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Assessment of Hatching and Emergence Success, Developmental Phases, and Pathology of Leatherback (*Dermochelys coriacea*) Embryos and Dead-in-Nest Hatchlings on St. Croix, US Virgin Islands

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ABSTRACT. – **Northwest Atlantic leatherback (*Dermochelys coriacea*) sea turtle populations are endangered and have low hatching success compared to other sea turtles. Hatchling survival is an important element of their conservation. This longitudinal study assessed developmental phase and pathology of leatherback embryos and hatchlings at Sandy Point National Wildlife Refuge (SPNWR) on St. Croix, US Virgin Islands, in 2019 to identify patterns in mortality and lesions across a nesting season, and to make regional comparisons. Hatching and emergence success averaged 63.6% and 56.6%, respectively, and both differed significantly by month. 'Breakout' analysis was conducted on 41 nests and showed a preponderance of unhatched eggs lacking grossly evident embryological development (52%). Necropsies were performed on 79 unhatched and dead in nest individuals from 34 nests, and most (58%) had lesions including inflammation associated with microorganisms (34%), renal mineralization (15%), mild multifocal skeletal muscle degeneration and necrosis (5%), and anatomic malformations (4%). Inflammatory lesions included chorioallantoitis, esophagitis, stomatitis, dermatitis, gastritis, and yolk sacculitis. These were associated with bacteria ($n = 13$), fungi ($n = 4$), or both ($n = 7$). Sex was determined histologically and was predominantly female (90%) with no males identified in nests laid after 3 April 2019. Although hatching success was higher in in situ relative to relocated nests, embryological development and lesion patterns were similar in both groups. Patterns of lesions observed in leatherback embryos and hatchlings did not differ across the season and are comparable to other Caribbean nesting sites. Future studies pairing 'breakout' and pathological analyses with assessments of potentially influential environmental and/or maternal factors could help develop targeted strategies for improving hatchling production.**

KEY WORDS. – ***Dermochelyidae*; disease; embryos; hatchlings; hatching success; emergence success; pathology; St. Croix**

Leatherback (*Dermochelys coriacea*) sea turtles are keystone species and bioindicators in the marine environment. Global leatherback populations are declining at a considerable rate worldwide (IUCN 2022). Since 2008, there has been a regional decline of nesting leatherback females and nests around the Caribbean; locations include but are not limited to French Guiana; Grenada; St. Kitts and Nevis; Costa Rica; and St. Croix, US Virgin Islands (Northwest Atlantic Leatherback Working Group 2018). It is estimated the population has decreased by ~67% as a result of fisheries bycatch, plastic pollution, poaching, and habitat loss (Pritchard 1982; Spotila et al. 1996; Sarti Martínez et al. 2007; Perrault et al. 2011; Eckert et al. 2012).

Leatherbacks have the lowest hatching and emergence success of all sea turtle species, with the majority of studies reporting less than 50% hatching success (Chan et al. 1985; Whitmore and Dutton 1985; Girondot et al. 1990; Boulon 1993; Arauz and Naranjo 1994; Chan and Liew 1995; Bell et al. 2003) and ~41% emergence success (Wallace et al. 2007). In comparison, other Caribbean sea turtle species such as hawksbills (*Eretmochelys imbricata*) have a hatching success of ~78.6% (Ditmer and Stapleton 2012) and emergence success of ~63% (Gulick et al. 2022), greens (*Chelonia mydas*) with ~84.2% (Broderick and Godley 1996) and ~45.6% (Zárate et al. 2013), respectively, and loggerheads (*Caretta caretta*) with ~79.1% (Broderick and Godley 1996) and ~63% (Peters et al. 1994), respectively. Emergence success is usually lower than hatching success because not all hatchlings will make it to the beach surface from the nest cavity.

With growing evidence that adult populations are decreasing, and fewer nests are being produced, the low hatching and emergence success of leatherback hatchlings means that future recovery of the population is in doubt. To combat this, increasing hatchling production is a key strategy used by sea turtle projects such as the St. Croix Sea Turtle Project at Sandy Point National Wildlife Refuge in the US Virgin Islands and is used as part of their management plan (Evans 2010). Nest protection methods include close monitoring for potential threats such as poaching, predation, and inundation during the incubation period and relocating nests from high-risk areas to low-risk areas. These methods are used to mitigate loss of whole nests and to increase numbers of hatchlings that reach the water (Dutton et al. 2005; Chaloupka et al. 2008; Evans 2010). More strategies are needed to address causes of in-nest and in-egg mortality. Nest incubation temperatures above 29°C have been linked to low hatching success (Howard et al. 2014), and humidity above 8% in green sea turtles (*Chelonia mydas*) nests was shown to reduce hatching success significantly (Yalçin Özdilek et al. 2007). Much of the literature addresses abiotic factors on hatching success, but there is little literature addressing diseases that may be fatal to embryos and hatchlings in the nest.

