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# Health Evaluation of Leatherback Turtles (*Dermochelys coriacea*) in the Northwestern Atlantic During Direct Capture and Fisheries Gear Disentanglement

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Abstract. – **Health evaluations were conducted in the northwestern Atlantic for 19 leatherback turtles, which included 12 turtles directly captured as part of a satellite telemetry study and 7 turtles entangled in fishing gear. Assessment included physical examination; determination of heart rate and respiratory rate; and hematologic, plasma biochemical, nutritional, toxicologic, parasitologic, and microbiological investigations. Significant differences were found between directly captured and entangled turtles for curved carapace length, curved carapace width, blood urea nitrogen, chloride, sodium, triglycerides, relative heterophil count, relative monocyte count, relative and absolute eosinophil count, pH, bicarbonate, lead, and beta-hydroxybutyrate. Directly captured turtles showed evidence of mild respiratory acidosis. Significant differences were found between sexes for curved carapace length, curved carapace width, total protein, globulin, sodium, relative monocyte count, gamma-globulin, and anion gap. Relatively high blood concentrations of selenium and cadmium were found in all turtles.**

Key Words. – **Reptilia; Testudines; Dermochelyidae, leatherback turtle; *Dermochelys coriacea*; health; entanglement; hematology; toxicology; biochemistry; parasitology; microbiology**

The leatherback sea turtle (*Dermochelys coriacea*) is the largest living species of turtle. It is listed as critically endangered by the World Conservation Union (IUCN 2009) and as an endangered species under the United States Endangered Species Act of 1973. Global threats to the species include collection of eggs and adults for human consumption (Eckert and Sarti 1997; Spotila et al. 2000), loss, degradation, and artificial lighting of nesting habitat (Lutcavage et al. 1997; Deem et al. 2007), ingestion of and entanglement in marine debris (Balazs 1985; Mrosovsky et al. 2009), climate change effects on ocean productivity (Wallace et al. 2006; Saba et al. 2008), and mortality because of fishery interactions (National Research Council 1990; Lewison et al. 2004a). Leatherback turtles migrate great distances between temperate foraging sites and tropical nesting sites, and thus are at risk from both pelagic fisheries (e.g., longline fisheries) (Witzell 1996; Lewison et al. 2004b; López-Mendilaharsu et al. 2009) and inshore fisheries (e.g., fixed-gear fisheries) (Lutcavage and Musick 1985; Godley et al. 1998; Dwyer-Dodge et al. 2002; James et al. 2005; Alfaro-Shigueto et al. 2007; López-Mendilaharsu et al. 2009).

In spite of its endangered status, the health of leatherback turtles remains largely uninvestigated (Turtle Expert Working Group 2007). Several physiologic and toxicologic investigations of leatherbacks have been conducted, but relatively few studies have described health data such as hematologic and plasma biochemical parameters, and nutritional values. Several recent studies have reported health information for nesting female leatherbacks from Gabon and Trinidad (Deem et al. 2006; Harms et al. 2007) and for nesting and foraging leatherbacks in the Pacific (Harris et al. in press).

This study was conducted to gather baseline health data about leatherback turtles at sea during their active

foraging season in the northwestern Atlantic. In addition, this study gathered medical data from live leatherbacks that were entangled in fishing gear, for comparison with directly captured conspecifics.

## METHODS

Health assessments were conducted on leatherback turtles in the northwestern Atlantic during direct capture or fisheries gear disentanglement. Direct capture was conducted off Georgia, USA, in 2007 and Massachusetts, USA, in 2008 to investigate the movement patterns, feeding ecology, and habitat use of leatherback turtles in the northwestern Atlantic. Veterinary personnel were deployed on each capture expedition as described by the National Marine Fisheries Service Procedures for Handling and Monitoring Leatherbacks During Capture-Related Work. (NMFS ESA Permit No. 1557–03)

For direct capture, turtles were spotted at sea by observers in a plane or boat. Upon locating a turtle at the surface, a break-away hoop net with a purse-string closure mechanism was used to capture the turtle from the bow of the boat. The turtle then was secured on a custom-built ramp deployed from the stern and was brought onto the deck for evaluation, satellite tag application, and diagnostic sample collection. Details of the capture and satellite tag attachment methodology will be reported elsewhere. Leatherback turtles entangled in fishing gear were assessed off Massachusetts in 2007 and 2008 (under the authorization of NOAA 50 CFR Part 222.310). Entangled turtles were brought onboard a boat for disentanglement, physical examination, diagnostic sample collection, and satellite tag application. Water temperature, depth, latitude, and longitude were recorded for each capture or entanglement location. For directly captured turtles, the time of initial capture, time of boarding, and time of release were recorded. For entangled turtles, the minimum duration of entanglement (time when the first report of entanglement was received to time of disentanglement), time of disentanglement, and time of release were recorded. The duration of each handling event was defined as the time between net capture and release for directly captured turtles and the time between initial disentanglement effort and release for entangled turtles.

Once on board the boat, each turtle was measured (curved carapace length [CCL] and curved carapace width [CCW]), photographed, and checked for external tags and internal passive integrated transponder (PIT) tags (Eckert et al. 1999). A hose was used to keep the turtle moist by using ambient seawater, and a moist cloth was placed over the eyes to decrease visual stimuli. A flexible digital temperature probe was inserted approximately 30 cm into the cloaca to record initial body temperature. For some turtles, a second body temperature measurement was obtained approximately 15 minutes after the first measurement. The difference between initial body temperature and sea surface temperature was calculated. The respiratory rate was recorded twice at a 20-minute interval by visual monitoring. Determination of heart rate was attempted by using an EC60 electrocardiograph monitor (Silogic Design Ltd, Stewartstown, PA) via alligator clips attached to the shoulders and proximal hind limbs, or by a Doppler blood flow detector (Pocket-Dop3; Nicolet Vascular, Madison, WI). Sex was assigned based on sexual dimorphism of the tail for turtles longer than 145-cm CCL (James et al. 2007). For 5 turtles that were less than 145 cm, sex was identified based on display of the penis during examination, subsequent necropsy, or confirmed subsequent nesting. All other turtles that were shorter than 145-cm CCL were classified as unknown sex.

A physical examination was conducted by the veterinary team while the satellite tag team attached the tag. Voided fecal samples were collected opportunistically. Nasal and cloacal aerobic bacterial and fungal culture samples were collected by using a sterile BBL Culture-Swab (Becton Dickinson and Company, Franklin Lakes, NJ) inserted 1–2 cm into the nares, and 5–10 cm into the cloaca, respectively. Upon completion of satellite tagging, venipuncture sites were disinfected by using sterile povidone iodine and isopropyl alcohol–infused gauze pads, and a 12–20-mL blood sample was collected from the dorsal cervical sinus or dorsal coccygeal vein by using a 1.5–3-inch, 18–21-gauge needle attached to a heparinized syringe. Syringes were prepared by using liquid sodium heparin (Heparin sodium, 1000 USP/mL; APP Pharmaceuticals, LLC, Schaumburg, IL), which was repeatedly expelled from the syringe until no visible heparin remained, which resulted in a heparin concentration of less than 10 USP per mL of blood. The time of blood collection relative to the time of capture was recorded. One milliliter of blood was immediately transferred into brain heart infusion broth (BBL Septicheck BHI; Becton Dickinson) by using a sterile needle for bacterial and fungal culture. After sampling, blood, fecal, and culture samples were placed in a cooler with ice until processed as described below.

As soon as possible after blood collection (median, 8.5 minutes; Table 1), whole blood was analyzed directly from the collection syringe for pH, partial pressure of carbon dioxide ($pCO_2$), partial pressure of oxygen ($pO_2$), bicarbonate ($HCO_3$), glucose, sodium, potassium, total carbon dioxide ($TCO_2$), ionized calcium, and lactate, by using a point-of-care analyzer (iSTAT [Heska Corporation, Loveland, CO]; i-STAT CG4+, and CG8+ cartridges [Abbott Point of Care Inc, Abbott Park, IL]) by following manufacturers' instructions. Whole blood was then transferred to lithium heparin blood collection tubes (BD Vacutainer; Becton Dickinson) and placed on ice for later hematologic and toxicologic studies. A portion of blood was centrifuged as soon as possible after collection (median, 35 minutes; Table 1) at $1500 \times g$ for 5 minutes, and the plasma was harvested and placed on ice for later biochemical, nutritional, and toxicological studies. Upon return to shore, microbiologic, hematologic, plasma

**Table 1.** Temperature, respiratory rate, heart rate, and time of handling events for 12 directly captured and 7 entangled leatherback turtles in the northwestern Atlantic.

