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PATHOLOGY OF HATCHLING HAWKSBILL SEA TURTLE (*ERETMOCHELYS IMBRICATA*) MORTALITIES OCCURRING WHILE UNDER REHABILITATIVE CARE, 2015–21

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ABSTRACT: Increasing hatchling survival is an important element of conservation of the critically endangered hawksbill sea turtle (*Eretmochelys imbricata*). Yet, there is little information regarding mortality-associated pathological states of hawksbill hatchlings. The aim of this study was to describe lesions affecting hawksbill hatchlings that died while under rehabilitative care. Forty-four turtles representing the nesting sites of two islands and a 7-yr study period were subjected to comprehensive postmortem examination. The most common lesions included dermatitis (34%), skeletal malformations (23%), and pneumonia (23%). Dermatitis and pneumonia were caused by a variety of presumptively opportunistic bacterial and fungal infections. Fungal infections affected 23% of study turtles, also causing rhinitis and esophagitis. Around half of the cases of dermatitis presented with history of skin lesions, and all those involving periocular areas had clinical history of eye lesions. Pneumonia was not predicted by clinical signs or time in rehabilitation. Malformations included carapace compressions, supra- or subnumerary scutes, and dysmelias with many of those affected having concurrent pathology involving other organs. Other lesions included bacterial yolk sacculitis (15%), skeletal muscle degeneration and necrosis (13%), and acute renal tubular necrosis (13%). The study population was female biased (93%), raising concern for skewed hatchling sex ratios and high incubation temperatures in the eastern Caribbean. The pathology described by this study improves our understanding of threats to hawksbill hatchlings and may be taken into consideration by clinicians when implementing strategies for rehabilitative care.

Key words: Chelonian, climate change, disease, hatchling, embryogenesis, posthatchling, reproduction, wildlife rehabilitation.

INTRODUCTION

Hawksbill sea turtles (*Eretmochelys imbricata*) are critically endangered and are one of the seven species of marine turtles included in the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (IUCN Species Survival Commission 2021). Globally they are found in coastal waters of more than 108 countries (Mortimer and Donnelly 2008). Prolonged exploitation of sea turtles for meat, tortoiseshell, and eggs, combined with destruction of nesting and foraging habitats, incidental capture, and marine pollution, have caused significant declines in sea turtle populations in the last century (Mortimer and Donnelly 2008). In the

Wider Caribbean Region, 38% of species-specific sea-turtle nesting sites host hawksbill turtles, with only 10 sites in Barbados, the Dominican Republic, Guadeloupe, Puerto Rico, and Panama supporting more than 1,000 crawls (beach approaches by nesting or nonnesting females) per year (Eckert and Eckert 2019). Most (72%) of the hawksbill nesting sites in the Wider Caribbean have less than 25 crawls per year. Along with the green (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*), the hawksbill is one of the most frequently encountered species of sea turtles in the West Indies.

Slow maturation (around 15–25 yr of age at sexual maturity; Avens et al. 2021) and a low proportion of hatchlings that survive until

adulthood are impediments to the recovery of endangered hawksbill populations. Therefore, increasing hatch success and hatchling survival is a crucial component in efforts to sustain healthy populations. Along with the establishment of protective legislation, nest protection strategies, and monitoring programs, other conservation strategies undertaken with the intent to aid in population recovery include hatcheries, head starting, and rehabilitation of debilitated hatchlings until they are fit for release (Pritchard 1980; Mortimer 1999). Head starting is the controversial captive rearing of wild-caught hatchlings to avoid the high mortality of early life stages to increase their chances of survival (Heppell et al. 1996; Burke, 2015; Bennet et al. 2017; Brei et al. 2019); it has been used for many species of sea turtles (Bell et al. 2005; Shaver and Rubio 2008; Okuyama et al. 2010; Abalo-Morla et al. 2018; Orós et al. 2020). In contrast, hatchling rehabilitation is the temporary provision of supportive veterinary care to hatchlings that fail to emerge from a nest or do not make it to the sea, for example, because of disorientation. Rehabilitation may be costly and there is little information on the extent to which it increases hatchling survival. However, it is one actionable option for sea-turtle programs and when done judiciously can be accompanied by educational programs promoting conservation. The usefulness of rehabilitation varies by individual; for example, turtles affected with disorientation are better candidates for release than those presenting with disease. Disease can reduce survival of hatchlings (Abalo-Morla et al. 2018; Orós et al. 2020), and must be characterized to comprehensively understand threats to hatchlings and to provide proper medical support while under human care.

Few studies have described diseases of hatchling sea turtles; most information that guides veterinary care during rehabilitation is derived from isolated case reports, gray literature, and extrapolations from other reptiles. Bacterial pneumonia, chorioallantitis, renal mineralization, and muscle necrosis are common lesions of dead-in-nest leatherback hatchlings in the eastern Caribbean (Hill

et al. 2019; Choi et al. 2020). Miller et al. (2009) documented the presence of muscle degeneration, renal tubular degeneration, pneumonia, and pulmonary edema in captive leatherback hatchlings in Florida. Various types of dermatitis, purulent or fibrinonecrotizing rhinitis, acute interstitial pneumonia, and fibrinonecrotizing stomatitis have been documented in head-started loggerhead sea turtle (*Caretta caretta*) hatchlings (Orós et al. 2020). There is comparably a paucity of information regarding diseases of hawksbill hatchlings.

The dual island nation of St. Kitts and Nevis serves as nesting and foraging grounds for the hawksbill. The St. Kitts Sea Turtle Monitoring Network (SKSTMN) and the Nevis Turtle Group (NTG) have monitored hawksbill nesting since 2006 and 2001, respectively. These programs excavate nests after emergence. Hatchlings found alive during excavation that are not fit for immediate release are taken in by the SKSTMN for rehabilitation. Clinical management of hawksbill hatchlings in rehabilitative care is challenging because there is little information regarding their diseases. Describing mortality-associated lesions is an important first step in the identification of potential factors impeding successful hatchling rehabilitation. We describe lesions affecting hawksbill hatchlings of St. Kitts and Nevis that died during rehabilitative care from 2015 to 2021.