Opening unhatched eggs to determine phase of development and pathological examination of dead embryos and dead hatchlings is a basic step to understanding poor hatching success in egg-laying vertebrates (Mauldin 2009). Studies conducted on St. Kitts and Grenada demonstrated low leatherback hatching success (18.7% and 30%, respectively), a preponderance of unhatched eggs lacking gross signs of development (no gross signs of development [NGSD]; 45.4% and 60%, respectively), and some degree of embryonal and in-nest hatchling mortality (early 31.4% and 8.3%, late 8.6% and 24%, hatchling 0.95% and 5%; Hill et al. 2019; Choi et al. 2020). Lesions affected 38% and 44% of embryos and dead-in-nest hatchlings on St. Kitts and Grenada, respectively, and included Gram-negative bacterial bronchopneumonia (7.7% and 12% respectively), Gram-negative bacterial chorioallantoitis (17.2%, was seen only on Grenada), skeletal muscle degeneration and necrosis (7% and 18.8% respectively), and renal mineralization (24%, was seen only on St. Kitts; (Hill et al. 2019; Choi et al. 2020). Bronchopneumonia and skeletal muscle degeneration and necrosis were also prevalent among Floridian dead-in-nest hatchlings (Miller et al. 2009; Perrault et al. 2011). Decaying nest environment and immunosuppression are important factors potentially conducive to opportunistic microbial infections and conducive to bacterial overgrowth on the egg surface or bacterial entry through the eggshell (Hill et al. 2019; Choi et al. 2020), and handling of eggs during nest relocation has a debatable role in introducing pathogenic bacteria to a nest (Hoh et al. 2020). Vitamin E and selenium deficiency may cause skeletal muscle degeneration and necrosis (Miller et al. 2009; Perrault et al. 2011), but Dennis et al. (2020) saw no association between selenium and hatching success or presence of skeletal muscle necrosis in St. Kitts' leatherback embryos. Renal mineralization often reflects host dehydration and is potentially associated with low nest humidity (Hill et al. 2019). Moreover, the role of detrimental effects from high nest incubation temperatures has been considered for most embryonal lesions, a concern bolstered by the concurrent observation of coagulated eggs and preponderance of eggs lacking embryological development (Hill et al. 2019; Choi et al. 2020). Similar prevalence of lesions suggested that their occurrence was unlikely to explain the differential hatching success observed among the 2 eastern Caribbean populations (Choi et al. 2020), and their overall contribution to the poor hatching success observed in the species remains uncertain.

This study was conducted at Sandy Point National Wildlife Refuge (SPNWR) on St. Croix, US Virgin Islands, in 2019, a location with the longest running monitoring program for leatherback sea turtle nesting in the Caribbean, with higher numbers of nesting females than other close islands such as St. Kitts and Nevis, and a higher hatching success than the global average at 58.5% (Garner et al. 2017). We hypothesized that mortality patterns and the prevalence of lesions would contrast with those of populations with lower hatching success, and that hatching and emergence success and the frequency of lesions influenced by nest incubation temperature would vary across a season. A longitudinal assessment of developmental phase and pathology of embryos and hatchlings was conducted over the 2019 season to address this hypothesis while improving our understanding of causes of in-nest mortality and low hatching success. A secondary objective was to determine if the occurrence of certain lesions was associated with nest relocation.

METHODS

This study took place at Sandy Point National Wildlife Refuge (SPNWR) from 21 May to 9 August 2019. Night patrols were conducted from 2000 hrs to 0400 hrs to mark and relocate nests, whereas day patrols were conducted to check for hatchling emergence and perform nest excavations.

Nests were marked by GPS and triangulation. If nests were laid in a high-risk location (prone to erosion), they were relocated. Nest relocations were done in accordance with SPNWR protocols. The dimensions of the relocated nests were kept as close to the original nest dimension as possible and relocated higher up on the beach in the same area as the original nest. Nest excavations were conducted 3 days postemergence and nest contents assessed. If there were no signs of emergence, unhatched nests were excavated on day 70. Most nests will hatch around 60 days (Eckert et al. 2012), thus the 70-day mark was used to ensure enough time had passed for any viable hatchlings to hatch and to prevent disturbing nests too soon. All unhatched eggs were opened, and contents described. An egg was categorized as hatched when greater than or equal to 50% of the eggshell was present. 'Breakout' analysis (opening and examining the unhatched eggs) was completed on all whole eggs; these were opened and categorized by phase of embryo development. Categories included no gross signs of development (NGSD), early, late, pipped, and hatched (Hill et al. 2019; Choi et al. 2020). Coagulated NGSD eggs had thick, congealed to dry, lumpy or crumbly texture, and white opaque albumin or pale yellow opaque yolk. Live hatchlings found in nest were counted and were released; no sampling of them was done. Hatching success and emergence success of the nest was calculated using the following calculations established by Miller (1999):

$$\text{Hatching success} = \text{no. hatched eggs (shells)}$$

$$\text{no. shells} + \text{NGSD} + \text{unhatched eggs}$$

$$\text{Emergence success} = \text{no. shells}– \\ (\text{dead-in-nest hatchlings} + \text{live hatchlings})$$

$$\text{no. shells} + \text{NGSD} + \text{unhatched eggs}$$

Up to 3 early, 3 late, and 3 pipped eggs, and 5 dead-in-nest hatchlings were collected from each nest during the excavation process. Preference was given to embryos/hatchlings that were not decomposed as indicated by softening/maceration of tissues and slippage/scaling of skin. Samples were placed in airtight bags and stored in a cooler box with ice packs until they were necropsied. Necropsies were performed in the field laboratory between 1 and 24 hr following collection. All remaining nest contents were placed back into the egg chamber and covered with sand so that they could decompose naturally on the beach.

