

1 Full title: Hematology, biochemistry, and toxicology of wild hawksbill turtles (*Eretmochelys*  
2 *imbricata*) nesting in mangrove estuaries in the eastern Pacific Ocean

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4 Short title: Blood values and toxicology of EP hawksbills

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6 Aubrey M. Tauer<sup>1</sup>, Michael J. Liles<sup>2,3,4</sup>, Sofía Chavarría<sup>2,3,5</sup>, Melissa Valle<sup>2,3,5</sup>, Sada Amaya<sup>5</sup>,

7 Gabriela Quijada<sup>5</sup>, Oscar Meléndez<sup>5</sup>, Stanley Rodríguez<sup>6</sup>, Eric F. Lock<sup>7</sup>, Ana V. Henríquez<sup>2,3</sup>,

8 Alexander R. Gaos<sup>3,9,10</sup> and Jeffrey A. Seminoff<sup>8</sup>.

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10 <sup>1</sup>Cūra Earth, Minneapolis, MN, USA;

11 <sup>2</sup>ProCosta, San Salvador, El Salvador

12 <sup>3</sup>Eastern Pacific Hawksbill Initiative, San Diego, CA, USA;

13 <sup>4</sup>Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, USA

14 <sup>5</sup>Departamento de Medicina Veterinaria, Universidad de El Salvador, San Salvador, El Salvador;

15 <sup>6</sup>Centro de Investigación y Desarrollo en Salud, Universidad de El Salvador, San Salvador, El  
16 Salvador;

17 <sup>7</sup>Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN,  
18 USA;

19 <sup>8</sup>National Oceanic and Atmospheric Administration – National Marine Fisheries Service,  
20 Southwest Fisheries Science Center, La Jolla, CA, USA

21 <sup>9</sup>Biology Department, San Diego State University, San Diego, CA, USA

22 <sup>10</sup>Graduate Group in Ecology, University of California at Davis, Davis, CA, USA

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30 **Abstract**

31 Sea turtles are a keystone species and are highly sensitive to changes in their environment,  
32 making them excellent environmental indicators. In light of environmental and climate changes,  
33 species are increasingly threatened by pollution, changes in ocean health, habitat alteration, and  
34 plastic ingestion. There may be additional health related threats and understanding these threats is  
35 key in directing future management and conservation efforts, particularly for severely reduced  
36 sea turtle populations. Hawksbill turtles (*Eretmochelys imbricata*) are critically endangered, with  
37 those in the eastern Pacific Ocean (Mexico–Peru) considered one of the most threatened sea turtle  
38 populations in the world. This study establishes baseline health parameters in hematology and  
39 blood biochemistry as well as tested for heavy metals and persistent organic pollutants in eastern  
40 Pacific hawksbills at a primary nesting colony located in a mangrove estuary. Whereas  
41 hematology and biochemistry results are consistent with healthy populations of other species of  
42 sea turtles, we identified differences in packed cell volume, heterophils and lymphocyte counts,  
43 and glucose when comparing our data to other adult hawksbill analysis (1), (2), (3). Our analysis  
44 of heavy metal contamination revealed a mean blood level of 0.245 ppm of arsenic, 0.045 ppm of  
45 lead, and 0.008 ppm of mercury. Blood levels of persistent organic pollutants were below the  
46 laboratory detection limit for all turtles. Our results suggest that differences in the feeding  
47 ecology of eastern Pacific hawksbills in mangrove estuaries may make them less likely to  
48 accumulate persistent organic pollutants and heavy metals in their blood. These baseline data on  
49 blood values in hawksbills nesting within a mangrove estuary in the eastern Pacific offer  
50 important guidance for health assessments of the species in the wild and in clinical rehabilitation  
51 facilities, and underscore the importance of preventing contamination from point and non-point  
52 sources in mangrove estuaries, which represent primary habitat to hawksbills and myriad other  
53 marine species in the eastern Pacific Ocean.



