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Potential impacts of historical disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll (Central Pacific)

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ABSTRACT

Palmyra Atoll, in the Central Pacific, is a unique marine ecosystem because of its remarkably intact food web and limited anthropogenic stressors. However during World War II the atoll was structurally reconfigured into a military installation and questions remain whether this may have impacted the health of the atoll's ecosystems and species. To address the issue we assessed green sea turtle (n = 157) health and exposure to contaminants at this foraging ground from 2008 to 2012. Physical exams were performed and blood was sampled for testosterone analysis, plasma biochemistry analysis, hematology and heavy metal exposure. Hematological and plasma chemistries were consistent with concentrations reported for healthy green turtles. Heavy metal screenings revealed low concentrations of most metals, except for high concentrations of iron and aluminum. Body condition indices showed that <1% of turtles had poor body condition. In this study, we provide the first published blood values for a markedly healthy sea turtle population at a remote Central Pacific Atoll.

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1. Introduction

Sea turtles are an integral part of diverse marine ecosystems and face numerous conservation challenges worldwide (Jackson et al., 2001; Chaloupka and Limpus, 2005; Hamann et al., 2010). The globally Endangered (IUCN, 2014) green sea turtle (*Chelonia mydas*) is generally found in tropical and subtropical waters in all major ocean basins. The Central Pacific supports several marine turtle stocks (Wallace et al., 2010), many of which are poorly studied with little known about their current or historical ecology and status (Balazs, 1995; Chaloupka et al., 2004). Demographic data from green turtles at foraging grounds in a few Pacific regions exist, but large parts of the Central Pacific are understudied. Green turtles spend a significant portion of their lives at foraging grounds, and studies of these areas are necessary for comprehensive population assessments, yet research lags behind that of more accessible nesting sites (Balazs et al., 1987; NRC, 2010).

Investigating the health status of protected species is important in population and conservation assessments, because health stressors and disease can be a threat to long-term persistence. Monitoring green turtle health status is of special relevance because fibropapillomatosis (FP), a debilitating herpes-virus linked disease characterized by benign tumors, has a circumtropical distribution and has reached epizootic concentrations in several sites across their range (Aguirre and Lutz, 2004). Further, baseline health information on green turtles may help to identify potential stressors. Exposure to contaminants has been acknowledged as a potential threat to sea turtles (Godley et al., 1999; Ikonomopoulou et al., 2009; Hamann et al., 2010), and as large bodied, long-lived marine vertebrates, green turtles are vulnerable to the effects of bioaccumulation (Caurant et al., 1999; Godley et al., 1999; Gardner et al., 2006; Barbieri, 2009; Todd et al., 2010). Numerous studies have found that while herbivorous green turtles feed at a lower trophic level than carnivorous sea turtles, adverse health impacts from contaminants still occur (Gardner et al., 2006; Komoroske et al., 2011). Collecting baseline information from hematology and metal screenings can advance our understanding of species health, and contribute to the development of conservation and management plans.

The Palmyra Atoll National Wildlife Refuge (PANWR), located approximately 1800 km south of Hawaii in the Central Pacific (Fig. 1a and b), is one of the most remote atolls in the world, but has a complex history. The atoll, among the most intact extant oceanic reef environments (Dinsdale et al., 2008; Sandin et al.,

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2008), is currently uninhabited except for limited research and management personnel, and free from most pervasive anthropogenic inputs. It has been described on several occasions as a unique ecosystem with a remarkably intact food web (Lafferty et al., 2008; Williams et al., 2008, 2011; Work et al., 2008).

However, during the Second World War (WWII), Palmyra was developed into a naval installation. Building on an undisturbed atoll made up of hundreds of small islets and distinct shallow water lagoons (Fig. 1b), the military increased land area by dredging, filling and constructing runways and causeways. Modifications also included dredging of a deep channel to allow ship access to the central lagoon (Dawson, 1959). The military base at Palmyra housed several thousand personnel from 1941 to 1945, thus generating sewage pollution and various toxic and hazardous chemicals, and leaving behind abandoned metal structures in the lagoons (Brainard et al., 2005).