Necropsies were done in accordance with the protocol established by Hill et al. (2019). Tissue samples collected in this study included sections from the heart, GI tract, gallbladder, liver, lungs, kidneys, gonads, chorioallantois, and cranium. Sex was determined in individuals histologically by examining the cortical epithelial cells of the gonads as outlined by Ceriani and Wyneken (2008).

Normality of the number of coagulated NGSD eggs per nest and hatching and emerging success were examined by plotting a histogram. Kruskal-Wallis tests were used to compare median number of coagulated NGSD eggs per nest, hatching success, and emerging success by month. A Z-test was used to determine if there were significant differences in hatching success and emergence success between in situ and relocated nests. Chi-square tests were used to compare the occurrence of turtles with lesions between in situ and relocated nests and by month, and to determine if the occurrence of renal mineralization was associated with month, or if the occurrence of microorganism-associated inflammatory lesions was associated with nest relocation. Finally, a binomial exact calculation using an online calculator (Kohn and Senyak 2021) was used to determine prevalence and 95% confidence intervals for microscopic and macroscopic lesions. Significance level was set at $p = 0.05$.

RESULTS

Sixty-eight clutches were laid from 12 March to 14 June 2019. Forty-one of these nests were excavated between 22 May and 13 August 2019. Hatching success for in situ nests (n = 33) ranged from 0% to 92.9%, and for relocated nests ($n = 8$) it ranged from 10.5% to 73.2%. Mean hatching success was higher in the in situ nests (67.6%) relative to relocated nests (46.9%; $Z = 2.85$, $p = 0.004$). Mean emergence success was also higher for in situ nests at 61.6% (± 21.5%) versus relocated nests, which was 36.1% (± 14.9%; $Z = 3.42$, $p = 0.0006$). Hatching success significantly varied by month ($H = 10.84$, $p = 0.013$), being the lowest for nests laid in May. Emergence success differed significantly by month ($H = 9.07$, $p = 0.028$) with the lowest for nests laid in May. The number of coagulated NGSD eggs per nest was highest for nests laid during June but was not considered significant (Table 1; $H = 6.73$, $p = 0.08$). Coagulated NGSD eggs of varying numbers were found within 29/41 (70.7%) nests (Table 1). 'Breakout' analysis also demonstrated 2 sets of twins, which were late embryos sharing a yolk sac (Fig. 1a). The 2 sets of twins were from different nests, 1 laid in March, the other in April.

Of the 105 early embryos, late embryos, and hatchlings that were necropsied, 26 were too underdeveloped or decomposed for meaningful pathological examination and were excluded from analysis. A total of 79 from 34 nests were examined by necropsy, including 24 early embryos (30.4%), 42 late embryos (53.2%), and 13 hatchlings (16.4%; Table 1). No pipped hatchlings were collected as a result of severe postmortem decomposition. Coagulated yolk within embryonal yolk sacs were of uncertain pathological significance and were excluded from lesion counts; they were identified in 9/24 early embryos and 11/42 late embryos. Macroscopic lesions were limited to single instances of anatomic malformations (Fig. 1b): meningoencephalocele, unilateral microphthalmia, and syndactyly. A total of 49 microscopic lesions were identified in 37/79 (47%) individuals. The most common lesions were chorioallantoitis (Fig. 2a–b), renal mineralization (Fig. 2c), and mild multifocal skeletal muscle degeneration and necrosis (Tables 1, 2). Eleven of the 20 cases (55%) of chorioallantoitis had intralesional bacteria, 2 had fungal hyphae (10%), and 7 had both intralesional bacteria and fungal hyphae (35%). Other inflammatory lesions associated with microorganisms included bacterial esophagitis ($n = 2$), fungal stomatitis ($n = 2$), fungal dermatitis ($n = 1$), fungal gastritis ($n = 1$), and yolk sacculitis associated with both fungi and bacteria ($n = 1$). Inflammatory lesions associated with bacteria affected 13 turtles, those associated with fungal hyphae or yeasts affected 4 turtles, and 7 turtles had lesions associated with both bacteria and fungi. The morphologic features of fungal hyphae within inflammatory lesions were consistent, in all instances consisting of slender (4–6 μm) septated hyphae with parallel wall, and right angle

Table 1. Phase of development and lesions observed by nest month and nest location in leatherback sea turtle (*Dermochelys coriacea*) nests at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands in 2019.