## 55 **Introduction**

56 Disease can cause declines in wildlife populations, especially those that are already threatened or  
57 vulnerable (4) (5) (6) (7). Baseline hematology and biochemistry blood parameters are useful  
58 indicators for the assessment of the health status of wild nesting sea turtle populations (8) and are  
59 especially helpful in clinical rehabilitation facilities (9). However, reference ranges for  
60 hematology and blood biochemistry are not widely available, with many reported values derived  
61 from captive animals that may not be representative of wild individuals. Additionally, data from  
62 one population of a species often are used as references for other populations, despite potential  
63 within-species variation (10) (11) (12) (13).

64 Hawksbill turtles (*Eretmochelys imbricata*) exemplify a species whose life history may  
65 vary widely among populations in distinct ocean basins (14) (15). In the Atlantic and Indo-  
66 Pacific, adult hawksbills primarily inhabit coral reef ecosystems (16) (17) (18) and can embark  
67 on long-distance (>2,000 km), offshore migrations between nesting and foraging areas (e.g., (19)  
68 (20)). Hawksbills in the eastern Pacific, however, often associate with mangrove ecosystems (21)  
69 (14) (22) (15) and undertake particularly short (<300 km) and neritic (<5 km) post-nesting  
70 migrations (23) (24). The marked difference in life history among hawksbills in these ocean  
71 basins could greatly influence general health parameters, which are largely unknown for adult  
72 hawksbills (3) and which have never been analyzed for individuals inhabiting mangrove  
73 estuaries. The availability of reference ranges is paramount for different populations of the same  
74 species and even subspecies, as values may even vary amongst a small population depending on  
75 diet and ecological variables (25).

76 Hawksbills are critically endangered globally according to the International Union for the  
77 Conservation of Nature's (IUCN) Red List (26) and the population in the eastern Pacific is  
78 among the most endangered Regional Management Units (27) for sea turtles worldwide (28).

79 Fewer than 700 adult female hawksbills are estimated to remain in the entire eastern Pacific  
80 Ocean (29) (15), where >80% of these individuals nest on beaches in mangrove estuaries of El  
81 Salvador and Nicaragua (30) (31) (15). These same mangrove ecosystems also provide important  
82 developmental habitat for juvenile and sub-adult hawksbills (14) (32). Known threats to this  
83 species in the region include incidental capture in coastal fisheries, human consumption of eggs,  
84 and alteration of nesting habitat (29) (31). An additional, albeit understudied potential threat to  
85 hawksbills inhabiting mangrove estuaries, is contamination by chemicals used in aquacultural  
86 and agricultural operations, including persistent pesticide residues from shrimp ponds (33) and  
87 toxic compounds discharged by surrounding rivers (34). These contaminants have been  
88 documented as negatively influencing myriad species, including estuarine fish species (35)  
89 mollusks (36) and marine turtles (37). If these contaminants are present in mangrove estuaries,  
90 reliance on such habitats could have direct impacts on health of hawksbills.

91 In this study, we measured blood biochemistry, hematology, and toxicological parameters  
92 in wild adult female hawksbills nesting in the Bahía de Jiquilisco mangrove estuary complex in  
93 El Salvador to establish baseline health data for one of the most important hawksbill nesting  
94 areas in the eastern Pacific. This information will establish a baseline for these parameters and  
95 aid in long-term evaluation of the health status of this severely depleted population and serve to  
96 guide future management and conservation efforts, as well as to facilitate comparisons among  
97 hawksbill populations in other oceanic basins.

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## 100 **Materials and Methods**

### 101 *Study site*

102 Bahía de Jiquilisco (13°13'N, 88°32'W) is located in the Department of Usulután on the south-  
103 central coast of El Salvador (Fig. 1), and is a National Conservation Area, RAMSAR wetland,  
104 and UNESCO Biosphere Reserve. It contains the largest mangrove forest in El Salvador (635  
105 km<sup>2</sup>), and includes numerous islands, channels, and estuaries, with moderate development at  
106 some nesting beaches (31). Bahía de Jiquilisco has 42.1 km of hawksbill nesting habitat that  
107 includes eight discernable fine grained sand beaches with fragmented second growth coastal  
108 forest and fruit tree plantations adjacent to the high water line (15) which host ~40% of hawksbill  
109 nesting activity in the eastern Pacific (29) (31) (38).

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111 Fig. 1. Locations of hawksbill nesting beaches with patrolled shoreline (black lines) at Bahía de  
112 Jiquilisco, El Salvador, 2013–2014.

