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ABSTRACT. – Hematologic and biochemistry ranges were established for 31 clinically healthy green sea turtles (*Chelonia mydas*) incidentally captured in artisanal fisheries in Sechura Bay, Peru. Postcapture stress may have influenced heterophil values and glucose concentration. Sechura Bay provides abundant dietary protein affecting urea and glucose values.

The green sea turtle (*Chelonia mydas*) is the most abundant of the 5 sea turtle species that use the Peruvian coast as a foraging and developmental habitat (Hays-Brown and Brown 1982). Because of fisheries interactions and other threats throughout their life cycle, *C. mydas* is listed as endangered on the IUCN Red List (Alfaro-Shigueto et al. 2011; International Union for Conservation of Nature [IUCN] 2004). In addition, *C. mydas* is the most incidentally captured sea turtle species in Peru especially

in the north (Alfaro-Shigueto et al. 2011). Constante port is located in Sechura bay in northern Peru, and is where most of the incidentally sea turtle captures occur (Alfaro-Shigueto et al. 2011). This port is characterized by trade and consumption of sea turtle meat for daily diet and medicinal uses (Santillán Corrales 2008). Hematology and biochemistry analyses are useful tools to evaluate health in wild populations and have been evaluated for many green turtle populations worldwide (Lewbart et al. 2014). However, research is scarce for the east Pacific *C. mydas* population (Labrada-Martagón et al. 2010b). The main objective of this study was to provide novel information on the hematological, morphometric and biochemical values for clinically healthy *C. mydas* from northern Peru. Such information can prove insights for monitoring the status of this green sea turtle population and long-term regional projects where *C. mydas* can serve as an indicator of the health of coastal marine ecosystems.

Methods. — The *C. mydas* individuals collected were incidentally captured by fishers from the port of Constante, in Sechura Bay, Piura (lat 5°67'S, long 80°84'W). The bay is characterized by the presence of primarily immature green sea turtles (Santillán Corrales 2008). There is high incidence of incidental captures by artisanal fisheries in this bay as well as habitat loss attributable to industrial and urban development and industrial contamination, which has resulted in increased turbidity (Santillán Corrales 2008). Therefore, incidental capture was the only technique available for the study. Sampling occurred in March, April, and May of 2012. Gillnets were set for approximately 16 hrs. Thirty-three of 50 animals were sampled after approximately 1 to 3 hrs of being recovered from nets. Fourteen individuals were recovered dead, having drowned in the nets. Two turtles were able to release themselves from the nets, and another one could not be sampled. Turtles were tagged with Inconel tags in both front flippers. Inclusion criteria for study animals were good body condition (Thomson et al. 2009), hydration status (no sunken eyes), swimming ability (no flipper amputation), and absence of external lesions. Curved carapace length (CCL) was measured using a flexible tape measure to estimate age.

For blood collection, individuals were placed in an inclined position with their head facing down. Blood was drawn from the dorsal cervical sinus (Owens 1999) and placed in vacutainer tubes® of lithium heparin and stored at 4°C. After 1 to 3 hrs, blood was processed at Tecno-medical Laboratory, Sechura. Hematocrit (HCT) and mean corpuscular volume (MCV) were manually measured. For HCT, blood was centrifuged for 5 min at 15,000 rpm. Red blood cell count (RBC) was performed using the Natt and Herrick method (Stacy et al. 2011). White blood cell count (WBC) was estimated using the formula number of cells/ μl = (average number of cells per field) \times (objective power)² (Strik et al. 2007). Two blood smears were prepared for each sample and stained with Wright to differentiate 100 leukocytes. Thrombocyte numbers were

subjectively assessed as reduced, normal or increased (Stacy et al. 2011) and expressed in a frequency table. To obtain plasma, blood was stored at room temperature for no more than 1 hr and centrifuged at 6000 rpm for 5 min and frozen at -20°C. Biochemistry panels were performed at Universidad Peruana Cayetano Heredia, Faculty of Veterinary and Zootechnics, Clinical Pathology Laboratory, Lima. These parameters were analyzed with semiautomatic analyzers Microlab 300 (Puteaux, France) and Rayto RT-1904C (Nanshan, China) using commercial kits (Human Diagnostics, Wiesbaden, Germany). Reference blood values were calculated with a 95% confidence level, and a Pearson's correlation was performed to assess the degree of association between read values and CCL. Both tests were performed using Microsoft Excel (Microsoft Corporation, 2010). Our results were statistically significant for probabilities $p < 0.05$. Reference blood values are expressed as mean, standard deviation, a range interval, and number of sample individuals (n). Our data are normally distributed as in the majority of researches about sea turtle hematology (Aguirre et al. 1995; Whiting et al. 2007; Casal et al. 2009; Deem et al. 2009; Labrada-Martagón et al. 2010b; Lewbart et al. 2014).

