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Baseline Health Parameters of East Pacific Green Turtles at Southern California Foraging Grounds

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Abstract. – Urban coastal ecosystems are unique intersections of human development and biodiversity, and monitoring populations in these areas is critical to understanding ecosystem health and function. Three highly urbanized estuary systems, San Diego Bay, San Gabriel River, and Seal Beach National Wildlife Refuge (all in California), are the northernmost foraging habitats for green turtles (*Chelonia mydas*) in the Eastern Pacific. Here, we report blood biochemistry and morphological parameters for these Southern California green sea turtle foraging aggregations to investigate the current health status of these animals and provide baseline values for future work. Morphometric and blood biochemistry parameters for green turtles captured in this study (n = 39) were clinically reasonable and were generally consistent with previously reported parameters for green turtles.

Key Words. – plasma biochemistry; marine turtle; wildlife health; urban ecology; *Chelonia mydas*

Urban coastal ecosystems can support high levels of biodiversity (Suchanek 1994) but can be impacted by human activities (Crain et al. 2009). The Southern California Bight (Fig. 1), for example, is adjacent to several of the United States' largest cities and supports high levels of biomass and biodiversity at every trophic level from phytoplankton to charismatic megafauna, such as cetaceans and sea turtles (McDonald and Dutton 1990; Dugan et al. 2003; Roch et al. 2011; Chow et al. 2013). Southern California coastal waters are affected by overharvesting (Jackson et al. 2001; MacCall et al. 2016), incidental bycatch (Lyons et al. 2013), urban runoff (Foster and Schiel 2010; Ensminger et al. 2013), and other factors that have altered community compositions in these coastal habitats (Kaustav et al. 2003; McClatchie et al. 2016). While some populations off the Southern California coast remain abundant, the health statuses of these populations are not always clear (Davison et al. 2015; Caron et al. 2017). Long-term monitoring can provide the information necessary to assess the health status of ecosystems, the populations within, and how they may respond to changes in urban coastal environments (Wolfe et al. 1987; Deem et al. 2006).

In Southern California, foraging aggregations of green sea turtles (*Chelonia mydas*) have been observed for years to decades in several coastal embayments. Green turtles in San Diego Bay were first monitored in the 1980s, principally in southern regions of the bay with abundant eelgrass beds (*Zostera marina*; Stinson 1984; McDonald and Dutton 1990). The entire San Diego Bay is highly urbanized and has been both historically and currently affected by dredging, urban runoff, trash accumulation, and other human activities (Fairey et al. 1998). The South Bay Power Plant emitted warm water into San Diego Bay from 1960 until it was decommissioned in 2010, artificially increasing habitat temperatures and growth rates of resident green turtles (Eguchi et al. 2012). Turtles have remained in San Diego Bay since the power plant closed in 2010 (Turner-Tomaszewicz and Seminoff 2012), but may have modified their fine-scale habitat utilization and behaviors in response to the closure of the power plant (MacDonald et al. 2012; Madrak 2016). More recently, foraging green turtles have been observed and monitored in the San Gabriel River and Seal Beach National Wildlife Refuge warm-water estuarine systems near Long Beach, California, where 2 functional power plants remain (Lawson et al. 2011; Crear et al. 2016).

Green turtles play important ecological roles in these coastal ecosystems through omnivorous foraging in seagrass beds (Bjorndal and Jackson 2003; Lemons et al. 2011). Maintaining and restoring healthy sea turtle populations is a high global conservation priority (Seminoff and Shanker 2008). Turtles are largely protected from the threat of direct harvest while residing in these

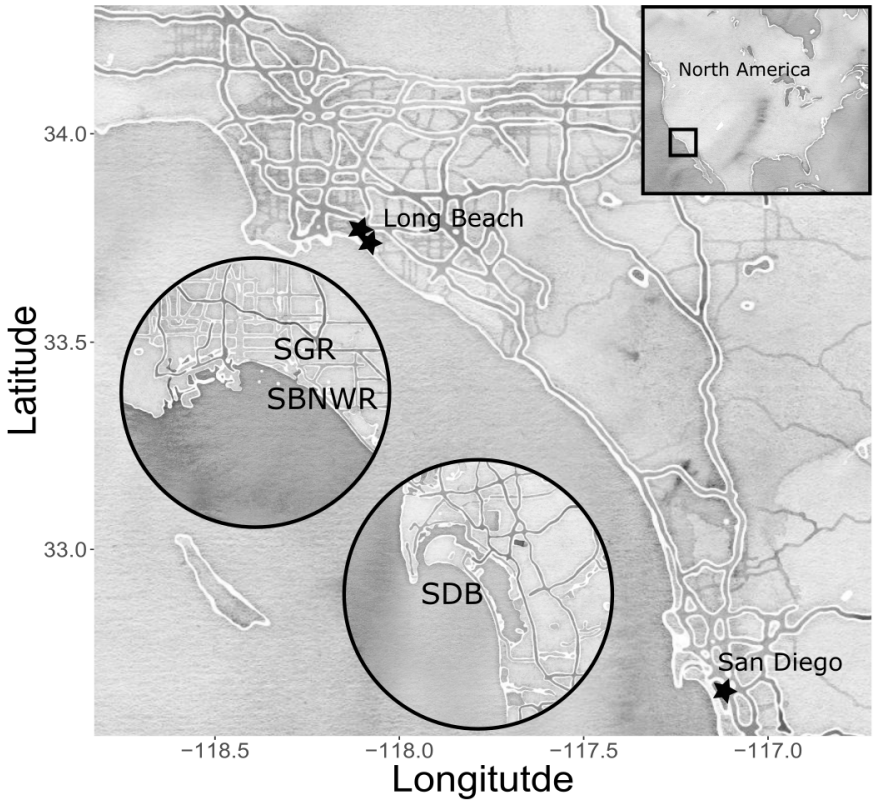

Figure 1. Locations of green turtle foraging habitats where sampling was conducted in Southern California coastal ecosystems. Turtles were captured in the San Gabriel River (SGR) and Seal Beach National Wildlife Refuge (SBNWR), as well as San Diego Bay (SDB).