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### 114 *Beach Monitoring and Turtle Measurements*

115 Hawksbill nesting occurs primarily between April and October, with a peak in June–July. We  
116 conducted beach patrols from 1 April to 15 October 2013–2014 at Bahía de Jiquilisco, where  
117 project personnel and an extensive network of >100 trained local egg collectors monitored  
118 nesting habitat from 18:00 to 06:00 daily by foot and boat in search of female hawksbills. We  
119 identified turtles by Inconel tags (Style 681, National Brand & Tag, Newport, KY, USA) located  
120 on the second proximal scale of both front flippers and internal passive integrated transponders  
121 (PIT tags; Biomark, Boise, ID, USA) in the right front flipper; Inconel and PIT tags were either  
122 present from application during previous tagging seasons or were applied after egg laying was

123 completed (15). For each female hawksbill encountered, we measured curved carapace length  
124 (nuchal notch to posterior-most tip of marginal scutes; CCL) and in 2013 we performed a  
125 complete visual and physical examination, noting all epi-biota on the turtle and body condition.

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### 127 *Sample Collection and Analyses*

128 We collected up to 12 ml of blood from the dorsal cervical sinus using a 10 ml syringe and 18  
129 gauge 1.5 inch needle and immediately transferred the sample into a red-top glass serum  
130 separator tube and sodium heparin vacutainer tubes. They were not refrigerated prior to  
131 processing. Blood smears were made in our field base camp from sodium heparin-treated blood  
132 and were fixed with 99% methanol on glass slides and air dried.

133 We initially processed the blood in the field within 6–8 hours of blood collection. Packed  
134 cell volumes were performed using a tabletop centrifuge and whole blood in sodium heparin  
135 tubes was transferred to 1 ml cryotubes and frozen in liquid nitrogen for heavy metal analysis.  
136 The remaining blood was spun for 10 minutes at 2000 RPMs and the serum separated and frozen  
137 in cryotubes in liquid nitrogen in the field, which were subsequently stored in  $-20^{\circ}$  C freezers at  
138 the University of El Salvador. Samples collected in 2013 were shipped in dry ice to the United  
139 States for hematology, serum biochemistry, heavy metal, and toxicology analyses, whereas in  
140 2014, plasma biochemistry analyses were conducted at Centro Scan (San Salvador, El Salvador).  
141 The results were pooled for determining biochemistry reference ranges after determining that  
142 there was no statistical difference between the two sample sets.

143 For hematology, blood films were stained at the Minnesota Zoo with DipQuick stain  
144 (Jorgenson Laboratories, Loveland, CO, USA) for manual differential accounts of circulating  
145 white blood cells and for hemo-parasite identification. Total white blood cell counts were  
146 estimated. Samples for serum biochemistry were shipped on dry ice for processing at Marshfield



147 Laboratories (Marshfield, WI, USA). The biochemical panel included alanine aminotransferase  
148 (ALT), aspartate aminotransferase (AST), alkaline phosphatase, cholesterol, CO<sub>2</sub>, creatine kinase  
149 (CK), glucose, lactate dehydrogenase (LDH), calcium, phosphorous, potassium, sodium,  
150 chloride, bicarbonate, total protein, anion gap and uric acid (UA). Reference intervals for  
151 biochemistry and hematology variables were computed using the package `referenceIntervals` for  
152 R (39) Parametric 95% reference intervals were computed, and the one-sample Kolmogorov-  
153 Smirnov test (40) was used to assess the distributional assumption. For variables with a  
154 Kolmogorov-Smirnov p-value less than 0.01, a non-parametric 95% reference interval was  
155 determined instead, with endpoints given by the 0.025 and 0.975 sample quantiles of the  
156 observed data.