MATERIALS AND METHODS

Study site and rehabilitative care

Hatchlings originated from hawksbill nesting beaches in St. Kitts and Nevis patrolled by the SKSTMN and NTG July to February in St. Kitts and year round in Nevis, 2015–21. Hatchlings that were considered not fit for immediate release were admitted for rehabilitative care provided by the SKSTMN. Clinical indications for admittance included signs of dehydration, weakness, or inability to move or swim, as well as musculoskeletal, integumentary, or ocular lesions that were considered severe enough to prevent the animals from crawling, entering the ocean, and swimming. Before 2020, only turtles from St. Kitts were admitted to rehabilitative care.

Animals were kept out of water until initial health assessments could be performed, a period of 1–12 h. During this time, ophthalmic ointment (Puralube ophthalmic ointment, Dechra Veterinary Products, Overland Park, Kansas, USA) was placed in the eyes and the animals were held on 2.5 cm of damp sand or paper towels within cooler boxes. Following the initial assessment, if strong enough, they were placed in plastic containers with approximately 10 cm of fresh saltwater obtained from the ocean. These containers were emptied and refilled once to twice daily on the basis of fecal output. Weaker animals were kept out of water until strength improved. Animals were fed in separate containers to prevent food debris buildup within the containers. Once hatchlings were eating on their own they were moved to larger enclosures ($1.8 \times 1.2 \times 0.9$ m deep) that received fresh saltwater circulation with one full turnover of the enclosures' water every 18 h. When indicated, systemic or topical antibiotics were administered. Nutritional support was provided in the form of preformulated slurry or pelleted diets or natural prey items. The majority of turtles brought in for rehabilitation had issues (typically mild dehydration or weakness) that resolved with supportive care for less than 72 h, after which the animals were released.

Postmortem examination

From 2015, all hatchlings that died while receiving rehabilitative care by the SKSTMN were subject to comprehensive postmortem examination. This totaled 44 hatchlings, 39 originating from St. Kitts and five from Nevis, including 27 (61%) that were found during excavation, 11 (25%) that had emerged from the nest, and six (14%) for which location when found was not recorded. Dead hatchlings were submitted to the Ross University School of Veterinary Medicine (RUSVM) Pathology Laboratory for complete postmortem examination. Tissues including yolk sac, heart, intestines, stomach, esophagus, skeletal muscle, lungs, liver, kidneys, skin, gonads, thyroid gland, pancreas, thymus, Rathke's gland, salt gland, and brain were fixed for at least 24 h in 10% buffered formalin and processed routinely for histology. Formalin-fixed, paraffin-embedded tissues were sectioned 4 μ m thick and stained with H&E. Other histochemical stains such as Gram, periodic acid-Schiff, and Gomori methenamine silver were performed where indicated by microscopic lesions.

Bacterial and fungal cultures of tissue specimens were performed when gross lesions were suggestive of an infectious etiology (i.e., presence of exudate or parenchymal nodules). Sex was determined histologically by conducting a qualitative assessment of the gonads (Ceriani and

Wyneken 2008): Specimens with disorganized medulla and cortex composed of a distinct layer of simple cuboidal to columnar cells were classified as female; those with cortex comprised of a simple layer of squamous cells and medulla containing seminiferous tubules were classified as male.

Statistics

A binomial exact calculation was used via an online calculator (Kohn and Senyak 2021) to determine 95% confidence intervals for the proportion of lesion-affected animals in the study population.

RESULTS

The number of deaths occurring while in rehabilitative care varied by year: Two in 2015–16, 10 in 2016–17, 22 in 2017–18, three in 2018–19, zero in 2019–20, and seven in 2020–21. Straight carapace length at death was mean 4.9 mm (SD 2.0 mm). The time spent in rehabilitative care before death was <72 h ($n=14$), 3–7 d ($n=10$), 1–4 wk ($n=13$), or >1 mo ($n=6$). Most turtles were found dead by the caregiver after a period of weakness or inactivity (23/44, 52%), or without premonitory signs of illness (7/44, 16%). Other clinical abnormalities observed before death included eye lesions (4/44, 9%), skin lesions (4/44, 9%), small size relative to other hatchlings (2/44, 4.5%), inappetence (2/44, 4.5%), skeletal malformations (5/44, 11%), ataxia or abnormal swimming (4/44, 9%), and floating (1/44, 2%). Gonad was histologically examined and sufficiently preserved to allow sex classification in 27 animals, including 25/27 (93%) females and 2/27 (8%) males.

Pathology

Most turtles (23/44, 52%) had grossly visible lesions, including cutaneous plaques, nodules, or ulcers indicative of dermatitis (Fig. 1A; $n=7$); skeletal malformations (Fig. 1B, C; $n=10$); pulmonary nodules or redness with consolidation indicative of pneumonia (Fig. 1D; $n=3$); and other lesions including coelomic serous effusion ($n=3$), periocular lesions (edema, erythema, ulceration, or plaques; $n=3$), pulmonary emphysema ($n=2$),

