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ORIGINAL ARTICLE

Blood parameters of the Eastern Pacific green turtle (*Chelonia mydas*) foraging in the Golfo Dulce, Costa Rica

Priscilla Howell[1] · Ana Meneses[1] · Marcela Suárez-Esquivel[1] · Andrea Chaves[2] · Didiher Chacón[3] · Kinndle Blanco-Peña[4,5,6]

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Abstract
The Eastern Pacific green turtle (EPGT) has been differentiating from the green turtle and is currently being considered a separate population. In this study, values for hematology and biochemistry of 85 green turtles captured from 2010 to 2014 were analyzed. These animals correspond to a resident foraging population at Golfo Dulce, Costa Rica. All of them underwent a physical examination and were deemed apparently healthy. Hematological analyses were done using manual methods, while seric metabolites were determined with automated and semi-automated spectrophotometry. According to the curved carapace length (CCL) measurements, the subadult individuals were the most frequent ($n=74$). The predominant cells were heterophils, followed by lymphocytes. Basophils were observed in 75% of the slides and in a proportion as high as 43%. Up to 80 samples were analyzed for the following seric parameters: total protein (2.6–6.2 g/dL), albumin (1–2.7 g/dL), globulin (1.2–4.1 g/dL), glucose (54–145 mg/dL), cholesterol (58–336 mg/dL), ureic nitrogen (0–12 g/dL), creatinine (0.1–0.7 mg/dl), phosphorus (3.4–17 mg/dl), calcium (4.9–10.2 mg/dl), magnesium (5.2–12.8 mg/dl), uric acid (0.1–2.8 mg/dL), triglycerides (14–743 mg/dl), alanine aminotransferase (0–13 U/L), aspartate aminotransferase (82–289 U/L) and seric alkaline phosphatase (41–1263 U/L). These data can aid health status and conservation programs for the EPGT in this and nearby, similar locations.

Keywords Hematological analyses · Sea turtles · Biochemistry · Seric metabolites

Introduction

Over the last decades, the number of reports of diseases in marine turtles, such as virus, fungal, bacterial, and parasite infections, renal oxalosis deposition, gastrointestinal disorders, and traumatic injuries, has been increasing (Stacy et al. 2008; Flint et al. 2009; Work et al. 2019; Mashkour et al. 2020). To contribute to conservation management efforts, nowadays, veterinarians play an important role in treating stranded turtles and diagnosing causes of diseases (Hamann et al. 2006; Whiting et al. 2007).

The Golfo Dulce, located within the marine area of the Piedras Blancas National Park in the southern Pacific of Costa Rica, is an important foraging area for different marine turtle species, including the Eastern Pacific green turtle (EPGT) (Chacón-Chaverri et al. 2015; Méndez-Salgado et al. 2020). This area is known for its high marine diversity, presenting 21.5% of the total species reported for the Pacific coast of Costa Rica (Morales-Ramírez 2011). However, anthropogenic activities are currently threatening, leading to water quality deterioration, reduced seagrass, and decreased number of species (Morales-Ramírez 2011; Sarmento et al. 2015), which can influence the animal health status.

When a sick animal is detected, it is necessary to reference its species physiology and biology to recognize

✉ Kinndle Blanco-Peña
kblanco@una.ac.cr

1 Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

2 Escuela de Biología, Universidad de Costa Rica, Montes de Oca, San Pedro, Costa Rica

3 WIDECAST, San José, Costa Rica

4 Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacional, Heredia, Costa Rica

5 Posgrado en Ciencias Veterinarias Tropicales (PCVET), Universidad Nacional, Heredia, Costa Rica

6 Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica

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pathologies and the most appropriate treatments. However, the lack of information on blood parameters makes it difficult to attend to clinical cases. Therefore, this study aimed to describe the hematological and biochemical parameters of the Golfo Dulce EPGT, which can be applied in conservation programs' medical care.

Methodology

Capture

EPGTs were captured in water at a foraging site located in the Golfo Dulce, Costa Rica (8° 40′ 31.01″ N, 83° 26′ 48.65″ O), using a simple 100 × 7 m net (Fig. 1). All institutional and national guidelines for the care of animals were followed. Any individual showing diminished reflexes, secretions, or injuries was not included in the study.