Results and Discussion. — Thirty-one individuals were considered fit for this study. Half of them presented diverse grades of depigmentation and scars from barnacles on the head and shell, but these did not obviously affect body condition. The mean CCL was 58.9 ± 8.9 cm (range: 47.3 to 91 cm). Reference ranges for hematology and biochemistry analytes are shown in Table 1. Plasma color was pale to medium yellow, and hemolysis was detected in one individual which was removed from the biochemistry results. The mean size of erythrocytes and leukocytes are shown in Table 2. Thrombocytes were within normal concentration with occasional clumps. In one individual, these values were considered as increased compared with our results. Analytes showed a direct correlation with size (CCL), especially eosinophils ($r = 0.55$; $p = 0.001$) and basophils ($r = 0.47$; $p = 0.007$).

According to the Central Limit Theorem, for populations with normal or abnormal distribution a sample size of 30 is sufficient to obtain a normal distribution (Salkind et al. 1998). Our research included 31 individuals; therefore, we consider our results to be statistically reliable. Furthermore, because *C. mydas* is an endangered species, obtaining a sample population over 30 is challenging.

HCT, RBC, and MCV values from this study were similar to *C. mydas* from other locations in the Pacific Ocean (Aguirre et al. 1995; Flint et al. 2009). The HCT value we report was 30% higher than that reported in the Galapagos Islands (Lewbart et al. 2014). Comparisons of WBC values were mixed, with some studies reporting similar results (Aguirre et al. 1995; Flint et al. 2009), whereas one showed levels 34% higher (Lewbart et al. 2014) and another studied reported 38% lower levels (Work et al. 1998). This variation is probably attributable

Table 1. Hematological and biochemical analytes in a wild population of green sea turtle (*Chelonia mydas*) in Sechura Bay, northern Peru.

Parameter ^a	Mean	Range	SD	n
Hematocrit (%)	33	23–45	5	31
RBC (10 ⁶ /μL)	0.52	0.21–0.97	0.18	31
MCV (fL)	716.51	319.59–1428.57	284.83	31
WBC (10 ³ /μL)	9.98	3.76–21.68	4.59	31
Heterophils (10 ³ /μL)	6.69	1.57–15.72	4.02	31
Lymphocytes (10 ³ /μL)	2.14	0.94–4.34	0.6	31
Monocytes (10 ³ /μL)	0.91	0.23–1.81	0.47	31
Eosinophils (10 ³ /μL)	0.12	0–0.48	0.13	31
Basophils (10 ³ /μL)	0.13	0–1.94	0.45	31
Alkaline phosphatase (UI/L)	38.04	17.3–74.32	14.85	30
Alanine aminotransferase (UI/L)	32.38	3.06–241.18	42.88	30
Aspartate aminotransferase (UI/L)	191.17	117–412	64.63	30
Urea (mg/dL)	64.31	13.93–173	37.84	30
Creatinine (mg/dL)	0.25	0.1–1.6	0.28	30
Glucose (mg/dL)	148	60–263	52	30
Total protein (g/dL)	4.2	2.6–5.9	0.7	30

^a RBC = red blood cell count; MCV = mean corpuscular volume; WBC = white blood cell count.

to different counting techniques. Heterophil values were 84% and 50% higher than Lewbart et al. (2014) and Work et al. (1998), respectively. These results could be attributable to postcapture stress (Aguirre et al. 1995). In the individuals from our study, this may have been caused by long-term entanglement, which could result in restricted access to air, intense struggling, and injuries to soft tissues (Snoddy et al. 2009). The mean values of basophils were sevenfold higher than the reference reported by Lewbart et al. (2014). Basophil counts are typically low in marine turtles and may depend on extrinsic and intrinsic factors such as seasonal, geographic, or age variation (Work et al. 1998).

In this study, 2 individuals were not included because one sample was contaminated with lymph and the other was oiled. This turtle was found inside the cabin of the boat to be used as bushmeat. It was restrained for 3 d. At clinical examination, it had sunken eyes but good swimming ability. It was sampled, rehabilitated with limited resources, and released the same day. Only enough blood to perform hematology evaluation could be obtained. The results were HCT 41%, RBC $0.83 \times 10^6/\mu\text{L}$, MCV 493.98 fL, WBC $13.12 \times 10^3/\mu\text{L}$, heterophils $9.64 \times 10^3/\mu\text{L}$, lymphocytes $1.34 \times 10^3/\mu\text{L}$, monocytes $2.13 \times 10^3/\mu\text{L}$, eosinophils and basophils 0. Comparing with our results for healthy green sea turtles, the red series clearly showed a dehydration state. WBC values are elevated principally because of heterophil values. Lymphocyte, eosinophil and basophil values are inside the range for healthy individuals. Monocyte values were outside the range interval expressing a monocytosis. Stacy et al. (2011) indicates this condition is attributable to a

Table 2. Morphometric values of erythrocytes, leukocytes, and thrombocytes in a wild population of green sea turtle (*Chelonia mydas*) in Sechura Bay, northern Peru.