157 Blood samples were screened at the California Animal Health and Food Safety  
158 Laboratory (San Bernadino, CA, USA) for heavy metals (arsenic [detection limit = 0.010 ppm],  
159 lead [0.050], and mercury [0.010]) and persistent organic pollutants (POP), including  
160 organochlorine insecticides (aldrin [0.010], alpha-BHC [0.010], gamma-chlordane [0.010],  
161 technical chlordane [0.050], pp-DDE [0.020], pp-DDD [0.020], pp-DDT [0.020], dicofol [0.020],  
162 op-DDE [0.020], op-DDD [0.020], op-DDT [0.020], dieldrin [0.010], endosulfan I [0.010],  
163 endosulfan II [0.010], endrin [0.010], HCB [0.010], heptachlor [0.010], heptachlor epoxide  
164 [0.010], lindane [0.010], methoxychlor [0.010]), mirex [0.010], toxaphene [0.400] and  
165 polychlorinated biphenyl (Arochlor 1221, 1232, 1242, 1248, 1254, 1260, 1262 [0.200 and  
166 0.400]). Mean toxicity levels were determined, with 95% confidence intervals, for arsenic, lead,  
167 and mercury. If observations were missing below the limit of detection, the mean and standard  
168 deviation were inferred via maximum likelihood under the assumption that the data have a log-  
169 normal distribution that is left-censored below the limit of detection using the package `censeReg`  
170 for R (41). All data and analysis is publicly available as an annotated reproducible R code file at

171 <https://doi.org/10.6084/m9.figshare.5702818> and

172 <https://doi.org/10.6084/m9.figshare.5702779>.

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## 174 **Results**

175 We encountered and examined 66 nesting hawksbills at Bahía de Jiquilisco in 2013–2014, which  
176 had a mean carapace length of 84.9 cm (SD 5.8, range = 71.0–96.6) and appeared in good general  
177 health. Physical exam findings in 2013 included two turtles that were covered in approximately  
178 5% of epi-biotic growth; nearly all other individuals were less than 1%. Six individuals exhibited  
179 carapace damage, including missing scutes, although all appeared to have healed from the  
180 injuries. One individual had a fairly large deformity of her distal carapace, but was mobile, in  
181 good body condition, and did not have difficulty depositing eggs. Additionally, one individual  
182 had a small tumor on the right rear flipper, but logistical limitations prevented biopsy collection.  
183 Hematologic values are presented in Table 1. No hemo-parasites were observed for the 28  
184 hawksbills evaluated in 2013. Table 2 provides the serum biochemistry reference ranges for  
185 blood collected in plain serum separator tubes in 2013 and blood plasma from sodium heparin  
186 tubes in 2014, including liver enzymes, total protein, electrolytes, and uric acid.

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200 Table 1. Hematology reference intervals for wild hawksbill turtles (*Eretmochelys imbricata*)  
 201 nesting at Bahía de Jiquilisco, El Salvador, 2013 (n = 28).  
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| Parameter <sup>a</sup>                                 | 95% reference interval |          | KS p-value <sup>b</sup> |
|--|------------------------|----------|-------------------------|
|  | Low                    | High     |                         |
| PCV (%)  | 23.0                   | 33.6     | 0.7130                  |
| WBC ( $\times 10^3/\mu\text{L}$ )                      | 1228.2                 | 10,871.8 | 0.0528                  |
| Heterophils (%)  | 43.4                   | 93.6     | 0.3306                  |
| Heterophils ( $\times 10^3/\mu\text{L}$ )              | 0                      | 8549.7   | 0.2120                  |
| Lymphocytes (%)  | 41.8                   | 42.6     | 0.8327                  |
| Lymphocytes ( $\times 10^3/\mu\text{L}$ )              | 87.5                   | 2517.7   | 0.8282                  |
| Monocytes (%)  | 0.3                    | 11.8     | 0.1358                  |
| Monocytes ( $\times 10^3/\mu\text{L}$ )                | 0                      | 748.3    | 0.4461                  |
| Basophils (%)  | 0                      | 7.1      | 0.5905                  |
| Basophils ( $\times 10^3/\mu\text{L}$ )                | 0                      | 398.4    | 0.8808                  |
| Eosinophils (%) <sup>c</sup>                           | 0                      | 5        | <0.0001                 |
| Eosinophils ( $\times 10^3/\mu\text{L}$ ) <sup>c</sup> | 0                      | 290      | <0.0001                 |

203 <sup>a</sup>PCV, packed cell volume; WBC, white blood cells.