urbanized coastal habitats, but are susceptible to boat strikes, pollutant exposure, and other negative anthropogenic impacts. Although still on the International Union for Conservation of Nature Red List of Threatened Species (Endangered) and listed under the US Endangered Species Act (Endangered or Threatened), the abundance of many green turtle nesting populations has increased in recent years (Seminoff 2004; Seminoff et al. 2015). At the same time, green turtle strandings in this region have markedly increased relative to previous years, but it is not yet understood if this is a transient anomaly because of changes in environmental conditions (e.g., water temperatures) or human activities, or if it is a consequence of increasing abundance and survival from over a decade of successful nesting beach conservation efforts (Eguchi et al. 2010; Delgado-Trejo and Alvarado-Figueroa 2012). Therefore, there is interest in assessing the health status of green turtles residing in Southern California urbanized habitats to better understand how they may be impacted by human activities and to establish baseline information for future monitoring efforts.

Health assessments including blood analytes can be used to monitor animal nutritional status, reproductive state, and life stage, and to characterize disease processes, as well as to determine exposure to contaminants, toxins, or parasites (Stacy and Innis 2017). Such analyses in wild vertebrates have the potential to serve as early indicators of a decline in the health of a population, before stressors lead to decreased survival or reproductive rates (Maceda-Veiga et al. 2015). However, the lack of baseline data can be a major limitation to using blood analyses for health diagnostics, and previous work on large marine vertebrates suggests significant differences in blood parameters between wild and captive populations (Greig et al. 2010; Maceda-Veiga et al. 2015). Blood biochemistry parameters have been reported for several other populations of green turtles inhabiting areas with varying levels of anthropogenic impacts (Bahamas, Bolten and Bjorndal 1992; Northern Australia, Hamann et al. 2006; Galapagos Islands, Lewbart et al. 2014), including San Diego Bay green turtles sampled from 2007 to 2009, prior to the decommission of the South Bay Power Plant (Komoroske et al. 2011). Here, we build on these resources by reporting blood analyte parameters in Southern California green turtles sampled from 2013 to 2016, and assess differences associated with location, water temperature, and morphometric measurements (in particular, size and body condition as proxies for age and overall health, respectively). This work serves to deepen our understanding of what a "healthy" urbanized population looks like and contributes to establishing baseline parameters for these highly urban foraging aggregations of green turtles.

METHODS

Study Sites. — Green turtles were captured from 2013 to 2016 as a part of larger population monitoring efforts in

3 Southern California foraging sites: San Diego Bay (SDB), San Gabriel River (SGR), and Seal Beach National Wildlife Refuge (SBNWR; Fig. 1). SGR and SBNWR are separated by less than 5 km, whereas SDB is 152 km south of SGR/SBNWR. The northern part of SDB has high levels of shipping and recreational boating activity, and the southern portion where turtles were captured is an ecological reserve. In SGR, we conducted sampling within the river, approximately 2.5 km inland and adjacent to 2 active power plant systems (Crear et al. 2016). At the SBNWR, the capture site was a dredged saltwater pond, which the turtles accessed through dredged tide channels within the Anaheim Bay estuary (Crear et al. 2016). Acoustic tagging has demonstrated that individuals move between SGR and SBNWR seasonally (Crear et al. 2016); therefore, individuals from SGR and SBNWR were grouped together for comparison with individuals from the San Diego study site. Only 1 green turtle to date has been documented to move between SGR/SBNWR and SDB (National Oceanic and Atmospheric Administration [NOAA], unpubl. data, 2018). Water temperatures within the SBNWR ponds vary seasonally owing to the shallow shoals (Jirik and Lowe 2012). All 3 capture sites are in the same climatic region, so we used SDB water temperatures as a general proxy for seasonality as well as water temperature fluctuation in Southern California over the study period. We extracted water temperature data from the NOAA National Data Buoy Center (Buoy 9410170; San Diego Bay; 2.3 m below mean lower low water level).

Sampling Methods. — Blood was collected from sea turtles as described in Allen et al. (2015) and Crear et al. (2016). Briefly, we captured turtles via entanglement netting year round from 2013 to 2016, as part of long-term monitoring of the foraging aggregations of green turtles in Southern California conducted by NOAA's Southwest Fisheries Science Center (SWFSC) according to methodologies permitted under National Marine Fisheries Service Permit 1591. For each turtle captured, we obtained morphometrics as in Eguchi et al. (2012), including measurements of size. We measured straight carapace length (SCL; from the nuchal notch of the carapace to the rear scutes) and straight carapace width (SCW; the largest lateral distance between edges of the carapace) with a forester's caliper. Curved carapace length (CCL) and curved carapace width (CCW) were also directly measured or estimated from SCL and SCW values, respectively, using the equation described by Eguchi et al. (2012). We measured mass with a 500-kg electronic balance, and quantified tail length from the trailing edge of the plastron to the tip of the tail with a measuring tape. We calculated body condition index (BCI) as mass (kg)/SCL^3 (cm) $\times$ 10,000 (Bjorndal et al. 2000; Labrada-Martagon et al. 2010; Stacy et al. 2018). Each turtle was assigned a unique identifier and tagged in 1 front flipper with an Iconel tag (Style 681, National Band and Tag Company, Newport, KY), followed by collection of blood samples for hematological and hormone analyses as described in Allen et al. (2015). In an effort to only make high confidence estimates for sex, turtles with tail length $>$ 30 cm and SCL $>$ 90.0 cm were classified as putative adult males, and those with short tails and SCL $>$ 90.0 cm were classified as putative adult females (Eguchi et al. 2010). We also used published data from testosterone enzyme-linked immunosorbent assays as described in Allen et al. (2015) to classify the sex of immature turtles (SCL $<$ 90.0 cm) when available. Immature turtles for which testosterone data were not available were classified as sex "unknown."