While it is believed that most of the military equipment and debris was removed at the end of WWII, little is known about what contaminants may remain in the marine environment at Palmyra (Collen et al., 2009, 2011). Studies examining trace metal contamination there showed no evidence of lead or other heavy metals in bivalve shells (Collen et al., 2011). However, preliminary X-ray fluorescence (XRF) screening of sand and sediment samples from five marine sites within the lagoons indicated that, based on NOAA's Screening Quick Reference Tables (Buchman, 2008), chromium and strontium in particular may occur in moderately and highly hazardous concentrations for biota, respectively (Papoulias et al., 2010). In contrast, concentrations of most heavy metals known to adversely impact wildlife health (e.g. mercury, lead, arsenic, cadmium, etc.) were below detection levels (Papoulias et al., 2010).

One aim of this study was to contribute to the body of literature evaluating if Palmyra may be considered a relatively intact ecosystem (Dinsdale et al., 2008; Sandin et al., 2008). Given our observational data that Palmyra green turtles appear in relatively good health (see Sterling et al., 2013), the goal of this study was to further constrain or support this hypothesis with quantifiable data. Quantifiable clinical data can add to our understanding of turtle health at this regionally important feeding ground, and to the effectiveness of Palmyra as a marine protected area. Additionally, the US Fish & Wildlife Service (USFWS), which co-manages the refuge, was interested in the health of the sea turtles at Palmyra, especially in light of debris left by the military and consideration of restoration plans for the lagoons. Baseline health data are noteworthy in that they can determine if health issues are present in a population, and help assess changes in health status that may occur in response to environmental pressures. In this study we report on comprehensive health assessments, and compare our findings across stage classes, sex, or site of capture within the atoll.

2. Materials and methods

2.1. Study area

The Palmyra Atoll National Wildlife Refuge (05°52′ N, 162°06′ W) is one of the Northern Line islands (Fig. 1a) in the Central Pacific Ocean. The atoll was purchased by the Nature Conservancy (TNC) in 2000, and the USFWS manages some of the islets and surrounding 15,000 acres of atoll waters. In 2009, it was designated as part of the Pacific Remote Islands National Monument.

The connection of islets and dredging of a channel for ship access during WWII created three artificially deep lagoons with reduced amounts of tidal flow (Maragos, 1993; Maragos et al., 2008). These lagoons vary in depth between 35 and 65 m and are geographically described as the Western, Central, and Eastern lagoons (Fig. 1b). In the northern and southern parts of the atoll, habitat consists of wide, shallow-water reef flats, while there are broad submerged reef terraces on the western and eastern ends (Collen et al., 2009). These reef flats and terraces are characterized by turf and macroalgal assemblages (McFadden et al., 2010). Steeply-sloped fore reefs surround the reef break atoll edges and these areas, combined with the shallow water reef habitats, serve as foraging habitat for green sea turtles (McFadden et al., 2010; Sterling et al., 2013).

2.2. Sampling

We captured green turtles at Palmyra Atoll from 2008 to 2012 using scoop nets, tangle nets, or by hand. We measured weight (kg) straight carapace length (SCL), and tail length (TL) (see Sterling et al. (2013) for methodological details). A body condition index (BCI) was calculated based on the commonly used relationship between mass and SCL ($BCI = [mass/SCL^3] \times 10^4$; Bjorndal et al., 2000). Each turtle was given a comprehensive physical examination including assessments of: plastron concavity/convexity; muscle tone and mass; weight; presence of ectoparasites or fibropapilloma tumors; bloody superficial lesions; and external abnormalities such as amputated flippers or injuries.

Approximately 20 ml of blood was collected from the dorso-cervical sinus using a 20-gauge 1.5-in. needle. Blood samples were visually examined for lymph clot contamination, and those with possible contamination were excluded. Samples were kept in a cooler with ice packs and were typically processed within 6 h of collection. Thin blood smears were made and fixed with 99% methanol. One hundred randomly selected blood smears were later examined by microscopy (100×) to assess potential hemoparasite infections. Heparinized whole blood mounts were made at the time of bleeding and later stained utilizing Diff Quick differential stain kits (Polysciences, Inc.). Leukocytes were then manually counted under 100× magnification and recorded as a percentage of the total leukocyte population. Estimated total leukocyte counts from a blood smear are not as reliable as manual counts using a hemocytometer, but they are far more practical for field situations. Packed cell volumes (PCV) were determined via hematocrit tubes and centrifugation. Serum total solids (g/100 ml) were determined by refractometer (Reichert VET 360) in the field.