Age estimation

Animals were classified into four age groups according to their curved carapace length (CCL) (Table 1) (Musick 2002).

Samples and blood analysis

A 10 ml blood sample was obtained from the cervical sinus and was separated in Nipro® collection tubes: one with heparin and another one without anticoagulant; later, the second one was centrifuged at 3000 rpm to separate the serum (Compact II centrifuge, Clay Adams® Brand, Decton Dickinson, Fisher Scientific, USA). Samples were stored in a 4 °C cooler during fieldwork, and later on, stored in a refrigerator, pending transport to the lab. All analyses were performed in 4 to 5 days from the sampling at the latest. Despite this, few samples were unfeasible and not analyzed.

The heparinized sample was used for the following analysis: packed cell volume (PCV), hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), total and differential white blood cell (WBC), and cellular morphology. Total protein (T.P.), albumin, globulin, glucose, cholesterol, blood urea nitrogen (BUN), creatinine, phosphorus, calcium, magnesium, uric acid, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and seric alkaline phosphatase (SAP) were measured from the serum sample. Any sample presenting clotting or severe hemolysis was discarded. Data analysis was performed using Excel and R with descriptive statistics. P-value was ≤ 0.05.

Results and discussion

Ninety-four green turtles were captured and sampled, but in only 85 cases, it was possible to obtain an appropriate blood quality. A physical examination performed on-site deemed them apparently healthy.

Age estimation

According to the age group size classification, the subadult individuals were the most frequent ($n = 74$; 78.8%) (Table 2). The carapace length is a common method to subdivide the sampled population into age groups (Aguirre and Balazs 2000; Seminoff et al. 2002). However, it is notoriously difficult to estimate whether an individual is sexually mature unless it has an evident sexual dimorphism. Only three individuals showed a tail length associated with males.

Some authors affirm that *C. mydas* from Eastern Pacific are smaller than *C. mydas* from the Caribbean Sea and the Atlantic Ocean (Márquez and Farías 2000). Likewise, the males' size is rarely reported since most of the studies are carried out with nesting females due to the ease of their capture (Musick 2002). For this reason, individuals classified as subadults despite having a long tail (the most unambiguous indication of sexually mature males and the only accurate dimorphism in sea turtles) were maintained in this study as such.

As there is a lack of equitable samples for each size, a comparative statistic of the hematological and biochemical results was not performed.

Hematology

The hematological analysis was carried out in 85 samples (Table 3). The hematocrit (PCV), hemoglobin, and CHCM results presented little dispersion, which coincides with previous studies in other regions (Jacobson et al. 2008; Bolten and Bjorndal 1992; Samour et al. 1998; Work et al. 1998; Work and Balazs 1999; Montilla Fuentemayor et al. 2006; Whiting et al. 2007; Flint et al. 2010; Page-Karjian et al. 2015a). The morphological description of erythrocytes and thrombocytes agreed with that reported in the literature (Samour et al. 1998; Work et al. 1998; Casal and Orós 2007; Stacy et al. 2011; Stacy and Boylan 2014). Erythrocytes with basophilic inclusions were observed, which is a normal finding (Fig. 2) (Stacy et al. 2011; Stacy and Boylan 2014).

The morphology found for all white blood cells proved constant amongst our samples and coincided with previous descriptions of *C. mydas* blood cells (Samour et al. 1998; Work et al. 1998; Montilla Fuentemayor et al. 2006; Casal and Orós 2007; Orós et al. 2010; Flint et al. 2010; Stacy et al. 2011; Stacy and Boylan 2014; Lewbart et al. 2014)