Blood Collection and Handling. — We used 3.81-cm $\times$ 0.82-mm (21-gauge) vacutainer needles (Becton, Dickinson and Company, Franklin Lakes, NJ) to collect blood samples from the dorsal cervical sinus (maximum volume $<$ 3 ml/kg; Owens and Ruiz 1980; Figueroa et al. 1992) in 6- or 10-ml sodium heparin vacutainer blood collection tubes (Becton, Dickinson and Company). Blood samples were collected within 1 hr of capture to minimize the influence of capture stress on blood chemistry parameters (Gregory et al. 1996). Samples were placed in a cooler with ice packs separated to avoid hemolysis and to keep cool until transport to the laboratory. Whole blood samples were delivered within 12 hrs either to SeaWorld's Animal Health Laboratory (San Diego, California) for immediate analysis, or brought to SWFSC (La Jolla, California) where they were subsampled for hematocrit determination followed by centrifugation (3000 $\times$ g for 10 min) to separate plasma, which was then aliquoted into 2-ml cryovials (Corning Inc., Corning, NY) and stored at –80°C until analysis for blood chemistry analysis at the SeaWorld laboratory at a later date. All samples were analyzed within 4 yrs.

Blood Chemistry Analysis. — Biochemistry panels were performed on plasma samples at SeaWorld (San Diego) for the following analytes: albumin, alanine aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride, cholesterol, creatine kinase, carbon dioxide, creatinine, globulin, glucose, iron, lactate dehydrogenase, phosphorus, potassium, sodium, total protein, triglycerides, and uric acid. The analytes were measured using an Olympus AU400E (Olympus, Center Valley, PA) automated blood analyzer. All reagents used were designed for and purchased from the distributor (Beckman Coulter, Inc., Diagnostics Division Headquarters, Brea, CA).

Statistical Analysis. — We calculated the medians, means, standard errors, and ranges (maximum–minimum) for the health parameters measured. Six adult turtles were captured twice during the duration of the study, over 30 d apart. Morphology parameters for the 6 recaptured individuals were averaged over the 2 events for estimations of descriptive statistics of morphometrics for our study aggregations. Because of sampling limitations, blood biochemical values were only measured at one of the capture events and hematocrit at the other capture event, so there are no repeat measures of blood parameters

for any individual turtle. Therefore, recaptures were treated independently for blood parameter analyses but were averaged for summarizing morphometric parameters. We compared blood analyte parameters from San Diego Bay measured in this study with previously reported blood analyte parameters for the San Diego Bay aggregation taken before the South Bay Power Plant closed (Komoroske et al. 2011), considering values that did not have overlapping 95% confidence intervals (calculated as mean $\pm$ 2 times the standard error) as statistically different.

We conducted 3 separate analyses of relationships of 1) sex, 2) site, and 3) body size, nutritional status, and seasonality with the blood analytes because of differences in size between sex classifications and across sites and multicollinearity. We evaluated each health parameter for differences based on sex (male, female, or unknown) using analysis of variance (ANOVA) and for differences based on capture site (SDB or SGR/SBNWR) using *t*-tests. Turtles in SGR/SBNWR were significantly smaller in body size (SCL) compared with those in SDB so we did not include SCL and site in the same model. We chose SCL as our metric for body size to include in our models of variance in blood parameters because it frequently used as a proxy for age class in sea turtles (Eguchi et al. 2012; Allen et al. 2015) and has been shown to have a strong relationship with age estimates from skeletochronology (Zug et al. 2002). We included BCI in our models as a metric of nutritional status (Hays 2000; Seminoff et al. 2003; Keller et al. 2005). We included 2- and 3-way interactions between SCL, BCI, and mean daily water temperature in our models, as it is possible for 2 or 3 of the variables to have a joint effect on blood parameters (e.g., a parameter may be low in individuals with smaller SCLs and low BCIs, but may remain at normal levels when an individual has a high SCL or BCI value). We graphically assessed estimates vs. residuals and QQ-normal plots to evaluate assumptions of homoscedasticity and normality, and found that our health parameter data met assumptions of normality and equality of variance, with the exception of creatinine. We centered and scaled (*z*-score normalization) all predictors (SCL, BCI, mean daily water temperature) prior to analysis. We used the R package *usdm* to calculate variance inflation factors (Naimi et al. 2014) and found no evidence of collinearity between SCL, BCI, and mean daily water temperature in San Diego Bay (variance inflation factors < 1.2). We used multiple linear regressions for each blood analyte parameter as a function of SCL used as a proxy for age, BCI as a metric of nutrition status, mean daily water temperature in San Diego Bay as an index of seasonality, and all 2-way and 3-way interactions of the 3 variables included. Finally, we used a polynomial regression to graphically represent the relationship between BCI and hematocrit. We used Pearson's correlation to test for associations between each pairing of blood analyte parameters. All analyses were conducted in R (v3.4.2; R Core Team 2016).

