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PATHOLOGIC FINDINGS IN HATCHLING AND POSTHATCHLING LEATHERBACK SEA TURTLES (*DERMOCHELYS CORIACEA*) FROM FLORIDA

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ABSTRACT: In an attempt to identify critical health issues affecting the survival of endangered leatherback sea turtles (*Dermochelys coriacea*), a prospective study was conducted in several dead-in-nest hatchlings and captive posthatchlings to examine pathologic changes and presence of pathogenic microorganisms. Numerous histopathologic changes were identified. Although bacterial etiologies were suspected in deaths of captive individuals, a single causative organism was not identified but rather, a mixed population of bacterial flora was cultured. Muscle degeneration observed in most samples implicates a potential environmental factor in species survival and needs future investigation.

Key words: *Dermochelys coriacea*, hatchling, histopathology, leatherback sea turtle, posthatchling.

INTRODUCTION

There are seven species of extant sea turtles; all are considered to be imperiled, and US populations are listed as threatened or endangered under the Endangered Species Act and are listed by the International Union for Conservation of Nature (IUCN) as endangered or critically endangered. Because of their predominantly pelagic nature, sea turtles can be challenging to study. Thus, the reasons for their limited numbers continue to elude us (Bell et al., 2003; Wallace et al., 2004). However, as we become familiar with the behavior and special needs of sea turtle species and with the advent of new and sophisticated laboratory and field testing, scientists are beginning to understand some of the factors that impede species recovery.

Because nesting females enter a cataleptic state once egg deposition commences, biologists can collect data (e.g., morphometrics, external body condition evaluation) during oviposition. During this time, the biologist can tag females for long-term monitoring and safely collect blood to assess health status with little or no detectable stress (Owens, 1999). After nest emergence, the dead-in-nest hatchlings or unhatched embryos may be collected to test for contaminants, infectious agents, deformities, and to assess the genetic structure of the population (Perrault et al., 2008). Dead hatchlings may become useful when paired with findings from the mother's blood sample and other descriptors of condition.

Leatherback sea turtles (*Dermochelys coriacea*) are the largest of the sea turtles. Their unique carapace is formed of fat and connective tissue embedded with thin dermal ossicles (Wyneken, 2001). In the western Atlantic, leatherbacks nest on several Caribbean islands and a recently growing population nests on Florida's east coast (Turtle Expert Working Group [TEWG], 2007). They have a particularly low hatchling emergence success (mean= $41.5\% \pm 23.3\%$) and nest success rates can be bimodal (ranges=30–39% and 50–59%; Perrault et al., 2008). In an attempt to refine estimates of hatchling recruit-

ment and understand the behavior and physiology of this pelagic species (e.g., Reina et al., 2002; Constantino and Salmon, 2003; Salmon et al., 2004; Jones et al., 2007; Gless et al., 2008), researchers collect and rear hatchlings (Jones et al., 2000) for weeks to months for study, and then later release them into the ocean. One such rearing facility, with over 10 yr of experience, noted and tracked recent survival issues with the hatchlings. Starting in 2005, hatchlings brought to the facility often appeared weak or eventually succumbed to opportunistic pathogens. Thus, we chose to investigate deaths as well as screen for significant changes in the dead-in-nest hatchlings in an attempt to identify critical health issues in this species and develop a hatchling screening protocol to maximize successful rearing. Herein, we describe pathologic findings from hatchlings (including dead in nest) and post-hatchlings (turtles >4 wk of age) from 2005 to 2007.

MATERIALS AND METHODS

Animals and study site

The leatherback sea turtle hatchlings involved in this investigation originated from nest sites on Juno Beach and Boca Raton, Florida on the western Atlantic coastline. The hatchlings examined were of two sources. The first source was dead-in-nest hatchlings ($n=56$) that were collected from 17 nests (one to eight hatchlings collected per nest) after nest emergence and during nest inventory. Dead-in-nest hatchlings included turtles that had successfully hatched from the egg but died while attempting to emerge from the nest. For three nests ($n=6$ hatchlings collected), the heads of the dead-in-nest hatchlings were at the surface at the time of death. All dead-in-nest hatchlings were shipped overnight on ice to the University of Georgia College of Veterinary Medicine, Veterinary Diagnostic and Investigational Laboratory (VDIL) in Tifton, Georgia, USA for necropsy.