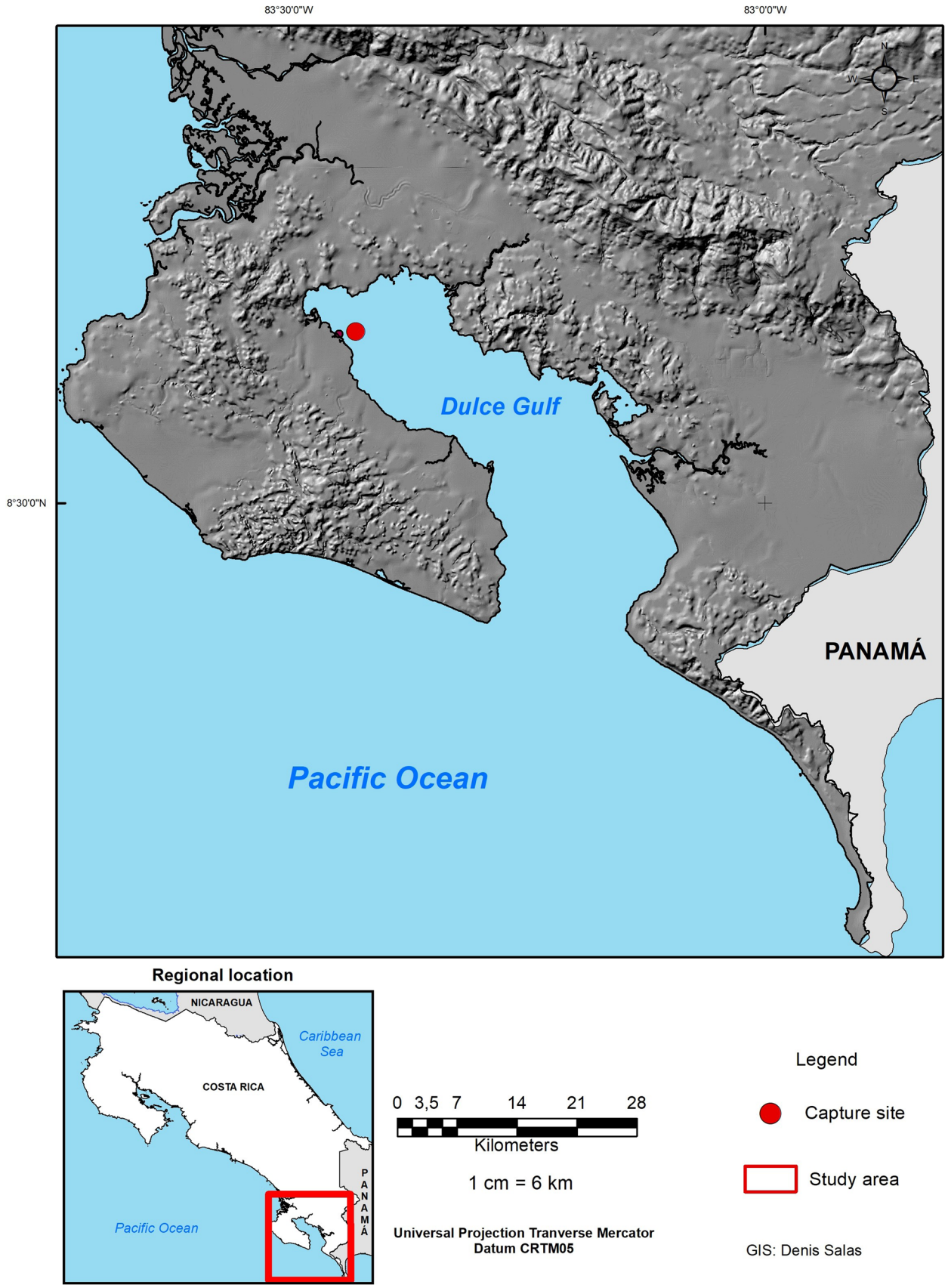

Fig. 1 Location of the foraging site in Golfo Dulce, Puerto Jiménez, Costa Rica, where green turtles were captured

Table 1 Parameters used to classify the Eastern Pacific Green turtle (*Chelonia mydas*) (EPGT) captured in the Golfo Dulce of Costa Rica in age groups

Age group	Curved carapace length (CCL)
Hatchling	< 38 cm
Juvenile	38–68 cm
Subadult	68–88 cm
Adult	> 88 cm

*According to Aguirre and Balazs (2000), following the adjustments suggested by Musick (2002)

(Fig. 2). We did not observe bilobed nuclei in any granulocyte, contrary to the case of *Caretta caretta* (Di Santi et al. 2013).