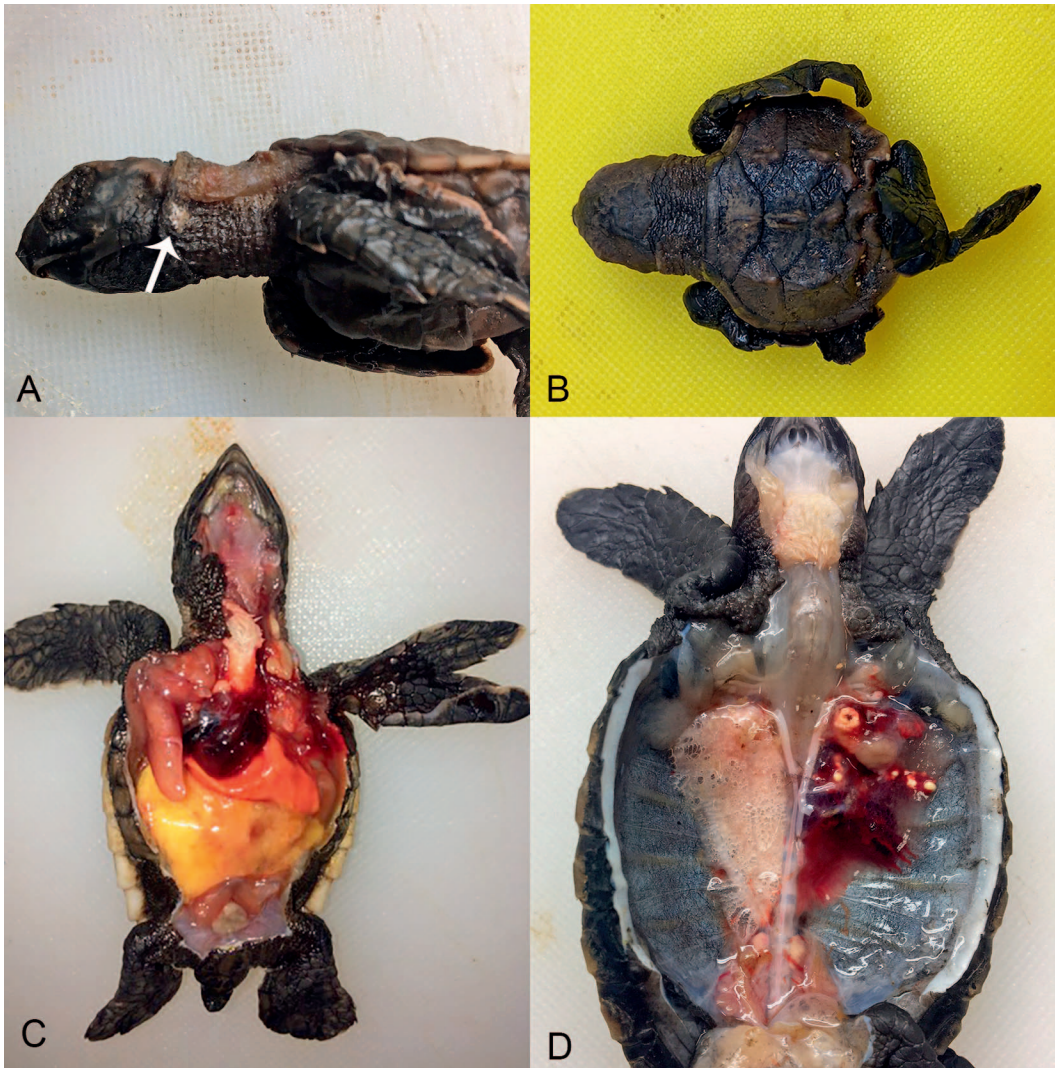


FIGURE 1. Examples of gross lesions observed in hawksbill sea turtle (*Eretmochelys imbricata*) hatchlings from St. Kitts and Nevis, West Indies that died during rehabilitative care from 2015 to 2020. A. White plaque on neck (arrow) that microscopically comprised fungal dermatitis. B. Carapace malformation (subnumerary scutes with osteoagenesis), dysmelia (malpositioning of hind limbs), and anury. C. Polymelia of the left forelimb. D. Pulmonary emphysema (right lung) and reddening with nodules (left lung) that microscopically consisted of heterophilic and granulomatous bronchopneumonia.

esophageal phytobezoar ($n=1$), emaciation ($n=1$), erythematous small intestinal mucosa ($n=1$), externalized yolk sac ($n=1$), coagulated yolk ($n=1$), impacted and distended jejunum ($n=1$), and forelimb traumatic compression injury ($n=1$).

Lesions were identified in 42/44 (95%) individuals and were categorized by system (Table 1). The most common microscopic

lesions included dermatitis (Fig. 2A, $n=14$), pneumonia (Fig. 2B, $n=10$), thrombosis ($n=6$), muscle necrosis (Fig. 2C, $n=5$), and acute renal tubular necrosis ($n=5$). No significant pathologic findings were identified in 2/44 (5%) turtles.

Dermatitis was observed in 14/41 (34%) turtles, all of which had been in rehabilitation for <1 mo (Table 2). Locations of dermatitis

TABLE 1. Prevalence of lesions affecting hatchling hawksbill sea turtles (*Eretmochelys imbricata*) from St. Kitts and Nevis that died while receiving rehabilitative care, 2015–21 (*n*=44).

System	Lesion	<i>n</i> (%)	95% CI ^a
Integumentary	Dermatitis	14 (34)	20.1–50.6
Respiratory			
Nasal passages	Rhinitis or rhinopharyngitis	2 (5)	0.6–15.8
Lungs	Pneumonia	10 (23)	11.8–38.6
	Emphysema	2 (5)	0.6–15.8
Cardiovascular			
Blood vessels	Thrombosis	6 (15)	5.7–29.8
Musculoskeletal			
	Skeletal malformation	10 (23)	11.5–37.8
	Muscle degeneration and necrosis	5 (13)	4.4–28.1
Excretory			
Kidneys	Acute tubular necrosis	5 (13)	4.4–28.1
Digestive			
Esophagus	Esophagitis	2 (5)	0.7–18.2
Intestines	Enteritis	3 (7)	1.5–19.1
	Impacted jejunum	2 (5)	0.6–15.8
Yolk sac	Bacterial yolk sacculitis	4 (15)	4.2–33.7
Coelomic cavity	Hydrocoelom	3 (17)	3.6–41.4
Total individuals with lesions		42 (96)	84.5–99.4

^a CI = confidence interval.

included neck (*n*=6), eyelids (i.e., blepharitis; *n*=3), head (*n*=2), and body (*n*=1). Two of 14 (14%) had dermatitis in more than one location, including head and neck (*n*=1) or limbs and head (*n*=1). Where dermatitis involved eyelids and surrounding skin, it was grossly evident in all three cases, with eye lesions including periocular swelling, erythema, ulceration, or plaques. Eye lesions were identified by the rehabilitator for all cases with blepharitis. In other locations, skin lesions were grossly indicative of dermatitis in 5/11 cases (45%), typically ulcerated plaques or nodules, a minority of which were evident to the rehabilitator (Table 2). Histologically, dermatitis was pustular (*n*=9), hyperkeratotic and pustular (*n*=2), hyperkeratotic (*n*=1), ulcerative (*n*=1), or chronic with stromal calcification (*n*=1). Affected individuals had intralesional bacteria (4/14; 29%), fungi (3/14; 21%), both bacteria and fungi (4/14; 29%), or no intralesional infectious agents seen (3/14; 21%). Topical antimicrobial therapy had been provided in three cases of dermatitis, two of which had intralesional fungi. Bacteria were gram-negative rods (2/8), gram-positive cocci

