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Chapter 3

Health Assessments in Free-Ranging Sea Turtles: Perspective of Animal Welfare in Wildlife

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Abstract

Sea turtle health is an important component of conservation since these taxa is susceptible to infectious diseases that can cause illness increasing its mortality. Threats to health, survival, and reproduction of sea turtles are increasingly documented; however, prevention and control has not yet been successfully achieved. Thus, the need to develop conservation strategies on an ecosystem scale is a growing concern. Information about health indicators of sea turtles is a useful tool to achieve the best possible conservation measures. The objective of this study was to establish a baseline of health assessments of free-ranging Eastern Pacific green turtles (*Chelonia mydas*) from developmental habitats in Baja California Sur. Here we contribute with a clinical exam for sea turtles and reference interval values of vital signs (Corporal temperature: subadults, 21.22 ± 3.43; juveniles, 22.2 ± 2.95. Heart rate: subadults, 37.41 ± 2.95; juveniles, 38.27 ± 4.09. Pulse rate: subadults, 15.07 ± 3.88; juveniles, 19.97 ± 0.82), that in conjunction with the complete blood count and plasma blood biochemistry, 100% of the turtles were classified as "healthy." Development of site-specific health indicators for wild, healthy sea turtle populations is an important factor in creating effective management protocols and thus enhances our ability to understand the effects of anthropogenic and environmental changes on sea turtle health.

Keywords: semiology, vital signs, hematology, blood chemistry, sea turtles welfare

1. Introduction

Sea turtle health is an important conservation component and is often overlooked. These species are susceptible to infectious diseases that can cause severe illness increasing its mortality [1]. In the case of the Eastern Pacific green turtle (EPGT) *Chelonia mydas* population, it is gradually recovering, yet much remains unknown about its health status due to its complex

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life cycle, especially its ethology, migratory conditions, and sometimes the inaccessibility to the study areas (places for study them). However, threats to health, survival, and reproduction of free-ranging sea turtles are being increasingly documented, and their prevention and control measures have not yet been successfully achieved [2]. That is why there is a growing concern about the need to develop conservation strategies to operate at the ecosystem scale [3] and, therefore, develop health assessments for marine turtles. It becomes a useful tool that can lead to the best possible conservation measures.

Oceanographic conditions of marine waters and coastal lagoons in Baja California Sur (BCS) provide diverse habitats and optimal conditions to carry out different stages of the life cycle for five of the seven species of sea turtles that exist in the world: the hawksbill turtle (*Eretmochelys imbricata*), green and Eastern Pacific green turtles (known locally as black turtle) (*Chelonia mydas*), loggerhead (*Caretta caretta*), olive ridley (*Lepidochelys olivacea*), and leatherback turtle (*Dermochelys coriacea*) [4], which are exposed to a wide range of factors specific to their environment and anthropogenic factors that can cause diseases and death. In the Baja California Peninsula, since 2016, health assessment programs have been applied and have been an integral part of management efforts to enhance the recovery of particularly the loggerhead turtle and the EPGT. These strategies include (1) on-site protection with health evaluations, (2) determination of their condition and distribution, (3) determination of the diet, (4) characterization of the population of origin and confirmation of species, (5) morphometric data collection, (6) individual sea turtle tagging, and (7) release. Initially, the monitoring efforts were intended to collect morphometric data and individual sea turtle tagging because in the past decades EPGT survival at BCS declined severely due to consumption [5]. Actually these efforts were notably successful, and these stocks of EPGT appear to be in the process of recovery [6]. However, in 2014 and 2016 there are disease reports and mortality associated with illness [7, 8]. Therefore, the idea of carrying out a program of health assessments to complement conservation efforts was proposed in order to minimize the threat of any potentially catastrophic disease outbreaks. Minimizing the risk of diseases and/or introducing diseases to a population can be accomplished by using the best available diagnostics to test turtles for evidence of infection or disease and taking a proactive approach to disease management (i.e., treatment or immunization as technology becomes available). The current impact on declining survival and the role of disease in the EPGT population are limited and in some cases unknown. Therefore, veterinarian research is needed to improve or provide animal welfare efforts. Internationally the information about sea turtle health is principally based on hematological values [9, 10, 11]. Through these methods, various aspects of health status of an individual are obtained.

