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COMPARATIVE HEALTH ASSESSMENT OF WESTERN PACIFIC LEATHERBACK TURTLES (*DERMOCHELYS CORIACEA*) FORAGING OFF THE COAST OF CALIFORNIA, 2005–2007

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ABSTRACT: Leatherback turtles (*Dermochelys coriacea*) are critically endangered, primarily threatened by the overharvesting of eggs, fisheries entanglement, and coastal development. The Pacific leatherback population has experienced a catastrophic decline over the past two decades. Leatherbacks foraging off the coast of California are part of a distinct Western Pacific breeding stock that nests on beaches in Indonesia, Papua New Guinea, and the Solomon Islands. Although it has been proposed that the rapid decline of Pacific leatherback turtles is due to increased adult mortality, little is known about the health of this population. Health assessments in leatherbacks have examined females on nesting beaches, which provides valuable biological information, but might have limited applicability to the population as a whole. During September 2005 and 2007, we conducted physical examinations on 19 foraging Pacific leatherback turtles and measured normal physiologic parameters, baseline hematologic and plasma biochemistry values, and exposure to heavy metals (cadmium, lead, and mercury), organochlorine contaminants, and domoic acid. We compared hematologic values of foraging Pacific leatherbacks with their nesting counterparts in Papua New Guinea ($n=11$) and with other nesting populations in the Eastern Pacific in Costa Rica ($n=8$) and in the Atlantic in St. Croix ($n=12$). This study provides the most comprehensive assessment to date of the health status of leatherbacks in the Pacific. We found significant differences in blood values between foraging and nesting leatherbacks, which suggests that health assessment studies conducted only on nesting females might not accurately represent the whole population. The establishment of baseline physiologic data and blood values for healthy foraging leatherback turtles, including males, provides valuable data for long-term health monitoring and comparative studies of this endangered population.

Key words: *Dermochelys coriacea*, domoic acid, health assessment, heavy metals, hematology, leatherback sea turtle, organochlorine contaminants, plasma biochemistry.

INTRODUCTION

Leatherback turtles (*Dermochelys coriacea*), the largest of the marine turtles, are a critically endangered species with a global distribution (Pritchard, 1997; IUCN, 2007). Leatherbacks undergo the longest migrations of any sea turtle species, crossing ocean basins to forage on cnidaria zooplankton such as jellyfish and salps (James and Herman, 2001; Hays et al., 2004; James et al., 2005; Benson et al., 2007a, b). Their large size and unique thermoregulatory adaptations allow them to inhabit tropical and temperate oceans (Frair et al., 1972; Greer et al., 1973). Major threats to leatherback turtles predominately are anthropogenic, including loss of nesting habitat due to coastal development, overharvest of eggs and meat, incidental bycatch in pelagic longline and gillnet fisheries, and ingestion of marine debris (Lutcavage et al., 1997; Spotila et al, 2000; Barreiros and Barcelos, 2001; Bugoni et al., 2001; Lewison et al., 2004).

The Pacific leatherback turtle population has experienced a catastrophic decline over the past two decades and might be on the verge of extirpation. The most conservative population estimates, derived from nest counts and numbers of females nesting annually, show that there could be as few as 2,300 adult females in the Pacific Ocean (Crowder, 2000). Others have proposed estimates of 1,690 adult females in the Eastern Pacific (Spotila et al., 2000) and 2,100–5,700 breeding females in the Western Pacific (Dutton et al., 2007). The progressive decline in numbers of nesting females in the Eastern Pacific, well documented at Playa Grande, Costa Rica (Spotila et al., 2000), and on the Pacific coast of Mexico (Sarti-Martinez et al., 2007), has been attributed to increased adult mortality associated with gillnet and longline fisheries and extensive egg harvesting (Spotila et al., 2000). In the Western Pacific, declines in nesting females have been documented in Papua, Indonesia (Hitipeuw et al., 2007) and Terengganu, Malaysia, where the nesting population virtually has collapsed as a result of intensive egg harvesting (Chan, 2006). Although consistent long-term nesting data are not available for most of the Western Pacific leatherback population, major nesting areas for leatherbacks recently have been identified, and conservation efforts to protect and monitor these sites are underway (Dutton et al., 2007).

Leatherback turtles that utilize critical foraging habitat along the western coast of the United States are part of a unique Western Pacific breeding stock, identified through satellite telemetry and mitochondrial DNA studies (Benson et al., 2007a; Dutton et al., 2007). Western Pacific leatherbacks nest in Papua Barat (Indonesia), Papua New Guinea, and the Solomon Islands and utilize different foraging areas, including temperate habitat in the eastern North Pacific Ocean (off California, Oregon, and Washington), the Sea of Japan, and tropical waters in the South China Sea (Benson et al., 2007a). In California, these leatherbacks typically occur along the central coast seasonally from July to November in retention areas characterized by sea surface temperatures of 14–17 C and large aggregations of jellyfish (*Scyphomedusae*), their preferred prey (Houghton et al., 2006; Benson et al., 2007b).

Although it has been proposed that the rapid decline of Pacific leatherback turtles is due to increased adult mortality (Spotila et al., 2000), little is known about the health of this species (George, 1997; Herbst and Jacobson, 2003). Compared to other species of sea turtles, few reports document disease in leatherback turtles, including bacterial infections (Ogden et al., 1981; Obendorf et al., 1987; Miller et al., 2009), metabolic disease (Davenport et al., 1993), and parasitic infestations (Threlfall, 1979). Heavy metals and persistent organic pollutants have been detected in low concentrations in the blood of Atlantic nesting female leatherbacks (Deem et al., 2006; Guirlet et al., 2008, 2010), but no comparative studies have been performed in the Pacific population or in foraging leatherbacks.