	Nest month				Nest location		
	March	April	May	June	In-situ	Relocated	Total
Nests excavated	7	22	6	6	33	8	41
Mean % hatch success ± SD (range)	77.8 ± 20 (35.9–91)	65.6 ± 20.9 (0–92.9)	42.6 ± 19.7 (10.5–71.4)	60.7 ± 9.2 (50–73.2)	67.6 ± 19.8[a] (0–92.9)	46.9 ± 18[a] (10.5–73.2)	63.6 ± 21.3 (0–92.9)
Mean % emergence success ± SD (range)	66.5 ± 29.8 (6.25–90.7)	60.5 ± 21.2 (0–88.3)	38 ± 19.5 (7.5–68.8)	49.2 ± 9.2 (31–54.4)	61.6 ± 21.5 (0–88.3)	36.1 ± 14.9 (7.46–54.4)	56.6 ± 22.6 (0–90.7)
Mean % phase of development ± SD (range)							
NGSD[a]	44.4 ± 21.4 (18–80)	52.5 ± 27 (8–99)	49.3 ± 31.5 (16–93)	37.6 ± 26.4 (8–82)	49.3 ± 25.2 (8–99)	45.1 ± 32.1 (16–93)	48.5 ± 26.3 (8–99)
Early embryo	13.7 ± 17.1 (0–50)	7 ± 6 (0–18)	12.1 ± 12.2 (0–28)	6 ± 5.5 (0–16)	9.8 ± 10.5 (0–50)	4.3 ± 2.8 (0–7)	8.8 ± 9.7 (0–50)
Late embryo	14.6 ± 11.2 (0–36)	7.8 ± 12.6 (0–56)	5.5 ± 5 (0–12)	2.6 ± 2.1 (0–5)	7.3 ± 8.1(0–36)	10.2 ± 19.1 (0–56)	7.9 ± 10.9 (0–56)
Pipped and dead hatchling	7.9 ± 10.8 (0–29)	18.2 ± 25.1 (0–92)	28.1 ± 27.7 (0–67)	33.9 ± 25.1 (5–68)	18 ± 23.1 (0–77)	29.54 ± 28.3 (0–67)	20.2 ± 24.3 (0–92)
Dead-in-nest hatchling	12 ± 28.7 (0–77)	9.7 ± 15.2 (0–52)	4.4 ± 4.3 (0–10)	6.6 ± 5.7 (0–14)	9.6 ± 17.6 (0–77)	5.9 ± 5.9 (0–14)	8.9 ± 16 (0–77)
Mean % coagulated NGSD eggs ± SD (range)	10.6 ± 23.6 (0–63)	28.8 ± 24.5 (0–75)	15.9 ± 23.8 (0–57)	33 ± 19.7 (8–64)	25.6 ± 24.6 (0–75)	16 ± 21.4 (0–64)	24.42 ± 24.2 (0–75)
No. individuals necropsied	16	40	15	8	64	15	79
No. lesions observed[b] (%)	8 (50)	25 (63)	8 (53)	5 (63)	38 (59)	8 (53)	46 (58)
Anatomic malformation (%)	0 (0)	2 (5)	1 (7)	0 (0)	3(5)	0 (0)	3 (4)
Renal mineralization (%)	3 (19)	6 (15)	1 (7)	2 (25)	10 (16)	2 (13)	12 (15)
Skeletal muscle necrosis (%)	0 (0)	1 (3)	2 (13)	1 (13)	3 (5)	1 (7)	4 (5)
Inflammation due to microbial infection (%)	5 (31)	16 (40)	4 (27)	2 (25)	22 (34)	5 (33)	27 (34)
No. males[c] (%)	5 (36)	2 (5)	0 (0)	0 (0)	7 (12)	0 (0)	7 (10)

[a] NGSD = no gross signs of development.

[b] Lesions included anatomic malformations, microophthalmia, occipital meningoencephalocele and syndactyly, renal mineralization, skeletal muscle necrosis, inflammatory lesions associated with microorganisms, bacterial esophagitis, fungal dermatitis, renal tubular degeneration and necrosis, yolk sacculitis, fungal gastritis, and stomatitis.

[c] Sex determined by histological examination of gonad.

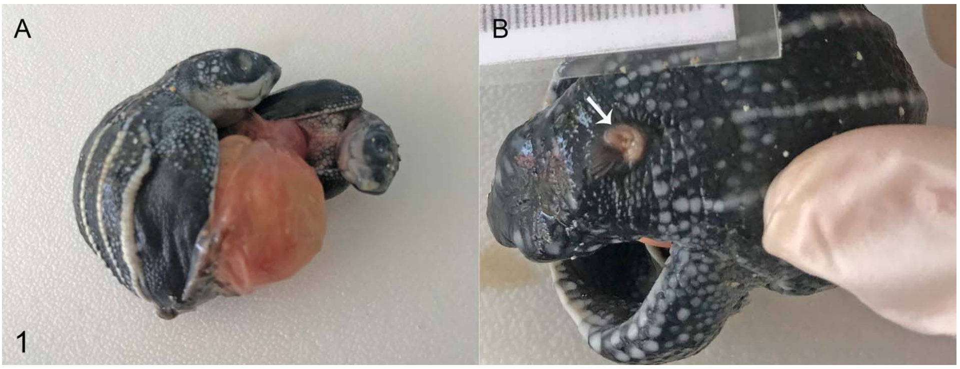

Figure 1. Gross pathology of leatherback sea turtle (*Dermochelys coriacea*) late embryos sampled from nests on Sandy Point Reserve, St. Croix, US Virgin Islands, 2019. (A) Twins. (B) Occipital meningoencephalocoele (arrow). Photos by Angela Picknell.