The results can be related and linked to anatomical, morphological, and functional changes as well as to nutritional and reproductive status [12]. However, descriptive hematological values and morphological characteristics of blood cells in sea turtles are regionalized and have been inconstant [13]. It is important to consider that blood elements can be affected by a number of factors such as age, sex, season, stress, diet, hormonal processes, oxygen pressure, body hydration [9], geographic area, and water temperature [11]. In order to document local health indices, health assessments, hematological values, and vital signs should be generated at baseline. In Mexico, there are several studies related to sea turtles hematology [14, 15, 16, 17];

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however, regionally, in BCS, there are still few published works [18, 7, 13, 19]. Thus, there is an urgent need to clearly indicate the health status and rate of diseases in regional sea turtle populations [8]. The present study was aimed at obtaining baseline information on selected health and disease indicators from the EPGT. The objectives of the study were to (1) assess the health of sea turtles via physical examination to document clinical signs, (2) generate their values of vital signs, (3) determine hematological values of EPGT from BCS, and (4) evaluate the results obtained to generate conservation strategies together with local authorities.

2. Materials and methods

During 2016, monthly field trips were carried out with captures of sea turtles in the pacific coast of Baja California Sur, particularly in coastal lagoons of feeding and development of *Chelonia mydas*. All captures were made using monofilament nylon gillnets. This method consists in the extraction of organisms from a 100-m-long net (locally known as "chinchorro"), with a drop of 5 m and a mesh size of 60 cm; this net is pulled from a small boat and left in periods of 6–8 h, covering day lapses in the areas considered transit of sea turtles and channels with constant flow of currents in the lagoons. The "chinchorro" net is reviewed in periods of an hour or an hour and a half to minimize the risk of death by asphyxiation or damage to organisms.

2.1. Clinical exam

Once the turtles were loaded onto the boat, identification of the species was carried out, each animal was allowed to rest for a few minutes alone and in a clear space; during this time, we observed the turtle in detail paying special attention to the behavior and movements, as well as the type and level of activity of each animal. The physical evaluation was carried out with the participation of two people to manipulate the animal and a third to record the observed changes and the data [20]. This process was carried out as gently and quietly as possible. First, one examiner held the animal on the back (placing his hands on the front flippers) to avoid excessive movement and reduce stress by struggle; the other performed a detailed systematic inspection with cranial-caudal and dorsal-ventral orientation. We carefully checked the skin and tegument for signs of excessive desquamation, abscesses, scars and wounds, the presence of injuries from hooks or nets, and the presence of leeches, barnacles, etc. Next, we also examined the position, shape, size, symmetry, color, and proportions of the head and skull as a whole with the intention of determining injuries, traumas, or pain. The visible structures of the eyes such as the conjunctiva, the sclera, the cornea, the iris, the pupil, and the ductal openings of the lacrimal sac were then checked, using a pocket medical scanning lamp, with which also we observe the trophism and symmetry of the eyes and their associated structures. Is important to note that in sea turtles, the eye is protected by the dorsal and ventral eyelids, which are keratinized and mobile; there is also a secondary eyelid on the lateral canthus that is keratinized but does not move [22]. In this step we put special attention in this area, since it is known that one of the main diseases in marine turtles (fibropapillomatosis) manifests initially in this zone [10]. In the nostrils it was checked if there was nasal discharge or congestion, traumatic injuries, and presence of foreign bodies. The ranphoteca was reviewed by the