Blood was dispensed directly into vacutainer tubes (Becton–Dickinson Diagnostics, Pre-Analytical Systems, Franklin Lakes, New Jersey, USA) containing no additive (for serum refractory analysis), lithium heparin (for hematology and plasma biochemistry analyses), or buffered citrate sodium (for heavy metal testing). Not all tests were performed on all individuals because the amount of material was limited for some of the turtles. Whole blood for heavy and trace metal analysis was collected from 2009 to 2012. Samples collected in the field were transferred to cryovials and stored frozen at −80 °C until analysis at RTI Laboratories (Research Triangle, NC). Plasma biochemistry samples were collected from 2008 to 2012. Samples from heparinized blood-containing tubes were centrifuged at approximately 3250 rpm (2078 g) for 5 min and 3–5 ml of plasma was subsampled and stored at −80 °C until analysis at IDEXX Laboratories (North Grafton, MA) for plasma chemistries analysis.

2.3. Sex determination

To assess sex ratios, subsamples of plasma were collected for testosterone radioimmunoassay procedures and androgen sexing (Owens et al., 1978; Wibbels, 1988; Wibbels et al., 1987, 1991). Radioimmunoassay procedures were conducted at Cornell University, and the testosterone assay's lower limit of detection was 3.12 pg. We used a size cutoff for adults of 80 cm SCL (Wibbels, 1988), and conservatively assigned as males turtles with: (1) >80 cm SCL; (2) tail length (TL) > 25 cm; and (3) testosterone concentrations >30 pg/ml. All other turtles with >80 cm SCL, <25 cm TL, and testosterone concentrations <20 pg/ml were con-