more frequently than acute angle branching. Renal mineralization was of varying severity, either mild ($n = 4$), moderate ($n = 3$), or severe ($n = 5$). Microscopically, coagulated yolks of embryos had foci where yolk was homogenized, dissociated into smaller particles, or hypereosinophilic, or was cleared with a basophilic rim, and contained fungal hyphae ($n = 2$; Fig. 2d), bacilli of varying size ($n = 4$), or no histologically evident microorganisms ($n = 14$).

Of nests examined for pathology, individuals with lesions were identified in 21/34 (62%), whereas no lesions were identified in individuals assessed from the remaining 13 (38%) nests. Lesions were more commonly identified in individuals from relocated nests (5/7 nests, 71%), relative to those from in situ nests (16/27, 59%), although this difference was not statistically significant ($\chi^2 = 0.34$, $p = 0.56$).

The number of individuals with microorganism-associated inflammatory lesions did not significantly vary between relocated (5/7, 71%) and in situ nests (10/27, 37%; $\chi^2 = 2.67$, $p = 0.102$). The number of embryos and hatchlings with lesions (all lesions pooled) did not vary by month ($\chi^2 = 2.89$, $p = 0.409$). The number of individuals with renal mineralization specifically also did not significantly vary by month ($\chi^2 = 1.17$, $p = 0.76$).

Sex was determined via histological examination of gonads. Gonads were sufficiently represented histologically for sex determination in 72 individuals. Of those, 65 (90%) were female and 7 (10%) were male (Fig. 2e–f). All male embryos and hatchlings were from 5 nests laid in the early months of the season: 12 March to 3 April 2019 (18 during this period were females). After 3 April 2019, all unhatched embryos and dead hatchlings examined for the remainder of the season (concluded on 13 August 2019) were female ($n = 46$).

DISCUSSION

This study longitudinally examined leatherback nests at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands, to identify patterns in mortality and lesions across a nesting season and to make regional comparisons. This study contributes information vital to establishing baseline pathology expected in populations with good hatching success. The findings help to improve our understanding of factors potentially detrimental to hatchling production with the ultimate goal of devising location-relevant conservation strategies to mitigate embryonal mortality.

Although hatching success reported in this study (63.6%) is higher than the global average of 50% (Bell et al. 2003) and considerably higher than values reported at other Caribbean sites including St. Kitts (~20%; Stewart 2023) and Grenada (~30%; Charles et al. 2023), we found that it varied significantly by month. Environmental variables that may fluctuate seasonally and be crucial to embryological development include temperature, humidity, and nest location on a beach (Garner et al. 2017). The lowest hatching and emergence success was observed in nests laid in May–June. Temperature loggers placed in nests in previous years have shown that nests laid after May–June were expected to be exposed to the hotter incubation temperatures (Sandy Point National Wildlife Refuge, pers. comm.). As a result of high incubation temperatures, it was thought all eggs laid after 3 April 2019 were female (not just the ones that were necropsied), the sex determined by higher incubation temperature (>29–30°C; Godfrey and Mrosovsky 2006). This is consistent with other studies that show nests laid during hotter times of the season will produce more females (Mrosovsky et al. 1984), but is in contrast to nests on St. Kitts where only females have been observed in nests laid from April to July (including 86 individuals analyzed from 2015–2017; Hill et al. 2019; Choi et al. 2020), suggesting consistently high incubation temperatures at that location. These observations highlight the need to more directly confirm links between site-specific differences in incubation conditions and hatching success and sex ratios on a broader regional and temporal scale.

The main basis for relatively low hatching success in Caribbean leatherbacks, as compared to other sea turtle species, appears to be a consistently high proportion of eggs lacking grossly evident embryological development. 'Breakout' analysis showed that these represented over 50% of unhatched eggs on St. Croix, similar to percentages observed on St. Kitts and Grenada (Hill et al. 2019; Choi et al. 2020), but much more frequent than observed