| | Minimum | Maximum | Mean | Median | SD | *n*[a] |
|---|---|---|---|---|---|---|
| Initial body temperature (°C) | 23.3 | 30 | 26.6 | 26.5 | 2.0 | 16 |
| Sea surface temperature (°C) | 17.7 | 25.1 | 20.3 | 19.8 | 1.9 | 19 |
| Delta body-water temperature (°C) | 2.6 | 9.9 | 6.2 | 5.8 | 2.3 | 16 |
| Second body temperature (°C) | 24.4 | 26.8 | 25.9 | 26.5 | 1.3 | 4 |
| Time of second body temperature (min) | 7 | 20 | 13.7 | 14.0 | 6.5 | 3 |
| Initial respiratory rate (bpm[b]) | 1 | 8 | 3.8 | 4 | 1.8 | 14 |
| Second respiratory rate (bpm) | 1 | 6 | 3.0 | 3 | 1.5 | 11 |
| Time of second respiratory rate (min) | 9 | 33 | 20.4 | 20.0 | 7.2 | 9 |
| Heart rate (bpm) | 24 | 36 | 30.9 | 32 | 5.5 | 8 |
| Time to collect blood (min) | 22 | 54 | 35.3 | 34.5 | 7.4 | 15 |
| Time to run i-Stat (min) | 2 | 33 | 9.4 | 8.5 | 7.4 | 14 |
| Time to centrifuge blood (min) | 5 | 900 | 124 | 35 | 236 | 15 |
| Minimum duration of entanglement (min) | 100 | 280 | 180 | 156 | 72 | 7 |
| Duration of event (min) | 40 | 100 | 49.9 | 49.0 | 13.2 | 19 |

[a] Number of turtles for which each parameter was recorded.
[b] bpm = breaths per minute (respiratory rate) or beats per minute (heart rate).

biochemical, and fecal samples were transferred on ice to a veterinary diagnostic laboratory, refrigerated, and analyzed within 24 hours (culture and blood testing) to 48 hours (fecal testing) of collection. Samples for nutritional and toxicologic studies were frozen at –80°C for 2–10 months before shipping on ice to analytical laboratories.

Before release, all the turtles were tagged with a single PIT tag in the dorsal shoulder musculature with a sterile syringe implanter (TX1440L 125kHz tags; Biomark, Inc, Boise, ID), and Inconel flipper tags (model 681; National Band and Tag Co, Newport, KY) were applied to the thin fold of skin between the tail and the rear flippers (Eckert et al. 1999). Two small (4–6 mm) skin samples were collected with sterile, disposable biopsy punches (Acuderm Inc, Fort Lauderdale, FL) from the posterior margin of the rear flippers for genetic and stable isotope analyses (Dutton 1996). All tagging and biopsy sites were cleaned and disinfected with sterile povidone iodine and isopropyl alcohol–infused gauze pads before tag application and sampling.

*Toxicology*. — Whole blood samples were analyzed for mercury, selenium, copper, lead, and cadmium (wet weight) at the Center for Environmental Sciences and Engineering at the University of Connecticut by using previously described methods (Innis et al. 2008). Briefly, samples were analyzed for copper, lead, selenium, and cadmium (limit of detection, 0.06, 0.01, 0.12, 0.01 μg/g, respectively) by using a Perkin Elmer/Sciex ELAN inductively coupled plasma mass spectroscopy after a nitric acid–hydrogen peroxide digestion. Blood samples were analyzed for mercury (limit of detection, 0.003 μg/g) by using cold vapor atomic absorption spectrophotometry by USEPA method 245.6 after sulfuric and nitric acid digestion. Standard quality assurance procedures were used, including analysis of duplicate samples, blanks, spikes, and standard reference materials. Plasma samples were analyzed for 20 organochlorine pesticides at the Virginia/Maryland Regional College of Veterinary Medicine Pesticide Residue Laboratory by using previously described methods (lower limit of quantification of 16 ppb; Innis et al. 2008). Chemicals included in the evaluation were aldrin, alpha-hexachlorocyclohexane (alpha-BHC), alpha-chlordane, beta-BHC, delta-BHC, dieldrin, endosulfan I and II, endosulfan sulfate, endrin, endrin aldehyde, endrin ketone, gamma-BHC (lindane), gamma-chlordane, heptachlor, heptachlor epoxide, methoxychlor, p,p′-dichlorodiphenyldichloroethylene; p,p′-dichlorodiphenyldichloroethane, and p,p′-dichlorodiphenyltrichloroethane.

*Microbiology*. — Microbiological analyses were initiated within 24 hours at a commercial veterinary diagnostic laboratory (Idexx Laboratories, North Grafton, MA). For bacterial isolation, nasal and cloacal swabs were plated onto Trypticase Soy Agar with 5% Sheep Blood (Becton Dickinson) and MacConkey II Agar (Becton Dickinson) by using standard methods at 25°C and 35°C for 18–24 hours. Subcultures included DNase Test Agar with Toluidine Blue (Becton Dickinson) for differentiation of *Serratia* spp.; BBL Selenite-F Broth (Becton Dickinson) for isolation of *Salmonella* spp., Hektoen Enteric Agar (Becton Dickinson) for isolation of *Shigella* spp., Campy CVA Agar (Becton Dickinson) for isolation of *Campylobacter jejuni*; Spot Indole Reagent (Remel Inc, Lenexa, KS) for isolation of *Escherichia coli*, Oxystrips (Hardy Diagnostics, Santa Maria, CA) for differentiation of *Pseudomonas* spp., and BBL DrySlide PYR Kit (Becton Dickinson) for the presumptive identification of *Enterococcus* spp. Differentiation was also accomplished by using BBL Crystal Identification Systems Enteric/Nonfermenter ID Kit (Becton Dickinson) or VITEK Colorimeter (bioMerieux Inc, Durham, NC). For cloacal samples, culture methods sought to identify only selected enteric zoonotic pathogens (*Salmonella* spp., *Campylobacter* spp., *Edwardsiella* spp., *Enterococcus* spp., *Pleisiomonas* spp., and *Shigella* spp.). For nasal and blood cultures, all isolates were identified and their antibiotic susceptibilities were tested. Antibiotic suscep-

tibility was determined by using VITEK Colorimeter susceptibility cards and Kirby-Bauer agar-disk diffusion, by following Clinical and Laboratory Standards Institute performance standards. Selection of antibiotics for sensitivity testing was based on whether the isolate was a gram-negative or gram-positive organism, as well as bacterial species–specific standard operating procedures of the diagnostic laboratory.

For fungal culture, nasal and cloacal swabs were plated onto inhibitory mold agar (Becton Dickinson) incubated at 22°–25°C and examined for fungal growth for 3 weeks. Yeast isolates were subcultured onto Sabaroud dextrose agar (Becton Dickinson) and incubated at 30°C for 24–48 hours. Yeasts were identified by using a IDS Rapid Yeast ID Kit (Remel) and a Vitek YBC card (bioMerieux). Filamentous fungal isolates were subcultured on potato flakes agar (Becton Dickinson) at 22°–25°C for 5–7 days and identified morphologically by using lactophenol cotton blue stain under light microscopy at ×100–400 magnification by following de Hoog et al. (2000), and Larone (2002).

Blood culture bottles were vented (BBL Venting Unit; Becton Dickinson) and incubated at 35°C for 8 days for aerobic bacterial isolation. In addition, broth was immediately subcultured onto blood agar (Becton Dickinson) for aerobic bacterial isolation at 25°C and 35°C, CDC anaerobe blood agar (Becton Dickinson) for anaerobic bacterial isolation at 25°C and 35°C, and inhibitory mold agar for fungal culture at 22°–25°C. Subcultures of broth were repeated at 72 hours for bacterial isolation and weekly for 3 weeks for fungal isolation. Gram stains of broth were prepared at the time of each subculture. Blood agar and CDC agar plates were observed daily for 5 days for bacterial growth. Inhibitory mold agar plates were examined for fungal growth for 21 days. Bacterial and fungal isolates were identified as described above. Blood cultures were reported as negative if no organisms were seen on broth Gram stains, no growth was seen on subculture plates, and culture broth remained clear for 30 days.

*Hematology and Chemistry*. — Hematologic and plasma biochemical assays were performed within 24 hours at a commercial veterinary diagnostic laboratory (Idexx Laboratories). Hematocrit was measured after centrifugation of blood at 13,000 × g for 5 minutes in heparinized capillary tubes. Differential leucocyte count was performed by a board-certified veterinary clinical pathologist. One hundred leucocytes were identified on fixed blood smears stained with a modified Wright-Giemsa stain (Fisher Scientific, Middletown, VA) by use of an automated stainer (HemaTek 2000, Bayer Health Care, Tarrytown, NJ). Total leucocyte count was performed manually by use of a manual, indirect leukocyte counting method. Briefly, 0.5 g of phloxine B stain was dissolved in 250 mL of propylene glycol and quantum sufficit to 500 mL in water. Twenty microliters of well-mixed whole blood was added to 620 µL of stain solution and incubated for 5 minutes. This mixture was then loaded onto a Neubauer hemacytometer, and the stained leukocytes were counted. Calculations for the total leukocyte count were performed by using previously described methods (Campbell and Ellis 2007). Plasma biochemistry values, including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, albumin, total protein, globulin, blood urea nitrogen (BUN), creatinine, cholesterol, glucose, calcium, phosphorus, chloride, potassium, sodium, uric acid, and triglycerides were measured by using an automated clinical chemistry analyzer (Olympus 5400; Olympus America, Melville, NY). Plasma protein electrophoresis was performed by using the Paragon SPEP II gel system (Beckman, Fullerton, CA) as described previously (Cray et al. 2007), with the exception that the total protein was determined by using an automated clinical chemistry analyzer as described above, rather than by refractometry.