Our study showed a total and differential white blood cell count fluctuating considerably, with wide ranges and big standard deviations. Heterophiles were the most abundant cells in most cases, followed by lymphocytes, eosinophils, basophils, and monocytes, in that order. Unexpectedly, basophils presented in 75% of the smears, as predominant as 43% of the differential, were the most common in six cases. Some authors suggest that these cells do not appear significantly in the differential counts (Samour et al. 1998; Work et al. 1998; Montilla Fuentemayor et al. 2006; Flint et al. 2010; Lewbart et al. 2014), while others argue a problem in the staining used (Stacy et al. 2011; Stacy and Boylan 2014).

The total leukocyte count was also lower than in other studies (Samour et al. 1998; Montilla Fuentemayor et al. 2006; Flint et al. 2010). Quantities of most cells varied greatly compared to previous reports, except for heterophils (Samour et al. 1998; Work et al. 1998; Montilla Fuentemayor et al. 2006; Casal and Orós 2007; Orós et al. 2010; Flint et al. 2010; Stacy et al. 2011; Stacy and Boylan 2014; Lewbart et al. 2014), eosinophils (Samour et al. 1998), and basophils (Work and Balazs 1999; Aguirre and Balazs 2000), whose values agreed with other studies.

The total leukocyte count varied widely, but it was lower than the results showed by other studies (Samour et al. 1998; Montilla Fuentemayor et al. 2006; Flint et al. 2010). The predominant cells were heterophils, followed by lymphocytes.

Table 2 Classification of the Eastern Pacific Green turtle (*C. mydas*) (EPGT) captured in the Golfo Dulce of Costa Rica according to their age groups

Age group	Hatchling	Juvenile	Subadult	Adult	Not evaluated
CCL range (cm)	< 38	38–68	68 – 88	> 88	ND
n	0	7	74	13	10
%	0	7.4	78.8	13.8	10

Basophils were observed in 75% of the slides and a proportion as high as 43% (Table 3).

The scarce studies in reptiles rarely specify hematological analysis methods, and the criteria for cell categorization are inconsistent among authors, possibly due to the unknown origin of the cell lines (Work et al. 1998; Casal and Orós 2007; Tavares-Dias et al. 2008). There are three possible methods for carrying out hematological analysis in reptiles: the Nat Herrick (N.H.) solution, floxin B, and the smear measurements. All of them have several sources of error (Sheldon et al. 2016). Besides, each laboratory and technical professional can show different results according to the species and the sample's handling (Campbell and Ellis 2007). So, the differences shown in our results could be justified by the method used, the environmental conditions, and the number of individuals analyzed.

Seric metabolites

Most analytes showed a statistical consistency with small S.D.s over the sampled population, specifically T.P., albumin, globulin, BUN, creatinine, uric acid, ALT, and AST, the latter despite its wide range of results. Glucose as well as all electrolytes showed a similar mean and median, in a broad spectrum and a relatively large S.D. On the contrary, A/G ratio, cholesterol, triglycerides, and SAP varied widely in their results and statistics (Tables 2 and 4).

Some parameters were like previous reports, varied slightly to moderately, or showed a broader range than research performed in different world areas. Such variations could be due to differences in diet, water conditions, seasonality, and the population sampled in each study, usually narrowed by age, sex, and/or the number of individuals sampled. Due to this study's nature, a broad, non-selective population was included from a gulf's small area.

It is likely to find variation between our results and data from Australia because of ecological factors. For example, differences between dietary components, food and predator abundance, and water conditions (e.g., temperature, proximity to marinas or crops) (Whiting et al. 2007; Flint et al. 2010), the Hawaiian Archipelago (Aguirre and Balazs 2000), Baja California (Labrada-Martagón et al. 2010), United Arab Emirates (Hasbún et al. 1998), Bahamas (Bolten and Bjorndal 1992), Puerto Rico (Page-Karjian et al. 2015b), and Florida (Jacobson et al. 2008) were other seric biochemistry researches conducted.