Because leatherback turtles are pelagic, opportunities to conduct postmortem examinations on fresh specimens or obtain baseline physiologic and clinical data from live animals during rehabilitation are few (Work and Balazs, 2002, 2010; Miller et al., 2009). Furthermore, such studies yield data that are difficult to interpret because they might suggest a higher disease prevalence or higher level of contamination than exists in the general population. To date, health assessments of live leatherbacks have focused on sampling females on nesting beaches, due to the relative ease of sampling these large sea turtles when they are on land (Work, 2002; Deem et al., 2006; Harms et al., 2007). Although these studies provide valuable biological information, sampling is biased by sex and physiologic state, so the applicability of the data to the population as a whole is constrained.

Our objectives were to assess the health of free-ranging, foraging Western Pacific leatherback turtles for the first time by evaluating baseline physiologic values, hematology, plasma biochemistries, and exposure to environmental contaminants including heavy metals (cadmium, lead, and mercury), organochlorines, and the biotoxin domoic acid. We hypothesized that blood values in foraging female Pacific leatherback turtles would differ from nesting females due to differences in physiologic and nutritional state. We also speculated that there might be differences in concentrations of contaminants in Pacific leatherbacks compared to values reported in Atlantic leatherbacks.

MATERIALS AND METHODS

Study sites and capture methods

Foraging Pacific leatherback turtles were captured off the coast of central California inside the 90 m isobath contour between Pigeon Point (37°10′56″N, 122°23′39″W) and Point Reyes (37°59′49″N, 123°01′37″W) during September 2005 and 2007 ($n=19$). Turtles were located via aerial surveys and captured from a small boat using a 1.5-m diameter break-away hoop net. Once secured in the net, turtles were brought aboard the capture boat for examination and sampling. To prevent injury, the boat was equipped with rubberized surfaces and rounded edges, and plastic air-filled floats were positioned around the turtles to cushion the body. A neoprene mat was placed over the eyes to minimize disturbance. A continuous flow of sea water was positioned at the neck and axillary regions to keep the turtles cool.

Nesting leatherbacks were sampled at three sites: The Western Pacific at the Kamiali Wildlife Management Area, Papua New Guinea, in December 2001 ($n=9$; 7°16′17″S, 147°07′46″E); the Eastern Pacific at Playa Langosta, Parque Nacional Marino Las Baulas de Guanacaste, Costa Rica, from January to February 2002 ($n=8$; 10°17′00″N, 85°51′02″W);, and the Caribbean/Atlantic at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands, in May 2006 ($n=12$; 17°41′12″N, 64°53′49″W). Nesting females were sighted during nightly beach patrols conducted by teams of local biologists and were observed from a distance until they had finished nest excavation. Once females had begun the process of oviposition and had entered a typical trance-like state, they were safely examined and sampled without disrupting their normal nesting behavior.

Physical examination

Physical examinations of foraging leatherback turtles captured at sea were performed to assess behavior, movement, mentation, body condition, the presence of ectobiota, and lesions on the skin and carapace. Healthy turtles were defined as those animals that were able to actively swim and dive, showed evidence of recent foraging activity (i.e., bits of jellies in or around mouth), demonstrated symmetrical use of the head and limbs, were mentally alert and in good nutritional condition, and had no evidence of recent debilitating traumatic injury or epibiont loads that compromised normal movement. Digital photos were taken of each animal to record wounds and characteristic markings. Morphologic measurements were recorded, including curved carapace length (CCL, measured from the nuchal notch to the tip of the pygal), curved carapace width (CCW, measured between the most lateral ridges at the widest part of the carapace), and body weight. Foraging turtles with CCL <145 cm were classified as subadults; all turtles with CCL ≥ 145 cm were considered to be adults (James et al., 2007). Sex for turtles ≥ 145 cm was determined using external sexually dimorphic features, such as the length of the tail relative to the pygal and position of the cloacal opening; direct visualization of the extruded penis was used for all age classes (Wyneken, 2001). To measure body weight, the turtle was supported in a harness of reinforced nylon webbing with stainless steel rings and lifted off the deck by a winch line suspended from the vessel's A-frame using a digital scale (Dyna-Line MSI-7200, Measurement Systems International, Seattle, Washington, USA).

Respirations were recorded from the time of capture until release and respiratory rate (respirations per minute) and respiratory interval (time between breaths) were calculated for each individual. Heart rate (beats per minute) and percentage oxygen saturation of hemoglobin (SPO_2 %) were recorded using a digital pulse oximeter (Heska Vet/Ox G2™, Heska Corporation, Loveland, Colorado, USA) with a probe held against the cloacal mucosa. Body temperature was monitored via pulse oximeter with a temperature probe inserted approximately 15 cm into the cloaca. Heart rate measurements were validated via electrocardiogram (PC-Based 12

lead Resting ECG, Welch Allyn, Inc., Skaneateles Falls, New York, USA). Some of the turtles were fitted with satellite transmitters as part of a concurrent study by the National Marine Fisheries Service (Eckert and Eckert, 1997; Benson et al., 2007a, b). Abbreviated physical examinations were performed on nesting females, including evaluation of symmetrical movement and documentation of traumatic injuries or lesions.

Sample collection

Blood was collected from one of two venipuncture sites: The dorsal cervical sinus using an 18-gauge, 8.9 cm spinal needle and syringe precoated with sodium heparin (Heparin sodium injection, USP, Baxter Healthcare Corporation, Deerfield, Illinois, USA) or the rear flipper nexus in the popliteal region using a 20-gauge, 3.8 cm multiple sample vacutainer needle (Dutton, 1996). A maximum of 60 ml of whole blood was drawn from each turtle (<5 ml/kg body weight). Blood was transferred or collected directly into lithium heparin, potassium EDTA, or trace element-free tubes (BD Vacutainer Systems, Franklin Lakes, New Jersey, USA). All blood samples were visually assessed for lymph contamination and, if observed, were not used for laboratory analyses. Samples were stored on ice in portable coolers and processed within 8 hr of collection.