204 <sup>b</sup>KS p-value, Kolmogorov-Smirnoff p-value.

205 <sup>c</sup>Indicates non-parametric 95% reference interval.  
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229 Table 2. Serum chemistry reference intervals for wild hawksbill turtles (*Eretmochelys imbricata*)  
 230 nesting at Bahía de Jiquilisco, El Salvador, 2013–2014 (n = 66).  
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| Parameter <sup>a</sup> | n  | 95% reference interval |        | KS p-value <sup>b</sup> |
|------------------------|----|------------------------|--------|-------------------------|
|                        |    | Low                    | High   |                         |
| Glucose (mg/dL)        | 51 | 57.5                   | 142.0  | 0.1771                  |
| AST (U/L)              | 51 | 18.3                   | 74.3   | 0.8810                  |
| ALT (U/L)              | 33 | 16.3                   | 78.1   | 0.8429                  |
| ALP (U/L)              | 51 | 25.7                   | 93.6   | 0.4668                  |
| CK (U/L) <sup>c</sup>  | 51 | 121                    | 1296.2 | <0.0001                 |
| LDH (U/L) <sup>c</sup> | 51 | 135.6                  | 1645.7 | 0.0009                  |
| Cholesterol (mg/dL)    | 51 | 107.1                  | 366.8  | 0.8260                  |
| TP (g/dL)              | 51 | 2.6                    | 5.0    | 0.6850                  |
| Phosphorus (mg/dL)     | 18 | 3.4                    | 16.1   | 0.6876                  |
| Calcium (mg/dL)        | 36 | 1.0                    | 21.2   | 0.0379                  |
| Sodium (mmol/L)        | 51 | 140.7                  | 169.4  | 0.8217                  |
| Potassium (mmol/L)     | 18 | 3.7                    | 5.7    | 0.9910                  |
| Chloride (mmol/L)      | 51 | 96.9                   | 148.5  | 0.1106                  |
| Bicarbonate (mmol/L)   | 18 | 9.8                    | 33.5   | 0.9538                  |
| Uric Acid (mg/dL)      | 33 | 1.0                    | 1.8    | 0.1846                  |
| Anion Gap (mmol/L)     | 18 | 7.6                    | 36.8   | 0.2089                  |

232 <sup>a</sup>AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase;  
 233 CK, creatine kinase; LDH, lactate dehydrogenase; TP, total protein.

234 <sup>b</sup>KS p-value, Kolmogorov-Smirnoff p-value.

235 <sup>c</sup>Indicates non-parametric 95% reference interval.

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237 Levels of arsenic, lead, and mercury are presented in Table 3. Arsenic had the highest level, with  
 238 a mean of 0.245 ppm (95% confidence interval = (0.10, 0.39)). Arsenic was detectable in all of  
 239 the samples collected (n = 28). Lead and mercury had lower mean levels of 0.045 (95%  
 240 confidence interval = (0.038, 0.056)) and 0.008 (95% confidence interval = (0.004, 0.017))  
 241 respectively. Samples from all turtles tested for POPs (n = 28) were below the detectable limits.

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250 Table 3. Heavy metal blood values for wild hawksbill turtles (*Eretmochelys imbricata*) nesting at  
251 Bahía de Jiquilisco, El Salvador, 2013 (n = 28).

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| Parameter | Mean  | 95% CI |       |
|-----------|-------|--------|-------|
|           |       | Low    | High  |
| Arsenic   | 0.245 | 0.100  | 0.390 |
| Lead      | 0.045 | 0.038  | 0.056 |
| Mercury   | 0.008 | 0.004  | 0.017 |

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## 255 Discussion

256 Our results provide the first assessment of hematology, biochemistry, heavy metal, and persistent  
257 organic pollutant levels in the blood of wild hawksbills nesting in mangrove estuaries and  
258 establish baseline values for mature female hawksbills in these habitats in the eastern Pacific  
259 Ocean. The population sampled in this study was rated overall as healthy, as nesting hawksbills  
260 were in good body condition, had minimal epibiota, and generally had normal physical exam  
261 findings. While interpreting the parameters in this study, it is important to note that it is common  
262 that highly contaminated reptiles show no acute signs of health distress, thus our results should  
263 not be misinterpreted as confirming the species is healthy in the region.