RESULTS

Thirty-nine unique green sea turtles, 19 from SGR/SBNWR (13 females, 1 male, and 5 unknowns) and 20 from SDB (10 females, 4 males, and 6 unknowns), were captured and sampled for regional sea turtle health assessment. Six turtles were captured and sampled twice (see "Methods"), resulting in a total sample size of 45. All turtles were ostensibly healthy upon capture, in that they were active, alert, ambulatory, and not visibly displaying any external wounds. Basic statistics for morphometric and blood analyte parameters are presented in Table 1. Comparisons of these data with previously published data from SDB before the San Diego power plant closed are presented in Table 2. Of the metrics measured in both studies, mean AST, BUN, and sodium values reported in this study were different (95% confidence intervals did not overlap) from those previously reported for the SDB population (Table 2; Komoroske et al. 2011).

We found significant differences based on sex (male, female, or unknown) in SCL ($F_{2,42} = 4.686$, $p = 0.0146$), CCL ($F_{2,42} = 3.77$, $p = 0.0312$), CCW ($F_{2,42} = 3.462$, $p = 0.0406$), mass ($F_{2,40} = 3.58$, $p = 0.0371$), chloride ($F_{2,36} = 5.158$, $p = 0.0107$), and globulin ($F_{2,36} = 4.319$, $p = 0.0208$). We found that green turtles captured in SGR/SBNWR were smaller than green turtles captured in SDB (SCL, $t_{40.48} = 6.32$, $p < 0.001$, Fig 2; SCW, $t_{37.44} = 4.51$, $p < 0.001$; CCL, $t_{40.91} = 6.39$, $p < 0.001$; CCW, $t_{42.85} = 5.67$, $p < 0.001$; mass, $t_{31.56} = 6.87$, $p < 0.001$). We also found differences between turtles captured in SDB and SGR/SBNWR in globulin ($t_{33.81} = 3.70$, $p < 0.001$), glucose ($t_{25.70} = -2.17$, $p = 0.0392$), and total protein ($t_{35.09} = 3.35$, $p = 0.002$).

Estimated parameters for the multiple regression models of each blood analyte parameter as a function of SCL, BCI, and mean daily water temperature are presented in Table 3. Calcium decreased with water temperature and increased with an interaction between SCL and water temperature ($\beta = 0.9151$, $p = 0.0361$); creatinine increased with an interaction between BCI and water temperature ($\beta = 0.02255$, $p = 0.0081$); hematocrit increased with BCI and decreased with SCL and an interaction between SCL and BCI ($\beta = -7.5406$, $p = 0.0162$); and triglycerides were increased with temperature (Table 3; Fig. 3). Finally, we discerned several correlations between blood analyte parameters, specifically between triglycerides and total protein ($R = 0.538$, $p < 0.001$; Fig. 4a), triglycerides and calcium ($R = 0.805$, $p < 001$; Fig. 4b), as well as phosphorous and sodium ($R = 0.571$, $p < 0.001$; Fig. 4c).

DISCUSSION

Assessment of blood analyte parameters contributes to our broader understanding of overall health of naturally occurring populations, especially as environmental and habitat conditions change due to human activities (e.g., climate change, pollution). Here, we report baseline health

Table 1. Basic statistics for morphometrics (body condition index [BCI], curved carapace length [CCL], curved carapace width [CCW], straight carapace length [SCL], straight carapace width [SCW], and mass) and blood analytes (albumin, alanine aminotransferase [AST], blood urea nitrogen [BUN], calcium, chloride, cholesterol, creatine kinase [CK], carbon dioxide [CO_2], creatinine, globulin, glucose, iron, lactate dehydrogenase [LD], phosphorus, potassium, sodium, total protein, triglycerides, and uric acid) for San Diego Bay (SDB) and San Gabriel River/Seal Beach National Wildlife Refuge (SGR/SBNWR). Sample size (n = 39 unique turtles sampled) varies slightly among parameters because of missing data.