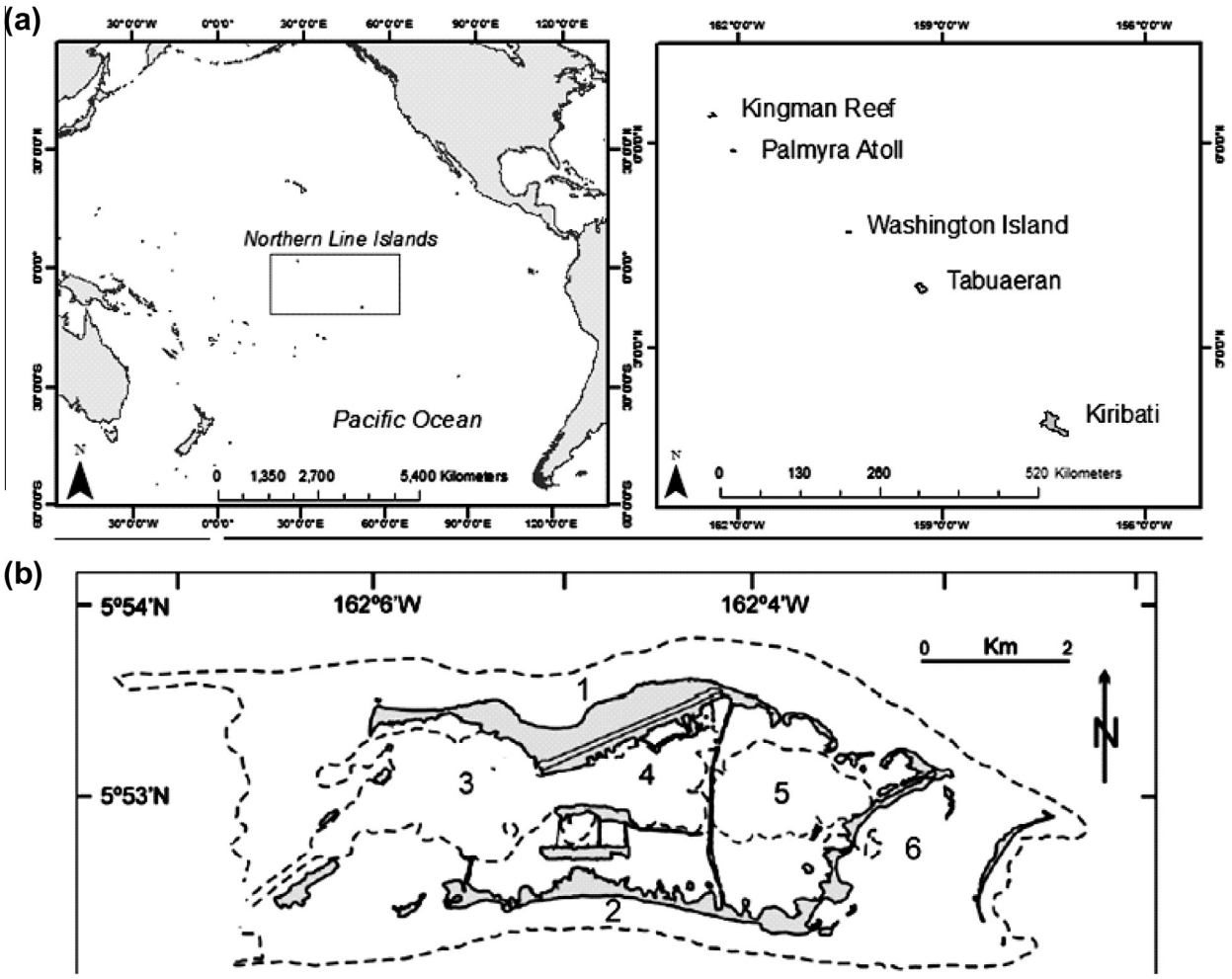

Fig. 1. (a) Map of Northern Line islands in the Central Pacific (top left) and location of Palmyra Atoll within the Line Islands (top right panel); (b) Geography of Palmyra Atoll and the different regions (1 = northern reef flats, 2 = southern reef flats, 3 = western lagoon, 4 = central lagoon, 5 = eastern lagoon, 6 = eastern terrace) within the atoll; modified from Collen et al. (2011) (bottom panel).

sidered females. In cases where blood samples were not collected for testosterone assays, we used a more conservative second set of morphological criteria for tentative gender assignment. Turtles >85 cm SCL and with a minimum tail length $\geqslant$30 cm were considered adult males (Wibbels, 2003; Hamann et al., 2003, 2006), and we tentatively assigned as females those >85 cm SCL with tails $\leqslant$21 cm long, with the understanding that without determinative techniques such as laparoscopy the sex identification was not definitive (Limpus and Reed, 1985; Limpus et al., 1994; Hamann et al., 2003, 2006). Turtles that did not meet these criteria were considered of "unknown" gender, and were excluded from analyses examining variation in health relative to sex. Further, as we did not use laparoscopy to definitively determine sex, we conservatively present testosterone results only for animals >80 cm SCL.

2.4. Chemical analyses

Samples for metal screening were prepared using a nitric acid digestion, and analyzed at RTI Laboratories (Livonia, Michigan, USA) by inductively coupled plasma-mass spectrometry (Thermo Element 2 SF-ICP-MS) and cold vapor atomic fluorescence for trace metals and mercury, respectively. Percent recovery of standard reference materials ranged from 85% to 117%. Due to high concentrations, iron, potassium, and magnesium were analyzed using a thermoinductively-coupled plasma Atomic Emission Spectrophotometer (iCAP 6500 ICP-AES).

Plasma biochemistry (aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), creatine phosphokinase (CPK), and lipase), metabolic indicators (total protein, albumin, globulin, glucose, uric acid, cholesterol, and triglyceride), and ions (sodium, potassium, calcium, inorganic phosphate, and magnesium) analyses were completed using Catalyst Dx Chemistry Analyzers at IDEXX Laboratories. Hematology based on blood smears (estimated leukocyte counts, and % heterophils, lymphocytes, monocytes, eosinophils, and basophils) was completed by reptilian specialists at IDEXX Laboratories using thorough manual counts at 100$\times$ microscopy.

Because stress can impact chemistry analyses, we also examined how CPK values differed depending on the capture methodology. In the initial years of this study, we mainly captured turtles via tangle net (2008–2009). In later years some tangle net captures continued, but hand capture with approach by boat was the primary method used. We used a one-way ANOVA to examine if CPK was related to the type of capture (hand capture versus nets) and the potential stress that one capture method might have with respect to another.