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### 265 *Hematology and Biochemistry*

266 The hematological and biochemistry results are generally comparable to those of other species of  
267 sea turtles sampled with healthy populations (42) (43) (44) (45) including hawksbill nesting  
268 females at open-coast beaches in Brazil (1) and for hawksbill foraging aggregations at coral reefs  
269 in the eastern Pacific (Table 4). Some differences are notable in comparing values between  
270 studies, for example glucose in the (3) study is significantly higher than that of all other studies  
271 and the Packed Cell Volume is lower in our study than in (1). Notably only eight individuals  
272 were sampled in the (3) study and the sea turtles were caught in the open water and brought on to

273 the beach instead of testing nesting females. The authors speculate that handling stress induced a  
274 stress hyperglycemia. This study also is the only other adult wild hawksbill study to include a  
275 white blood cell differential count, which varies from ours in numbers of heterophils and  
276 lymphocytes. The other two studies, (2) and (1) have similar values for biochemistries to our  
277 study.

278 One female in our study had white blood cell count and heterophil count twice as high as  
279 the lowest WBC and heterophil count sampled, so occult illness in one or more individuals of our  
280 studied population may be possible (46). Biochemistry reference ranges were established (2) for  
281 juvenile hawksbills occupying a coral reef ecosystem off the Pacific coast of Colombia, with  
282 calcium, total protein, phosphorus, glucose values similar to our data, but with much wider  
283 ranges of LDH, AST, and cholesterol. Some differences were noted between several  
284 hematological and biochemistry values when compared to published data from juvenile  
285 hawksbills undergoing rehabilitation in the United Arab Emirates (47). For example, juvenile  
286 hawksbills had lower mean PCVs, lower total white blood cell counts, and higher AST, CK, and  
287 uric acid levels. Additionally, mean calcium, phosphorus, and total protein levels were lower in  
288 the rehabilitated animals when compared to our study sample. These differences may be due, at  
289 least in part, to the impaired health of animals in rehabilitation, as well as possible geographic  
290 variation in environmental variables or in the life-history characteristics of hawksbills in distinct  
291 oceanic regions. Variation in biochemistry ranges may reflect differences in physiological  
292 requirements between life stages (i.e. juvenile vs. adults) and/or behavior/habitats (nesting in  
293 mangrove estuaries vs foraging at coral reef ecosystems) of each studied hawksbill population  
294 (15).

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296 Table 4. Available blood values for hawksbill turtles.