The second source was from captive hatchlings ($n=45$) that represented a subset of individuals collected from 16 nests (one to four hatchlings per nest) at nest emergence and transported to a captive facility. These collections were conducted under Florida Fish and Wildlife Conservation Commission Sea Turtle Permit No. 073 and US Fish and Wildlife Service permit TE05217-2. Animals were cared for in strict accordance with the guidelines set forth by Florida Atlantic University's Institutional Animal Care and Use Committee under authorization No. A0026. Leatherbacks are unusually difficult to maintain in captivity, as the species are gelatinavores (prey on jellyfish, comb jellies, pyrosomas, and pelagic tunicates; Den Hartog and Van Nierop, 1984; Davenport and Balazs, 1991; Frazier et al., 1995) and require oceanic quality water (Jones et al., 2000). It is an open ocean species that seldom learns to avoid barriers. Despite these difficulties, the sea turtle facility had a history of successfully rearing the hatchlings. Captive turtles were to be reared for generally no more than 3 mo as part of ongoing studies of hatchling behavior and gonadal development. This time period allowed sufficient growth for turtles to reach a size conducive for laparoscopic examination of gonads, after which hatchlings were released.

Captive turtles were housed indoors in an open-flow sea water system with UVA-UVB fluorescent lighting 45 cm above the tanks and on a 12:12-hr cycle. The water was filtered with the use of two series of filter socks, passed through a protein skimmer, treated with ultraviolet light, and sent through a chiller to bring temperatures to 21–24 C. In 2005, five of 32 (16%) 3–6-wk-old posthatchlings became lethargic, anorexic, and bloated. Small white to yellow epidermal plaques, pink flipper edges, and pale (gray) patches of skin were observed. Turtles with external epidermal lesions (small yellow plaques, necrosis of flipper edges, and or small pale patches of skin) were treated daily with 1% povidone iodine ointment, or, if no improvement was observed, then with 1% silver sulfadiazine cream (Par Pharmaceuticals, Spring Valley, New York, USA). Turtles with gastrointestinal signs were treated initially with oral simethicone liquid (1–2 mg/kg q 24 hr, Johnson & Johnson, Merck Consumer Pharmaceuticals Co., Fort Washington, Pennsylvania, USA). If the condition did not improve, turtles were treated with intramuscular injection of ceftazidime (20 mg/kg q 72 hr, GlaxoSmithKline, Research Triangle Park, North Carolina, USA) amikacin (2.5 mg/kg q 72 hr, Vedco, Phoenix Scientific, Inc., St. Joseph, Missouri, USA), oral nystatin (100,000 U/kg q 24 hr, Morton Grove Pharmaceuticals, Morton Grove, Illinois, USA; compounded to working strength). Despite therapeutic attempts, the five turtles died approximately 2 days after the onset of clinical signs. In subsequent years, all hatchlings were quarantined in separate tanks,

TABLE 1. Leatherback sea turtles (*Dermochelys coriacea*) that died in quarantine.

Year	Loss in quarantine
2005	5/32 (16%)
2006	4/45 (9%)
2007	25/40 (63%)

until they began feeding (generally 5–10 days), before entering the colony. Turtles that died in quarantine never entered the study colony (Table 1). Within the first hours to several days in the water, 10 (four in quarantine) and 30 (25 in quarantine) turtles died in 2006 and 2007, respectively, and were shipped overnight on ice to the VDIL.