*Parasitology*. — Fecal samples were evaluated for the presence of metazoan and protozoan parasites at the University of Florida, College of Veterinary Medicine. Samples were received fresh and unfixed on ice packs. Fecal flotation was performed for one-half of each sample by using Fecasol (Vetoquinol USA Inc, Buena, NJ). Feces were mixed well with 15 mL of flotation solution and then strained through 1 layer of cheesecloth into a 15-mL conical glass centrifuge tube until a slight positive meniscus was formed. A cover slip was placed onto the surface for 15 minutes. The cover slip was carefully removed and placed onto a glass microscope slide and the entire area under the coverslip was examined by light microscopy at ×100 magnification. Any helminth eggs or protozoan oocysts seen were measured and identified at ×400 magnification. The remainder of each fecal sample was used for fecal sedimentation to detect trematode eggs and helminth eggs that did not float in standard flotation solution. The sample was mixed well with 50-mL dilute detergent solution (0.1% solution of household liquid detergent and water), strained through one layer of cheesecloth into a standard 50-mL conical glass centrifuge tube, and allowed to stand vertically for 5 minutes. The supernatant was decanted, the tube was refilled with tap water, mixed with the sediment, and allowed to stand for 5 minutes. This process was repeated several times until the supernatant remained clear. When a clear supernatant was achieved, it was decanted and the entire volume of the sediment was examined for eggs, oocysts, cysts and larvae in a petri dish by using a dissecting microscope at ×20-40 magnification. Any parasite eggs, larvae, etc. that were observed were pipetted from the dish onto a glass slide, a coverslip was placed, and identification was performed by light microscopy as described above.

*Nutrition Assays*. — Plasma vitamin A (retinol), vitamin E (alpha-tocopherol), 25-hydroxyvitamin D, and beta-hydroxybutyric acid (BHB) analyses were performed

at the Diagnostic Center for Population and Animal Health at Michigan State University. Vitamins A and E were extracted from plasma with ethanol and hexane in volumetric ratios to plasma of 1:1 and 2:1, respectively. Concentrations of vitamins A and E were determined simultaneously by high-pressure liquid chromatography on a 75-mm Waters Symmetry C18 column (Waters Chromatography, Milford, MA) by using a mobile phase of 70:20:10 (v:v:v) acetonitrile:methylene chloride:-methanol. Detection was at 325 nm for retinol and 292 nm for alpha tocopherol. BHB concentrations were determined enzymatically via the BHB dehydrogenase reaction on an auto analyzer (Olympus 640e, Beckman Coulter, Inc, Brea, CA) by using a commercial mammalian kit (Catachem C442-0A, Catachem Inc, Bridgeport, CT). Circulating concentrations of 25-hydroxyvitamin D were determined with a commercially available radioimmunoassay kit validated for mammals (25-Hydroxyvitamin D $^{125}$RIA Kit, DiaSorin, Inc, Stillwater, MN). The procedures for acetonitrile extraction of the sample and performance of the assay were followed as described in the manufacturer's protocol. The manufacturer reports 100% crossreactivity of the 25-hydroxy-metabolites of both vitamin $D_3$ and vitamin $D_2$ and 0.8% crossreactivity with vitamin $D_3$ and $D_2$.

*Statistical Analysis*. — The effects of sex and entanglement on morphometric and blood data were determined. Statistical significance ($p < 0.05$) was tested with a 2-way analysis of variance (ANOVA) (sex and entanglement) with pairwise multiple comparisons determined by the Holm-Sidak method (SigmaStat 3.1; Systat, Richmond, CA). Those data failing to meet the assumptions of normality and equal variance were analyzed with a Kruskal–Wallis 2-way ANOVA on ranks ($p < 0.05$).

Results for blood pH, $pCO_2$, and $pO_2$ were mathematically corrected for each turtle's body temperature ($pH_{TC}$, $PCO_{2TC}$, $pO_{2TC}$); and pH-corrected ionized calcium values ($iCa_{cor}$) were calculated by using pH $_{TC}$ (Chittick et al. 2002; Innis et al. 2007). A corrected $HCO_3$ value ($HCO_{3cor}$) was calculated by using the Henderson-Hasselbalch equation, $pH_{TC}$, $pCO_{2TC}$, and values of $\alpha CO_2$ and dissociation constant (pK) were calculated for each turtle (Stabenau and Heming 1993).

## RESULTS

Nineteen leatherback turtles were evaluated. Two turtles were directly captured off Georgia in 2007, and 10 turtles were directly captured off Massachusetts in 2008. Five entangled turtles were assessed off Massachusetts in 2007, and 2 turtles in 2008. Of directly captured turtles, 6 were male, 4 were female, and 2 were of unknown sex. Of entangled turtles, 3 were male, 1 was female, and 3 were of unknown sex. Because of the small numbers of entangled turtles in each sex category, the relationship between sex and entanglement was not statistically assessed. Temperature, respiratory rate, heart rate, and temporal data are presented in Table 1.

**Figure 1.** Posterior view of the right elbow of a leatherback turtle (*Dermochelys coriacea*) after removal of an entangled rope. The animal's head is to the right, the right forelimb is protracted, and the elbow is extended. There are multiple areas of skin abrasion and ulceration, as well as a central full-thickness skin defect, exposing the fascia of the distal biceps muscle (Photo by CI).

Results of a physical examination revealed mild-to-moderate previously healed injuries in 10 turtles, including partial flipper amputations, an eyelid injury, and carapace abrasions. All entanglements were caused by linear rope. Entangled turtles had variable external injuries, which ranged from mild skin abrasions to severe skin and muscle lacerations of the forelimbs, neck, and carapace. One entangled turtle had exposure of the right distal biceps muscle (Fig. 1). The majority of turtles were active on the deck of the capture boat, often moving several meters during the examination and sampling. Males often displayed their penis during examination. Four directly captured turtles had superficial skin abrasions caused by the turtles' attempts to extricate themselves from the capture net and ambulate on the deck of the capture boat. Direct capture also resulted in unilateral loss of 1.5 cm of the keratin point on the rhamphotheca of 1 turtle, presumably caused by biting the capture net while under tension. Two directly captured turtles had moderate dermatitis of the perineal region, and several turtles had superficial, 2–10-cm, semilunar, bleeding wounds along the posterior margins of the front and rear flippers Several turtles had small numbers of unidentified barnacles and remoras on the carapace, with no associated lesions.

For many of the measured parameters, there were no significant differences between entangled and directly captured turtles, or between sexes (Table 2). Data for parameters that differed significantly between sex or entanglement groups are presented in Tables 3 and 4. Entangled turtles had significantly lower values for CCW, CCL, sodium, chloride, BUN, triglycerides, and relative and absolute eosinophil count and had significantly

**Table 2.** Blood values of leatherback turtles in the northwest Atlantic for which there were no significant differences between sexes or entanglement status.[a]