2.5. Statistical analyses

A Shapiro–Wilk statistic, skewness, and kurtosis were used to assess for normality. Data that were not normally distributed were log transformed. Mean, standard deviation (SD) and range were

determined for each toxicological, plasma biochemistry and hematological parameter. Levene's test for equality of variances was used to determine data homogeneity, and comparisons were made between size class, and when possible, sex, using an ANOVA. Specific between-group differences (between size or sex) were evaluated using a Tukey's b post hoc test. A one-way ANOVA was used to determine the differences between large (either adult or large immature, SCL ⩾ 65 cm) and small (SCL < 65 cm) turtles for each metal value and plasma biochemistry parameter. Values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Morphology and condition

A total of 157 green sea turtles received physical examinations, and were weighed and measured. Mean straight carapace length (SCL) was 67.02 ± 14.8.1 cm (range 39.2–105.5 cm SCL). Small turtles (SCL < 64.9 cm) were more frequently captured (39.6%), followed by medium (SCL 65–84.9 cm, 35%) and large turtles (SCL ⩾ 85 cm, 25.4%). Seven of the 157 animals sampled had either full or partial front or rear flipper amputations. Barnacles were recorded on eleven animals. No fibropapilloma tumors were observed on any of the turtles. The mean body condition index was 1.42 ± 0.28 ($n = 144$) (Fig. 2).

The mean (±SD) of packed cell volume (PCV) was 32.2% (±7.8) (Table 1). One turtle had a PCV of 56% and was considered emaciated based on low BCI values and a concave plastron. The next closest PCV value was 49%. As has been reported in other studies, packed cell volume was significantly related to SCL (mean = 36%) ($F = 3.69$, $df = 2$, $p = 0.001$) (Frair, 1977).

Radioimmunoassay analysis resulted in adult sex assignment of 14 female and 14 male green turtles (representing 18% of all weighed animals). An additional 3 individuals were assigned based on SCL measurements ⩾85 cm and TTL ⩾ 30 cm (3 males). The mean SCL did not significantly vary between males (86.14 ± 4.62 cm) and females (88.71 ± 5.55 cm) ($t = -1.37$, $df = 29$, $p = 0.179$).

3.2. Plasma chemistries and hematology

When comparing the plasma chemistry values of Palmyra turtles and healthy free-ranging green turtles from the literature (Bolten and Bjorndal, 1992; Aguirre and Balazs, 2000; Hamann et al., 2006; Flint et al., 2010; Fong et al., 2010; Komoroske et al., 2011), all measures except creatine phosphokinase (CPK) were within previously published ranges. CPK values in Palmyra green turtle blood (Table 1) were somewhat higher than for animals in Australia caught using hand capture by boat methods (Hamann et al., 2006), but within the 95% confidence interval (CI) for green turtles caught in nets in San Diego (Komoroske et al., 2011). Values for CPK did not differ amongst years ($F = 2.59$, $df = 4$, $p = 0.55$). Method of capture (hand capture via boat versus tangle nets) was not significantly correlated to CPK values ($R^2 = 0.230$, $p = 0.56$).

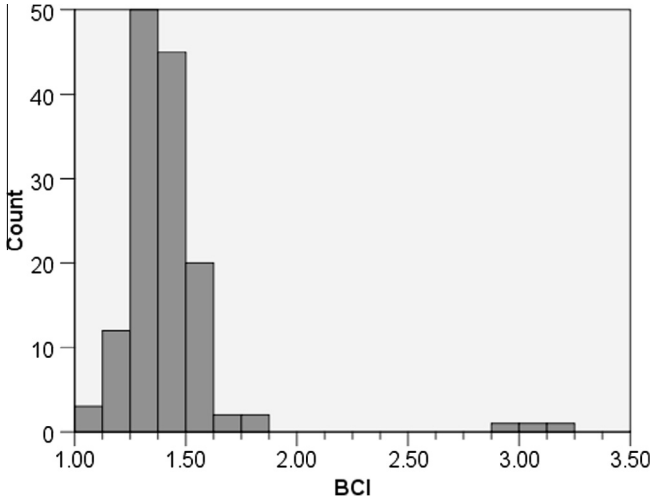

Fig. 2. Proportion of individuals and their respective body condition index (BCI) values for green turtles at Palmyra Atoll (2008–2012).

When examining plasma chemistry values relative to BCI, no significant differences were identified. When examining plasma chemistry values relative to size, cholesterol and glucose significantly differed based on turtle SCL size categories (small, medium, or large turtles). Cholesterol levels were highest in turtles ⩾85 cm SCL ($F = 12$, $df = 2$, $p < 0.05$). Glucose levels were on average 20% higher in small turtles compared to medium or large sized turtles ($F = 8.56$, $df = 2$, $p < 0.05$). No difference in any chemistry value was found related to sex.