Specimen collection

Full necropsies were performed on all turtles. Sections of multiple tissues (skin, muscle, brain, eye, heart, lung, miscellaneous connective tissue and glands, tongue, trachea, esophagus, pancreas, stomach, intestines, kidney, gonads, adrenal glands, cloaca) were collected and placed in 10% buffered formalin for histologic examination. Swab specimens collected from the choana and cloaca, and fresh tissue specimens (liver, esophagus, kidney, spleen, small intestine, large intestine, stomach, coelomic cavity, lung, skin lesion) were collected for microbial culture and virus isolation. Fecal specimens were collected for electron microscopic examination and fecal parasite screening.

Diagnostic testing

Formalin-fixed tissues were routinely processed and embedded in paraffin. One or more 4-µm-thick sections were cut from each paraffin block and placed on glass slides. The slides were stained with hematoxylin and eosin (H&E), covered with a coverslip, and viewed by light microscopy for histolopathologic changes in tissues.

Fresh tissue specimens from all organs were used for virus isolation. In brief, a 10% tissue homogenate was made and filtered directly onto confluent monolayers of a variety of cell lines, including fathead minnow (FHM), white sturgeon skin (WSSK), channel catfish ovary (CCO), and epithelioma papilloma cyprini cells (EPC). Cultures demonstrating viral cytopathic effect (CPE) were harvested and random isolates verified by electron microscopy.

Swabs and tissues were tested for the presence of bacterial pathogens. Samples were inoculated into blood agar, MacConkey agar,

and Sabouraud dextrose agar. Isolates were speciated either by using an automated bacterial identification system (Sesititer, Trek Diagnostic Systems, Westlake, Ohio, USA) or conventional biochemical testing.

Feces were mixed with Sheather's sugar solution in a conic tube, covered with a coverslip, and allowed to sit for approximately 1 hr. The coverslip was then placed on a slide and examined for parasite ova and oocysts with the use of light microscopy.

Fecal samples were examined for virus by negative stain electron microscopy. Grids were examined for viruses or viruslike particles with the use of a Zeiss EM 900 TEM at 12,000 power magnification or greater.

RESULTS

Gross necropsy findings

Significant gross changes were not always noted, but when present included mild external and internal changes, and were primarily observed in the captive group. External changes included minimally (barely noticeable) sunken eyes and mild (pale pink) erythema around the cloaca, along the inferior trailing edge of the flippers, around the umbilical scar, and occasionally elsewhere. Fat reserves and skeletal musculature were minimal. Internal changes included wet congested lungs, mottled red/tan kidneys, pale hearts and livers, distended gall bladders, and mildly to moderately (up to 2×) distended (with gaseous contents) stomachs and intestines (Fig. 1A). Intussusceptions were seen in the mid to distal intestines of two captive posthatchlings and were characterized by fibrinous adhesions and erythema, and were friable (Fig. 1B).

Histopathologic findings

Histopathologic changes were primarily observed in the kidneys, heart, skeletal muscle, lungs, and alimentary tract. In general, pulmonary edema and thrombosed vascular spaces were more common in the dead-in-nest group, whereas renal tubular and cardiac degeneration were most pronounced in the captive group (Table 2). Granulopoiesis was common in
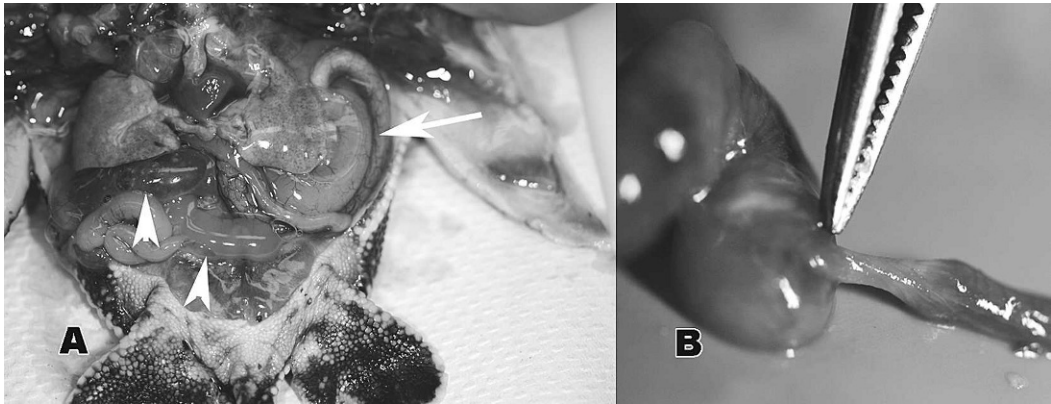
FIGURE 1.   Photographs of gross lesions observed in leatherback sea turtle (*Dermochelys coriacea*) hatchlings. A. Stomach (arrow) and intestines (arrowhead) were filled with gas in the captive-reared hatchlings. B. Intussusceptions were seen in two captive-reared hatchlings.