Blood smears were prepared from whole blood with lithium heparin and air dried. A portion of whole blood in lithium heparin tubes was set aside for hematology and the remainder was centrifuged for 15 min (Cole-Parmer Model 17250-10, Vernon Hills, Illinois, USA). The plasma was transferred to cryotubes (Corning Incorporated, Corning, New York, USA) for biochemical and domoic acid analyses. Aliquots of whole blood from EDTA tubes were transferred to cryotubes for heavy metal analysis. Whole-blood samples in trace-element-free tubes were allowed to clot, centrifuged for 10 min, and the serum transferred into cryotubes for contaminant analysis. Blood samples and smears from California and St. Croix were shipped within 24 hr of collection for hematologic analyses, and samples from Papua New Guinea and Costa Rica were processed for complete blood counts in the field. Biochemical analysis was performed on plasma samples stored on ice from California turtles within 48 hr of collection and on frozen plasma samples from nesting turtles within 12 mo of collection. All remaining cryotubes from foraging and nesting turtles were frozen at −20 C or placed in liquid nitrogen in the field for up to 2 wk and were subsequently stored in a −80 C freezer until laboratory analyses could be performed.

Laboratory analyses

Hematologic analysis for samples from California and St. Croix was performed at IDEXX Laboratories (West Sacramento, California, USA) by the same technician. Red blood cell (RBC) counts were estimated manually with a hemacytometer using the RBC Unopette® System (BD, Franklin Lakes, New Jersey, USA) and white blood cell (WBC) counts were made using the Eosinophil Unopette® System (BD). A small amount of whole blood was transferred to a microhematocrit tube and centrifuged to determine packed cell volume (PCV). Blood smears were fixed in methanol, stained with Wright-Giemsa, and examined under light microscopy for differential cell counts, including heterophils, lymphocytes, monocytes, azurophils, eosinophils, and basophils (Work et al., 1998). Cells classified as azurophils were pooled with monocytes, because they are closely related cell types based on their cytochemical and ultrastructural characteristics (Campbell, 2006). Blood samples collected in Costa Rica and Papua New Guinea were processed manually in the field for hematology by the same veterinarian (TMW). All field methods were the same as above, except that erythrocyte counts were completed using Natt-Herrick's solution with hemacytometer (Natt and Herrick, 1952).

Plasma samples were analyzed at IDEXX Laboratories with the same automated Olympus AU 5431 biochemistry analyzer (Olympus America Inc., Center Valley, Pennsylvania, USA) using standard reagents to minimize variability in results (Wolf et al., 2008). Biochemical parameters included alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), total protein (TP), albumin, globulin, cholesterol, glucose, calcium, phosphorus, potassium, sodium, and uric acid.

Whole blood was analyzed for cadmium, lead, and mercury at the California Animal Health and Food Safety (CAHFS) Laboratory (School of Veterinary Medicine, University of California, Davis, California, USA). Whole blood samples for metal analyses were only available from leatherback turtles sampled in California and St. Croix. Cadmium and lead were measured using atomic absorption spectroscopy (AAS). Reporting limits were 0.002 parts per million (ppm) for cadmium and

0.06 ppm for lead. Mercury was analyzed using hydride generation inductively coupled plasma-atomic emission spectroscopy (ICP-AES), with a detection limit of 0.020 to 0.005 ppm, which varied with sample volume available.

Serum organochlorine (OC) pesticides and polychlorinated biphenyl (PCB) analytes were measured by gas chromatography with electron capture detection (GC-MCD) at CAHFS. Samples were extracted following Sundberg et al., (2006). Individual isotopically labeled internal standards of two OC and two PCB congeners were utilized for isotope dilution analysis. Analysis was performed on a 60 m DB-XLB (0.250 mm internal diameter) column in an Agilent 5975/6890 GC-MSD (Agilent Technologies, Palo Alto, California, USA). The nominal reporting limit for all OCs (alpha-benzene hexachloride, hexachlorobenzene, lindane, heptachlor, aldrin, dicofol, heptachlor epoxide, o,p′-DDE, chlordane, endosulfan I, trans-nonachlor, dieldrin, p,p′-DDE, o,p′-DDD, endrin, o,p′-DDT, cis-nonachlor, endosulfan II, p,p′-DDD, p,p′-DDT, methoxychlor, mirex) was 5 parts per billion (ppb). The nominal reporting limits for PCBs were 1 ppb for total tetrachloro-PCBs, total pentachloro-PCBs, total hexachloro-PCBs, and total heptachloro-PCBs; 5 ppb for total octachloro-PCBs and total nonachloro-PCBs; and 10 ppb for decachloro-PCB. Reporting limits were doubled for samples with limited volume or poor quality that resulted in difficulties in the extraction process.

Domoic acid was analyzed at CAHFS in plasma and feces from foraging California leatherbacks by liquid chromatography-mass spectrometry (LC-MS; Tor et al., 2003). This method involved the extraction of toxin from plasma and feces using an Oasis HLB solid phase extraction column and analysis by positive electrospray ionization LC-MS. Plasma used for this assay included nine additional samples from foraging Pacific leatherback turtles acquired in 2003 to 2004 from Monterey Bay, California that had been frozen at −80 C since collection. Plasma samples from nesting female leatherbacks in St. Croix were used as negative controls for domoic acid analysis in California turtles because the biotoxin has not been reported in the Caribbean. The nominal reporting limit was 5 ppb for plasma samples and 500 ppb for feces.

Statistical analyses

Descriptive statistics for morphometric measurements, physiologic parameters, and blood values were performed in Microsoft Excel™ (Microsoft Corporation, Redmond, Washington, USA). Median and range were reported as the most representative measures of central tendency for clinical use; however, mean±SD were included for several parameters to facilitate comparison with other studies. Due to the small sample sizes for each group ($n<30$), nonparametric statistical tests were used (SPSS, version 16.0, Chicago, Illinois, USA). Mann-Whitney *U*-tests were used to compare standard measurements, physiologic parameters, and blood values between foraging males and females from California (Daniel, 2005) and to assess differences in blood values between foraging and nesting females from all populations (Daniel, 2005). Kruskal-Wallis one-way analysis of variance (KWANOVA) was used to detect differences in blood values among nesting females by location (Daniel, 2005). Where differences were found, Mann-Whitney *U*-tests were performed for each pair and significance was determined using Holm's sequential Bonferroni adjustment of the *P* value (Holm, 1979). Groups comprised of less than five samples were not included in statistical analyses and statistical significance for all analyses was defined as $P \leq 0.05$.