Hematologic tests showed no significant differences between sexes. Hematological variables were more similar to some previously published studies (Flint et al., 2010) than others (Work et al., 1998; Work and Balazs, 1999). Low levels of basophils (1%) were found in three individuals. No hemoparasites were observed in any blood smear. The turtle with the PCV of 56% was not one in which plasma chemistries or metal screening was performed. However, this individual's differential cell count was suggestive of leukopenia and lymphopenia (counts two standard deviations below the mean). The individual with the abnormally low PCV of 18% showed no indication of abnormal metal exposure, but exhibited levels suggestive of leukocytosis (WBC = $18.4 \times 10^3/\mu l$) and heterophilia (Table 1). Plasma chemistries for this individual also showed low triglycerides and glucose concentrations.

A one-way ANOVA followed by a Tukey's b post hoc test identified several variables that significantly varied by capture location in each of the four regions of the atoll. A one way ANOVA indicated significant variation in the distribution of size class amongst the four capture sites ($F = 12.39$, $df = 3$, $P < 0.05$) with animals in the east being smallest (mean SCL = 58.72 cm) and those in the west being largest (mean SCL = 76.35 cm). Cholesterol concentrations were highest in turtles captured in the west ($F = 3.30$, $df = 3$, $p = 0.024$), where on average the largest animals were captured. A Sherman correlation test indicated significant correlation of cholesterol and SCL ($r^2 = 423$, $p < 0.05$). SGOT was highest in the east, where we on average have captured smaller turtles ($F = 7.55$, $df = 3$, $p < 0.05$). A Spearman correlation test indicated SGOT was also highly correlated with SCL ($r^2 = 0.32$, $p < 0.05$). While CPK was lowest in the west ($F = 4.40$, $df = 3$, $p < 0.05$), there was no significant correlation with this variable and SCL.

3.3. Heavy metal concentrations

While studies have examined both heavy and trace/essential metals in sea turtle tissue, several trace metal values (Table 2) from our extended metal scan are, to our knowledge, the first known reported blood values in a free ranging green turtle population. Heavy and trace metal screenings revealed low concentrations of most metals. However Aluminum (Al) and Iron (Fe) were above the range detected in previous studies of free ranging green turtles (Table 2). We know of only one study reporting Al in green turtle blood (Komoroske et al., 2011), where blood concentrations were ten times lower than for Palmyra turtles. Mean Fe concentrations (237,968 ± 52,977 ng/g) in Palmyra green turtles were within the range of values found in green turtle liver samples in Japan (Sakai et al., 2000) and Hawaii (Aguirre et al., 1994), but an order

Table 1
Differential cell counts and plasma chemistry assay values (minimum, maximum, mean and standard deviation (SD)) from green turtles (*Chelonia mydas*) at Palmyra Atoll from 2008 to 2012. Abbreviations as follows: WBC = white blood cells, PCV = packed cell volume, creatine phosphokinase (CPK), alkaline phosphokinase (ALP), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). Symbol (†) indicates variables for which 95% CIs were different in this study compared to published studies of healthy green turtles (Bolten and Bjorndal, 1992; Aguirre and Balazs, 2000; Hamann et al., 2006; Flint et al., 2010; Komoroske et al., 2011).