Parameter	Site	n	Median	Mean	SE	Range
BCI (kg/cm^3 × 10,000)	SGR/SBNWR	19	1.32	1.31	0.00	1.10–1.58
	SDB	18	1.40	1.41	0.00	1.28–1.55
CCL (cm)	SGR/SBNWR	19	71.3	70.8	2.23	50.8–93.8
	SDB	20	98.5	94.0	3.79	61.6–118.3
CCW (cm)	SGR/SBNWR	19	68.4	67.0	2.26	48.2–93.3
	SDB	20	89.9	85.2	3.15	58.8–101.5
SCL (cm)	SGR/SBNWR	19	67.4	66.2	2.10	47.5–87.3
	SDB	20	93.3	88.1	3.63	55.9–109.3
SCW (cm)	SGR/SBNWR	19	53.2	52.5	1.38	39.3–65.0
	SDB	20	68.4	63.4	2.87	26.4–78.0
Mass (kg)	SGR/SBNWR	19	38.0	40.4	4.48	15.0–105
	SDB	18	122	107	10.9	33.0–176
Albumin (gm/dl)	SGR/SBNWR	19	1.80	1.80	0.10	0.80–2.70
	SDB	20	2.00	2.00	0.09	1.10–2.80
Albumin:globulin	SGR/SBNWR	19	0.60	0.58	0.03	0.27–0.82
	SDB	20	0.51	0.54	0.02	0.41–0.79
AST (U/l)	SGR/SBNWR	19	171	180	11.5	84.0–293
	SDB	20	186	182	11.9	107–313
BUN (mg/dl)	SGR/SBNWR	19	7.00	15.1	3.97	1.00–62.0
	SDB	20	13.0	16.8	3.05	2.00–57.0
Calcium (mg/dl)	SGR/SBNWR	19	8.80	8.60	0.39	5.80–13.0
	SDB	20	8.40	9.30	0.64	5.90–16.9
Chloride (mEq/l)	SGR/SBNWR	19	113	113	1.22	104–125
	SDB	20	113	112	1.77	97.0–125
Cholesterol (mg/dl)	SGR/SBNWR	19	187	208	25.9	89.0–606
	SDB	20	187	219	23.5	118–528
CK (U/l)	SGR/SBNWR	19	993	955	86.8	139–1725
	SDB	20	850	913	91.8	310–1864
CO_2 (mEq/l)	SGR/SBNWR	19	25.0	26.8	1.70	18.0–44.0
	SDB	20	21.5	24.4	2.24	6.00–46.0
Creatinine (mg/dl)	SGR/SBNWR	19	0.10	0.10	0.001	0.00–0.10
	SDB	20	0.10	0.10	0.001	0.00–0.10
Globulin (g/dl)	SGR/SBNWR	19	3.00	3.10	0.11	2.20–3.90
	SDB	20	4.00	3.80	0.16	2.10–5.00
Glucose (mg/dl)	SGR/SBNWR	19	112	124	9.07	76.0–223
	SDB	20	101	102	4.28	65.0–142
Hematocrit (%)	SGR/SBNWR	6	34.8	34.7	1.35	30.0–38.0
	SDB	14	40.2	40.1	0.77	35.0–47.0
Iron (mcg/dl)	SGR/SBNWR	19	47.0	53.7	7.70	16.0–145
	SDB	20	28.0	43.9	7.10	0.00–131
LD (U/l)	SGR/SBNWR	19	164	180	15.2	96.0–311
	SDB	20	180	215	25.1	104–597
Phosphorous (mg/dl)	SGR/SBNWR	19	8.80	8.70	0.52	5.30–12.9
	SDB	20	8.60	8.70	0.32	5.30–10.9
Potassium (mEq/l)	SGR/SBNWR	19	4.30	4.60	0.20	3.60–7.00
	SDB	20	4.30	4.50	0.18	3.00–6.30
Sodium (mEq/l)	SGR/SBNWR	19	158	160	1.73	148–177
	SDB	20	158	159	1.75	148–175
Total protein (gm/dl)	SGR/SBNWR	19	4.90	4.90	0.18	3.80–6.60
	SDB	20	6.00	5.80	0.23	3.70–7.80
Triglycerides (mg/dl)	SGR/SBNWR	19	182	240	46.3	29.0–706
	SDB	20	261	409	110	55.0–2102
Uric acid (mg/dl)	SGR/SBNWR	19	1.10	1.30	0.16	0.60–3.30
	SDB	20	0.80	1.00	0.11	0.20–1.80

parameters for foraging aggregations of green turtles in highly urbanized settings and compare them with previously reported parameters in order to contribute to the long-term monitoring of anthropogenically influenced coastal ecosystems. Although samples taken at a single time point offer only a snapshot in time of individuals, observed changes in blood values from a monitored population over time can provide insight into external impacts that may affect overall health of the population.

This is the first reporting of blood analyte parameters from the SGR/SBNWR green turtle foraging aggregation, as well as the SDB aggregation after the power plant closed in 2010. Overall, the most recent blood analyte and hematocrit parameters are consistent with previously

Table 2. Blood analyte values from green turtles captured at San Diego Bay compared with values from a previous study in San Diego Bay (Komoroske et al. 2011) (see Table 1 for abbreviations).

	Present study		Komoroske et al. 2011	
Parameter	Mean	SE	Mean	SE
Albumin (gm/dl)	2.00	0.09	2.16	0.07
AST (U/l)	182[a]	11.9	153.3	6.74
BUN (mg/dl)	16.8[a]	3.97	22.38	1.59
Calcium (mg/dl)	9.30	0.39	8.24	0.26
Chloride (mEq/l)	112	1.77	111.5	2.77
Cholesterol (mg/dl)	219	23.5	228.6	23.63
CK (U/l)	913	91.8	1312	312.6
Globulin (gm/dl)	3.80	0.16	3.65	0.12
Glucose (mg/dl)	102	4.28	95.94	3.07
Hematocrit (%)	40.1	0.77	38.19	1.64
LD (U/l)	215	25.1	239.0	40.21
Phosphorous (mg/dl)	8.70	0.32	8.98	0.24
Potassium (mEq/l)	4.50	0.18	4.53	0.17
Sodium (mEq/l)	159[a]	1.75	155.0	0.89
Total protein (gm/dl)	5.80	0.23	5.81	0.18
Uric acid (mg/dl)	1.00	0.11	1.08	0.10

[a] Mean falls outside of the 95% confidence interval (mean ± 2 SE) reported in Komoroske et al. (2011).

reported parameters for this and other green turtle populations (Bolten and Bjorndal 1992; Aguirre and Balazs 2000). However, there were 13 hypernatremic (sodium levels > 160 mEq/l) turtles as well as 1 individual that was both hyperglycemic and hypernatremic (sodium level > 160 mEq/l; glucose level > 200 mg/dl). Sodium and other electrolytes levels can vary with disease, hydration, reproductive state, activity, sex, season, and anorexia or feeding state (Heatley and Russell 2019). Glucose can vary with physiological changes (e.g., stress), metabolic rate (e.g., level of food consumption), environmental changes (e.g., seasons), and diet (Heatley and Russell 2019). Hyperglycemia is most often associated with stress, but can also be present immediately after feeding following long periods of fasting (Stacy and Innis 2017). The mean values for a few blood analyte parameters of the SDB aggregation increased (AST and sodium) or decreased (BUN) since the previous SDB study (Komoroske et al. 2011). AST values can be affected by liver disease and can be elevated immediately after feeding or as a result of muscle damage (Heatley and Russell 2019). BUN can fluctuate in response to hydration, renal disease, liver disease, postprandial effects, or anorexia/fasting (Heatley and Russell 2019). However, the majority of blood analyte parameters measured in SDB turtles did