the livers and kidneys in both groups (Table 2) but appeared increased in organs displaying histopathologic changes (e.g., degeneration or inflammation).

Although changes were noted in most lungs ($n=82$, Table 2), moderate to marked changes were observed less often ($n=31$). Affected lungs contained pale eosinophilic amorphous material (edema) that filled airways and falveoli and expanded the interstitium along with infiltrating heterophils. In 13 hatchlings, falveoli were expanded by cellular debris admixed with heterophils, macrophages,

and bacteria and surrounded by bands of macrophages with early granuloma formation (Fig. 2A). Similar inflammatory cells were found infiltrating and expanding the interstitium surrounding these areas.

Rare foci of necrosis were observed in the livers ($n=2$ captive and 3 dead in nest) and kidneys ($n=5$ captive and 2 dead in nest). These areas presented as granulomas with central regions of cellular debris and bacteria surrounded by heterophils and macrophages. When bacteria were observed in the tissues, intravascular margination of heterophils (Fig. 2B) and associated perivascular infiltration by heterophils were noted.

All turtles had some degree of epithelial cell degeneration noted in the renal tubules (Table 2). However, severe degeneration and necrosis were only observed in turtles ($n=9$) from the captive group. Captive posthatchlings that died after longer duration (days) of clinical illness had marked (>50% of the organ affected) disruption, vacuolar degeneration and fragmentation of the epithelial lining cells. These individuals often presented with renal tubular mineralization and deposited calculi (Fig. 2C).

Heart and skeletal muscle fibers had mild (25% fibers affected) to moderate (25–50% of fibers affected) and occasionally marked (>50% of fibers affected)

TABLE 2.   Histopathologic changes observed in tissues collected from captive (C) and dead-in-nest (D) leatherback sea turtles (*Dermochelys coriacea*). Numbers in parentheses represent the percent of turtles affected in the respective group.