RESULTS

Physical examination

Nineteen Pacific leatherback turtles were captured and sampled off the coast of California during the study period; seven in September 2005 and 12 in September 2007. Of these, 13 were females and six were males. All foraging turtles were classified as adults, except for the smallest male that was considered to be a subadult (CCL=144.0 cm, body weight: 380 kg). One foraging male leatherback captured off California in September 2002 also was included for evaluation of morphometric and hematology values. In addition, 11 nesting females were sampled from Papua New Guinea in December 2001, eight from Costa Rica in January to February 2002, and 12 from St. Croix, US Virgin Islands in May 2006. All nesting females were classified as adults based on sexual maturity.

All foraging leatherback turtles were alert and active at capture and were considered clinically healthy on physical

TABLE 1. Morphometric measurements and physiologic values for foraging Pacific leatherback turtles captured off the coast of California, 2005–2007.

Measurement	*n*	Mean±SD	Median	Range
Body weight (kg)	19	499.4±63.1	518	380–607
Curved carapace length (cm)	19	158.04±7.32	158.0	144.0–172.0
Curved carapace width (cm)	19	117.57±12.02	116.0	100.0–155.5
Heart rate (bpm)[a]	17	80.0±14.2	79	42–120
Percent oxygen saturation (%)	15	88.6±10.2	91	62–100
Respiratory interval (min:sec)[b]	18	0:39±0:34	0:30	0:03–5:45
Body temperature (C)	10	22.89±2.26	22.8	18.8–26.7
Temperature differential (C)[c]	10	7.65±2.88	8.9	1.9–10.2

[a] bpm = beats per min.

[b] Respiratory interval refers to the time interval between breaths, recorded from the time of capture until release.

[c] Temperature differential represents the difference between the core body temperature (using the first temperature recorded after capture) and the sea surface temperature. Both values were measured with the same probe using a Heska pulse oximeter and calibrated with the boat's temperature sensor.

examination, with the exception of one female captured in California in September 2007. This turtle was foraging on jellyfish at the time of capture and maintained a stable heart rate and respiratory rate throughout application of the satellite-linked transmitter. However, after approximately 40 min on deck, she became lethargic, apneic, and regurgitated several times and was returned to the water and monitored for several hours. She continued a trend of increasing activity in the subsequent hours until dark. Satellite data obtained during the following weeks showed that she was actively swimming and diving as she moved offshore in the direction of the Hawaiian Islands. Results of monitoring data and clinical blood work from this individual were removed from summary calculations, because her clinical presentation did not fit the criteria for a healthy, stable individual. One blood sample from an apparently healthy turtle revealed a hematocrit below 10% and total protein less than 1.0 g/dl; this sample was considered to be of poor quality with potential lymph contamination and was excluded from statistical analyses for hematology and plasma biochemistries.

Physical examinations revealed old traumatic wounds in 17 of 19 (89%) turtles, including superficial lacerations and scarring on the top of the head overlying the pineal gland ($n=11$), flippers ($n=11$), carapace ($n=9$), face ($n=3$), and eye or adnexal structures ($n=3$). One turtle was missing the distal third of the left front flipper with multiple exposed phalanges, but the wound appeared to be healing well and the turtle was able to maneuver and swim normally. Another turtle had a deep circular wound filled with granulation tissue penetrating into the musculature of the left shoulder, a presumptive shark bite. Two turtles had multiple healed parallel lacerations in the carapace consistent with boat propeller wounds. Barnacles were reported on two turtles at the lateral border of the plastron and the base of the hind flipper. None of these lesions compromised normal movement or behavior.

All nesting females appeared healthy according to our physical examination criteria and were strong enough to navigate from the water to their nesting sites and undergo the rigorous process of digging, laying, and nest covering without incident. Many of the nesting turtles had evidence of old scars and healed injuries, none of which appeared to affect their behavior.

Morphometric measurements and physiologic values for foraging Pacific leatherback turtles captured off the coast of

TABLE 2. Hematology values for Pacific leatherback turtles foraging off the coast of California, 2005–2007, and nesting in Papua New Guinea and Costa Rica, 2001–2002.

	Foraging				Nesting	
	Males (n=5)		Females (n=7)		Females (n=17)	
Measurement[a]	Median	Range	Median[b]	Range	Median	Range
RBC ($\times 10^6$/μl)	0.58	0.47–0.93	0.72*	0.34–1.06	0.38*	0.14–0.59
PCV (%)	57	50–71	54*	48–63	37*	30–45
WBC ($\times 10^3$/μl)	12.3	8.3–16.0	12.3*	9.0–20.3	8.4*	4.5–9.5
Heterophils ($\times 10^3$/μl)	7.360	5.859–9.102	7.380*	4.068–9.135	2.582*	1.404–5.572
Lymphocytes ($\times 10^3$/μl)	2.551	1.577–5.986	3.857	1.820–6.215	4.130	2.270–6.607
Monocytes ($\times 10^3$/μl)	0.146	0.123–0.498	0.450*	0–1.260	0.068*	0–0.306
Eosinophils ($\times 10^3$/μl)	0.898	0.083–2.720	1.620	0–6.496	0.086	0–0.419

[a] RBC = red blood cell; PCV = packed cell volume; WBC = white blood cell.

[b] Medians with an asterisk are significantly different between foraging and nesting females ($P \leq 0.05$).

California are summarized in Table 1. There were no differences in standard measurements and weights of foraging males and females, so data were combined for presentation. Foraging males had a median CCL of 155.2 cm (range: 144–160 cm), a CCW of 110.1 cm (100–132 cm), and weighed 450 kg (380–576 kg). Foraging females had a CCL of 158.4 cm (146–172 cm), a CCW of 118.0 cm (110–155.5 cm), and weighed 528 kg (399–607 kg).