presence of injuries and traumas mainly. Afterwards, we checked the position and continuity of the maxilla and mandible to discard or confirm fractures or dislocations; it is important to emphasize that the maxilla and mandible vary according to each species; nevertheless, they maintain similar anatomical and functional characteristics [22]. We put special attention in the area between the maxilla and the mandible because it is a place of development of fibropapillomatosis [10]; also the interior of the mouth and lumen of the esophagus were carefully reviewed to evaluate the color of the mucous membranes and identify abnormalities such as oral plaques, ulcerations, and abnormal odor and the presence of foreign bodies such as hooks or ropes. Afterwards, the entire neck was inspected, exploring its shape (short, long, and normal), volume (wide, thin, and normal), position (central, with lateral deviation, in flexion, and in extension), its mobility, and the presence or absence of injuries and neoplasms, as well as the presence of foreign bodies (ropes and hooks) and epibionts, leeches, and ectoparasites. Likewise, the musculoskeletal structures and the mobility of the neck were explored, as well as the parotid, submaxillary, and sublingual regions and the region close to the structures of the shoulders and neck. Then the glands were inspected by palpation. After that, the flippers were reviewed in detail (skin, muscles, bones, and joints) looking for lesions, the presence of epibionts, inflammations, fractures, etc. [20]. When evaluating the carapace we look for evidence of abnormal keratinization, changes in firmness and flexibility, injuries caused by boats and propellers, ulcerations, vesicles, osteomyelitis, shark bites, deformities, fibropapillomas, fractures, or the presence of epibionts, leeches, algae, and ectoparasites [20], and finally all the anatomical, morphological and functional changes observed were recorded in a health format. During this review, the registration of vital signs (carapace temperature, plastron, inguinal area, cloaca, and heart rate record) and the exploration of sensitivity and reflections of each animal were included. To generate vital signs, the carapace temperature was measured three times with a digital infrared thermometer type gun Steren® HER-425, placing it at a distance of 10–20 cm from the carapace and pointing in the central surface of the carapace (using a scute index as a reference). Subsequently, an average of the three temperatures was calculated and recorded in a database. The organism was reviewed in detail ventrally, with special attention to the plastron and cloaca following the same dorsal revision methodology. The body temperature was taken three times in the center of the plastron and in the left inguinal area; an average of these was calculated following the same methodology. During the physical examination, in the ventral review, the turtles were auscultated with an IUMED® model 400 stethoscope, which was placed on the skin between the neck and the proximal frontal flipper. Then, deep pressure was made to locate the resonance of the heartbeat; once identified, the heart rate (HR) was recorded for 30 s and multiplied by 2. Next, the deep cloacal temperature was measured with an electronic thermometer model SureTemp® Plus 690 (Veterinary) Welch Allyn; this was introduced gently (approximately 1.5 cm) into the cloaca of the turtles, and then, the probe was tilted so that the tip of it was in contact with the tissue and when the final temperature was reached and appeared on the thermometer screen, it was registered. After completing the temperature measurement, the probe was gently removed from the cloaca of the turtle. The next vital sign measured was pulse rate, in marine turtles it is very difficult to measure the pulse in a practical way for its anatomical and morphological characteristics; therefore, the pulse rate in relation to their body weight was measured using the following equation: $X * (Y^{-0.25})$, where X = beats per minute (lat/min) and Y = weight in kilograms (kg) [21].

After the examination, turtles were sexed according to the criteria of sexual dimorphism described by [22] (classified as females, males, and undefined based on the size of the tail in relation with the size of the turtle). Then, to estimate the size of sexual maturity of the animals, the morphometric data was collected using a flexible measuring tape [23]. The morphometric data collected were curved carapace length and curved carapace width; immediately after, the turtles were tagged with metallic marks of monel 400/inconel 625 in the back flippers following the methodology described by Balazs and finally were released. All values obtained during the clinical examination of turtles evaluated were recorded in **Table 1**.

2.2. Sample collection

2.2.1. Blood samples

At the end of the physical examination, the individuals were positioned in ventral recumbency on a flat and fixed surface; the eyes of the turtles were covered with a wet cloth to avoid stress by manipulation. The head of the turtle was placed below the body and protruding from the flat surface on which the turtle was placed. Approximately 10 ml of blood was collected from the dorsal cervical sinuses, which are located on both sides of the midline of the neck between 1/3 and 1/2 of the distance between the back of the head and the anterior edge of the carapace, approximately 2.5 cm lateral to the midline, 2–3 cm below the surface of the skin. Once located, the needle was inserted perpendicular to the dorsal surface of the neck at 1–3 cm depth into the vein [24], using double pointed needles (1.5 inches, 32 mm caliber) connected to a Vacutainer© holder; finally, the needle was removed from the vein and the venipuncture site was covered with the index finger by pressing to avoid the formation of

	EPGT (*Chelonia mydas*) subadult (n = 59)	**EPGT (*Chelonia mydas*) juveniles (n = 20)**
	Mean ± SD	**Mean ± SD**
CCL	75.61 ± 4.48	51.37 ± 3.71
Weight	40.94 ± 18.92	18.39 ± 1.50
Temperature (°C)		
Carapace	23.22 ± 3.97	22.76 ± 3.91
Plastron	22.61 ± 3.40	23.37 ± 4.10
Inguinal	21.22 ± 3.43	22.2 ± 2.95
Cloacae	19.85 ± 2.25	22.44 ± 3.13
HR	37.41 ± 2.95	38.27 ± 4.09
Pulse	15.07 ± 3.88	19.97 ± 0.82
Sex	34(U) 23(F) 2(M)	19(U) 1(F)

CCL: curved carapace length; cm: centimeters; SD: standard deviation; °C: degree celsius; HR: heart rate; F: female; M: male; U: undefined.