| Histopathologic change	Captive vs. dead in nest
Liver degeneration	C (82), D (95)
Liver granulopoiesis	C (96), D (89)
Kidney granulopoiesis	C (73), D (75)
Nephritis	C (38), D (45)
Renal tubular degeneration	C (58), D (41)
Renal mineralization	C (13), D (7)
Cardiac degeneration	C (58), D (41)
Skeletal muscle degeneration	C (24), D (32)
Gastrointestinal inflammation	C (36), D (46)
Pulmonary edema	C (18), D (23)
Pneumonia	C (78), D (80)
Pulmonary thrombosis	C (0), D (5)
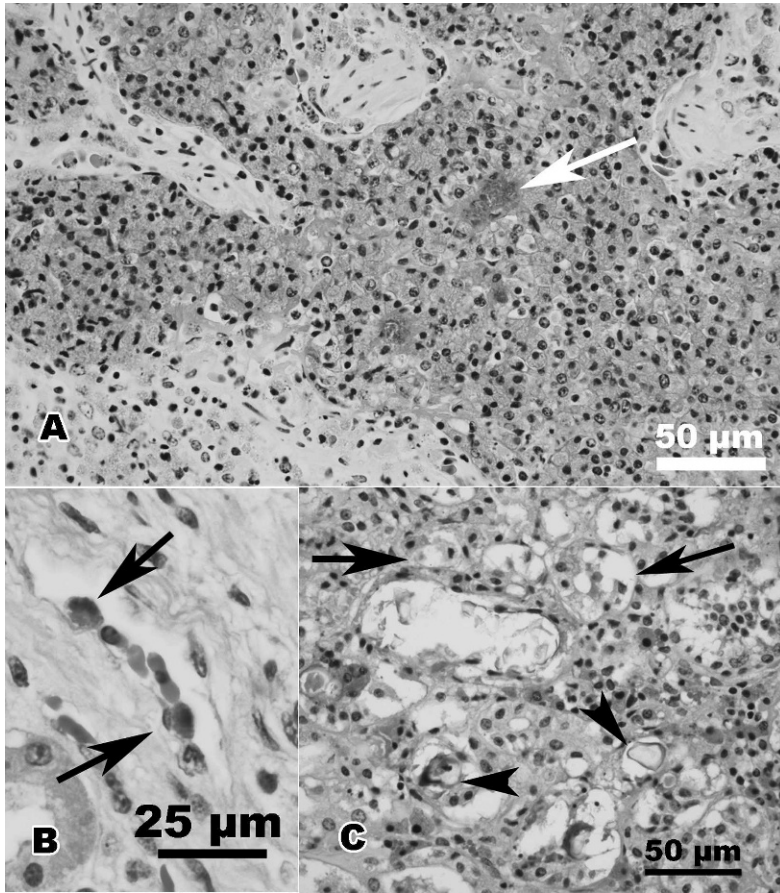
FIGURE 2.   Hematoxylin and eosin–stained sections from captive leatherback sea turtle (*Dermochelys coriacea*) hatchlings. A. Lung with falveoli expanded by cellular debris admixed with heterophils, macrophages, and bacteria (arrow). B. Blood vessel showing intravascular margination of heterophils. C. Kidney with vacuolar degeneration and fragmentation of the epithelial lining cells (arrows) and calculi (arrowheads).

vacuolar degeneration, fragmentation, loss of cross striations, and wavy fibers (Fig. 3, Table 2). The most severe changes were only observed in the captive posthatchlings. However, mild to occasionally moderate changes were found in both groups.

There were a few other miscellaneous but significant changes noted. In three hatchlings, skin sections had erosion of the epidermis with transepidermal and dermal infiltration of heterophils and bacteria (Fig. 4). One posthatchling presented with severe mycotic dermatitis from which *Fusarium* spp. was isolated, but not from internal organs. Bone-marrow sections of three captive animals revealed a slight predominance of granulocytic cells. Submucosal heterophilic infiltrates with vacuolation of the epithelial lining cells and dilation (1.5×) of crypts were noted in the intestinal sections of turtles with bloating and intussusceptions. The lymphoid tissues (including thymus) were within normal limits.