(1/8), or a mixture of gram-negative rods and gram-positive cocci (5/8). Fungi always consisted of hyphae <10 µm wide with parallel walls. Dichotomous branching was observed in 2/7, whereas 5/7 were poorly septated and rarely branched. Fungal culture was attempted for two turtles with fungal dermatitis that was grossly evident, both of which yielded *Penicillium* spp. and *Paecilomyces* spp., plus *Aspergillus ochraceopetaliformis* in one case. Pneumonia was identified in 10/43 (23%) individuals. Pneumonia was grossly evident as reddened lungs with multifocal, white to yellow nodules in 3/10 of affected turtles. Pneumonia was categorized as bronchopneumonia (i.e., when infiltrate concentrated within airways) in 7/10 instances and embolic (i.e., multifocal infiltrate involving interstitium and airways) in 3/10. Affected individuals had intralesional bacteria (5/10), fungi (1/10), bacteria and fungi (2/10), or no agents identified (2/10). Intralesional bacteria included gram-negative bacilli (*n*=4), gram-positive cocci (*n*=2), and gram-positive bacilli (*n*=1). Two cases had intralesional keratinized squamous cells and extracellular brown granular

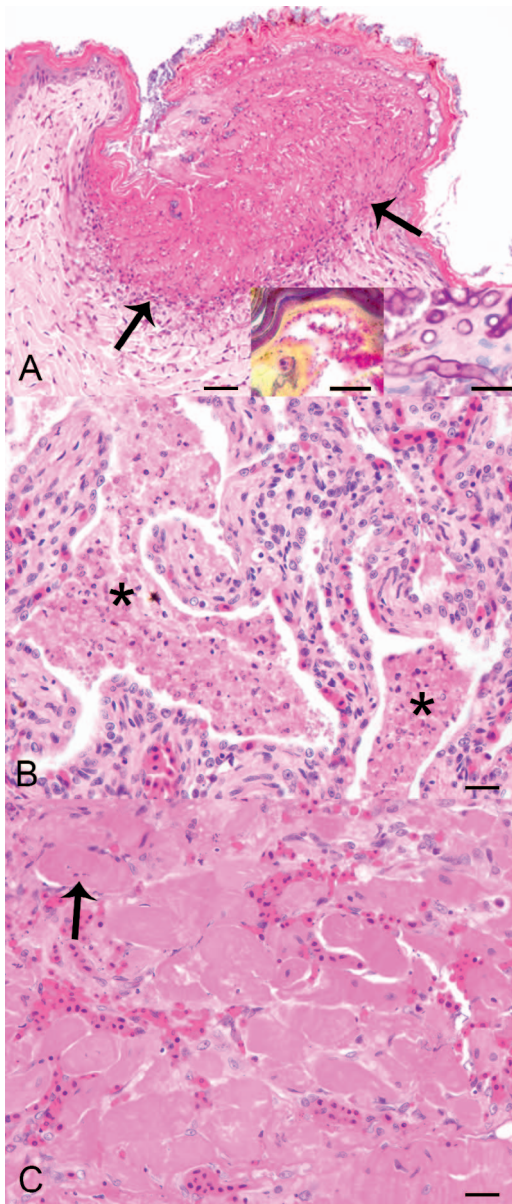


FIGURE 2. Examples of microscopic lesions observed in hawksbill sea turtle (*Eretmochelys imbricata*) hatchlings from St. Kitts and Nevis, West Indies that died during rehabilitative care from 2015 to 2020. A. Pustular dermatitis. The epidermis is expanded by heterophilic exudate (arrows), containing intralesional colonies of gram-negative rods (inset left, Brown-Hopps Gram stain) and fungal hyphae (inset right, periodic acid-Schiff stain), H&E stain, bar=20 μ m, inset bars=10 μ m. B. Heterophilic exudate fills ediculae (asterisks). H&E stain, bar=20 μ m. C. Subacute skeletal muscle necrosis. Myofibers have hyalinized or vacuolated sarcoplasm with nuclear pyknosis (arrow). H&E stain, bar=20 μ m.

material, suggestive of aspiration. Microbial cultures were performed on two cases with grossly evident pneumonia. One case of fungal pneumonia grew *Phialemonium* sp., whereas aerobic culture yielded rare (1+) *Pseudomonas* sp., and rare (1+) mixed bacterial growth of nonrecognizable pathogens. One case of bacterial pneumonia produced mixed growth of *Aeromonas hydrophila*, *Enterococcus faecalis*, *Alcaligenes faecalis*, and *Providencia stuartii*. Two cases of bacterial pneumonia also had bacterial yolk sacculitis. One case of fungal pneumonia also had fungal dermatitis.

Skeletal malformations were observed in 10/44 (23%) turtles. Malformations included carapace compression ($n=6$), dysmelias ($n=3$; including polydactyly [Fig. 1C] and malpositioning of limbs [Fig. 1B]), carapace malformation ($n=2$; one with supranumery scutes and one with subnumery scutes and osteoagenesis [Fig. 1B]), anury ($n=1$; Fig. 1B), and palatoschisis and cheiloschisis ($n=1$). On postmortem examination, 9/10 individuals with skeletal malformations were affected by concurrent lesions, including 4/10 with dermatitis and 2/10 with pneumonia. Of those with dermatitis, 3/4 had a compressed carapace.

Thrombosis was identified in 6/40 (15%) individuals from St. Kitts. All affected individuals had lesions in other organs, particularly respiratory and excretory system lesions, including pulmonary congestion and edema ($n=4$), acute renal tubular necrosis ($n=4$), bacterial pneumonia ($n=2$), bacteremia (i.e., histologically evident bacterial emboli; $n=1$), and carapace compression ($n=1$).

Skeletal muscle degeneration and necrosis were seen in 5/40 (12.5%) turtles, typically monophasic, acute-subacute, mild to moderate in severity, and recognized within sections of neck and spine. All affected turtles were brought into rehabilitation for failure to emerge from the nest and weakness that failed to improve during rehabilitation. Two of five (40%) cases had concurrent dermatitis (one with intralesional bacteria, one with no etiology detected).

TABLE 2. Clinical signs, time in rehabilitation, and island of origin associated with most common lesions observed in hawksbill sea turtles (*Eretmochelys imbricata*) that died while receiving rehabilitative care from 2015 to 2021.