| | Minimum | Maximum | Mean | Median | SD | *n* |
|---|---|---|---|---|---|---|
| ALKP (U/L) | 13 | 199 | 85 | 65 | 54 | 18 |
| ALT (U/L) | 0 | 44 | 11 | 10 | 10 | 18 |
| AST (U/L) | 97 | 1312 | 286 | 229 | 269 | 18 |
| CK (U/L) | 40 | 13,262 | 1388 | 479 | 3092 | 18 |
| LDH (U/L) | 276 | 1632 | 649 | 497 | 375 | 18 |
| Albumin (g/dL) | 0.9 | 2.3 | 1.7 | 1.8 | 0.3 | 18 |
| Creatinine (mg/dL) | 0.0 | 1.7 | 0.4 | 0.1 | 0.5 | 18 |
| Cholesterol (mg/dL) | 142 | 438 | 285 | 284 | 79 | 18 |
| Glucose (mg/dL) | 58 | 130 | 87 | 85 | 21 | 18 |
| Calcium (mg/dL) | 1.8 | 9.3 | 6.0 | 6.1 | 1.7 | 18 |
| Phosphorus (mg/dL) | 6.5 | 11.6 | 9.0 | 8.8 | 1.7 | 18 |
| Potassium (mEq/L) | 3.7 | 7.0 | 5.0 | 4.8 | 0.9 | 18 |
| Uric acid (mg/dL) | 0.5 | 3.9 | 1.3 | 1.1 | 0.8 | 1 |
| Bile acids (μmol/L) | 0.0 | 15.9 | 5.2 | 5.3 | 4.7 | 11 |
| Hematocrit (%) | 24 | 49 | 42 | 43 | 7 | 18 |
| Leucocytes (cells/μL) | 2800 | 22,000 | 8544 | 6700 | 6041 | 18 |
| Lymphocytes (%) | 5 | 51 | 31 | 29 | 14 | 18 |
| Basophils (%) | 0 | 1 | 0 | 0 | 0 | 18 |
| Heterophils (cells/μL) | 504 | 9240 | 4369 | 4456 | 2547 | 18 |
| Lymphocytes (cells/μL) | 335 | 5500 | 2756 | 2739 | 1536 | 18 |
| Monocytes (cells/μL) | 0 | 970 | 290 | 139 | 314 | 18 |
| Basophils (cells/μL) | 0 | 194 | 17 | 0 | 48 | 18 |
| TP (g/dL) | 2.6 | 6.2 | 4.6 | 4.5 | 1.0 | 18 |
| alpha1 globulin (g/dL) | 0.13 | 0.81 | 0.52 | 0.61 | 0.24 | 18 |
| alpha2 globulin (g/dL) | 0.00 | 0.90 | 0.40 | 0.34 | 0.30 | 18 |
| beta1 globulin (g/dL) | 0.41 | 1.00 | 0.76 | 0.75 | 0.18 | 18 |
| Albumin (g/dL) | 0.94 | 2.44 | 1.76 | 1.76 | 0.37 | 18 |
| $pCO_2$ (torr) | 60.4 | 127.9 | 81.1 | 74.5 | 18.6 | 16 |
| $pO_2$ (torr) | 54.0 | 124.0 | 78.4 | 74.5 | 17.7 | 16 |
| $HCO_3$ (mEq/L) | 20.9 | 39.6 | 32.1 | 32.8 | 5.0 | 16 |
| Glucose (i-Stat, mg/dL) | 52 | 110 | 76 | 81 | 16 | 14 |
| Potassium (i-Stat, mEq/L) | 3.4 | 6.2 | 4.7 | 4.6 | 0.8 | 14 |
| Total $CO_2$ (mmol/L) | 23.0 | 43.0 | 34.9 | 35.0 | 5.4 | 15 |
| Ionized calcium (mmol/L) | 0.54 | 1.01 | 0.77 | 0.79 | 0.14 | 13 |
| Lactate (mmol/L) | 0.8 | 15.8 | 9.2 | 8.7 | 3.6 | 16 |
| $pCO_{2TC}$ (torr) | 36.5 | 71.4 | 49.3 | 45.3 | 10.4 | 15 |
| $pO_{2TC}$ (torr) | 46.0 | 108.8 | 68.8 | 67.1 | 15.5 | 15 |
| Ionized $calcium_{cor}$ (mmol/L) | 0.49 | 0.93 | 0.70 | 0.69 | 0.14 | 13 |
| Copper (μg/mL) | 0.69 | 1.19 | 0.99 | 1.03 | 0.14 | 16 |
| Selenium (μg/mL) | 3.95 | 13.43 | 7.55 | 6.59 | 2.83 | 16 |
| Cadmium (μg/mL) | 0.05 | 0.12 | 0.07 | 0.07 | 0.02 | 16 |
| Mercury (μg/mL) | 0.01 | 0.02 | 0.01 | 0.01 | 0.00 | 16 |
| Vitamin A (ng/mL) | 79.00 | 404.00 | 173.63 | 167.50 | 76.96 | 16 |
| Vitamin E (μg/mL) | 0.05 | 14.01 | 5.14 | 3.16 | 5.26 | 16 |
| 25-OH-Vitamin D (nmol/L) | 3.00 | 40.00 | 22.00 | 20.50 | 8.41 | 16 |

[a] ALKP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; LDH = lactate dehydrogenase; TP = $pCO_2$ = partial pressure of carbon dioxide; $pO_2$ = partial pressure of oxygen; $HCO_3$ = bicarbonate; $pCO_{2TC}$ = temperature-corrected partial pressure of carbon dioxide; $pO_{2TC}$ = temperature-corrected partial pressure of oxygen.

greater values for relative heterophils count, relative monocyte count, pH, $pH_{TC}$, $HCO_{3cor}$, and BHB. Male turtles had greater sodium values than females and greater total protein, globulin, gamma globulin, and anion gap values than turtles of unknown sex. Relative monocyte counts were different among all 3 sex groups. Only 1 blood parameter, relative monocyte count, demonstrated a significant sex and entanglement interaction. There was no significant sex effect on relative monocyte count when the turtles were directly captured; however, among entangled turtles, males had significantly lower relative monocyte counts than females or unknown sex animals.

Entangled turtles had significantly higher blood lead concentrations than directly captured turtles. Adequate sample volumes for organochlorine assays were only obtained for 4 turtles in 2007, and organochlorines were not detected in any sample. Thus, additional organochlorine assays were not conducted in 2008.

Nasal cultures were obtained from 19 turtles. Two turtles had negative nasal cultures, whereas 17 turtles had positive cultures for bacteria or fungi, with 1 turtle having positive results for both bacteria and fungi. Nasal fungal cultures grew *Cladosporium* sp. from 1 turtle, and *Rhodotorulla* sp. from 1 turtle. Forty-four bacterial isolates were obtained from the nares of 16 turtles. Nasal cultures yielded 1 bacterial isolate from 2 turtles, 2 isolates from 5 turtles, 3 isolates from 5 turtles, 4 isolates from 3 turtles, and 5 isolates from 1 turtle. Nasal bacterial

**Table 3.** Morphometric and blood data of leatherback turtles in the northwest Atlantic for which there were significant differences between sexes ($p < 0.05$).[a]

| | Male | | | | | | Female | | | | | | Unknown | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | Median | SD | *n* | Minimum | Maximum | Mean | Median | SD | *n* | Minimum | Maximum | Mean | Median | SD | *n* | *p* |
| CCW (cm)[b] | 98.7 | 117.6 | 106.8 | 105.5 | 5.9 | 9 | 99.5 | 113.3 | 106.9 | 108.5 | 5.3 | 5 | 87.5 | 101.0 | 96.7 | 98.0 | 5.5 | 5 | 0.029 |
| CCL (cm)[b] | 138.5 | 154.0 | 147.8 | 149.5 | 5.8 | 9 | 136.0 | 161.5 | 147.6 | 146.0 | 9.2 | 5 | 123.0 | 140.4 | 134.9 | 137.5 | 7.2 | 5 | 0.031 |
| Total protein (g/dL)[b] | 4.3 | 6.2 | 5.0 | 4.5 | 0.7 | 9 | 4.4 | 5.9 | 5.2 | 5.2 | 0.6 | 4 | 2.6 | 4.7 | 3.9 | 4.1 | 0.9 | 5 | 0.042 |
| Globulin (g/dL)[b] | 2.6 | 4.0 | 3.2 | 3.0 | 0.4 | 9 | 2.9 | 3.9 | 3.3 | 3.2 | 0.4 | 4 | 1.7 | 3.0 | 2.4 | 2.3 | 0.5 | 5 | 0.031 |
| Sodium (mEq/L)[c] | 155 | 162 | 157 | 156 | 3 | 9 | 145 | 158 | 153 | 155 | 6 | 4 | 152 | 161 | 156 | 153 | 4 | 5 | 0.030 |
| Monocytes (%)[c] | 0 | 5 | 1 | 1 | 2 | 9 | 1 | 11 | 4 | 3 | 5 | 4 | 1 | 13 | 5 | 3 | 5 | 5 | 0.029 |
| Gamma1 globulin (g/dL)[b] | 0.79 | 1.91 | 1.52 | 1.60 | 0.37 | 9 | 1.40 | 1.85 | 1.64 | 1.65 | 0.22 | 4 | 0.80 | 1.39 | 1.04 | 1.00 | 0.23 | 5 | 0.027 |
| Sodium (i-Stat, mEq/L)[c] | 148 | 161 | 153 | 153 | 5 | 6 | 146 | 149 | 148 | 148 | 1 | 4 | 147 | 155 | 152 | 153 | 4 | 4 | 0.023 |
| Anion gap (mEq/L)[b] | 10.4 | 18.5 | 13.0 | 11.4 | 3.0 | 7 | 7.1 | 18.0 | 11.1 | 8.1 | 6.0 | 3 | −2.8 | 13.3 | 5.4 | 4.9 | 6.1 | 5 | 0.048 |

[a] CCW = curved carapace width; CCL = curved carapace length.
[b] Male values significantly different than unknown sex values.
[c] Male values significantly different than female values.