Microbial findings

Microbial cultures resulted in a mixed growth of opportunistic organisms (Table 3). A common organism was not consistently isolated from captive or dead-in-nest turtles. Given the postmortem interval likely varied from less than
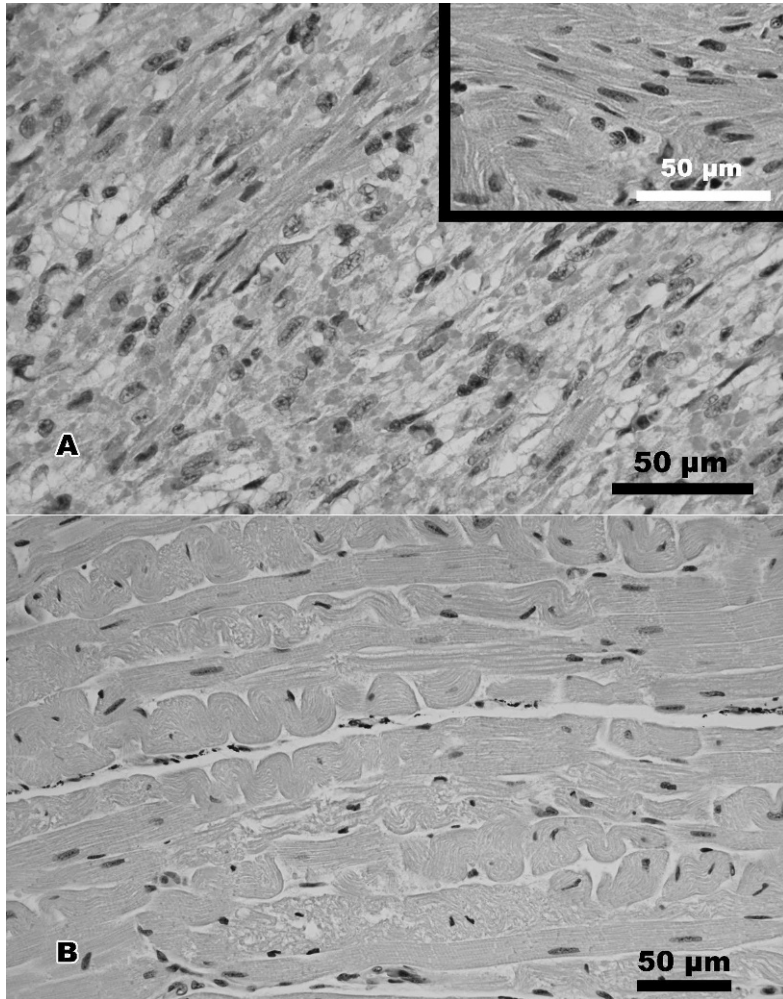
FIGURE 3.   Hematoxylin and eosin–stained sections from captive leatherback sea turtle (*Dermochelys coriacea*) hatchlings. A. Cardiac muscle fibers showing vacuolar degeneration, fragmentation, and loss of cross striations. Normal cardiac muscle is shown for comparison (inset). B. Skeletal muscle fibers showing similar changes.

24 hr to possibly a few days (for dead-in-nest turtles), bacterial culture results may be skewed by postmortem microbial growth and thus are not included. No viruses were isolated. Neither ova nor parasites were detected in the feces.