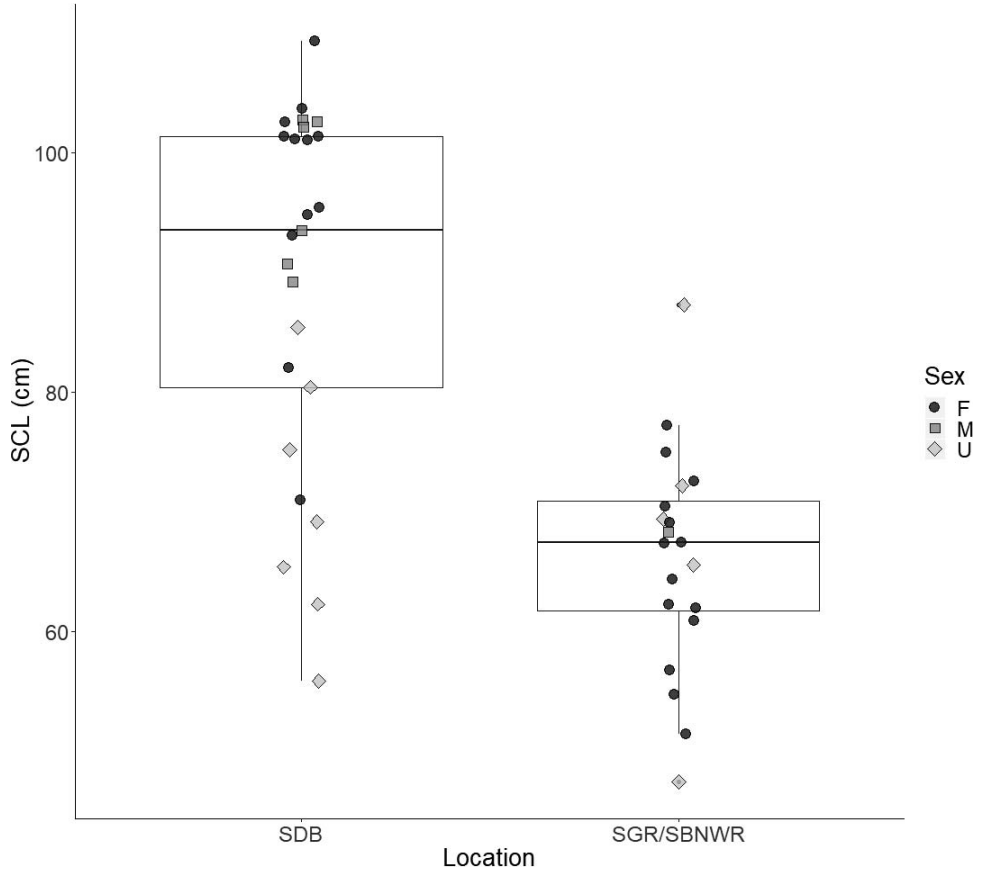

Figure 2. Turtles captured in San Diego Bay (SDB) were significantly larger than those captured in San Gabriel River/Seal Beach National Wildlife Refuge (SGR/SBNWR) ($t_{40.48} = 6.32$, $p < 0.001$; *t*-test). Boxplots represent the first quartile, median, and third quartile, ± 1.5 times the interquartile range of green turtle body size (SCL), and outliers for individuals captured in SGR/SBNWR and SDB. Points represent individual turtles and the shape represents sex of the individual (female [F], male [M], or unknown [U]).

Table 3. Parameter estimates from multiple regression models for each blood analyte in samples collected from 2 green sea turtle foraging aggregations (San Gabriel River/Seal Beach National Wildlife Refuge and San Diego Bay) in Southern California. Predictors used were SCL, BCI (centered and scaled), and San Diego Bay water temperatures (centered and scaled) as an index of seasonality, and their interactions. Statistically significant parameter estimates for interaction terms are noted in the text. Adjusted R^2 values and model p-values are also shown. Bold parameter estimates and R^2 values are significant at * $p < 0.05$ and at ** $p < 0.01$ (see Table 1 for abbreviations).

Blood analyte	n	SCL	BCI	Temperature	Adjusted R^2	Model p-value
Albumin	39	0.057	0.136	0.139	0.205	0.052
AST	39	−15.254	8.568	7.628	−0.071	0.706
BUN	39	−2.2894	4.144	−2.257	−0.108	0.827
Calcium	39	0.339	−0.154	**1.068***	0.143	0.114
Chloride	39	−2.235	0.737	0.947	0.059	0.275
Cholesterol	39	12.715	6.044	5.674	−0.170	0.966
CK	39	−82.018	83.172	−133.477	−0.134	0.898
CO_2	39	0.619	−0.690	−2.498	−0.002	0.457
Creatinine	39	0.001	−0.008	0.009	0.0928	0.198
Globulin	39	0.260	0.159	0.173	0.096	0.191
Glucose	39	−11.814	2.324	5.199	0.017	0.396
Hematocrit	20	**−2.621***	**2.842***	−2.149	**0.490***	0.042
Iron	39	−6.139	4.996	11.886	0.030	0.357
LD	39	−5.563	5.408	20.743	−0.055	0.645
Phosphorus	39	−0.558	0.069	0.023	−0.006	0.473
Potassium	39	−0.174	−0.132	0.262	0.076	0.234
Sodium	39	−2.853	1.463	2.259	0.077	0.232
Total protein	39	0.317	0.295	0.311	0.115	0.156
Triglycerides	39	50.000	−8.725	**140.731***	0.0722	0.243
Uric acid	39	−0.176	−0.175	0.008	0.012	0.412

not show differences between pre– and post–power plant closure. Prior to the power plant closure, it was unknown how the closure of the power plant would affect the behavior and physiology of the green turtles foraging in San Diego Bay, and our data suggests there have been few physiological impacts of the power plant closure.