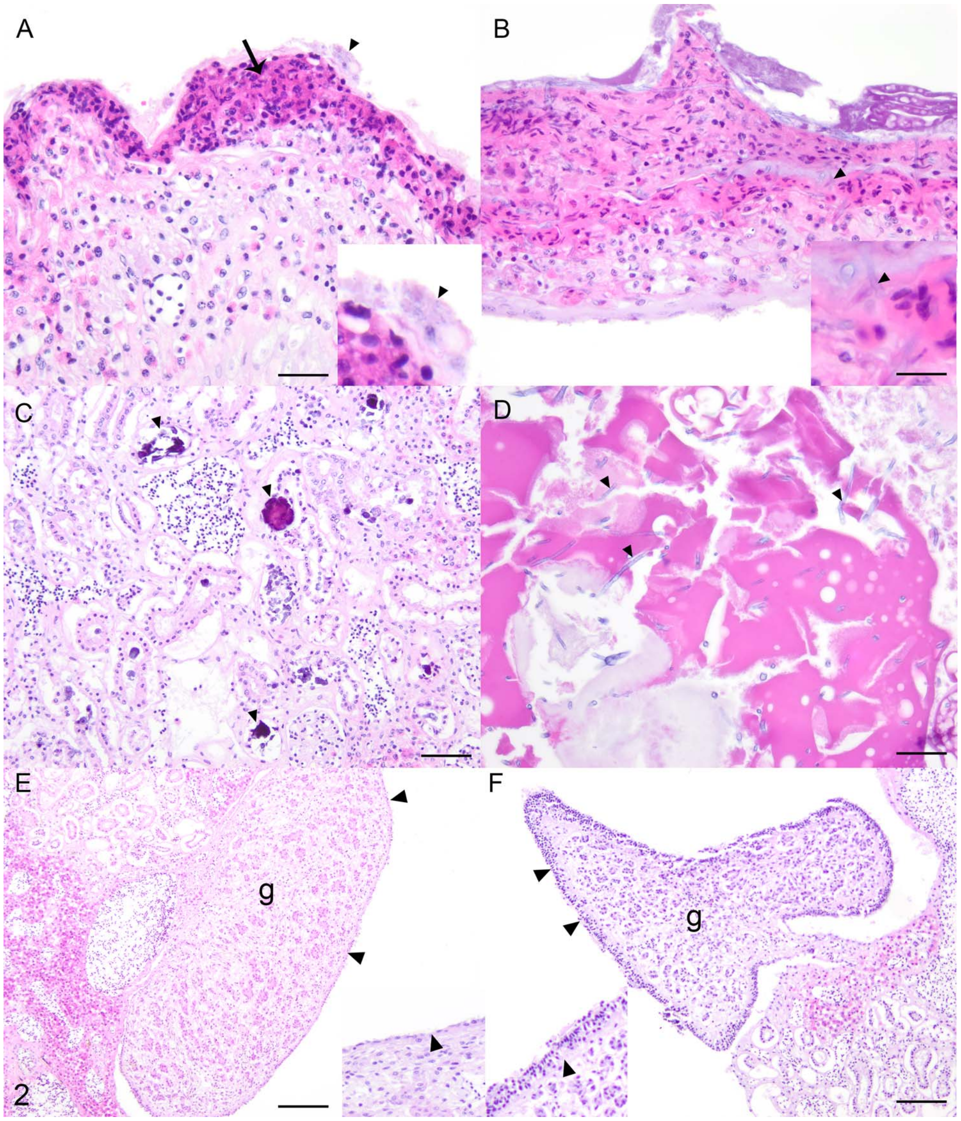

Figure 2. Microscopic findings of leatherback sea turtle (*Dermochelys coriacea*) embryos sampled from nests at Sandy Point National Wildlife Refuge, St. Croix, 2019. (A) Bacterial chorioallantoitis in a late embryo. A heterophilic pustule (arrow) has colonies of fine bacilli (arrowhead, inset). Hematoxylin and eosin (H&E). Bar = 50 µm. (B) Fungal chorioallantoitis in an early embryo. Necrotic heterophils are aggregated around fungal hyphae (arrowhead, inset). H&E. Bar = 50 µm. (C) Severe renal mineralization in an early embryo. Mineral deposits (arrowheads) are within tubular epithelium and lumina. H&E. Bar = 100 µm. (D) Coagulated yolk in an early embryo. Yolk is homogenized and contains fungal hyphae (arrowheads). H&E. Bar = 50 µm. (E) Male gonad (g) in a late embryo, as indicated by simple squamous epithelium on the outer cortex (arrowheads, inset). H&E. Bar = 100 µm. (F) Female gonad (g) in a late embryo, as indicated by tall cuboidal epithelium on the outer cortex (arrowheads, inset). H&E. Bar = 100 µm. Photos by Michelle M. Dennis.

in hawksbill nests on St. Kitts (around 20%, Mau et al. 2024). These eggs are either unfertilized or represent very early embryo mortality, with only 1 study demonstrating the latter by artificial incubation (Bell et al. 2003). Infertile eggs would indicate a much different set of causes and potential interventions than embryonic mortality (Foster 2021), and it is possible that a certain number of nonfertilized eggs is normal for the species. For example, explanations for infertility range from unsuccessful mating encounters to diseases of the gonads and reproductive tracts of the parents. In contrast, explanations for embryonal mortality involve a suite of infectious (bacteria, viruses, protists, etc.) and noninfectious (environmental stress such as temperature derangement, nutritional imbalance, toxicoses, genetic abnormalities, etc.) insults to the embryo. Although artificial incubation is not practical on a large-scale level, protocols for accurately distinguishing an infertile egg from early embryo death postincubation have been recently developed for endangered bird populations with similarly problematic hatching success (Assersohn et al. 2021). There would be much value in developing similar methods appropriate for reptile eggs, particularly for leatherbacks.