**Table 4.** Morphometric and blood values of leatherback turtles in the northwest Atlantic for which there were significant differences between directly captured and entangled turtles ($p < 0.05$).[a]

| | Directly captured | | | | | | Entangled | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | Median | SD | *n* | Minimum | Maximum | Mean | Median | SD | *n* | *p* |
| CCW (cm) | 100.8 | 117.6 | 107.6 | 107.8 | 5.3 | 12 | 87.5 | 105.5 | 98.3 | 98.7 | 5.7 | 7 | 0.010 |
| CCL (cm) | 133.8 | 161.5 | 148.0 | 149.5 | 7.7 | 12 | 123.0 | 146.4 | 138.1 | 140.0 | 7.5 | 7 | 0.035 |
| BUN (mg/dL) | 81 | 174 | 128 | 127 | 29 | 11 | 40 | 135 | 83 | 82 | 31 | 7 | 0.009 |
| Chloride (mEq/L) | 110 | 130 | 121 | 123 | 5 | 11 | 102 | 119 | 113 | 117 | 7 | 7 | 0.001 |
| Sodium (mEq/L) | 153 | 161 | 157 | 157 | 3 | 11 | 145 | 162 | 154 | 155 | 5 | 7 | 0.030 |
| Triglycerides | 523 | 1104 | 768 | 758 | 196 | 9 | 124 | 357 | 241 | 241 | 165 | 2 | 0.017 |
| Heterophils (%) | 18 | 62 | 36 | 34 | 12 | 11 | 48 | 68 | 60 | 60 | 7 | 7 | 0.001 |
| Monocytes (%) | 0 | 5 | 2 | 1 | 1 | 11 | 0 | 13 | 5 | 3 | 5 | 7 | 0.005 |
| Eosinophils (%) | 9 | 47 | 29 | 29 | 12 | 11 | 1 | 23 | 7 | 1 | 8 | 7 | 0.002 |
| Eosinophils (cells/µL) | 360 | 8924 | 4006 | 2948 | 3362 | 11 | 42 | 1541 | 542 | 84 | 653 | 7 | 0.030 |
| pH | 7.08 | 7.25 | 7.16 | 7.14 | 0.06 | 10 | 7.23 | 7.36 | 7.30 | 7.31 | 0.05 | 6 | 0.002 |
| $pH_{TC}$ | 7.21 | 7.40 | 7.31 | 7.29 | 0.07 | 11 | 7.40 | 7.56 | 7.47 | 7.48 | 0.06 | 6 | 0.003 |
| $HCO_{3cor}$ | 20.9 | 34.7 | 29.5 | 30.3 | 4.3 | 11 | 33.5 | 41.4 | 37.3 | 37.6 | 3.2 | 6 | 0.005 |
| Lead (µg/mL) | 0.07 | 0.14 | 0.11 | 0.12 | 0.02 | 10 | 0.08 | 0.20 | 0.14 | 0.15 | 0.05 | 6 | 0.015 |
| BHB (mg/dL) | 0.57 | 2.32 | 1.33 | 1.23 | 0.54 | 10 | 12.90 | 55.41 | 32.66 | 35.11 | 16.49 | 6 | 0.001 |

[a] BUN = blood urea nitrogen; $pH_{TC}$ = temperature-corrected pH; $HCO_{3cor}$ = corrected bicarbonate value; BHB = beta-hydroxybutyric acid.

species and percentage of antibiotic sensitivity for each species are shown in Table 5 (gram-negative species) and Table 6 (gram-positive species).

Blood culture was completed for 16 turtles (10 directly captured, 6 entangled) and had bacterial growth for 3 turtles (1 directly captured, 2 entangled; Tables 5 and 6). Blood cultures were negative for fungi for all turtles.

Cloacal cultures were collected from 19 turtles. Cloacal culture was positive for *Salmonella* sp. for 3 turtles (2 directly captured, 1 entangled, Table 5). Cloacal cultures were negative for *Shigella* spp., *Pleisiomonas* sp., *Edwardsiella* spp., and *Campylobacter* spp. Fungal culture of the cloaca was positive for 3 turtles (2 directly captured, 1 entangled), with 1 of the directly captured turtles yielding 2 fungal isolates. *Rhodotorulla* sp. was isolated from the cloaca of 2 turtles, *Stemphyllium* sp. from 1 turtle, and *Candida* sp. from 1 turtle.

Fecal samples were obtained from 8 turtles. Fecal parasite testing revealed no parasites for 3 turtles. *Enodiotrema* sp. ova were seen in 4 samples, *Pyelosomum renicapite* ova were seen in 1 sample, and *Calycodes anthos* ova were seen in 1 sample.

Detailed presentation of satellite telemetry results, description of the gear involved in entanglements, genetic data, and stable isotope data is beyond the scope of this article and will be described elsewhere. Briefly, satellite tags transmitted for an average of 156 days (range, 16–272 days; median, 181 days). One disentangled turtle died approximately 18 days after release because of subsequent entanglement. A second disentangled turtle died of unknown causes approximately 35 days after release. Its body was recovered in a salt marsh approximately 20 days after death, and necropsy revealed an 83 × 35-cm piece of plastic in its stomach. Autolysis prevented histopathology. Retrospective analysis of video footage provided by the Provincetown Center for Coastal Studies indicated that this turtle had been entangled 11 days before its second entanglement but was able to free itself before arrival of the tagging and veterinary teams. For both mortalities, the estimated date of death was based on cessation of diving in the archival record of the recovered satellite tags. The long-term fate of the remaining turtles is unknown, but 1 adult female directly captured in August, 2008 was witnessed nesting in Costa Rica and Panama in March and April 2009, having shed its satellite tag, and appeared to be in good health.

## DISCUSSION

Directly captured turtles were in good physical condition, although many of them had evidence of past injuries that had completely healed. Similar healed injuries have been seen in Pacific leatherbacks (Harris et al. in press). The cause of the perineal dermatitis and fresh flipper skin wounds observed in several turtles is unknown. Diagnostic biopsy of these sites was beyond the

**Table 5.** Identity and percentage of antibiotic sensitivity for gram-negative bacteria isolated from the nares, blood, and cloaca of leatherback sea turtles.[a]

| | *n* | AMK | AMP | AM/CL | CFZ | CFR | CPHLO | CHLO | CIPR | ENRO | GENT | PIP | TET | TIC | TOB | TMS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nares | | | | | | | | | | | | | | | | |
| *Aeromonas* sp. | 1 | 100 | 0 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 100 | NE | 100 | 0 | 100 | 0 |
| *Brevundimonas vesicularis* | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Citrobacter freundii* | 1 | 0 | NE | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | NE | 100 | 100 | 100 | 100 |
| *Citrobacter braaki* | 1 | 0 | NE | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | NE | 100 | 100 | 100 | 100 |
| *Citrobacter koseri* | 1 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | NE | 100 | 100 | 100 | 100 |
| *Delftia acidovorans* | 1 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Enterobacter* sp. | 1 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | NE | 100 | 100 | 100 | 100 |
| *Flavobacterium odoratum* | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Nonenteric gram-negative rod* | 7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Photobacterium damselae* | 8 | 100 | 87 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 87 | 100 | 100 |
| *Proteus vulgaris* | 1 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Pseudomonas* sp. | 3 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 67 | 100 | 100 | 67 | 0 | 100 | 0 |
| *Shewanella putrefaciens* | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Shewanella* sp. | 1 | 100 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Vibrio alginolyticus* | 8 | 27 | 12 | 100 | 100 | 100 | 75 | 100 | 75 | 75 | 50 | 62 | 100 | 14 | 27 | 100 |
| Blood | | | | | | | | | | | | | | | | |
| *Vibrio alginolyticus* | 1 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 |
| *Providencia rettgeri* | 1 | 100 | NE | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | NE | 0 | 100 | 100 | 100 |
| Cloaca | | | | | | | | | | | | | | | | |
| *Salmonella* sp. | 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | NE | 100 | 100 | 100 | 100 | 100 |

[a] *n* = number of isolates; AMK = amikacin; AMP = ampicillin; AM/CL = amoxicillin-clavulanate; CFZ = ceftazidime; CFR = ceftiofur; CPHLO = cephalothin; CHLO = chloramphenicol; CIPR = ciprofloxacin; ENRO = enrofloxacin; GENT = gentamicin; PIP = piperacillin; TET = tetracycline; TIC = ticarcillin; TOB = tobramycin; TMS = trimethoprim/sulfadiazine; NE = not evaluated.