Parameter (μm)	Mean	Range	SD	n
Erythrocyte length	19.6	17–22	1.5	7
Erythrocyte width	13.8	13.1–14.8	0.8	5
Heterophil diameter	15.9	12.8–20.7	2.8	7
Lymphocyte diameter	7.5	5.4–10.8	1.8	8
Monocyte diameter	13.4	9.4–15.5	2.3	5
Eosinophil diameter	18	12.6–21.9	3.1	8
Basophil diameter	14.4	11.8–15.7	1.3	4
Thrombocyte length	11.2	9–14.5	2.1	6
Thrombocyte width	7.5	6.5–8.6	0.8	6

chronic antigenic stimulation, chronic inflammation, or bacterial or parasitic disease. This turtle probably suffered long-term entanglement and was maintained inside the boat without food and water for 3 d. In addition, it got oiled from the engine oil. Chronic stress is evidence enough for the monocytosis.

A positive correlation between biochemical parameters and size for green sea turtles has been reported (Whiting et al. 2007; Labrada-Martagón et al. 2010b). In our study, hematological analytes are also correlated with size, in particular eosinophils and basophils values. Omnivorous green sea turtles are more commonly infected with spirorchids and other parasites affecting the presence of eosinophils in blood (Stacy et al. 2011). Nevertheless, this study did not look for internal parasite infestation.

ALP values were 6% lower than Labrada-Martagón et al. (2010b) and Aguirre et al. (1995) except for the 3–4 hrs postcapture result which was similar (38.8 UI/L). ALT and AST values were similar to Labrada-Martagón et al. (2010b) and 82% and 26% lower than Aguirre et al. (1995). These enzymes can be detected in muscle damage and related to capture stress (Aguirre et al. 1995). However, both Aguirre et al. (1995) and Snoddy et al. (2009) did not find a correlation between AST activity and capture stress. Also, Labrada-Martagón et al. (2010b) indicated AST is related to hepatocellular damage, and higher concentrations of transaminases in adult green sea turtles can be a physiological response to also high concentrations of some organochlorines (OC) contaminants. In this study, such comparisons could not be performed because our sampled individuals were exclusively immature turtles. However, one individual from our study presented 87% higher concentration for ALT than our mean value. This would indicate some contaminant exposure but should be taken with caution. It would be informative to do further research related to OC contaminants and heavy metals in tissue and blood of green turtles in Sechura Bay and other foraging areas with minor human impact.

The mean value for urea, when compared with green sea turtles in the Hawaiian Islands, was 93% higher than Aguirre et al. (1995) and 80% higher than Aguirre and

Balazs (2000), probably because that *C. mydas* population is herbivorous (Arthur and Balazs 2008), whereas in Sechura Bay they have an omnivore diet (Santillán Corrales 2008). Whiting et al. (2007) suggested that high concentrations of urea in omnivorous sea turtles may be attributable to an increased protein diet, starvation, or higher residual levels in animals that are beginning an herbivore phase. The mean glucose concentration value we report was 41% higher than the zero-hour postcapture result but almost equal to the 3–4-hr postcapture result reported by Aguirre et al. (1995) and also similar to Labrada-Martagón et al. (2010b) who indicated that elevated levels are attributed to stress and high ingestion of carbohydrates and protein. Snoddy et al. (2009) found high blood glucose concentrations in entangled sea turtles 60–120 min after capture. Our individuals were entangled for many hours and sampled after another 1–3 hrs. This long period of stress may have affected the glucose levels in our individuals. Furthermore, *Caretta caretta* also showed elevated glucose values (Casal et al. 2009; Deem et al. 2009) possibly by its carnivorous diet. Labrada-Martagón et al. (2010a) found a positive correlation of body index and season. In summer, when there is good food availability, animal body condition improves and is reflected in high glucose, protein, and lipids parameters. In our study, the sampling period occurred in the cold season when vegetable food is scarce and *C. mydas* tend to have a carnivorous diet (Santillán Corrales 2008). It can be inferred, therefore, that Sechura Bay has a good availability of dietary protein.

There are no previous published reports of hematological and biochemical values in *C. mydas* from Peru. Our research helps improve our understanding of the health of the east Pacific *C. mydas* population and may prove useful for local and regional conservation. It also provides a starter information for diseases monitoring as it has been proved gillnets may affect some analytes in blood. We recommend expanding this research to include comparisons by sex, age, season, and geographical location.

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