Parameters such as T.P., albumin, cholesterol, triglycerides, BUN, uric acid, glucose, and phosphorus are heavily influenced even by changes in diet components and abundance (Ho et al. 1982; Bolten and Bjorndal 1992; Campbell 2006; Goldberg et al. 2011; Hasbún et al. 1998; Jessop 2001; Lutz et al. 2003; Wyneken 2008; Anderson et al. 2011; Meneses and

Table 3 Hematology results for the Eastern Pacific Green turtle (*C. mydas*) (EPGT) of the Golfo Dulce, Costa Rica

Parameter	*n*	Min	Max	Mean	Med	SD	Wilcoxon
PCV (%)	82	22	50	37.15	38	5.53	36–38.5
Hemoglobin (g/dl)	78	8	15.2	11.49	11.05	1.79	11–11.95
MCHC (g/dl)	78	22	37	30.59	31	3.8	29.5–31.5
WBC (/µl)	70	124	1930	810	740	458.91	670–912.5
Heterophils (/µl)	70	42	1366	498.6	395	358.89	362–578.5
Eosinophils (/µl)	70	0	200	45.55	31	50.59	32.5–60.5
Basophils (/µl)	70	0	352	78.8	28.5	97.87	59.5–120.5
Lymphocytes (/µl)	70	15	378	117.5	77	101.01	75.5–138
Monocytes (/µl)	70	0	97	26.06	20.5	26.9	24–39.5
Erithrocytes (µm)	495	15.8	21.48	18.46	18.45	1.13	18.34–18.55

Bouza 2015). Given the peculiar behavior of pre-migratory aphagia (Lutz et al. 2003; Jessop et al. 2004), variations are expected to occur depending on the migratory stage each individual is undergoing and the energetic reserves accumulated.

Changes in globulin, A/G ratio, calcium, cholesterol, and triglycerides could be expected to show differences between males and females due to the internal dynamics female experiences while developing eggs, which would be shown in most studies that sample a female nesting population (Ho et al. 1982; Bolten and Bjorndal 1992; Jessop 2004; Wyneken 2008; Anderson et al. 2011; Meneses and Bouza 2015; Wilkinson 2004; Campbell 2006; Goldberg et al. 2011). Furthermore, creatinine, globulin, A/G ratio, and SAP are also correlated to muscle and bone growth and condition, as well as age and activity of the immune system (Cray et al. 2001; Meneses and Bouza 2015), so individuals