We found significant differences in morphometrics based on sex. Of the blood parameters measured we found sex-based differences in globulin and chloride, which differs from sex-based differences in uric acid and cholesterol found in a previous study (Bolten and Bjorndal 1992). Female sea turtles that have active vitellogenesis (yolk formation during egg development) or have just laid eggs will have elevated globulin levels (Heatley and Russell 2019). While we found few sex-based differences in blood parameters, this may be a result of sampling a mix of adults and sexually immature individuals. It is also possible that length of time spent in the foraging ground and reproductive status contribute to variation in blood parameters. Deem et al. (2009) found a significant difference in blood parameters between nesting and foraging turtles. Some individuals in this study may have nested or been preparing to nest, but we did not assess reproductive state in this study. Very few of the juvenile turtles captured were males, in agreement with other recent studies of female-biased green turtle foraging aggregations at this and other locations (Allen et al. 2015; Jensen et al. 2018). However, the sexes of some juveniles are part of a long-term hormone study and have not yet been analyzed, so determining the sex of these "unknown" turtles could alter our findings and warrants further study. We also found significant differences in morphometric values between the sampling sites but few differences in blood parameters between these locations. This seems reasonable as these foraging aggregations live in similar environments in the same climatic region. There is still an active power plant in the San Gabriel River (Crear et al. 2016). Both the comparison of the pre– and post–power plant closure blood analytes of the San Diego Bay aggregation and the comparison between sites indicate minimal immediate impacts of nearby power plants on blood analyte parameters in foraging green turtles.

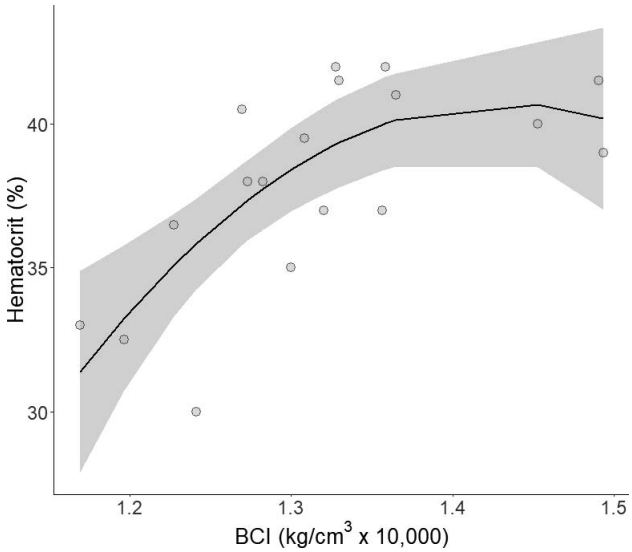

Figure 3. Hematocrit was positively affected by body condition index (BCI). Each point represents an individual turtle. Line represents polynomial regression model fit with 95% confidence intervals shaded in grey (adjusted $R^2 = 0.485$).

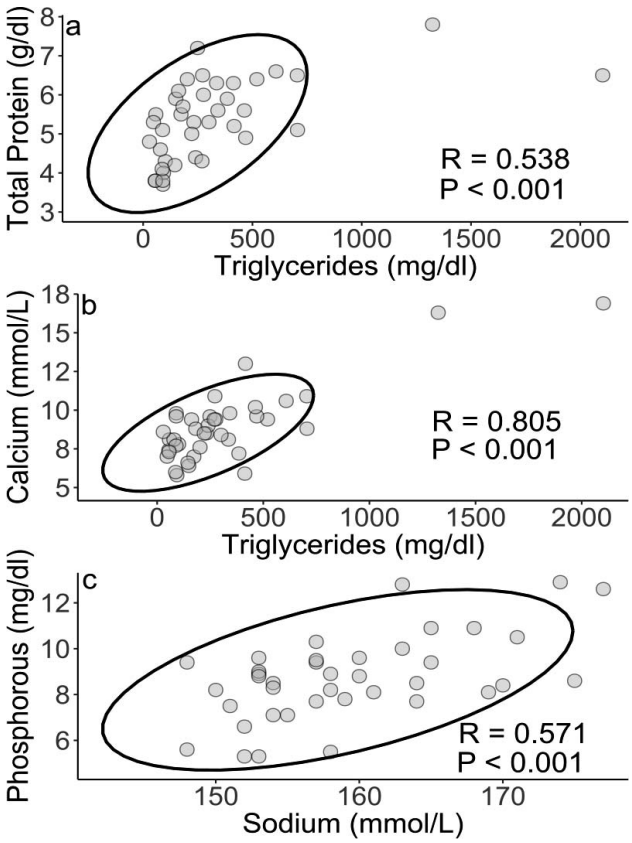

Figure 4. Several blood analytes were significantly correlated in Southern California green turtle foraging aggregations. Each point represents one turtle. Ellipses are 95% confidence ellipses calculated assuming a multivariate *t* distribution. (a) Triglycerides and total protein are significantly correlated. (b) Triglycerides and calcium are significantly correlated. (c) Sodium and phosphorous are significantly correlated.