Laboratory analyses

Blood samples were collected from 51 leatherback turtles: 20 from California, 11 from Papua New Guinea, eight from Costa Rica, and 12 from St. Croix; however, not all tests were run on all individuals due to limited sample quantity. The dorsal cervical sinus was used as the sole blood collection site for turtles from Papua New Guinea and Costa Rica, the popliteal vein was used exclusively in St. Croix, and both sites were employed for foraging turtles in California (dorsal sinus: n=13; rear flipper nexus: n=7). Approximately half (9/19, 47%) of blood samples from foraging turtles had mild to moderate hemolysis due to the difficulty associated with sampling animals on a boat with limited physical restraint.

Results of hematologic tests are provided in Table 2. Hematologic data from St. Croix were excluded from statistical comparison due to the small number of samples that were viable upon arrival at the laboratory (n=2). There were no significant differences in any of the hematology values between foraging males and females. Foraging females had higher WBC (P=0.001), RBC (P=0.007), PCV (P<0.001), heterophils (P=0.004), heterophil to lymphocyte ratio (P<0.001), and monocytes (P=0.007) than nesting females. Lymphocyte count and eosinophil count were not different between foraging and nesting females, or between foraging and nesting females from the same Western Pacific metapopulation (California and Papua New Guinea). No differences in hematology values were detected between the two nesting populations (Papua New Guinea and Costa Rica). No basophils were detected in any of the leatherback blood samples examined. Thrombocytes were determined to be adequate in all turtles evaluated.

Plasma biochemistries for foraging and nesting leatherback turtles are presented in Tables 3 and 4. There were no differences in any biochemical parameter between foraging males and females, with the exception of ALP, which was higher in males (P=0.023). The majority of biochemical values differed significantly between foraging and nesting females, with the exception of CK, albumin, glucose,

TABLE 3. Plasma biochemistry values of Pacific leatherback turtles foraging off the coast of California, 2005–2007, and nesting in Papua New Guinea, Costa Rica, and St. Croix, 2001–2007.

	Foraging				Nesting	
	Males (n=5)		Females (n=9)		Females (n=31)	
Measurement[a]	Median	Range	Median[b]	Range	Median	Range
ALP (U/l)	817	80–4,098	79*	50–290	44*	25–240
AST (U/l)	166	121–185	165*	111–731	115*	71–242
CK (U/l)	272	65–1,366	535	14–5,278	524	8–5,139
LDH (U/l)	394	338–855	584*	311–3,640	345*	134–1,351
Albumin (g/dl)	2.1	1.2–2.4	1.9	1.4–2.7	1.7	1.2–2.3
Total protein (g/dl)	5.3	3.8–5.9	4.9*	3.7–5.9	4.1*	2.8–5.2
Globulin (g/dl)	3.1	2.6–3.8	3.1*	2.1–3.6	2.3*	1.6–2.9
Cholesterol (mg/dl)	424	267–567	396*	267–596	352*	195–525
Glucose (mg/dl)	92	72–98	87	71–117	90	64–136
Calcium (mg/dl)	7.3	2.3–7.9	7.9*	1.7–15.0	11.0*	7.4–14.5
Phosphorus (mg/dl)	12.1	9.9–15.2	14.3	10.1–17.9	12.8	10.5–16.4
Potassium (mEq/l)	6.7	6.4–8.8	6.3*	4.2–12.0	3.5*	2.9–4.8
Sodium (mEq/l)	151	150–158	151*	146–163	146*	135–151
Uric acid (mg/dl)	1.8	0.7–3.7	1.0*	0–2.1	0.4*	0.3–1.1

[a] ALP = alkaline phosphatase; AST = aspartate aminotransferase; CK = creatine kinase; LDH = lactate dehydrogenase.

[b] Medians with an asterisk are significantly different between foraging and nesting females ($P \leq 0.05$).

and phosphorus. Foraging female leatherbacks had higher values for ALP (P=0.001), AST (P=0.001), LDH (P=0.023), TP (P=0.006), globulin (P=0.001), cholesterol (P=0.028), potassium (P<0.001), sodium (P=0.001), and uric acid (P=0.002). Calcium was the only parameter that was significantly higher in nesting leatherbacks (P=0.023). There were fewer differences in plasma biochemistry values

TABLE 4. Plasma biochemistry values of nesting female Pacific and Atlantic leatherback turtles from Papua New Guinea, Costa Rica, and St. Croix, 2001–2006.

	Nesting Females					
	Papua New Guinea (n=11)		Costa Rica (n=8)		St. Croix (n=12)	
Measurement[a]	Median[b]	Range	Median[b]	Range	Median[b]	Range
ALP (U/l)	61 A	36–240	40 B	25–53	41 B	27–61
AST (U/l)	107 A	82–164	135 A	97–242	100 A	71–153
CK (U/l)	765 A,B	11–3,601	1,022 A	134–4,263	44 B	8–5,139
LDH (U/l)	234 A	134–431	432 B	225–881	456 B	297–1,351
Albumin (g/dl)	1.9 A	1.5–2.3	1.7 A	1.4–2.0	1.7 A	1.2–2.0
Total Protein (g/dl)	4.2 A	3.5–5.2	3.9 A	3.6–4.4	4.1 A	2.8–4.7
Globulin (g/dl)	2.3 A	2.0–2.9	2.4 A	2.0–2.5	2.3 A	1.6–2.7
Cholesterol (mg/dl)	367 A	294–525	348 A	284–415	341 A	195–454
Glucose (mg/dl)	113 A	64–136	93 A	79–114	82 A	64–105
Calcium (mg/dl)	12.4 A	8.1–14.5	10.9 A	7.8–11.2	9.9 A	7.4–14.4
Phosphorus (mg/dl)	12.9 A	10.8–16.4	13.3 A	10.5–15.6	12.6 A	10.6–15.4
Potassium (mEq/l)	3.5 A	2.9–4.6	3.6 A	3.0–4.8	3.6 A	2.9–4.7
Sodium (mEq/l)	148 A	146–150	147 A	142–151	141 B	135–149
Uric acid (mg/dl)	0.5 A,B	0.3–1.1	0.6 A	0.4–0.9	0.4 B	0.3–0.5

[a] ALP = alkaline phosphatase; AST = aspartate aminotransferase; CK = creatine kinase; LDH = lactate dehydrogenase.