Many of the eggs lacking embryological development had coagulated albumin or yolk, such as those described in other studies (Choi et al. 2020). Consistent with the hypothesis that leatherback eggs are influenced by high

Table 2. Microscopic lesions observed in 79 leatherback (*Dermochelys coriacea*) early embryos, late embryos and hatchlings at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands, from 22 May to 13 August 2019.

			Phase of development		
Lesion	Total nests affected (prevalence; 95% CI)	Total individuals affected (prevalence; 95% CI)	Early ($n = 24$)	Late ($n = 42$)	Hatchling ($n = 13$)
Inflammation due to microbial infection[a]	15 (36.6; 22.1–53.1)	24 (34.2; 23.9–45.7)	11	12	1
Renal mineralization	8 (19.5; 8.8–34.9)	12 (15.4; 8.2–25.3)	11	1	0
Skeletal muscle necrosis	4 (9.8; 2.7–23.1)	4 (5.1; 1.4–12.6)	0	1	3
Renal tubular degeneration and necrosis	2 (4.9; 6–16.5)	2 (2.5; 3.1–8.9)	0	0	2
Anatomic malformations[b]	3 (7.3; 1.5–19.9)	3 (3.8; 0.79–10.7)	2	1	0
Multiple-organ mineralization	2 (4.9; 0.6–16.5)	3 (3.9; 0.8–10.8)	3	0	0
Total with lesions	21/41 (51.2; 35.1–67.1)	46/79 (58.2; 46.6–69.2)	20/24 (83.3; 62.6–95.3)	21/42 (50; 34.2–65.8)	5/13 (38.5; 13.9–68.4)

[a] Inflammation due to microbial infection includes bacterial or fungal chorioallantoits ($n = 20$), bacterial esophagitis ($n = 2$), fungal stomatitis ($n = 2$), bacterial and fungal yolk sacculitis ($n = 1$), fungal gastritis ($n = 1$), and fungal dermatitis ($n = 1$).

[b] Anatomic malformations includes 1 instance each of anophthalmia, meningoencephalocele, and syndactyly.

incubation temperatures, these were most commonly observed during the hottest timeframe of the study period, although not statistically significant. However, Charles et al. (2023) observed similar eggs in nests without high incubation temperatures and made parallels to inspissated chicken eggs infected with venereally transmitted viral or bacterial disease. Alternatively, these eggs may reflect a form of decomposition, but the variation in their occurrence suggests otherwise, and further research is needed to determine their significance in leatherbacks. Some embryos had similarly coagulated yolk sac, but the morphological basis for coagulation was unclear histologically. Some of the observed microscopic changes were also seen in the yolk sac of decomposed embryos lacking gross coagulation (data not shown). Less than half of the coagulated yolk sacs had histologically evident bacteria or fungi not associated with inflammatory response, indicative of post-mortem microbial growth. Although coagulated yolk may be a gross change associated with yolk sacculitis (Rodríguez et al. 2023), these observations indicate it is a nonspecific gross change and the need for caution when attributing it to microbial etiology.

The most common developmental phase observed in unhatched eggs was pipped hatchlings (mean 20%), more common than was observed on St. Kitts and Grenada (mean less than 5%) where early and late embryos were relatively more frequent (Hill et al. 2019; Choi et al. 2020). There is little information on developmental phase-specific mortality patterns for reptiles compared to birds. However, in birds, temperature, humidity, ventilation, egg shell quality, and embryo malpositioning are considerations for chick mortality during the critical period near hatching (Romanoff 1949; Assersohn et al. 2021). Making similar parallels for sea turtle embryos will probably require compiling detailed observations of incubation conditions paired with 'breakout' analysis.

Exploring the pathology of embryos can help explain poor hatching success resulting from mortality occurring in ovo. Lesions observed on St. Croix were similar to those of St. Kitts or Grenada, including chorioallantoitis, renal mineralization, and mild skeletal muscle necrosis. The similarities across studies and within a season suggest these lesions are common background issues that account for an undetermined baseline level of embryo and hatchling mortality in wild populations. To date there have not been sufficient differences in lesion distributions to explain differential hatching success among locations. Curiously, bacterial bronchopneumonia was not observed on St. Croix despite consistent observations in the Lesser Antilles (Hill et al. 2019; Choi et al. 2020; Charles et al. 2023). Regardless, these studies together document potentially common life-threatening lesions of free-living leatherback hatchlings that are highly relevant to those managing their rehabilitation.