Although seasonality is known to affect physiological processes and biochemical analyte parameters in ectotherms (Laube et al. 2016; Hofmeyr et al. 2017), we found an effect of water temperature as an index of seasonality in only 2 of the blood parameters measured (calcium and triglycerides). Both foraging populations in this study were sampled in geographically protected estuarine warm water areas that can have lowered levels of temperature fluctuation relative to coastal waters (Delgadillo-Hinojosa et al. 2008; Crear et al. 2016), and the SGR/SBNWR water temperatures are likely steadied by the nearby power plants. Additionally, the years during which sampling took place had unusually high water temperatures starting with the "warm water blob" in late 2013 through early 2014, and ending in 2 yrs of El Niño during 2014–2016 (Peterson et al. 2015; Jacox et al. 2016). Thus, whereas the parameters revealed by this study should provide robust reference parameters for these aggregations generally, future studies that include sampling during normal years may need to account for this difference in interpreting blood analyte parameters.

Interestingly, body condition positively affected hematocrit values, but turtle SCL alone, which is correlated with age, negatively affected hematocrit in contrast to results from Stacy et al. (2018) that revealed a significant correlation between SCL and hematocrit in immature loggerhead turtles (*Caretta caretta*). We found that an interaction between SCL and BCI has a negative effect on hematocrit, indicating that turtles with large SCLs and high BCIs still tended to have lower hematocrit values. Higher hematocrit values are expected in older turtles (with larger SCLs) because they are required for size-dependent increases in diving behaviors in leatherbacks (*Dermochelys coriacea*) and loggerhead turtles (Stamper et al. 2005; Perrault et al. 2016). Previous studies have also found higher total protein, albumin, lactate dehydrogenase, iron, and cholesterol, and lower chloride and phosphorous values in larger green turtles (Hasbún et al. 1998). In this study, we did not find significant effects of size or nutritional status (measured by BCI) on the blood parameters measured, except for hematocrit. We found that an interaction between size and mean daily temperature had a positive effect on calcium, indicating that calcium levels tended to be higher in larger individuals sampled during warmer periods. Seasonal changes in calcium and phosphorus during vitellogenesis in females have been reported in most reptile species (Heatley and Russell 2019), and our population is heavily female biased (Allen et al. 2016). Lastly, we found that an interaction between BCI and mean daily water temperature positively affected creatinine, indicating that creatinine levels are high in individuals with high BCI during warm periods. The adjusted R^2 values for our multiple regression models for all blood analytes except hematocrit explained a small proportion of the variation in the blood parameter modeled ($< 50\%$) and were statistically insignificant. This suggests that there may be other factors not measured in our study that influence variation in blood biochemistry in Southern California foraging aggregations of green turtles. Discrepancies between our findings and previous studies may be because of our small sample size, temporal differences, the highly omnivorous diets of southern California green turtles (Lemons et al. 2011), or spatial differences. Triglycerides were weakly correlated with total protein and calcium. Both triglycerides and total protein have been shown to decrease throughout nesting seasons and reflect nutritional status in green turtles and leatherback turtles, respectively (Hamann et al. 2002; Honarvar et al. 2011; Perrault et al. 2014). When diet is not known, triglyceride levels are more reflective of reproductive status than nutritional status (Heatley and Russell 2019). Although differences in calcium levels between captive and wild green turtles have been reported (Stringer et al. 2010), calcium levels as a single metric does not reveal information about a population's health.

The method of capture of wild animals will inherently cause a variable stress-induced response resulting in elevated glucose, electrolytes, and other physiologic changes not measured in the current study. Analyte ranges for species have been established and

interpretation of how the blood was obtained must be viewed with circumspection. Blood biochemistry can be affected by seasonal variation, reproductive state, life stage, diet preference, activity, restraint, and quality of the sample obtained (Heatley and Russell 2019). However, hematological and blood chemistry analysis is a necessary piece of wildlife health assessment and evaluating individuals within a population to determine overall health status will better characterize the sex, size of animals, reproductive state, and foraging status of sea turtles occupying these urban marine ecosystems. Despite limitations of sample size, diversity of age class, and reproductive state, this study contributes to our knowledge of what clinical blood panels of wild foraging populations look like and bolsters ongoing monitoring of this population to assess the impacts of the power plant closure, other anthropogenic impacts, and natural environmental conditions. In particular, we found that the measured health parameters of green turtles in Southern California were similar to other wild foraging populations, including those in less urbanized habitats (Bahamas, Bolten and Bjorndal 1992; Hawaii, Aguirre and Balazs 2000). Body condition and other external metrics for the turtles captured seemed normal (Tristan and Norton 2017), and there were few correlations with BCI. All but 3 of the health parameters we report are consistent with previously reported parameters for the SDB aggregation (Komoroske et al. 2011). Detected differences between groups have minimal clinical significance, suggesting that overall the health status of this population has not changed. However, we stress that further analysis of other metrics such as contaminant accumulation, stress hormone concentration, biotoxins, necropsies of the increasing number of green turtle strandings in the area, and linking to reproductive success are needed to provide further insight into the health, survival, and reproductive potential of animals that rely on urbanized areas for critical foraging habitats. These values provide key insight of baseline parameters in the wild population to compare with those of live-stranded sea turtles, and will be valuable references for monitoring of the SGR/SBNWR and SDB foraging aggregations as their habitat quality changes in the future (e.g., power plant closing, climate change). Blood biochemistry and other tools can be used to detect changes in health status of long-lived species such as sea turtles and it is important to continue monitoring health parameters, which can be informative for management decisions.

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