**Table 6.** Identity and percentage of antibiotic sensitivity for gram-positive bacteria isolated from the nares, blood, and cloaca of leatherback sea turtles.[a]

| | *n* | AMP | AM/CL | CFR | CPHLO | CHLO | ENRO | GENT | TET | TMS | CLIN | ERY | OXA | VANCO | PEN | G-SYN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nares | | | | | | | | | | | | | | | | |
| *Enterococcus durans* | 1 | 100 | NE | NE | 100 | NE | NE | NE | 100 | NE | NE | NE | NE | 100 | 100 | 100 |
| *Enterococcus faecalis* | 1 | 100 | NE | NE | 100 | NE | NE | NE | 100 | NE | NE | NE | NE | 100 | 100 | 100 |
| *Enterococcus* sp. | 1 | 100 | NE | NE | 100 | NE | NE | NE | 100 | NE | NE | NE | NE | 100 | 100 | 100 |
| *Streptococcus* sp. (gamma) | 1 | 0 | 100 | 100 | 0 | 100 | NE | 0 | 100 | 0 | 100 | 0 | NE | NE | 0 | NE |
| *Staphylococcus* sp. (NHCN) | 1 | 0 | 0 | 0 | 0 | NE | 0 | 0 | NE | 0 | 0 | 0 | 0 | 100 | 0 | NE |
| *Staphylococcus* sp. (HCN) | 2 | 100 | 100 | 50 | 100 | NE | 100 | 100 | NE | 100 | 100 | 100 | 100 | 100 | 100 | NE |
| Blood | | | | | | | | | | | | | | | | |
| *Staphylococcus* sp. (NHCN) | 1 | 0 | 100 | 100 | 100 | NE | 100 | 100 | NE | 100 | NE | 0 | 100 | 100 | 0 | NE |

[a] *n* = number of isolates; AMP = ampicillin; AM/CL = Amoxicillin/clavulanate; CFR = ceftiofur; CPHLO = cephalothin; CHLO = chloramphenicol; ENRO = enrofloxacin; GENT = gentamicin; TET = tetracycline; TMS = Trimethoprim/sulfadiazine; CLIN = clindamycin; ERY = erythromycin; OXA = oxacillin; VANCO = vancomycin; PEN = penicillin; G-SYN = penicillin in synergy with gentamicin; NHCN = nonhemolytic, coagulase negative; HCN = hemolytic, coagulase negative; NE = not evaluated.

scope of the permitted activities of this study. On several occasions, the spotter plane pilot and capture net handler saw schools of bluefish (*Pomatomus saltatrix*) biting at these areas before capture; however, it is unclear if such biting was the cause of the skin lesions or if the fish were feeding on previously damaged skin. Parasitism of whales by gulls has been documented (Thomas 1988), and it is possible that bluefish similarly parasitize leatherbacks. Histologic evaluation of these lesions would be useful to rule out primary infectious etiologies. Entangled turtles were in variable physical condition, and the injuries that were present in some turtles were severe. It is possible that such injuries could lead to reduced mobility, pain, reduced foraging, secondary infection, reperfusion injury, and muscle necrosis, which could compromise the long-term health of an individual.

Consistent with previous observations of this species, body temperatures were on average 6°C greater than ambient sea surface temperature (Frair et al. 1972; James and Mrosovsky 2004; Harris et al. in press). The average body temperature of leatherbacks in this study was approximately 2°C warmer, and the average sea surface temperature was approximately 4°C warmer than that reported for 4 female leatherbacks off Nova Scotia, Canada (James and Mrosovsky 2004). In these relatively warmer waters, several turtles had body temperatures of 28°–30°C, which is 2°–4°C higher than the highest temperature of the turtles off Nova Scotia.

Respiratory rates in this study were similar to those previously documented for leatherbacks (Lutcavage et al. 1992; Paladino et al. 1996; Harms et al. 2007; Harris et al. in press). Heart rates were similar but slightly greater than surface heart rates reported for free-swimming female leatherbacks in Costa Rica (mean, 25 bpm; $n = 5$; Southwood et al. 1999) and Grenada (range, 26–30 bpm; $n = 1$; Myers and Hays 2007). It is likely that the higher heart rates in some turtles were because of the stress of capture and handling. Mean heart rate of 80 bpm ($n = 17$) has been documented in directly captured leatherbacks in the Pacific, which is substantially higher than heart rates in this study (Harris et al. in press). The heart rates in the Costa Rica study were measured without restraint via implanted electrocardiogram recorders and thus likely reflected a more normal physiologic state. Electrocardiograms were successfully obtained for only 3 turtles in this study. In most cases, electrocardiogram tracings were adversely affected by the turtle's movements and were illegible. In 1 previous study, successful electrocardiograms were obtained for sedated postnesting female leatherbacks by using the same instrument used in this study (Harms et al. 2007). It is likely that the sedated females were moving much less than the turtles in this study, which resulted in legible electrocardiograms. Although subcutaneously implanted electrodes have been shown to provide valid electrocardiograms for unrestrained leatherbacks (Southwood et al. 1999), the use of such electrodes was beyond the scope of the permit for the

present study. Adhesive electrocardiogram pads were attached to the carapace of several turtles in an attempt to obtain better results, but they were not effective. For the 3 turtles for which legible electrocardiograms were recorded, the tracing was of good enough quality to determine heart rate (e.g., discernible R waves) but not of good enough quality to consistently discern other electrocardiographic details. Use of a mouth sensor for detection of heart rate was not within the scope of permitted activities for this study (Myers and Hays 2007).

Doppler blood flow monitoring was more effective than electrocardiogram for determining heart rate. In most sea turtle species, Doppler instruments easily detect blood flow when positioned over the carotid artery and heart base, between the shoulder and the neck. This site was not consistently effective in leatherbacks, possibly because of the depth of blood vessels in this very large species. The Doppler instrument was also used over the postoccipital sinus, eyes, interdigital, posterior femoral, and dorsal coccygeal vasculature with minimal success. The most consistent site for Doppler blood flow detection was dorsal to the hip, anterior to the tail base, under the margin of the carapace, with the probe directed dorsomedially toward the kidney. It is unclear exactly which artery was detected at this site, but, in this region, there are a number of large arteries, such as the dorsal aorta, renal arteries, and common iliac arteries that may have been detected by Doppler (Wyneken 2001). Heart rate was detected successfully in 6 leatherbacks at this site, and the ability to detect the heart rate with this method improved with experience. Harris et al. (in press) report successful acquisition of leatherback heart rates with per cloaca pulse oximetry. This method was not attempted in the present study, but is worthy of further investigation.

Total leucocyte counts of turtles in this study were similar to those previously documented for leatherbacks and were within ranges reported for sea turtles in general (Bradley et al. 1998; Stamper et al. 2005; Deem et al. 2006; Kakizoe et al. 2007; Innis et al. 2009; Harris et al. in press). As typically found in sea turtles, heterophils and lymphocytes were the most numerous leucocytes (Bradley et al. 1998; Deem et al. 2006; Kakizoe et al. 2007; Innis et al. 2009; Harris et al. in press). However, directly captured turtles in this study generally had higher relative and absolute eosinophil counts compared with nesting female leatherbacks and entangled leatherbacks in this study (Deem et al. 2006; Harris et al. in press). Relatively high eosinophil counts have also been documented in some directly captured Pacific leatherbacks (Harris et al. in press). Although no significant differences in eosinophil counts were seen between stranded, nesting, and foraging loggerhead turtles (Deem et al. 2009), other studies have shown that eosinophil counts of turtles vary with time after capture, temperature, and exogenous epinephrine administration (St. John and Nardone 1956; Aguirre et al. 1995). Thus, physiologic differences among nesting female, directly captured, and entangled leatherbacks may explain the discordant eosinophil counts among these groups.

Plasma biochemistry results were generally similar to previously documented values for leatherbacks (Deem et al. 2006; Harms et al. 2007; Harris et al. in press); however, there were several notable differences. BUN values were much higher compared with nesting females, which have very low BUN values (Deem et al. 2006; Harms et al. 2007). In general, sea turtles have relatively high BUN values, and it is likely that the relatively high BUN values seen in leatherbacks in this study are typical during foraging periods. Lower BUN values for nesting female vs. foraging turtles has previously been noted in loggerhead turtles (Deem et al. 2009). Similarly, triglyceride values of directly captured leatherbacks in this study were approximately twice those of nesting females (Deem et al. 2006). Although there is debate about whether female leatherbacks fast during the nesting season (Myers and Hays 2006; Fossette et al. 2008), lower BUN and triglyceride values seen in nesting female leatherbacks could be related to reduced protein and lipid intake because of fasting. It is also possible that reduced BUN and triglyceride values of nesting females may be caused by changes in hepatic protein and lipid metabolism during ovulation and egg production. Lower triglyceride values have been noted in postnesting female green turtles (*Chelonia mydas*) in comparison with vitellogenic females (Hamman et al. 2002).

There were several differences between entangled turtles and directly captured turtles. The lower BUN, triglycerides, sodium, and chloride values of entangled turtles were likely because of reduced food and seawater ingestion during entanglement. Reduced BUN values of debilitated sea turtles have been previously documented (Deem et al. 2009; Innis et al. 2009). Hematologic changes of entangled turtles were likely because of a generalized stress response as well as inflammatory response. Hematologic differences between healthy and physiologically stressed or ill sea turtles have previously been described (Aguirre et al. 1995; Deem et al. 2009). Although the CCW and CCL of entangled turtles were significantly lower than those of directly captured turtles, the reasons for this are unclear and may reflect sampling bias for this relatively small number of animals. For example, it is possible that larger turtles were easier to visually locate than smaller turtles, thus, more likely to be directly captured.