Parameter	*n*	Minimum	Maximum	Mean	SD
Basophil × 10^3/μl (%)	138	0 (0)	127 (1)	3.78 (0.04)	18.57 (0.20)
Eosinophil × 10^3/μl (%)	138	112 (1)	6149 (56)	1313 (15.2)	968 (9)
Heterophils × 10^3/μl (%)	138	1176 (14)	9752 (76)	3951 (46.1)	1790 (12)
Lymphocytes × 103/μl (%)	138	330 (6)	6954 (69)	2782 (33.2)	1240 (12)
Monocytes × 10^3/μl (%)	138	0 (0)	2040 (26)	476 (5.4)	430 (4.5)
WBC count (×10^3/μl)	138	3	18.4	8.5	2.9
PCV (%)	131	9	56	32.2	7.8
CPK (μ/L)†	103	112	15,157	1045	1671
ALP (μ/L)	103	9	58	24	9.00
Lipase (U/l)	103	0	129	17	15.2
AST (U/l)	103	81	403	186	63.3
LDH (U/l)	103	46	712	173	99
Total protein (g/dl)	103	1.9	6	4.1	0.7
Serum protein (g/dl)	115	2	6.3	4.3	0.8
A/*G* ratio (g/dl)	103	0.2	0.7	0.5	0.1
Albumin (g/dl)	103	0.4	1.9	1.3	0.2
Globulin (g/dl)	103	1.5	4.1	2.8	0.5
Cholesterol (mg/dl)	103	24	343	164.9	55
Triglycerides (mg/dl)	103	10	449	152.8	97.5
Calcium (mg/dl)	103	3.5	16.9	9.7	2.4
Glucose (g/dl) †	103	34	133	96.1	21.5
Potassium (mmol/l)	103	3.2	7	4.3	0.6
Phosphorus (mg/dl)	103	2.7	10.1	5.1	1.3
Sodium (mmol/l)	103	131	173	151.7	6.4
Uric acid (mg/dl)	103	0	2.5	0.9	0.5

Table 2
Concentration (mean ± SD, minimum and maximum) of metal elements in whole blood (ng/g, wet mass) of green turtles (*Chelonia mydas*) from 2009 to 2012 at Palmyra Atoll. ∗ Symbol indicates variables for which 95% CIs were different in this study compared to published studies of healthy green turtles (van de Merwe et al., 2010; Komoroske et al., 2012).

Palmyra green turtle metal descriptive statistics (2009–2012) (ng/g)						Komoroske et al. (2012)		van de Merwe et al., 2010	
Parameter	*N*	Minimum	Maximum	Mean	SD	Mean	SE	Mean	SE
Al*	79	164	2870	1057	935	146	34		
Ar	100	6.5	1650	191	211	157	26	2719	883
Ba	87	182	1214	556	228				
Be	87	4.6	6.1	<5	0.4				
Bo	48	870	2160	1466	297				
Cd	87	2.78	113	27	25	13.2	4.2	32	8
Cr	87	5.1	107	38	38				
Co	87	4.6	39.2	<6	3.7			32	6
Cu	87	85.2	1050	432	177	749	46	957	84
Fe*	87	68,900	377,000	237,968	59,277				
Pb	105	3.3	73.2	18	12	1260	222	21	4
Mg	87	71,100	180,000	106,779	26,743				
Mn	87	5.6	173	28.3	25.6	463	89		
Hg	54	1	24.9	1.9	3.8	1	0.2	1.9	0.3
Mo	87	5.1	63	14	8.6				
Ni	87	5.1	118	17.1	19.6				
K	39	565,000	1,830,000	1,196,744	238,001				
Se	87	39.6	19,900	1579	3528.7	776	253	2202	630
Si	87	4.66	6.14	<6	0.3	1.6	0.5		
St	87	775	4670	1881	934				
Va	87	5.1	122	13.8	23				
Z	87	1680	12,200	7501.3	2349			7639	526

of magnitude larger than green turtle muscle samples in Mexico (Gardner et al., 2006).

Iron concentrations were highly correlated with PCV ($r^2 = 0.80$, $p = 0.001$, $n = 86$) but not significantly correlated to SCL. A one-way ANOVA indicated that mean (±SD) Fe concentrations were significantly higher in male turtle blood samples (296,000 ± 53,193 ng/g) than in females (249,000 ± 37,469 ng/g) ($F = 5.04$, $df = 1$, $p = 0.038$). Chi-square tests found no significant differences in any other metal value related to sex.

4. Discussion

The overall health of green turtles at Palmyra appears very good based on physical exam findings, body condition indices, and blood values compared to other populations of healthy green turtles. None of the individuals we captured appeared to be impaired, although at least two animals were emaciated, showed poor body condition or abnormal plasma chemistry values, and a small fraction of the population displayed evidence of amputated flippers.

The study provides the first baseline health data and reference values for what appears to be a healthy green turtle population. Anthropogenic stressors have significantly impacted most marine ecosystems around the world, and this study may serve as a reference for turtles in more disturbed areas. Regional differences in green turtle blood biochemistry analyses have been previously documented, and these highlight the need for population-level baseline reference values (Aguirre and Balazs, 2000; Whiting et al., 2007; Arthur et al., 2008).