	Dermatitis	Pneumonia	Skeletal malformation	Thrombosis	Skeletal muscle necrosis	Acute renal tubular necrosis	Total
Clinical signs							
Weakness	5	6	4	3	5	3	23
None	2	1	0	2	0	2	7
Malformed	2	2	5	1	0	0	5
Eye lesions ^a	3	1	0	0	0	0	4
Skin lesions ^a	2	0	2	0	0	0	4
Abnormal gait or swimming	1	2	1	0	0	0	4
Inappetence	0	0	1	0	0	0	2
Small size	2	0	2	0	0	0	2
Time in rehabilitation							
<72 h	4	4	6	1	1	1	17
3 d to 1 wk	5	1	1	2	0	2	12
1–4 wk	5	3	2	3	4	2	20
>1 mo	0	2	1	0	0	0	3
Island							
St. Kitts	14	10	7	6	4	4	39
Nevis	0	0	3	0	1	1	5
Total	14	10	10	6	5	5	

^a Indicates lesions observed by caregiver and described in history.

Other lesions observed included acute renal tubular necrosis (5/40; 13%), bacterial yolk sacculitis (4/27; 15%), enteritis (3/40; 8%), and esophagitis (2/40; 5%). None of the turtles with acute renal tubular necrosis received any medical treatment before death. Bacterial yolk sacculitis occurred in four cases from St. Kitts; all showed weakness and inactivity before death. Intralesional gram-negative bacilli were seen in all instances of yolk sacculitis. Bacterial yolk sacculitis occurred concurrently with thrombosis ($n=1$) and bacterial pneumonia ($n=2$). All three cases of enteritis were from the island of St. Kitts and each was in rehabilitative care >1 mo. Clinical signs observed before death included abnormal swimming ($n=1$) or floating ($n=1$). Histologically, enteritis was either heterophilic and ulcerative ($n=2$) or necrohemorrhagic ($n=1$). All had concurrent hydrocoelom. Other concurrent lesions included jejunal impaction ($n=1$), esophagitis ($n=1$), rhinitis ($n=1$), and fungal pneumonia

($n=1$). Esophagitis was seen in two turtles from St. Kitts that were in rehabilitative care for >1 mo. In both cases, the lesion was focused at the gastroesophageal junction; there was concurrent rhinitis/nasopharyngitis and intralesional fungal hyphae and yeasts. Overall, 10/44 (23%) hatchlings had fungal infections and 16/44 (36%) had bacterial infections; of these, 6 were mixed bacterial and fungal infections.

Lesions observed only once included cystitis with squamous metaplasia, renal tubular urate stasis, esophageal phytobezoar, squamous metaplasia of the gastric mucosa, necrogranulomatous hepatitis, hepatocellular atrophy, meningeal hemorrhage, forelimb traumatic compression injury, and proptosis. The turtle with an esophageal phytobezoar had been in rehabilitation for months and was being kept in the aforementioned tank environment with a diet of natural prey items. It was found dead without premonitory clinical signs.

DISCUSSION

This study improves the collective understanding of pathological states that threaten hawksbill sea-turtle hatchlings and may impede survival in the wild or during rehabilitation. This information may facilitate the establishment of prophylactic measures and treatment protocols for certain conditions, with the goal to maximize hatchling survival. Conditions observed in hatchlings that died during rehabilitation included presumptively opportunistic bacterial and fungal infections (associated with dermatitis, pneumonia, and less frequently, blepharitis, yolk sacculitis, rhinitis, or esophagitis), skeletal malformations, skeletal muscle degeneration and necrosis, and acute renal tubular necrosis. For many of these conditions, clinical signs identified by the rehabilitators were nonspecific, making antemortem diagnosis challenging.

The most prevalent lesion that we found was dermatitis (34%), similar to head-started loggerheads (41%, Orós et al. 2020), and a common lesion among captive reptiles (Palmeiro and Roberts 2013). Dermatitis affected all age groups, suggesting that skin infections are potentially acquired both from the nesting environment and during rehabilitative care. Mycotic dermatitis has been documented in leatherback embryos (Choi et al. 2020); thus some infections may develop before hatching. The heterogeneity of microbial organisms observed in dermatitis is consistent with opportunistic, environment-derived infections. Sun exposure or ant predation are common among hawksbill hatchlings stranded on the beach and may serve as predisposing factors for dermatitis. Orós et al. (2020) hypothesized that dermatitis in head-started loggerhead hatchlings was a consequence of conspecific aggression. Although this behavior has been observed in rehabilitated hawksbill hatchlings, mechanisms are in place to keep them separated as much as possible to minimize potential interactions while in rehabilitative care. Less than half of the cases with dermatitis were reported to have grossly evident skin lesions by their rehabilitator.

This emphasizes a need for more powerful visual assessment tools when conducting physical examinations on hatchlings. Blepharitis seemed to be the most clinically apparent location affected; eye lesions were identified by the rehabilitator in all three cases.

Pneumonia caused by bacteria or fungi was also highly prevalent in hawksbills that died during rehabilitation (23.3%), and could not be predicted by clinical history or time in rehabilitation. Many (40%) individuals with pneumonia spent <72 h in rehabilitation, consistent with the view that respiratory infections may be acquired in egg or in nest (Al-Bahry et al. 2009; Hill et al. 2019). Pneumonia was grossly evident as reddened lungs or lung nodules in a minority of cases, and most cases consisted of bronchopneumonia suggestive of inhaled route of infection, as seen with leatherback, green, and loggerhead hatchlings (Glazebrook et al. 1993; Miller et al. 2009; Hill et al. 2019; Choi et al. 2020), but in contrast to the interstitial pneumonia reported in loggerhead hatchlings (Orós et al. 2020). However, there were several with an embolic pattern of pneumonia or infectious lesions in other organs, consistent with a hematogenous route of pulmonary infection; few with intralesional foreign material consistent with aspiration; and many with intralesional cocci, fungal hyphae, or mixed microbial agents suggesting more diverse etiology than the gram-negative bacilli described in leatherback hatchling pneumonia (Choi et al. 2020). A wide range of bacteria have been isolated from hatchling bronchopneumonia in this study and others (Glazebrook et al. 1993; Miller et al. 2009; Hill et al. 2019), supporting the view that pulmonary bacterial infections are typically opportunistic.