Entangled turtles had venous pH values that were similar to those of healthy postnesting female leatherbacks but generally higher $HCO_3$ values. Directly captured turtles were mildly acidotic, as demonstrated by significantly lower venous pH and $HCO_3$ values compared with entangled turtles and healthy postnesting females. Interestingly, the relatively lower pH seen in directly captured turtles was similar to that reported for sedated postnesting female leatherbacks (Harms et al. 2007). Although there were no statistical differences

between entangled and directly captured turtles for lactate or $pCO_2$ values, the highest lactate and $pCO_2$ values were seen in directly captured turtles, and these values were higher than previously reported for nesting females of this species. There are several possible explanations for these observations. It is possible that these derangements are caused by the turtles' response to capture, i.e., increased muscle exertion generating increased $CO_2$. However, it is also possible that it is a reflection of the turtles' physiologic status just before capture. Because captures were conducted by locating turtles that were resting at the surface, it is likely that such turtles may have been actively diving and foraging just before capture. Active and prolonged dives could induce the mild respiratory acidosis that we noted (Lutz and Bentley 1985). Because it is not possible to routinely sample blood from diving sea turtles, it will be difficult to differentiate between these possibilities (Lutcavage and Lutz 1991). It is possible that future technological developments may allow for remote monitoring of blood pH and $pCO_2$ values during diving, but such instruments are not available at this time.

BHB is a ketone produced during periods of fasting and exercise to provide an alternate energy source when glucose reserves may be insufficient (Laffel 1999). Significant elevations of BHB may be associated with deranged metabolic conditions, e.g., ketoacidosis (Laffel 1999). BHB has been previously evaluated as a measure of metabolic status in the desert tortoise (*Gopherus agassizzi*) and yellow mud turtle (*Kinosternon flavescens*), in which BHB levels increase during periods of fasting and drought (Chilian 1976; Christopher et al. 1994). BHB values have not previously been determined for sea turtles, and its use as a diagnostic assay for sea turtles will require validation. BHB values for entangled leatherbacks were an order of magnitude greater than those of directly captured turtles. In conjunction with reduced BUN and triglycerides values, elevated BHB values suggest that the metabolic status of entangled leatherbacks was significantly different than that of directly captured animals. Although this might be predicted given the high metabolic demand of this species, these data provide objective evidence of the potential adverse physiologic effects of entanglement. The exact duration of entanglement could not be determined but, in most cases, was suspected to be several hours to several days, with the minimum duration ranging from approximately 1.5 hours to 4 hours. It is notable that measurable metabolic changes occurred during this relatively short time, and it is possible that entanglement-induced mortality could occur relatively quickly because of significant metabolic derangements.

There were few sex-related differences in blood values of leatherbacks in this study. Although the reasons for these differences cannot be determined from the available data, these observations are consistent with several previous studies that found sex- and age-related differences in sea turtle blood values (Bradley et al. 1998; Hasbún et al. 1998; Kakizoe et al. 2007). For example, Hasbún et al. (1998) found significant differences in plasma iron, calcium, lactate dehydrogenase, and aspartate aminotransferase values between male and female green turtles from the United Arab Emirates.

Selenium and copper are essential elements that serve a variety of biological functions, whereas mercury, cadmium, and lead have no known biological function and are considered contaminants. These elements are generally acquired through environmental and dietary sources, and can have adverse health effects at elevated concentrations. Blood mercury concentrations in this study were similar to those previously reported in several sea turtle species, including leatherbacks in French Guiana, California, and St. Croix (Kenyon et al. 2001; Day et al. 2005; Guirlet et al. 2008; Harris et al. in press). However, mercury concentrations were 10 times lower than concentrations reported for nesting female leatherbacks in Gabon (Deem et al. 2007). The significance of mercury exposure for leatherback turtles has not yet been investigated, but studies of loggerhead turtles (*Caretta caretta*) indicate that even modest mercury exposure may have adverse immunologic effects (Day et al. 2007). In humans, blood mercury concentrations in the range of those documented in leatherbacks are associated with increased risk for neurodevelopmental abnormalities (Mahaffey et al. 2004).

Blood selenium concentrations in the present study were very similar to those found in nesting female leatherbacks (Guirlet et al. 2008). These concentrations are very high compared with other reptiles and mammals (Elsey and Lance 1983; Lockitch 1989; Stowe and Herdt 1992; Liu et al. 1998; Hopkins et al. 2001; Campbell et al. 2005; Burger et al. 2006; Chen et al. 2006; Burger et al. 2007). The reason for relatively high selenium concentrations in leatherbacks is unclear, and it is unknown whether such concentrations are required for normal physiologic processes for this species or represent an abnormal condition. While elevated selenium concentrations may be protective against mercury toxicity, the blood mercury concentrations in this study were moderate and were not likely high enough to explain the high selenium values. Additional study of selenium sources and utilization in leatherbacks is warranted. Blood copper and lead concentrations in this study were similar to those previously reported for leatherbacks and Kemp's ridley turtles (*Lepidochelys kempii*; Kenyon et al. 2001; Deem et al. 2006; Guirlet et al. 2008; Innis et al. 2008; Harris et al. in press). Copper concentrations are within the expected biologically relevant range for vertebrates (Burger et al. 2006; Lech and Sadlick, 2007). Although there was a significant difference in lead values between directly captured and entangled turtles, these lead concentrations were not likely high enough to have any adverse health effect on the turtles (Deem et al. 2006). The reason for

higher lead values in entangled turtles is unknown, and additional data are required to explore this question.

Blood cadmium concentrations in this study were very similar to those previously reported in leatherbacks (Guirlet et al. 2008; Harris et al. in press) and are similar to those reported for red-eared slider turtles (*Trachemys scripta elegans*) from a cadmium-contaminated wetland (Hays and McBee 2007). In comparison with snakes, these concentrations are approximately an order of magnitude greater (Burger et al. 2007). High cadmium tissue concentrations have been noted in leatherback turtles in the northeast Atlantic, and limited data suggest that this could be because of relatively high cadmium concentrations in the jellyfish diet of leatherbacks (Godley et al. 1998; Caurant et al. 1999). High trophic transfer coefficients for cadmium have previously been noted for green and hawksbill turtles (*Eretmochelys imbricata*; Anan et al. 2001). The source and possible effects of cadmium in leatherbacks should be investigated further because this metal may be carcinogenic and teratogenic, and may have toxic effects on the kidneys and respiratory, endocrine, and reproductive systems in a variety of species (Goering et al. 1995; Stoica et al. 2000). The absence of detectable concentrations of organochlorines in this study is consistent with previous findings for leatherback turtles (Godley et al. 1998; McKenzie et al. 1999; Deem et al. 2006; Harris et al. in press).

This is the first report of plasma concentrations of 25-hydroxyvitamin D for free-ranging sea turtles, and the documented concentrations are presumed to be normal for this species. Although not validated specifically for reptiles, the 25-hydroxyvitamin D assay used in this study has previously been used in reptiles, including sea turtles, and appears to produce accurate results (Acierno et al. 2006; Purgley et al. 2009). Plasma 25-hydroxyvitamin D concentrations in leatherbacks were in the low end of ranges reported for captive green turtles (Purgley et al. 2009) and were lower than those of red-eared slider turtles and several species of lizards (Gymiesi and Burns 2002; Acierno et al. 2006). Vitamin A and E values were similar to those of nesting female leatherbacks (Deem et al. 2006), and it is likely that these concentrations are normal for the species.

Protein electrophoresis results showed similar ranges to those of nesting female leatherbacks; however, turtles in this study had higher mean albumin, $\alpha$-1 globulin, and gamma-globulin (Deem et al. 2006). Male leatherbacks had greater total protein concentrations than turtles of undetermined sex because of greater gamma-globulin values. It is possible that this is because of a recent immune response or overall greater maturation of the immune system of adult males. The significance of these findings is unclear because protein electrophoresis has not been widely used for sea turtle health assessment. Age- and sex-related differences in plasma protein fractions have been documented in loggerhead turtles (Gicking et al. 2004; Deem et al. 2009). Although nesting female loggerheads had significantly greater albumin values than stranded and foraging turtles, there were no significant differences in other protein fractions among these groups (Deem et al. 2009). Additional studies of protein electrophoresis patterns for larger numbers of healthy and ill sea turtles are needed to determine the usefulness of protein electrophoresis in assessing sea turtle health.