While we categorize the population as a whole as healthy by clinical standards, a closer examination of a few individuals does indicate that, not surprisingly, some abnormalities exist, reflecting the range of variation expected even in a healthy population. While at least two animals had chemistry or hematological results suggestive of stress or illness, we found no evidence from blood biochemical assays that Palmyra green turtles were chemically different from healthy free-ranging green turtles from published studies (Bolten and Bjorndal, 1992; Aguirre and Balazs, 2000; Hamann et al., 2006; Flint et al., 2010; Fong et al., 2010; Komoroske et al., 2011). The PCV of 56% found on one turtle may be a result of many factors including, but not limited to, health or unconfirmed lymph contamination.

When examining chemistry values relative to sex and size, glucose concentrations for small turtles were higher than for medium or large turtles. This is consistent with other studies of free ranging green turtles and may suggest ontogenetic variation in diet, improved digestive efficiency of larger turtles (Hamann et al., 2006), microenvironmental factors in each capture site, or size specific capture response. Chemistry variables such as cholesterol and SGOT significantly differed based on capture location within the atoll, where average turtle size also varied. Cholesterol is known to increase in larger animals of reproductive age (Divers, 2000). It is not clear however, why liver enzyme (SGOT) were higher in the eastern zone, where smaller turtles are found. Given that some regions within the atoll tend to contain smaller animals (i.e. eastern flats), the differences in plasma chemistries between turtles captured in different zones likely reflect the variation in mean turtle size/stage class of each zone and not abnormal physiological status.

While data on known heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) exist for some sea turtle populations (see Storelli and Marcotrigiano, 2003), little is known about baseline trace metal element concentrations. However, we collected these data because of the host of anthropogenic factors (i.e. lagoon dredging, changes in tidal flow due to dredging, and the possible presence of military debris in the lagoons) that have the potential to impact sea turtle health at Palmyra. Previous studies have shown that some clinical chemistry parameters in sea turtles have broad normal ranges (Aguirre and Lutz, 2004), and the effects of these elements are generally unknown. Further study comparing sea turtle populations in different ecosystems are needed to determine whether the values observed here are remarkable or within normal ranges.

In large part, all metal exposure was consistent with previous studies (see Komoroske et al., 2012). However aluminum (Al) levels were higher than those detected in blood (but not carapace) from healthy green turtles in an urbanized bay ecosystem (Komoroske et al., 2012) and from dead green turtles stranded in Australia (van de Merwe et al., 2010) (Table 2). A review paper of chemical elements in loggerhead (*Caretta caretta*) turtles (D'Ilio et al., 2011), which examined different tissue and organs in the Mediterranean Sea, and the Atlantic and Pacific Oceans, had much lower Al values than those found in our study. Aluminum is the single most abundant naturally occurring metal and its concentration is generally not influenced by anthropogenic sources (Schropp and Windom, 1987). While this does not exclude the possibility that Palmyra green turtle values may be abnormally high, little information exists about Al concentrations in green turtle blood in other ecosystems or its potential effects on sea turtle health (Sparling et al., 2010).

Mean iron (Fe) concentrations were significantly higher in male than female turtles. Due to the conservative criteria we used in assigning sex, the analysis of differences in Fe concentrations relative to sex was conducted only on large, and putatively reproductively mature animals. Thus, the differences in Fe level may be attributed to factors related to reproduction, such as the maternal transfer of metals via egg deposition (Ramsay and Campbell, 1954), which has been documented in sea turtles (Komoroske et al., 2011; Ikonomopoulou et al., 2013).

One main limitation of the heavy metal evaluation in this study is that a complete and thorough evaluation should include not only circulating/blood levels, but also assess other tissues, such as liver, spleen, skin, muscle, bone, and/or fatty tissue. An examination of organs and other tissues is needed to detect not only recent intake of heavy metals, but also elements that accumulate through the life cycle (McArthur et al., 2004). However, due to the protected status of this species, we were not able to sample other tissue types.

In conclusion, data from this study indicate that the green turtle population at Palmyra Atoll appears clinically healthy and without indication of any major metal contamination known to adversely impact health in sea turtles. These data provide baseline information for future surveys and for comparisons of health and contaminants in other biota from Palmyra Atoll to sea turtles in other tropical foraging grounds.

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