Fungal infections were more frequent in hawksbill hatchlings (23%) relative to cases represented in reports of disease in hatchlings of other sea turtles (Miller et al. 2009; Hill et al. 2019; Choi et al. 2020; Orós et al. 2020). The fungi found in this study are ubiquitous saprophytes that opportunistically cause cutaneous or systemic mycoses in captive aquatic reptiles, favored by conducive environmental conditions, stress, and host immunosuppres-

sion (Pare and Conley 2020). These fungi (*Penicillium* and *Aspergillus* spp., *Paecilomyces* spp. and the closely related *Purpureocillium lilacinum*, and *Fusarium* spp.) have been documented causing a variety of lesions in a large cohort of head-started Kemp's ridley sea turtles (*Lepidochelys kempi*; Clary and Leong 1984); pneumonia and dermatitis in captive loggerhead hatchlings (Arpini et al. 2019); pneumonia in captive loggerhead and green hatchlings and juveniles (Glazebrook et al. 1993); and dermatitis in rehabilitated adult loggerheads (Cafarchia et al. 2020). Although there are many studies evaluating the fungal organisms present in unhatched eggs or failed nests (Sarmiento-Ramírez et al. 2010, 2014; Rosado-Rodríguez and Maldonado-Ramírez 2016; Candan 2018; Gleason et al. 2020), the role saprophytic isolates play in perinatal mortality rather than nest decomposition is challenging to disentangle, and there is a growing need for identification of fungi associated with lesions in sea-turtle embryos and hatchlings. Preventative measures such as turtle washing with mild soap upon admittance and repeated washing over the course of hospitalization might be useful for reducing the occurrence of opportunistic fungal and bacterial infections in susceptible rehabilitated hatchlings.

Malformations in sea turtles are generally considered rare, affecting <1% of eggs (Bárcenas-Ibarra et al. 2015). Reports of their occurrence in the Caribbean are limited to green turtles and hawksbills in Costa Rica and the Gulf of Mexico (Fowler 1979; Bárcenas-Ibarra et al. 2015), with around 2% prevalence in hawksbills. The comparatively high frequency of skeletal malformations in our rehabilitated hawksbill mortalities (23%) suggests that either they are more prevalent in the study population, or that they affect hatchling fitness and ability to emerge from the nest. Similar to our study, the most common malformation type affecting hawksbills in the Gulf of Mexico was carapacial compression (Bárcenas-Ibarra et al. 2015)—deformation caused by external mechanical forces during embryogenesis. The high prevalence in hawksbills relative to other sea

turtles might be explained by the high density of eggs in hawksbill nests, or by maternal nesting behavior where there is a preference for sites close to the vegetation line, and frequent invasion of nests by roots that may compress eggs. Carapacial compressions typically resolve during rehabilitation (personal observation, SKSTMN), and were therefore considered unlikely to affect hatchling survival. Although other minor malformations, such as anury or supra- or subnumerary scutes, might not affect hatchling fitness (Hewavisenthi and Parmenter 2001; Bently et al. 2021; Maffucci et al. 2020), many animals with malformations in this study had concurrent lesions, suggesting a broader effect on health than might be expected, or that malformations are a result of unsuitable conditions that may instigate concurrent lesions in other systems. Dismelias were the next most common malformations both in the study by Bárcenas-Ibarra et al. (2015) and our study. The pathogenesis of dismelias and other observed malformations is unknown in hawksbills, but probably involves genetic and environmental factors (Martin Del Campo et al. 2021). The possibility that certain malformations result in embryonal mortality or inability to hatch or emerge from the nests (Kaska and Downie 1999; Bárcenas-Ibarra et al. 2015) underscores the need for further research to determine the prevalence of malformations in hawksbills in the Wider Caribbean and their associated effects on hatch and emergence success and hatchling health. In particular, there would be much value in investigating the relationship between high nest temperature and low nest humidity with the occurrence of malformations in hawksbills (Telemeco et al. 2013; Zimm et al. 2017).

The diversity of other lesions occurring at lower frequencies in this study reflects the heterogeneity in reasons for rehabilitation failure in hawksbills. Yolk sacculitis was always associated with gram-negative bacilli. In reptiles, bacterial yolk sacculitis may develop as a consequence to improper yolk internalization or resorption, patent urachus, unsanitary umbilicus, or primary chorioallantoitis. No hatchling with yolk sacculitis in the

present study was clinically identified as having yolk, urachal, or umbilical problems. Preventative measures for this lesion undertaken by the SKSTMN include cleaning externalized yolk sacs when hatchlings are admitted to rehabilitation. Skeletal muscle necrosis, which affected 12% of hawksbills dying during rehabilitation, is common among Caribbean leatherback hatchlings (Miller et al. 2009; Hill et al. 2019; Choi et al. 2020), and has been observed in net-entangled juvenile green and loggerhead sea turtles (Orós et al. 2005; Phillips et al. 2015). In our study, this lesion was associated with weakness, although weakness was common regardless of underlying disease. Affected individuals had acute to subacute lesions and many were in rehabilitative care >1 wk, suggesting the myopathy may be acquired during rehabilitation rather than a result of exertion during nest emergence. Rehabilitation conditions lacked features likely to pose risk for exertion or capture myopathy (i.e., restraint, confinement). The role of nutritional factors in the development of necrosis during rehabilitation is unclear, as is whether vitamin E and selenium supplementation may reduce its occurrence. Although enteritis was uncommon in our study, it occurred only in hatchlings that were in rehabilitative care for more than 1 mo, consistent with other reports of enteritis involving post-hatchling sea turtles (Chuen-Im et al. 2010; Ryan et al. 2016), and could be recognized by problems with buoyancy.

Similar to eastern Caribbean leatherback embryos and hatchlings (Hill et al. 2019; Choi et al. 2020), our population was overwhelmingly female (93%). Sex determination is temperature dependent in sea turtles, with most species needing to reach a pivotal temperature of approximately 29 C to produce a 1:1 male:female sex ratio (Godley et al. 2002). Although hatchling sex ratios may be normally female biased (Hawkes et al. 2013), and the extent to which sex biases hatchling fitness and survival is unclear (Janzen et al. 2000; Booth and Astill 2001), these findings warrant confirmation of sex ratios and incubation temperatures in eastern Caribbean hawksbills. Female predominance in hatch-

lings of multiple studies bolsters concern for rising incubation temperatures and for climate change threatening regional population extinctions long term (Jensen et al. 2018; Blechschmidt et al. 2020).