DISCUSSION

Although a variety of significant changes were observed, the muscular changes are of particular interest. These changes may suggest an underlying nutritional etiology, toxic etiology, or be secondary to systemic disease (i.e., septicemia/toxemia) or hypoxia during failure to emerge. It was noted that the cardiac changes also were reminiscent of changes seen with selenium deficiency, such as in bovine neonates born to selenium-deficient cows (Enjalbert et al., 1999). Given the current concern regarding mercury levels in our oceans (Booth and Zeller, 2005), its potential effects on marine animals (Caurant et al., 1996; Dietz et al., 2000; Cardellicchio et al., 2002), and our
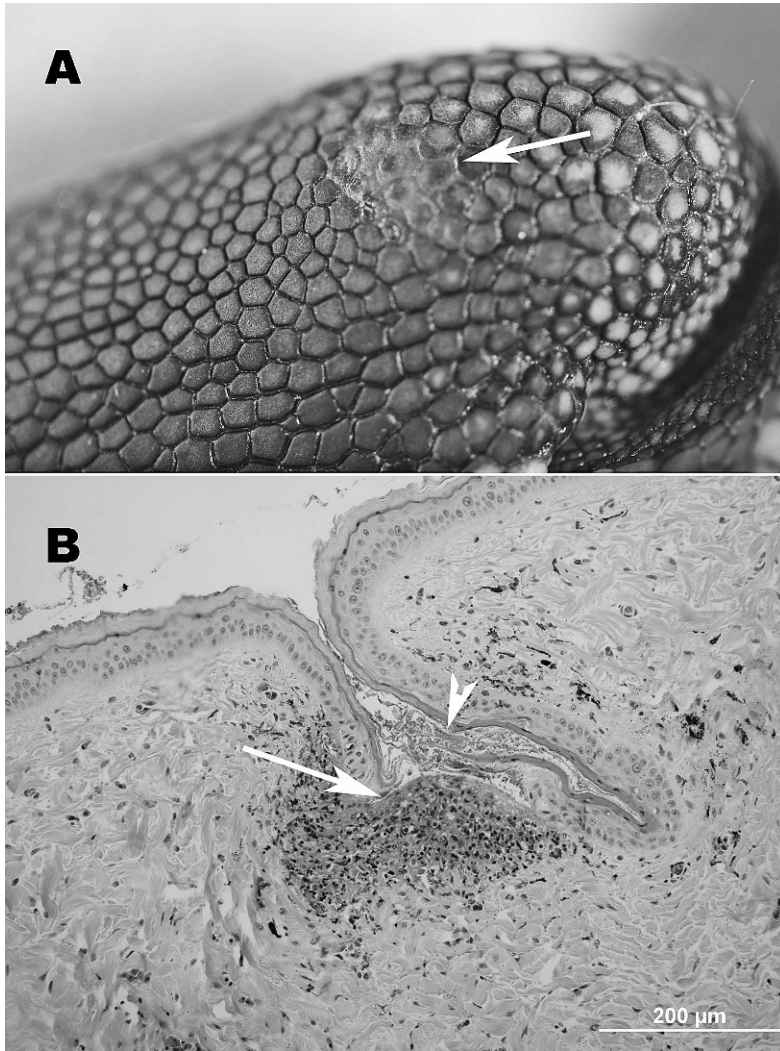
FIGURE 4.   A. Photograph of a lesion observed on the flipper of a leatherback sea turtle (*Dermochelys coriacea*) hatchling that died at emergence. B. Hematoxylin and eosin–stained section showing erosion of the epidermis with transepidermal and dermal infiltration of heterophils (arrow) and bacteria (arrowhead).

understanding that the body may use selenium to detoxify mercury, these changes provide the impetus for future investigation into mercury and selenium levels in sea turtles. Currently, mercury levels have only been reported for adult sea turtles (Day et al., 2005; Deem et al., 2006) and levels in hatchlings remain unknown. Thus, a focus of our future study is to correlate mercury and selenium levels in nesting females and their hatchlings.

Another consistent finding was renal tubular degeneration. The renal changes were severe in some turtles but these tended to be the turtles that had experienced prolonged (greater than 1 day) illness. Thus, these changes may be secondary to bacterial septicemia/toxemia along with suspected dehydration. Further, we cannot rule out the possibility that our therapeutic attempts (e.g., amikacin administration), in light of the suspected dehydration, may have contrib-

TABLE 3.   Microbial organisms cultured from 30 captive turtles (C) and four hatchlings that died at emergence and were considered dead-in-nest (D) turtles. Culture results are not provided for animals dead greater than 24 hr. Numbers in parentheses represent percent of turtles affected.