Chorioallantoitis (prevalence range across studies: 18%–27%) has been seen in a range of development phases involving either bacteria or fungi, presumptively representing environmental microorganisms that penetrate the eggshell (Al-Bahry et al. 2009; Choi et al. 2020). It is unclear if immunosuppression or the microbial growth frequently observed in decomposing excavated leatherback nests represent important risk factors for chorioallantoitis or other inflammatory lesions involving microorganisms. However, in other reptiles, unhatched eggs (i.e., either infertile eggs or those containing dead embryos) in a clutch are believed to promote colonization by potentially pathogenic saprophytic fungi, such as *Fusarium* spp. (Moreira and Barata 2005), leading to collateral mortality in adjacent viable eggs. Of individuals necropsied in this study, 11/79 (13.9%) had inflammatory lesions associated with fungi, comparatively less frequent in embryos and hatchlings of St. Kitts or Grenada (prevalence range across studies: 0%–5%), but still accounting for a minority of mortality-associated lesions. Histologically, the fungal morphology was nonspecific, but consistent with *Fusarium* spp. among other fungi (Hoffmann et al. 2023). Although sea turtle egg fusariosis has been touted as an emerging disease threatening sea turtle nests worldwide (Gleason et al. 2020), the low prevalence of fungal lesions observed in Caribbean leatherbacks argues against this view and is more consistent with sporadic mortality caused by saprophytic and ubiquitous opportunists.

Renal mineralization (prevalence range across studies: 15%–24%) is more common among early embryos and is poorly understood as is mineral homeostasis more broadly in reptile embryos. It may reflect dehydration, maternal calcium derangements, or low nest humidity or temperature (Miller et al. 2009; Hill et al. 2019). Skeletal muscle necrosis (prevalence range across studies: 5%–17%) is more common among late embryos and hatchlings, and proposed pathogenesis has included exertion during hatching and emergence, conducive nest factors (such as depth, sand accretion, or high nest temperatures), or trace mineral status (Miller et al. 2009; Hill et al. 2019; Choi et al. 2020; Dennis et al. 2020; Charles et al. 2023).

Relocation of nests laid in areas at high risk for erosion or inundation is sometimes a necessary practice on Sandy Point (Eckert 1987; Boulon et al. 1996) and a common practice regionally (Dutton et al. 2005; Garner and Garner 2010). Relocated nests have lower hatching success than in situ nests (Boulon et al. 1996; Garrett et al. 2010; Stewart et al. 2023), but relocation is preferred over the loss of all of the embryos contained in the nest. While hatching success was significantly lower in relocated relative to in situ nests (47% vs. 68%, respectively), the reasons for this were not apparent in the 'breakout' or pathological analyses. The similar proportions of eggs lacking grossly evident embryological development (NGSD eggs) among in situ and relocated nests suggest that egg relocation does not typically cause deleterious disturbance of vulnerable early developmental phases. Additionally, the similar frequency of lesions in turtles of in situ and relocated nests supports the view that handling eggs during relocation is not typically harmful to the embryos nor a common route of pathogen introduction, although best confirmed by larger scale studies in the future. These findings encourage the use of nest relocation when implemented properly.

This study increases knowledge regarding anatomic malformations of leatherbacks, including lesions that have been infrequently reported in the species (Eckert et al. 2012; Báracenas-Ibara et al. 2015). Previously reported malformations have included craniofacial malformations and limb deformities (Tucker 1988; Eckert et al. 2012; Choi et al. 2020). We also documented microphthalmia, meningoencephalocele, and syndactyly in this study. Malformations in sea turtles are thought to be influenced by morphogenesis errors during embryonic development, female health including stress, genetics, and maternal offloading of pollutants, and nest conditions including temperature, humidity, and gaseous exchange (Bárcenas-Ibarra et al. 2015). Some of these factors are relevant to hatching success and other lesions observed in this study, and future studies should attempt to more closely tie suspected causative maternal and environmental factors to 'breakout' and pathological analyses. Similarly, instances of twinning are uncommon among chelonians (De Carvalho et al. 2020) but were documented in leatherbacks at Sandy Point in this and previous studies (Eckert 1990).

In conclusion, hatching success in leatherback nests at Sandy Point, St. Croix, fluctuated across the 2019 season and overall was higher than hatching success reported in other eastern Caribbean locations. However, the reasons for this were not readily explained by 'breakout' or pathological analysis. Their nests conform to trends elsewhere showing a high proportion of eggs lacking grossly evident embryological development, and a similar variety of infectious and noninfectious lesions that are poorly understood. Pairing 'breakout' and pathological analyses with assessments of potentially influential environmental or maternal factors is necessary to better explain these observations and identify conservation strategies for improving hatchling production, but is likely to be complicated by multifactorial causes. Most abnormalities documented in leatherback embryos and hatchlings are suspected to be associated with high incubation temperature and/or deranged humidity. Future studies are needed to determine if strategies for reducing incubation temperature, such as shading, relocation, or hatchery translocation, can effectively reduce prevalence of embryonic and hatchling pathology.

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