Although most hawksbill hatchlings from St. Kitts and Nevis are successfully released within 72 h of admittance, a clear understanding of health threats to hatchlings that require longer-term or more intensive care may help alleviate mortality occurring during rehabilitation. Future studies are needed determine effective mitigations for lesions that may impede hatchling survival; the effect and pathogenesis of skeletal malformations; and the extent to which rising incubation temperatures are influencing embryo mortality, hatchling diseases, and female sex bias in the species.

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LITERATURE CITED

- Abalo-Morla S, Marco A, Tomás J, Revuelta O, Abella E, Marco V, Crespo-Picazo JL, Fernández C, Valdés F, et al. 2018. Survival and dispersal routes of head-started loggerhead sea turtle (*Caretta caretta*) post-hatchlings in the Mediterranean Sea. *Mar Biol* 165: 51.
- Al-Bahry S, Mahmoud I, Elshafie A, Al-Harthy A, Al-Ghafri S, Al-Amri I, Alkindi A. 2009. Bacterial flora and antibiotic resistance from eggs of green turtles *Chelonia mydas*: An indication of polluted effluents. *Mar Pollut Bull* 58:720–725.
- Arpini CM, Nóbrega YC, Casthelo VD, Neves DS, Tadokoro CE, da Costa GL, Oliveira MME, Santos

- MRD. 2019. *Purpuricillium lilacinum* infection in captive loggerhead sea turtle hatchlings. *Med Mycol Case Rep* 23:8–11.
- Avens L, Ramirez MD, Goshe LR, Clark JM, Meylan AB, Teas W, Shaver DJ, Godfrey MH, Howell L. 2021. Hawksbill sea turtle life-stage durations, somatic growth patterns, and age at maturation. *Endanger Species Res* 45:127–145.
- Bárcenas-Ibarra A, de la Cueva H, Rojas-Lleonart I, Abreu-Grobois FA, Lozano-Guzmán RI, Cuevas E, García-Gasca A. 2015. First approximation to congenital malformation rates in embryos and hatchlings of sea turtles. *Birth Defects Res A Clin Mol Teratol* 103:203–224.
- Bell CD, Parsons J, Austin TJ, Broderick AC, Ebanks-Petrie G, Godley BJ. 2005. Some of them came home: The Cayman Turtle Farm headstarting project for the green turtle *Chelonia mydas*. *Oryx* 39:137–148.
- Bennett AM, Steiner J, Carstairs S, Gielens A, Davy CM. 2017. A question of scale: Replication and the effective evaluation of conservation interventions. *Facets* 2:892–909.
- Bentley BP, McGlashan JK, Bresette MJ, Wyneken J. 2021. No evidence of selection against anomalous scute arrangements between juvenile and adult sea turtles in Florida. *J Morphol* 282:173–184.
- Blechschmidt J, Wittmann MJ, Blüml, C. 2020. Climate change and green sea turtle sex ratio—Preventing possible extinction. *Genes* 11:588.
- Booth DT, Astill K. 2001. Incubation temperature, energy expenditure and hatchling size in the green turtle (*Chelonia mydas*), a species with temperature-sensitive sex determination. *Aust J Zool* 49:389–396.
- Brei, M, Pérez-Barahona A, Strobl E. 2019. Protecting species through legislation: The case of sea turtles. *Am J Agric Econ* 102:300–328.
- Burke RL. (2015). Head-starting turtles: Learning from experience. *Herpetol Conserv Biol* 10:299–308.
- Cafarchia C, Paradies R, Figueredo LA, Iatta R, Desantis S, Di Bello AVF, Zizzo N, van Diepeningen AD. 2020. *Fusarium* spp. in loggerhead sea turtles (*Caretta caretta*): From colonization to infection. *Vet Pathol* 57:139–146.
- Candan ED. 2018. Molecular identification of fungal isolates and hatching success of green turtle (*Chelonia mydas*) nests. *Arch Microbiol* 200:911–919.
- Ceriani SA, Wyneken J. 2008. Comparative morphology and sex identification of the reproductive system in formalin-preserved sea turtle specimens. *Zoology* 111:179–187.
- Choi E, Charles KE, Charles KL, Stewart KM, Morrall CE, Dennis MM. 2020. Leatherback sea turtle (*Dermochelys coriacea*) embryo and hatchling pathology in Grenada, with comparison to St. Kitts. *Chelonian Conserv Biol* 19:111–123.
- Chuen-Im T, Areekijserree M, Chongthammakun S, Graham SV. 2010. Aerobic bacterial infections in captive juvenile green turtles (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) from Thailand. *Chelonian Conserv Biol* 9:135–142.
- Clary JC III, Leong JK. 1984. Disease studies aid Kemp's Ridley sea turtle headstart research. *Herpetol Rev* 15: 69–d70.
- Eckert KL, Eckert AE. 2019. *An atlas of Sesa turtle nesting habitat for the Wider Caribbean Region*. Revised Ed. WIDECAST Technical Report No. 19. Godfrey, Illinois. 232 pp., plus electronic Appendices.
- Fowler LE. 1979. Hatching success and nest predation in the green sea turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. *Ecology* 60:946–955.
- Glazebrook JS, Campbell RSF, Thomas AT. 1993. Studies on an ulcerative stomatitis—obstructive rhinitis—pneumonia disease complex in hatching and juvenile sea turtles *Chelonia mydas* and *Caretta*. *Dis Aquat Organ* 16:133–147.
- Gleason FH, Allerstorfer M, Lilje O. 2020. Newly emerging diseases of marine turtles, especially sea turtle egg fusariosis (SEFT), caused by species in the *Fusarium solani* complex (FSSC). *Mycology* 11:184–194.
- Godley BJ, Broderick AC, Glen F, Hays GC. 2002. Temperature-dependent sex determination of Ascension Island green turtles. *Mar Ecol Prog Ser* 226:115–124.
- Hawkes LA, McGowan A, Godley BJ, Gore S, Lange A, Tyler CR, Wheatley D, White J, Witt MJ, et al. 2013. Estimating sex ratios in Caribbean hawksbill turtles: Testosterone levels and climate effects. *Aquat Biol* 18:9–19.
- Heppell SS, Crowder LB, Crouse DT. 1996. Models to evaluate headstarting as a management tool for long-lived turtles. *Ecol Appl* 6:556–565.
- Hewavisenthi S, Parmenter CJ. 2001. Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). *Copeia* 2001:668–682.
- Hill K, Stewart KM, Rajeev S, Conan A, Dennis MM. 2019. Pathology of leatherback sea turtle (*Dermochelys coriacea*) embryos and hatchlings from nests in St. Kitts, West Indies (2015–16). *J Wildl Dis* 55:782–793.
- Janzen FJ, Tucker JK, Paukstis GL. 2000. Experimental analysis of an early life-history stage: Selection on size of hatchling turtles. *Ecology* 81:2290–2304.
- Jensen MP, Allen CD, Egnuchi T, Bell IP, LaCasella EL, Hilton WA, Hof CAM, Dutton PH. 2018. Environmental warming and feminization of one of the largest sea turtle populations in the world. *Curr Biol* 28:154–159.
- Kaska Y, Downie R. 1999. Embryological development of sea turtles (*Chelonia mydas*, *Caretta caretta*) in the Mediterranean. *Zool Middle East* 19:55–69.
- Kohn MA, Senyak J. 2021. *Sample size calculators* [website]. UCSF CTSI. Available at <https://www.sample-size.net/> Accessed January 2023.
- Maffucci F, Pace A, Affuso A, Ciampa M, Treglia G, Pignalosa A, Hochscheid S. 2020. Carapace scute pattern anomalies in the loggerhead turtle: Are they