Organism	Captive vs. dead in nest	Sample cultured
Bacteria		
Acinetobacter baumannii	C (6)	Intestines, coelomic swab, lung, liver, kidney, heart, skin
Alcaligenes faecalis	C (13), D (25)	Coelomic swab, lung, liver, kidney, heart, skin
Bacillus spp.	C (6)	Lung
Brevundimonas dimunuta	C (17)	Skin
Brevundimonas vesicularis	C (3)	Coelomic swab, lung, stomach, liver, intestine
Burkholderia cepacia	C (3)	Skin
Chryseobacterium meningosepticum	C (6)	Liver, lung, liver, kidney, heart, skin
Chryseobacterium spp.	C (6), D (100)	Carapace, heart, skin, coelomic swab
Citrobacter freundii[a]	C (6)	Liver, kidney, spleen, heart, intestine, carapace, bone
Enterobacter aerogenes	C (3), D (100)	Lung, liver, kidney, heart, skin, coelomic swab
Enterobacter cloacae[a]	C (17), D (25)	Stomach, intestine, lung, bone, carapace, esophagus, liver, kidney, heart, skin, coelomic swab
Enterococcus faecium[a]	C (57)	Lung, liver, kidney, stomach, bone, muscle, intestine, heart, skin, esophagus, coelomic swab
Morganella morganii	C (3)	Coelomic swab
Mycobacterium spp.	C (17)	Skin, kidney, coelomic swab, esophagus
Pseudomonas aeruginosa[a]	C (20)	Heart, carapace, lung, coelomic swab
Pseudomonas fluorescens	C (3)	Stomach, intestine, lung, bone, carapace, esophagus, coelomic swab, heart, liver, skin
Pseudomonas mendocina[a]	C (40), D (25)	Coelomic swab, heart, lung, kidney, liver, intestine, carapace, bone, esophagus, stomach, skin
Shewanella putrefaciens[a]	C (67), D (25)	Coelomic swab, heart, lung, kidney, liver, intestine, carapace, bone, spleen, stomach, skin, esophagus
Sphingomonas paucimobilis	C (3)	Skin
Streptomyces spp.	C (3)	Coelomic swab
Alpha Streptococcus spp.[a]	C (13)	Coelomic swab, heart, lung, kidney, liver, yolk sac, intestine, carapace, bone, muscle
Vibrio fluvialis	C (20)	Coelomic swab, kidney, skin, lung
Fungi		
Candida spp.	C (6)	Stomach
Fusarium spp.	C (3)	Skin, carapace

[a] Organisms cultured from five of the turtles suspected to be septic.

uted to these changes during the perimortem period. Additionally, these changes are similar to those reported in birds with "chick nephropathy" (McMullin, 2004), where renal tubular necrosis and tophi are seen and theorized to perhaps be due to dehydration secondary to low humidity during incubation. Another possible cause for these changes in birds is viral infection (Shirai et al., 1992). Virus isolations were negative in the leatherbacks.

Pulmonary changes may be secondary to other pathology. In captive individuals, the pulmonary edema may have been secondary to cardiac compromise in individuals with myocardial degeneration. Necrosis in the lungs was similar to that seen in the liver and kidneys and may have indicated bacterial septicemia, especially

when vascular thrombosis was present. For dead-in-nest hatchlings, the degree of pulmonary edema and inflammation may be reflective of the length of time that the hatchlings were in the nests attempting to emerge.

The vascular changes suggest a systemic process (e.g., septicemia/toxemia). Although bacterial etiologies were suspected as factors in the deaths of many of the captive group, the fact that mixed populations of organisms were generally cultured suggests that these bacteria were opportunistic invaders. Further, the skin lesions observed in the dead-in-nest hatchlings suggest a possible route of entry for opportunistic organisms. Given that similar bacteria were cultured from both the captive and dead-in-nest groups, it is possible that the opportunistic bacteria isolated from the captive group may have been brought to the tanks from the nests on the external surfaces of the hatchlings.

Because of these findings, all hatchlings considered for entrance into the captive colony are now examined for dermal lesions and washed with a mild antimicrobial soap prior to entrance into quarantine. Hatchlings are closely monitored for onset of normal behaviors before being transferred to the study colony (generally 5–10 days). Minimal criteria for entrance into the study colony include normal swimming and breathing patterns, and initiation of feeding and defecation.

The results of this investigation provide us with direction for future investigation. Currently, studies are under way to attempt to identify possible environmental factors contributing to hatchling emergence success and survival. Ultimately, the results of these studies may aid in the development of management strategies for this and other sea turtle species.

ACKNOWLEDGMENTS

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