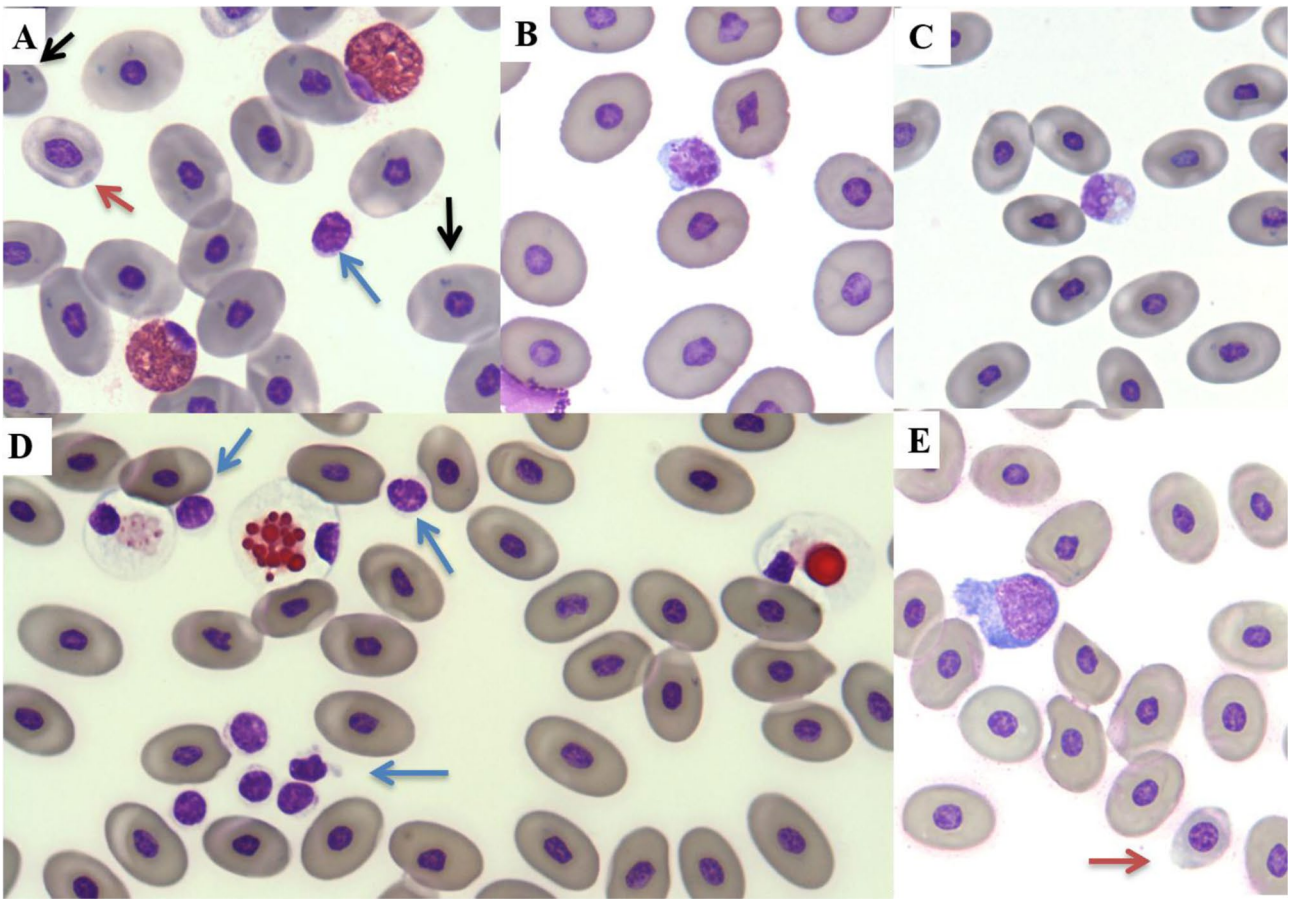

Fig. 2 Cells evaluated during the differential count: **A** two heterophiles; **B** a lymphocyte; **C** a basophil; **D** three eosinophils with markedly reddish variegated granules; **E** a monocyte. Normal erythrocytes can be seen in photos **A**, **B**, **C**, **D**, and **E**. Black arrows indicate basophilic stippled erythrocytes, blue arrows show thrombocytes, and the red ones, immature red cells

Table 4 Seric metabolites results of the Eastern Pacific green turtle (*Chelonia mydas*) (EPGT) from Golfo Dulce, Costa Rica

Parameter	*n*	Min	Max	Mean	Med	SD	Wilcoxon
TP (g/dl)	75	2.6	6.2	4.35	4.4	0.78	4.15–4.55
lbumin (g/dl)	78	1	2.7	1.79	1.8	0.36	1.7–1.85
Globulin (g/dl)	77	1.2	4.1	2,54	2,6	0.63	2.4–2.7
A/G Ratio (g/dl)	75	0.28	1,11	0.68	0.64	0.16	0.63–0.7
Glucose (mg/dl)	61	54	145	88.93	84	20.58	81.5–93.5
Cholesterol (mg/dl)	66	58	336	163.6	153	61.99	144.5–178
Triglycerides (mg/dl)	60	14	743	150.3	115.5	113.29	112–161
BUN (g/dl)	75	0	12	4.1	3.7	2.91	3.2–4.5
Creatinine (mg/dl)	72	0.1	0.7	0.43	0.4	0.14	0.4–0.45
Uric Acid (mg/dl)	76	0.1	2.8	1.21	1.1	0.6	1.05–1.3
Phosphorus (mg/dl)	80	3.4	17	8.66	8.5	2.27	8.1–9.05
Calcium (mg/dl)	76	4.9	10.2	7.38	7.15	1.29	7–7.6
Magnesium (mg/dl)	65	5,2	12.8	9.02	9,3	1,64	8.7–9.55
SAP (U/l)	55	41	1263	316	195	284.8	191.5–350
ALT (U/l)	65	0	13	3.97	3	3.33	3–5
AST (U/l)	76	82	289	184.8	187,5	62.62	175–194.5

likely repairing or growing bone show some expected variations (Bolten and Bjorndal 1992; Aguirre and Balazs 2000; Whiting et al. 2007; Page-Karjian et al. 2015b). The turtle's size has been correlated to higher cholesterol, triglycerides, creatinine, and ALT levels in bigger animals (Hasbún et al. 1998; Anderson et al. 2011; Meneses and Bouza 2015).