- indicative of hatchling's survival probability? *J Zool* 310:315–322.
- Martín Del Campo R, Calderón-Campuzano MF, Rojas-Leonart I, Briseño-Dueñas R, García-Gasca A. 2021. Congenital malformations in sea turtles: Puzzling interplay between genes and environment. *Animals* 11:444.
- Miller DL, Wyneken J, Rajeev S, Perrault J, Mader DR, Weege J, Baldwin CA. 2009. Pathologic findings in hatchling and posthatchling leatherback sea turtles (*Dermochelys coriacea*) from Florida. *J Wildl Dis* 45: 962–971.
- Mortimer JA. 1999. Reducing threats to eggs and hatchlings: Hatcheries. In: *Research and management techniques for the conservation of sea turtles*. Eckert KL, Bjørndal KA, Abreu-Grobois FA, Donnelly M, editors. IUCN/SSC Marine Turtle Specialist Group Publication No. 4. Blanchard, Pennsylvania, pp. 169–174.
- Mortimer JA, Donnelly M (IUCN SSC Marine Turtle Specialist Group). 2008. *Eretmochelys imbricata*. In: *The International Union for Conservation of Nature red list of threatened species*. <https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T8005A12881238.en>. Accessed January 2022.
- Okuyama J, Shimizu T, Abe O, Yoseda K, Arai N. 2010. Wild versus head-started hawksbill turtles *Eretmochelys imbricata*: Post-release behavior and feeding adaptations. *Endanger Species Res* 10:181–190.
- Orós J, Suárez-Saavedra A, Liria-Loza A, Arencibia A. 2020. Lesions observed post mortem in post-hatchling loggerhead sea turtles (*Caretta caretta*) from a head start programme. *J Comp Pathol* 174:73–80.
- Orós J, Torrent A, Calabuig P, Déniz S. 2005. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998–2001). *Dis Aquat Organ* 63:13–24.
- Palmeiro BS, Roberts H. 2013. Clinical approach to dermatologic disease in exotic animals. *Vet Clin North Am Exot Anim Pract* 16:523–577.
- Pare JA, Conley KJ. 2020. Mycotic diseases of reptiles. In: *Infectious diseases and pathology of reptiles: Color atlas and text*. 2nd Ed. Jacobson ER, Garner MM, editors. CRC Press, Boca Raton, Florida, pp. 795–825.
- Phillips BE, Cannizzo SA, Godfrey MH, Stacy BA, Harms CA. 2015. Exertional myopathy in a juvenile green sea turtle (*Chelonia mydas*) entangled in a large mesh gillnet. *Case Rep Vet Med* 2015: article ID 604321.
- Pritchard PC. 1980. The conservation of sea turtles: Practices and problems. *Am Zool* 20:609–617.
- Rosado-Rodríguez G, Maldonado-Ramírez SL. 2016. Mycelial fungal diversity associated with the leatherback sea turtle (*Dermochelys coriacea*) nests from Western Puerto Rico. *Chelonian Conserv Biol* 15: 265–272.
- Ryan PG, Cole G, Spiby K, Nel R, Osborne A, Perold V. 2016. Impacts of plastic ingestion on post-hatchling loggerhead turtles off South Africa. *Mar Pollut Bull* 107:155–160.
- Sarmiento-Ramírez JM, Abella E, Martín MP, Tellería MT, López-Jurado LF, Marco A, Diéguez-Urbeondo J. 2010. *Fusarium solani* is responsible for mass mortalities in nests of loggerhead sea turtle, *Caretta*, in Boavista, Cape Verde. *FEMS Microbiol Lett* 312: 192–200.
- Sarmiento-Ramírez JM, Abella-Pérez E, Phillott AD, Sim J, West PV, Martín MP, Marco A, Diéguez-Urbeondo, J. 2014. Global distribution of two fungal pathogens threatening endangered sea turtles. *PLoS ONE* 9: e85853.
- Shaver DJ, Rubio C. 2008. Post-nesting movement of wild and head-started Kemp's Ridley sea turtles *Lepidochelys kempii* in the Gulf of Mexico. *Endanger Species Res* 4:43–55.
- Telemeco RS, Warner DA, Reid MK, Janzen FJ. 2013. Extreme developmental temperatures result in morphological abnormalities in painted turtles (*Chrysemys picta*): A climate change perspective. *Integr Zool* 8:197–208.
- Zimm R, Bentley BP, Wyneken J, Moustakas-Verho JE. 2017. Environmental causation of turtle scute anomalies in ovo and in silico. *Integr Comp Biol* 57:1303–1311.

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