| Parameter <sup>a</sup>      | Wrobel Goldberg et al. 2013 |      |                | Tobón-López & Amorocho Llanos 2014 |       |                | Muñoz-Pérez et al. 2017 |       |                | Tauer et al. this study |        |                |
|-----------------------------|-----------------------------|------|----------------|------------------------------------|-------|----------------|-------------------------|-------|----------------|-------------------------|--------|----------------|
|                             | Mean                        | SD   | n <sup>b</sup> | Mean                               | SD    | n <sup>c</sup> | Mean                    | SD    | n <sup>d</sup> | Mean                    | SD     | n <sup>e</sup> |
| <i>Hematology</i>           |                             |      |                |                                    |       |                |                         |       |                |                         |        |                |
| PCV (%)                     | 39.4                        | 2.9  | 41             | –                                  | –     | –              | –                       | –     | –              | 28.34                   | 7.71   | 28             |
| RBC (× 10 <sup>12</sup> /L) | –                           | –    | –              | –                                  | –     | –              | 0.35                    | 0.09  | 8              | –                       | –      | –              |
| WBC (× 10 <sup>9</sup> /L)  | –                           | –    | –              | –                                  | –     | –              | 5.31                    | 3.86  | 8              | –                       | –      | –              |
| Heterophils (%)             | –                           | –    | –              | –                                  | –     | –              | 32.3                    | 6.9   | 8              | 65.5                    | 12.81  | 28             |
| Lymphocytes (%)             | –                           | –    | –              | –                                  | –     | –              | 45.9                    | 6.1   | 8              | 22.21                   | 10.4   | 28             |
| Monocytes (%)               | –                           | –    | –              | –                                  | –     | –              | 3.6                     | 1.9   | 8              | 6.04                    | 2.92   | 28             |
| Basophils (%)               | –                           | –    | –              | –                                  | –     | –              | 0.1                     | 0.2   | 8              | 2.82                    | 2.20   | 28             |
| Eosinophils (%)             | –                           | –    | –              | –                                  | –     | –              | 18.5                    | 4.5   | 8              | 0.36                    | 0.99   | 28             |
| <i>Biochemistry</i>         |                             |      |                |                                    |       |                |                         |       |                |                         |        |                |
| Glucose (mg/dL)             | 98.6                        | 14.6 | 41             | 103.5                              | 16.6  | 11             | 1567.6                  | 180.2 | 7              | 99.71                   | 21.56  | 51             |
| AST (U/L)                   | 55.4                        | 7.1  | 41             | 132.6                              | 111.2 | 11             | 196                     | 54    | 8              | 46.33                   | 14.29  | 51             |
| ALT (U/L)                   | 6.6                         | 2.4  | 41             | –                                  | –     | –              | 38                      | 15    | 8              | 47.18                   | 15.75  | 33             |
| ALP (U/L)                   | 15.9                        | 3.7  | 41             | –                                  | –     | –              | 53                      | 26    | 8              | 56.63                   | 17.33  | 51             |
| LDH (U/L)                   | –                           | –    | –              | 136.5                              | 78.9  | 11             | –                       | –     | –              | 394.41                  | 288.12 | 51             |
| Cholesterol (mg/dL)         | 287                         | 42   | 41             | 84.5                               | 30.9  | 11             | –                       | –     | –              | 236.92                  | 66.24  | 51             |
| TP (g/dL)                   | 5.45                        | 0.63 | 41             | 2.5                                | 0.7   | 11             | 4.8                     | 0.7   | 8              | 3.79                    | 0.62   | 51             |
| Phosphorus (mg/dL)          | 11.3                        | 1.4  | 41             | 6.7                                | 2     | 11             | –                       | –     | –              | 9.76                    | 3.23   | 18             |
| Calcium (mg/dL)             | 11.6                        | 1.5  | 41             | 7.8                                | 1.8   | 11             | –                       | –     | –              | 11.09                   | 5.14   | 36             |
| Sodium (mmol/L)             | 139.6                       | 3.5  | 41             | –                                  | –     | –              | 157                     | 2     | 7              | 155.04                  | 7.33   | 51             |
| Potassium (mmol/L)          | 5.09                        | 0.76 | 41             | –                                  | –     | –              | 4.2                     | 0.4   | 7              | 4.68                    | 0.51   | 18             |
| Chloride (mmol/L)           | –                           | –    | –              | –                                  | –     | –              | –                       | –     | –              | 122.71                  | 13.14  | 51             |
| Biocarbonate (mmol/L)       | –                           | –    | –              | –                                  | –     | –              | –                       | –     | –              | 21.61                   | 6.05   | 18             |
| Uric Acid (mg/dL)           | 0.95                        | 0.17 | 41             | 3.7                                | 2.7   | 11             | –                       | –     | –              | 1.39                    | 0.20   | 33             |
| Anion Gap (mmol/L)          | –                           | –    | –              | –                                  | –     | –              | –                       | –     | –              | 22.22                   | 7.45   | 18             |

297 <sup>a</sup>PCV, packed cell volume; RBC, red blood cells; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline  
 298 phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase; TP, total protein.

299 <sup>b</sup>Hawksbills nesting on open-coast beaches in Brazil, Atlantic Ocean.

300 <sup>c</sup>Hawksbills foraging at coral reefs in Colombia, Pacific Ocean.

301 <sup>d</sup>Hawksbills foraging at coral reefs in Ecuador, Pacific Ocean.

302 <sup>e</sup>Hawksbills nesting on beaches within mangrove estuaries in El Salvador, Pacific Ocean.

