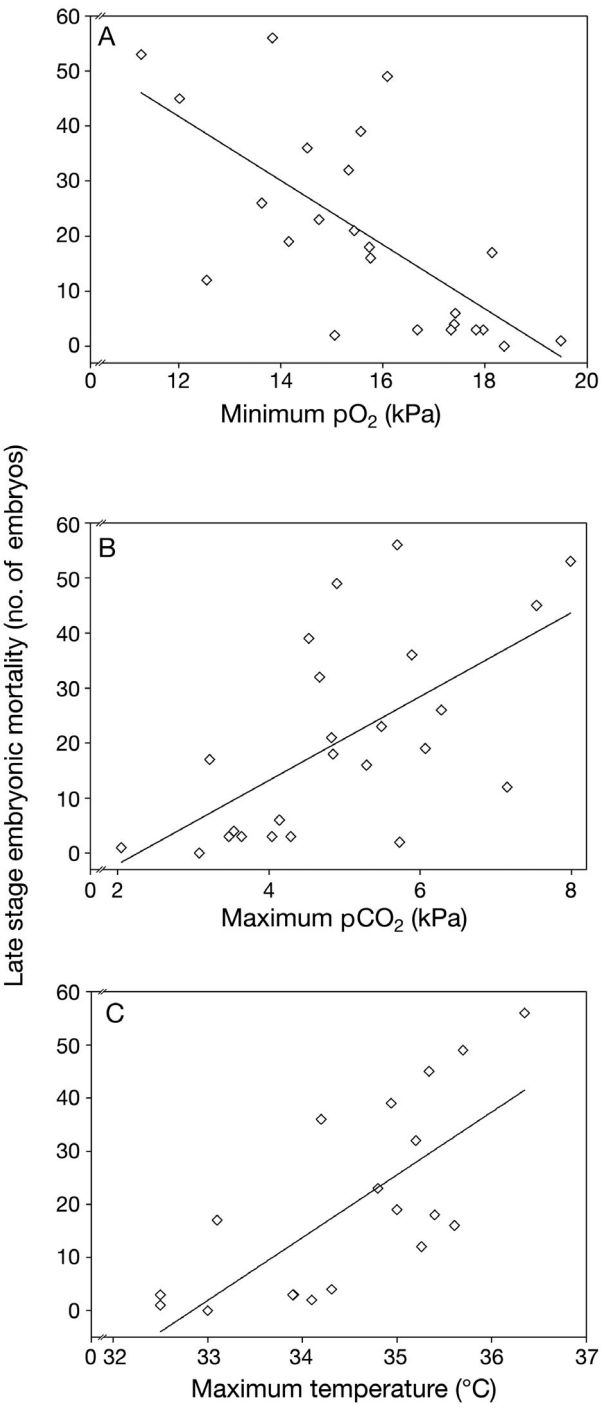

Fig. 4. *Dermochelys coriacea*. Late stage embryonic mortality correlated negatively with (A) minimum partial pressures of oxygen (pO_2; $r^2 = 0.471$, n = 24) and positively with (B) maximum partial pressures of carbon dioxide (pCO_2; $r^2 = 0.385$, n = 24) and (C) temperatures ($r^2 = 0.519$, n = 20) measured in leatherback nests at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Data shown are observed values, but analyses were performed on residual values, following fit of intercept models (female and clutch size effects removed). Each data point represents a single nest

Variation in sand composition and vegetation could have caused differences in environmental parameters that we measured in leatherback nests. Sand particle size is known to influence all of the parameters that we measured (Ackerman 1980, Speakman et al. 1998). Mortimer (1990) found that embryonic mortality is positively correlated with mean particle size, suggesting that conditions within nests in coarser sand are detrimental to embryonic development. Although preliminary data on sand particle size at SPNWR did not reveal a significant relationship with hatching success (Site A: $p = 0.992$; Site B: $p = 0.473$; Site C: $P = 0.550$; across all sites: $p = 0.593$), further research is necessary to determine if factors such as silt and organics and the presence and abundance of vegetation influence the clutch environment and/or hatching success at SPNWR.

In this study, we found intra-beach variation in nest gas concentrations and temperatures, as well as relationships between these variables and hatching success at SPNWR. In particular, we measured the lowest pO_2 and highest pCO_2 values at Site B, and these variables were significantly correlated to hatching success across clutches. Based on these findings, we conclude that the current strategy for relocating eggs at SPNWR from Site B (erosion zone) to Site A (accretion zone) is justified as a conservation practice, particularly in light of the high degree of potential egg loss by leaving eggs *in situ* at Site B. Correlations between nest environmental conditions and patterns of embryonic mortality at SPNWR warrant further study of these factors at other nesting beaches to explain variation in hatching success among leatherback populations.

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