Glucose is also affected by water temperature since insulin and glucagon are temperature-dependent in reptiles (Mader and Campbell 2006; Campbell 2007). Likewise, water salinity is involved in the workings of the excretion organs — kidneys and salt glands — that maintains plasma osmolarity, so dysfunctions in either, or perhaps changes in salinity, could be associated with different results of uric acid, phosphorus, and magnesium. Uric acid, the best indicator for kidney function (Bolten and Bjorndal 1992; Campbell 2006; Goldberg et al. 2011), was one of the steadiest analytes statistically, both within this study and others.

Phosphorus varied widely; as mentioned before, this could be related to diet and handling of the sample in field conditions, as a delayed separation of the serum from the clot could have allowed a release of phosphorus from the erythrocytes. The magnesium case, broader and higher in our study than in previous reports (Jacobson et al. 2008; Hasbún et al. 1998; Whiting et al. 2007; Flint et al. 2010), is more difficult to pinpoint since it is not measured frequently, and neither acceptable levels nor hypermagnesemia etiology is known for sea turtles (Innis et al. 2007). It has been reported in cold stun cases and organochlorine pesticide exposure, likely due to salt gland and/or kidney dysfunctions (Keller et al. 2004; Lutz and Musick 1996; Lutz et al. 2003; Campbell 2006). However, there is no data on pesticide levels in this region, and it is improbable that these animals suffered freezing in Costa Rica. Differences between our and other studies could also be related to the methods used or nutritional and environmental conditions.

The SAP also showed unusually disperse and elevated results (Jacobson et al. 2008; Bolten and Bjorndal 1992; Hasbún et al. 1998; Aguirre and Balazs 2000; Whiting et al. 2007; Flint et al. 2010; Labrada-Martagón et al. 2010; Page-Karjian et al. 2015b). It was not possible to correlate this to any normal or abnormal conditions or circumstances, so environmental factors, methods, or handling of the sample should not be discarded as possible causes of variations. Similarly, although to a lesser degree, ALT presented scattered and narrower data than other previous studies (Bolten and Bjorndal 1992; Aguirre and Balazs 2000; Whiting et al. 2007; Labrada-Martagón et al. 2010). However, some authors have previously described a positive correlation between carapace length and width with liver enzymes ALT and AST, suggesting that animals with larger hepatic volume show more significant enzymatic activity (Goldberg et al. 2011). None of the measured enzymes are liver-specific (Campbell 2006; Meneses and Bouza 2015). AST is considered a good indicator of hepatocellular damage (particularly if it is complemented with lactate dehydrogenase analysis) (Campbell 2006). No alterations were found for this enzyme, both in this study and compared to literature data.

Conclusions

This work provides descriptive hematology and blood biochemistry parameters of the EPGT from the Golfo Dulce, Costa Rica. Since the sample was vast, it was possible to provide a statistically significant analysis.

These results can aid conservation programs and rescue and rehabilitation efforts of animals in this and nearby, similar locations to identify pathologies and appropriate treatments.

Recommendations

It is highly recommended to unify the hematological criteria to decrease the variability between researchers.

It is suggested to re-examine blood parameters at least every 5 years to monitor the population's health status.

It is highly recommended to develop testosterone testing to validate the morphometric method and monitor the population, especially in subadult individuals.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval The study was conducted following the current guidelines for animal welfare stipulated by the School of Veterinary Medicine, Universidad Nacional, Costa Rica. Samples were collected according to the following permit numbers: MINAET-SINAC-Costa Rica: SINAC-SE-GASP-PI-202.

Conflict of interest The authors declare no competing interests.

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