The bacterial flora of turtles in this study were similar to those previously described for sea turtles, including leatherbacks, and were dominated by gram-negative rods (Kelly et al. 2006; Santoro et al. 2006; Santoro et al. 2008; Foti et al. 2009). The fungal species isolated from nasal and cloacal swabs are widely distributed in nature and may act as opportunistic pathogens (de Hoog et al. 2000; Larone 2002). In the absence of confirmed pathology, their presence in leatherback turtles likely represents colonization or contamination rather than infection. The reported bacteria and fungi are those that grew under the described culture methods, but it is likely that additional species would be identified through more sensitive culture or molecular methods. As expected for gram-negative organisms, many isolates were resistant to amoxicillin-clavulanate, ampicillin, and first-generation cephalosporins but susceptible to advanced penicillins, third-generation cephalosporins, fluoroquinolones, and aminoglycosides. Only 1 isolate had notable antibiotic resistance. A coagulase-negative, nonhemolytic *Staphylococcus* sp. was isolated from the nares of 1 turtle and was resistant to all assayed antibiotics except for vancomycin. Although it is possible that this organism was introduced as a contaminant during the collection or culture process, antibiotic resistant bacteria from anthropogenic sources have been previously documented in marine species (Johnson et al., 1998). The significance of such bacteria for the health of marine species is unclear at this time. *Salmonella* spp. have been widely reported as potential pathogens that can be transmitted from reptiles to humans, and there are many reports of *Salmonella* spp. colonizing the digestive tract of turtles (e.g., Dickinson et al. 2001; Brenner et al. 2002). In light of our results, individuals who handle leatherback turtles should use personal protective equipment to prevent exposure to these potential pathogens.

The significance of the positive blood cultures for 3 turtles is unclear. The methods of skin disinfection used in this study have been shown to be valid for field collection of shark blood cultures (Mylniczenko et al. 2007). However, the possibility of contamination cannot be ruled out because field conditions could have resulted in contamination of the skin, needle, etc. (e.g., wind-blown seawater spray). Although a positive blood culture is generally considered to be an indicator of significant illness in most vertebrates, positive blood cultures have been documented in apparently healthy monitor lizards and sharks (Hanel et al. 1999; Mylniczenko et al. 2007). One of the turtles with a positive blood culture also had the highest white blood cell count in this study (22,000

cells/µL); however, statistical comparison of blood values of turtles with positive blood cultures to those with negative blood cultures could not be conducted because of the very small number of positive blood cultures. To our knowledge, no other study has assessed blood cultures of leatherbacks, and additional studies should be conducted to determine whether healthy leatherback turtles may sometimes demonstrate a nonpathologic bacteremia.

The 3 trematode parasite species detected in fecal specimens in this study have previously been reported in leatherback turtles, but they have not yet been associated with disease (Threlfall 1979; Manfredi et al. 1996).

The ability to collect a full suite of samples and data for each turtle was influenced by activity of the animal, experience of personnel, success of venipuncture, equipment function, human error, and field conditions. In some cases, particular observations or data were not recorded because personnel were completing other parts of the examination and assisting with restraint. However, there was general improvement of complete data collection over time because of experience and retrospective review of each event. For example, the ability to consistently obtain heart rate improved in the second year of the study when a reliable site for Doppler detection was discovered.

The methods of blood collection in this study were generally appropriate, but several limitations were noted. The average time between capture and blood collection from each animal was approximately 35 minutes, which likely resulted in some degree of stress-induced change in certain parameters. This delay in blood collection was intentional, because the first priority of each event was to attach a satellite tag, and the satellite tag team requested that simultaneous blood collection not be conducted. The venipuncture location was not consistent among all the turtles, and, in some cases, the venipuncture location was not recorded. In some chelonian species, blood samples obtained from different venipuncture sites produce discordant results, but this has not been investigated in marine turtles (Gottdenker and Jacobson 1995; Crawshaw and Holz 1996). Determining the effects of venipuncture site was not a goal of the present study. In some cases, 1 site was abandoned after an unsuccessful attempt, and the second site was used, whereas, in other cases, attempts were made at both sites simultaneously. The success or failure at 1 site was often determined by the animal's movements and position on the boat. To minimize the duration of the event, we accepted blood samples from the first successful venipuncture regardless of location. Grossly obvious lymph contamination was not noted during the collection of any blood sample. Future studies to investigate serial blood samples and effects of venipuncture site should be considered for leatherbacks.

Sodium heparin was used in the blood collection syringe to ensure anticoagulation under conditions of unpredictable collection and processing times. In addition, heparinizing the syringe permitted point-of-care analysis directly from the collection syringe, which ensured valid results for pH and blood gases. Although point-of-care analysis was conducted quickly in most cases, we could not ensure that samples would not clot before analysis without the use of heparin. For subsequent hematologic and plasma biochemical testing, samples were transferred to lithium heparin tubes, which are commonly used for storage of reptile blood samples. Although concern is often raised over possible discrepancies in results when using these 2 different types of heparin, no significant differences in plasma biochemical values were seen between samples collected in sodium heparin vs. lithium heparin in a previous study of leatherback turtles (Deem et al. 2006). Harris et al. (in press) also used syringes coated with sodium heparin, followed by transfer to lithium heparin collection tubes during health assessments of leatherbacks. The sodium heparin concentration of blood samples in this study is consistent with manufacturer's guidelines for the point-of-care analyzer (iSTAT).

Consistent with current recommendations, point-of-care blood analysis was conducted within 10 minutes of sample collection for the majority of turtles (Harms et al. 2003; Wolf et al. 2008), but analysis was not conducted until 13 minutes after sample collection for 1 entangled turtle, and 33 minutes for 1 directly captured turtle. The significance of this delay has not been studied in marine turtles, but it could result in artifactual changes in some parameters. For the 2 turtles for which analysis was delayed, results were generally similar to other turtles of similar status (i.e., entangled or directly captured). Presentation of both raw data (generated by the point-of-care analyzer at 37°C) and temperature-corrected data for pH, $pCO_2$, $pO_2$, and $HCO_3$, and pH-corrected data for ionized calcium has become a standard part of sea turtle physiology and health studies (Chittick et al. 2002; Harms et al. 2003; Harms et al. 2007; Innis et al. 2007). Mathematically corrected data are thought to provide a more accurate assessment of the true physiologic status of sea turtles (Chittick et al. 2002).

Processing and storage of blood samples for hematologic and biochemical analysis were appropriate based on current clinical recommendations for sea turtles and were similar to methods used previously in leatherback turtle health studies (Deem et al. 2006; Harms et al. 2007, Eisenhawer et al. 2008). In many cases, centrifugation and harvest of plasma was conducted at sea within 30 minutes of blood collection. In other cases, a lack of electricity on some boats and prolonged travel times prevented immediate centrifugation. However, samples were kept cold by using ice, and even the longest delay in centrifugation (15 hours) was within the time that is believed to avoid artifactual changes for marine turtle plasma biochemical values (Eisenhawer et al. 2008) Several blood analytes (e.g., glucose, sodium) were measured by both the point-of-care analyzer and the commercial diagnostic laboratory. In-depth comparison of these results was beyond the scope of this study, and it has

been shown that sea turtle biochemical values obtained from different analyzers may be different (Wolf et al. 2008). In general, however, results for these analytes were very similar between the 2 methods, and differences would not likely have affected clinical decisions. For example, there was only a 3% difference in mean sodium values obtained by the 2 methods.

Direct capture events, disentanglements, satellite tag attachments, and health assessments were conducted safely and efficiently, which required an average of 49 minutes to complete. The maximum event duration of 100 minutes for 1 entangled turtle involved a turtle with a complex disentanglement operation. It has previously been shown that leatherback turtles can be safely captured at sea, equipped with satellite tags, and subsequently resume activity and migratory patterns that are thought to be normal for the species (James et al. 2005; Benson et al. 2006). Our data also support this conclusion. However, based on the relatively low venous pH, high $pCO_2$, and high lactate values seen in some directly captured turtles, additional studies of the immediate physiologic effects of direct capture are warranted. Specifically, future direct capture studies should consider immediate blood sampling, as well as sampling upon completion of the event, to differentiate the immediate effects of capture from the effects of restraint and exertion during tagging and health assessment. With regard to the safety of direct capture of leatherbacks, our data provide justification for monitoring physiologic parameters during capture studies, because more severe derangements may provide objective grounds for termination of an individual capture and tagging event.

Similar to the findings of López-Mendilaharsu et al. (2009), our data suggest that at least some disentangled leatherback turtles are able to resume normal behavior and migratory patterns. However, 2 of the leatherbacks in this study were entangled at least twice, while a third disentangled turtle had significant forelimb skin and muscle injuries (Fig. 1). Based on these data, we suggest that disentanglement efforts for individual leatherback turtles are warranted but that disentanglement may not result in long-term survival in all cases. In addition, the risk of subsequent re-entanglement may be significant. Although turtle excluder devices have been developed to reduce leatherback bycatch in trawl fisheries (National Oceanic and Atmospheric Administration 2003), efforts to minimize the risk of leatherback entanglement in fixed-gear fisheries should continue.

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