303

304 *Heavy Metals*

305 Heavy metal values appear variable amongst species and subpopulations in loggerhead (*Caretta*  
306 *caretta*), kemp's ridley (*Lepidochelys kempii*), and green (*Chelonia mydas*) turtles (48) (49) (50),  
307 and are likely related to environmental effects, diet, age, and geography. While hawksbills are  
308 omnivorous, their diet worldwide is primarily composed of sponges (51), which are of low  
309 trophic level and may explain lower levels of contaminants than sea turtle species that eat items  
310 higher up on the food chain, such as olive ridley (*Lepidochelys olivacea*) and kemp's ridley  
311 turtles (48). Adult hawksbills have been documented having relatively low concentrations of the  
312 heavy metals in their blood, although maternal transfer of heavy metals from adult hawksbills to  
313 their eggs is known to occur (43).

314 Higher levels of arsenic were found in adult hawksbill tissues in Japan, particularly in  
315 muscle, than compared to adult green turtles (52). Additionally, arsenic levels of marine sponges  
316 were found to range from 0.8–157 mgm/gram of dry weight, suggesting that sponges may be a  
317 significant source of arsenic in adult hawksbills. It is unclear the role that sponges may play in  
318 accumulation of other heavy metals or persistent organic pollutants, such as the low levels of lead  
319 and mercury found in our study population.. Importantly, hawksbills in our study area utilize  
320 mangrove estuaries and are believed to feed predominantly on mangrove seeds and roots (M.  
321 Liles, pers. obs.), indicating that they may feed at an even lower trophic level than populations of  
322 hawksbills in other regions. The tendency to feed at low trophic levels may enable eastern Pacific  
323 hawksbills to avoid higher levels of blood pollutants seen in conspecifics in other habitats, as  
324 well as other sea turtle species.

325



## 326 *Persistent Organic Pollutants*

327 Organic and inorganic pollutants have been more frequently studied in loggerheads than other sea  
328 turtle species (53) (54) (55). Studies on loggerheads have found detectable POP and PCB results  
329 in which several of the individual contaminants had correlations with changes in clinical  
330 parameters such as packed cell volume (56). Further studies are needed on all sea turtle species to  
331 determine the individual and population level effects on health and reproductive outcomes in  
332 animals exposed to inorganic and organic pollutants.

333         The trophic level of food items consumed by sea turtle species at different life stages may  
334 impact levels of POP and PCBs. For instance, green turtles consume marine invertebrates as  
335 juveniles before transitioning to primarily algae and sea grass as adults, whereas adult  
336 leatherback and hawksbills forage on jellyfish and primarily marine sponges, respectively (57).  
337 For hawksbills and leatherbacks, this may mean they tend to accumulate more pollutants. More  
338 recently, however, leatherback turtles (*Dermochelys coriacea*) in Gabon with evaluated levels of  
339 POP and PCB in the blood of nesting and all turtles had levels below the detectable limit (42), a  
340 recent study (58) north of the west coast of Senegal in the Cape Verde Islands comparing POP and  
341 PCB levels in juvenile green and hawksbill turtles found detectable levels in both species,  
342 although green turtles had both higher levels and a greater prevalence of contamination. Trophic  
343 levels might not reflect higher levels of POP in adult green and hawksbill turtles and viable turtle  
344 eggs (59).

345

## 346 **Conclusions**

347 Our study provides baseline health data for hawksbills nesting at a primary rookery located in a  
348 mangrove estuary in the eastern Pacific Ocean, which can provide a starting point for long-term  
349 monitoring of health status of hawksbills in the region and offer diagnostic indications for

350 treatment of individuals in clinical rehabilitation. Additional studies between healthy juvenile and  
351 adult hawksbills in both mangrove estuaries and other habitats should be conducted to delineate  
352 size or age related differences in biochemistry and hematologic values in this species, as apparent  
353 health status may not reflect contaminant loads. We suggest that future research determine  
354 contaminant loads of marine sponges and mangrove vegetation in the Bahía de Jiquilisco and the  
355 potential role they play in accumulation of toxins in the environment. It is possible ecosystem  
356 processes are occurring that prevent uptake of toxins in the environment to the sea turtles  
357 themselves, or through their diet, which, contrary to most hawksbill populations, includes  
358 substantially more vegetation (60). Further studies at Bahia de Jiquilisco utilizing skin, muscle,  
359 carapace, fat and liver may provide different results than those obtained in this study.

360

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372

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