[b] Medians values in a row that share a common letter are not significantly different at α=0.05; significance assessed using Holm's sequential Bonferroni adjustment ($P \leq 0.017$).

TABLE 5. Concentrations of heavy metals in whole blood from free-ranging leatherback turtles foraging off the coast of California and nesting in St. Croix, 2005–2007.

	Foraging				Nesting	
	Males ($n=3$)		Females ($n=9$)		Females ($n=11$)	
Metal	Median	Range	Median[a]	Range	Median	Range
Cadmium (ppm)	0.077	0.069–0.085	0.078*	0.043–0.182	0.042*	0.014–0.063
Lead (ppm)	0.200	0.200–0.220	0.190	0.090–0.310	0.150	0.080–0.190
Mercury (ppm)	0.019	0.014–0.035	0.022*	0.007–0.048	<DL* [b]	<DL–0.013

[a] Medians with an asterisk are significantly different between foraging and nesting females ($P \leq 0.05$).

[b] <DL = below detection limit.

between foraging and nesting females from the same Western Pacific stock (California and Papua New Guinea), including AST ($P=0.003$), LDH ($P=0.001$), globulin ($P=0.020$), calcium ($P=0.020$), potassium ($P<0.001$), sodium ($P=0.042$), and uric acid ($P=0.020$). Among the three nesting locations, differences in several biochemical analytes were detected, including ALP ($P=0.007$), CK ($P=0.017$), LDH ($P=0.001$), sodium ($P=0.001$), and uric acid ($P=0.015$). Although a consistent pattern was not evident to explain the variability among nesting locations, most values from the two Pacific sites, Papua New Guinea and Costa Rica, were similar, but one or both of these locations differed from the Atlantic site, St. Croix, for all parameters.

Whole blood concentrations of cadmium, lead, and mercury are presented in Table 5. Metals only were evaluated in leatherback turtles from two locations: California ($n=12$) and St. Croix ($n=11$). Differences between foraging males and females could not be assessed because adequate sample quantity was available for only three males, so data from males were not included in analyses. Pacific foraging female leatherbacks had significantly higher concentrations of cadmium ($P=0.001$) and mercury ($P<0.001$) than nesting Atlantic females; however, there was no difference detected in lead levels ($P=0.092$).

None of the OC or PCB analytes were detected in the serum of leatherback turtles from California ($n=14$) or St. Croix ($n=10$) at or above the nominal reporting limits of 5 ppb and 1–10 ppb, respectively. Six samples were assigned reporting limits two times higher than nominal due to limited volume or poor quality of the sample which resulted in difficulties with the extraction process. Domoic acid was not detected in any of the plasma samples from foraging Pacific leatherback turtles at or above 5 ppb ($n=20$) or in fecal samples at or above 500 ppb ($n=7$).

DISCUSSION

This study provides the most comprehensive assessment to date of the health status of leatherback turtles in the Pacific, including a comparison of foraging and nesting leatherbacks from the same Western Pacific metapopulation. We found significant differences in blood values between foraging and nesting female leatherbacks, which suggests that health assessment studies conducted only on nesting females might not accurately represent the whole population. The establishment of baseline physiologic data and blood values for healthy foraging leatherback turtles, including males, provides valuable data for long-term health monitoring and comparative studies of this endangered population.

The traumatic wounds observed in both foraging and nesting leatherback turtles are seen frequently in long-lived, free-ranging wildlife and did not appear to

impact normal behavior or movement. Traumatic wounds from boat strikes, as evidenced by parallel scarring on the carapace in two of 19 turtles, are commonly observed in many species of sea turtles and marine mammals, such as sea otters and sea lions, that spend time at the surface (Kreuder et al., 2003; Greig et al., 2005; Deem et al., 2006). Injuries from boat strikes can result in death; however, such mortalities are rarely documented because few leatherback carcasses are recovered in a fresh postmortem condition.

Values for curved carapace length and width in foraging leatherbacks in this study were similar to those reported for nesting female leatherbacks in Costa Rica (Southwood et al., 1999, 2005), Gabon (Deem et al., 2006), Papua New Guinea (Benson et al., 2007c), Trinidad (Harms et al., 2007), and French Guiana (Guirlet et al., 2008). However, mean body weights of foraging female leatherbacks in California (512.4±6.82 kg; 380–607 kg; $n=13$) were significantly higher than those previously reported in nesting adult female leatherbacks in Trinidad (287±26 kg; 242–324 kg; $n=10$) and Costa Rica (268±44 kg; 196–308 kg; $n=6$), where there was no overlap of ranges (Wallace et al., 2005; Harms et al., 2007). We found a marked distinction between the body condition of foraging and nesting turtles, which likely reflects differences in nutritional status. Foraging turtles had a markedly rounder carapace and a thicker neck and hind end compared with nesting females, which appeared emaciated with prominent dorsal ridges.

The mean heart rate (beats per min) in foraging leatherback turtles in this study (80.0±14.2 bpm) was nearly four times higher than values reported for swimming (24.9±1.3 bpm), diving (17.4±0.9 bpm), and nesting (range=18.3–22.3 bpm) leatherbacks in Costa Rica (Southwood et al., 1999). This likely was a result of the stress and physical exertion associated with capture and restraint. Diving turtles experience a mild dive-associated bradycardia similar to marine mammals and nesting turtles are in a hyporesponsive trance-like state (Southwood et al., 1999). These heart rate values obtained with a pulse oximeter were validated with the use of a simultaneous electrocardiogram.

The median respiratory interval (30 sec) equates to a respiratory rate of 1–2 respirations per minute (rpm), which is similar to respiratory rates from nesting females during nest excavation and oviposition in the tropics (1.5–6.0 rpm; Harms et al., 2007). Respiratory interval is a useful monitoring tool for leatherbacks at capture and can be an early indicator of distress. In the clinically compromised turtle, respiratory interval slowly increased with the longest period of apnea measured at 13 min 20 sec. Routine dive durations for leatherbacks range from 5–14 min, with a maximum recorded dive duration of 67 min (Southwood et al., 1999). The long respiratory interval observed in the clinically compromised turtle is an outlying value compared to other turtles in this study but fell within the boundaries of a normal breathhold during a routine dive for the species. Anesthetized leatherbacks have been reported to experience periods of apnea after induction with ketamine and medetomidine with a maximum respiratory interval of 28 min and became hypoxic, hypercapnic, and acidotic, according to blood gas analysis (Harms et al., 2007). Unique diving physiology might allow leatherback turtles to tolerate longer periods of apnea, similar to the dive reflex in marine mammals. These findings confirm the importance of monitoring vital signs throughout the capture process and should be considered when establishing guidelines for emergency response for the species.

Body temperatures for foraging leatherbacks in California (22.89±2.26 C) were similar to foraging turtles from the cold waters off Nova Scotia (24.33±1.87 C) and were lower than temperature ranges in swimming and diving (30.2±0.7 C) and anesthetized (31.1±0.4 C) leatherbacks in

tropical climates (James and Mrosovsky, 2004; Southwood et al., 2005; Harms et al., 2007). As expected, all foraging leatherback turtles maintained a higher core body temperature than the ambient water temperature (13.8–16.9 C), with a mean differential temperature of 7.65± 2.88 C and a maximum differential of 10.2 C. These findings are similar to those recorded in leatherbacks from Nova Scotia (8.2±2.4 C; James and Mrosovsky, 2004) and higher than the median of 3 C above water temperature reported in the tropics (Harms et al., 2007). The higher temperature differential seen in leatherbacks in temperate waters likely is a result of the colder ambient water and air temperatures in northern latitudes. Leatherback turtles exhibit inertial endothermy as a result of their large body size, countercurrent heat exchangers, insulating fat layers, and continuous movement, which allows them to thrive in cold temperate waters (Frair et al., 1972; Greer et al., 1973; Spotila et al., 1997).

Blood collection techniques were variably successful due to the challenges associated with rapid blood clotting in this species and our minimal physical restraint of the turtles during sample collection. Lymph contamination occurred at both bleeding sites, although the dorsal sinus typically had a reduced risk compared to peripheral veins (Hernandez-Divers, 2006). Effects of bleeding site on blood values are not well understood in leatherback turtles; however, significant differences between hematologic and plasma biochemical values from the jugular vein and occipital sinus in desert tortoises were attributed to hemodilution of the occipital region samples with extravascular fluid or lymph (Gottdenker and Jacobson, 1995).

Packed cell volumes, significantly elevated in foraging turtles, can vary with physiologic state, nutritional status, and sex, all of which could contribute to the differences observed (Campbell, 2006). Hematology values in nesting females from this study were similar to values in nesting females from Gabon (Deem et al., 2006). Leukocytosis characterized by heterophilia, lymphopenia, monocytosis, and an elevated heterophil to lymphocyte ratio can be an indicator of physiologic stress response in reptiles (Gross and Siegel, 1983), which could explain the elevations seen in captured foraging turtles relative to nesting turtles. Differences in hematology also might reflect variability in methods and laboratory personnel.

Nutritional status can influence plasma biochemical values and might explain the differences observed between foraging and nesting turtles in this study. Uric acid, cholesterol, total protein, and potassium can increase postprandially and decrease with fasting (Campbell, 2006). There were no differences observed in plasma glucose levels between foraging and nesting turtles, which is not surprising because fasting animals must continue to maintain blood glucose for normal function. Malnourished sea turtles in negative energy balance might exhibit anemia, hypoproteinemia, and hypoglycemia, and can have a concave plastron, sunken eyes, muscular atrophy, and decreased body weight (George, 1997), some of which were observed in nesting females in this study. Although elevated AST, LDH, and potassium in foraging leatherbacks could indicate cellular muscle damage or increased metabolic activity, they were more likely associated with gross hemolysis during blood collection (Christopher et al., 1999). Creatine kinase also may be elevated in cases of difficult sampling (Campbell, 2006), but differences could not be detected here due to the wide range of values for both groups and large degree of variability associated with the measurements.

The elevated plasma calcium measured in nesting compared to foraging females is most likely a result of calcium mobilization during vitellogenesis. Female leatherback turtles maintain fairly constant plasma calcium levels throughout the nesting season and their ovaries contain a full

complement of preovulatory follicles at the start of the season, suggesting that vitellogenesis is complete prior to arrival at the nesting beach (Rostal et al., 1996, 2001). Ratios of calcium to phosphorus were less than one for foraging males (0.60), foraging females (0.55), and nesting females (0.86) in this study, similar to nesting leatherbacks from Gabon (0.73; Deem et al., 2006) and Trinidad (0.61; Harms et al., 2007).

Biomagnification of trace elements via trophic transfer might be limited in leatherbacks due to their diet of cnidarian zooplankton. Female leatherback turtles can reduce contaminant burdens in blood through maternal transfer to eggs, whereas males lack a comparable physiologic mechanism for excretion of toxicants (Guirlet et al, 2008). Although the sample size for males was too small for statistical comparison, a subjective assessment of the data indicates that the metal concentrations are similar for both sexes, with overlap of the ranges for all metals. Blood cadmium levels were highest in foraging Pacific female leatherback turtles (0.10±0.05 ppm), as compared to Atlantic nesting turtles from St. Croix (0.04±0.02 ppm) and French Guiana (0.08±0.03 ppm; Guirlet et al., 2008). Unfortunately, comparisons were not possible between foraging and nesting females from the same metapopulation or within the same ocean basin. Lead levels were not different between foraging (0.20±0.08 ppm) and nesting female leatherbacks (0.15±0.03 ppm) in this study, and they appear similar to nesting leatherbacks from French Guiana (0.18±0.05 ppm; Guirlet et al., 2008) and slightly higher than levels measured in nesting leatherbacks in Gabon (0.087±0.031 ppm; Deem et al. 2006). Cadmium and lead might compete with essential metals for binding sites on metalloenzymes and both are carcinogenic and teratogenic; however, the toxicokinetics and threshold for toxic effects of metals in reptiles have not been determined (Linder and Grillitsch, 2000). Using guidelines established for birds, lead exposure in foraging California leatherback turtles (0.202±0.067 ppm) falls at the boundary between background level (<0.2 ppm), and recent exposure (0.2–0.590 ppm) to lead (Brown et al., 2006).

Mercury levels in the blood of foraging females from California (0.021±0.010 ppm) were moderate compared with high levels in nesting females from Gabon (0.200±0.200 ppm; Deem et al., 2006) and lower values in nesting female leatherbacks from French Guiana (0.011±0.003 ppm; Guirlet et al., 2008) and St. Croix (0±0.01 ppm), and nesting loggerhead turtles (*Caretta caretta*) from the southeastern United States (0.009±0.008 ppm; Day et al., 2005). For comparison, Pacific harbor seals (*Phoca vitulina*) living in the polluted waters of San Francisco Bay had blood mercury concentrations that increased with age in pups (0.093±0.023 ppm), juveniles (0.284±0.026 ppm), and adults (0.302±0.023 ppm; Brookens et al., 2007). Blood concentrations of mercury reflect both dietary intake and long-term accumulation. Mercury bioaccumulates in food chains and has been associated with the suppression of lymphocyte proliferation in loggerhead and Kemp's ridley (*Lepidochelys kempi*) sea turtles (Day et al., 2007). Although the effects of metals on leatherbacks are largely unknown, these results have important implications for human health where people consume leatherback eggs, meat, and internal organs (Aguirre at al., 2006).

None of the OC or PCB analytes were detected in the blood of leatherbacks in this study with the available detection limits (5 ppb for OCs and 1–10 ppb for PCBs). These negative findings are consistent with a previous study on nesting female leatherbacks from Gabon with similar detection limits for OCs (20 ppb; Deem et al., 2006). However, a recent study that utilized more sensitive methods with lower limits of quantification (0.08 ppb) showed that nesting female leatherbacks from French Guiana have measurable levels of OCs and PCBs in

blood and eggs (Guirlet et al., 2010). These differences in leatherback blood concentrations could reflect differences in the quality of foraging habitat, the physiologic state of the turtle at the time of sampling, or the use of more sensitive laboratory detection methods. In juvenile loggerhead and Kemp's ridley sea turtles, OCs were detected in blood at a similarly lower limit of detection (10 pg/g wet mass=0.01 ppb) and were significantly correlated with levels in paired fat samples, suggesting that blood might be a reasonable and less invasive alternative to fat biopsies in live turtles (Keller et al., 2004). Leatherbacks feed at a lower trophic level compared with loggerheads and Kemp's ridleys, both of which eat crustaceans and bivalves that can bioaccumulate toxicants more effectively than cnidarian zooplankton. Indeed, loggerhead turtles consistently have higher levels of PCBs and DDE than green turtles (*Chelonia mydas*) due to dietary differences (George, 1997). In general, blood concentrations of lipophilic contaminants represent recent exposures whereas concentrations in fat typically reflect long-term chronic exposures. However, blood concentrations can increase with lipid mobilization during egg production (Keller et al., 2004) and during periods of fasting, as observed in pinnipeds with weight fluctuations during rehabilitation (Hall et al., 2008). The complex and dynamic relationship between these variables in leatherback turtles is not well understood and the biological significance of these low concentrations of organic contaminants is unknown.

Domoic acid, a potent marine algal toxin that causes neurologic disease in marine mammals and seabirds off the coast of California, was not detected in the plasma or feces of any turtles in this study, although it is present seasonally in the coastal waters off California where leatherbacks forage. Domoic acid bioconcentrates in the tissues of small fish such as anchovies and sardines that feed on the toxic diatoms, and affects top level predators, including humans (Work et al., 1993; Gulland et al., 2002). Although leatherback turtles share the same nearshore marine environment as seabirds and marine mammals that are commonly affected by domoic acid, jellyfish might not concentrate the toxin as effectively as fish. However, we did detect domoic acid in a leatherback turtle for the first time in a fresh dead specimen with propeller wounds that died off the coast of California in August 2008. This turtle had trace levels of domoic acid in the urine (<5 ppb), but plasma and feces were both negative for the toxin. The fact that domoic acid was not detectable in the blood is not surprising, given the rapid renal clearance of the toxin from the blood within several hours in primates and rats (Truelove and Iverson, 1994). Urine and stomach contents are better samples for evaluation of exposure to domoic acid in marine mammals (Tor et al., 2003), but these samples are not easily obtainable from live sea turtles. This important finding illustrates that although the leatherbacks in this study were all negative for domoic acid in plasma and feces, the potential for exposure to the toxin in foraging leatherback turtles can not be ruled out and should be explored further.

In conclusion, this study represents the first look at the health status of leatherback turtles in the Pacific. The establishment of baseline physiologic data and blood values for healthy foraging leatherback turtles of both sexes provides valuable data for long-term health monitoring of this population. Leatherbacks share the coastal California foraging habitat with other sensitive marine species and could serve as good biological indicators of ocean health. In regions where leatherback eggs, meat, and internal organs are consumed, the health of these turtles also might have larger implications for human health. The integration of population health data should be a vital component of ecological studies that address the conservation and recovery of this endangered species.

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