Table 1. Mean and standard deviation of the curved carapace length, weight, vital signs (carapace temperature, plastron, groin and cloaca, heart rate, and pulse) and subadult and juvenile EPGT sex.

bruises until it was sure that no more blood came out. Blood samples were stored in two 7 ml Vacutainer© tubes (Becton Dickinson, Franklin Lakes, New Jersey). The first tube lacked an anticoagulant in order to recover serum later and perform a blood chemistry panel [25], and the second tube included lithium heparin (He/Li) as an anticoagulant to determine complete blood count [10, 26]. Blood samples were transported in a cooler at 4°C to the marine Botany Lab and Oceanography Lab at the Autonomous University of Baja California Sur (UABCS) where they were immediately processed.

2.3. Hematology

2.3.1. Complete blood count and blood chemistry

Hematocrit (HCT) was manually measured filling a capillary of the micro-hematocrit, supporting one of the ends on one blood drop; subsequently, the end closest to the blood was covered with plasticine and the capillary was introduced into a microcentrifuge for 5 min at 15,000 rpm in an automatic microcentrifuge model ECOspin III® (medical ECONET®); with a metric ruler the length of the column formed by sedimented red blood cells was measured in the capillary and was referenced in percentage to the total length occupied by the blood that fills the capillary with the next equation—hematocrit (%): A /B (100) with (A) total length of blood in the capillary and (B) length of the cell fraction (100). The total red blood cell count (erythrocytes) and white blood cell count (leukocytes) was made in a Neubauer chamber, using Natt and Herrick methodology [27]; 10 μL of blood was drawn from the anticoagulant tube and used to obtain a 1:100 dilution with 990 μL of Natt-Herrick diluent [27]. This was incubated for 3 min and then a drop was deposited in a contrast Neubauer chamber (Improved Neubauer, BOECO®, West Germany) of 0.1 mm depth and 0.0025 mm^2. Next, the camera was placed in an optical microscope (Olympus® CX31) for 2 min until the solution stabilized. Observation under a minor objective (10×) was started to locate the primary quadrant of leukocyte count. Hemoglobin assay (Hb) was determined by commercial Labtest® kit. Erythrocyte indices (MCV, means cell volume; MCH, corpuscular hemoglobin; MCHC, corpuscular hemoglobin concentration) were calculated from the total erythrocyte count, hematocrit, and hemoglobin. In the case of thrombocytes, the number of these cells per 1000 erythrocytes was counted. Leukocyte count was carried out through the blood smears made at the time of blood sample collection (two blood smears were prepared, air dried, and then fixed with methanol). Later on, they were stained with a rapid blood staining kit Hemacolor® (Merck Millipore®) at the Marine Botany Laboratory in the Autonomous University of Baja California Sur (UABCS). Blood smear slides were reviewed for leukocytes and classified based on the morphological features [26, 28] using a microscope Olympus® CX31 with 40× and 100× lens and a Neubauer hemocytometer, according to Natt and Herrick method [27]. For cell identification, we followed the criteria established by [26]. Plasma was separated by an automatic centrifuge model ECOspin III® (medical ECONET®) at 3000 rpm for 15 min and pipetted into 2 ml sterile vials and stored in a freezer at −4°C until transfer for further chemical analysis. Blood plasma values were processed at Fidelis labs with the routine technique; these samples were analyzed using a chemistry analyzer and commercial kits. Selected blood values from biochemical analysis were albumin, globulin, urea, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP) (except in juveniles), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and total protein. In addition,

Variable	Mean ± SD	Range	Mean ± SD	Range
	Subadults (n = 59)		**Juvenile (n = 20)**	
HGB	11.07 ± 0.80	10–13.5	8.72 ± 0.69	7.5–9.9
HCT	31.75 ± 1.39	28–34.7	39.6 ± 1.67	37.4–42.25
MCV	62.84 ± 2.79	50.1–73.1	61.91 ± 2.38	53–70
MCH	19.04 ± 1.60	150–218.6	19.10 ± 1.90	16.9–23
MCHC	17.07 ± 2.08	12–19.3	11.82 ± 1.06	10.1–13.2
E	1.17 ± 0.48	0.4–4.62	0.48 ± 0.04	0.42–0.54
TH	16.11 ± 3.75	13.3–24.9	18.76 ± 0.72	17–20
LEU	8.48 ± 0.50	7.1–11.1	2.13 ± 0.19	1.8–2.4
LYM	22.81 ± 0.80	20–27	31.38 ± 2.75	28–36
MOs	1.9 ± 0.56	1–6	2.23 ± 1.30	1–6
EOS	1.43 ± 0.23	1–2	0.23 ± 0.43	0–1
Het	66.61 ± 2.42	59–73	62.38 ± 1.98	60–65
BA	0	0	0	0

HGB: hemoglobin (g/dl); HCT: hematocrit (%); MCV: mean corpuscular volume (fl); MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (gr/dl); E: erythrocytes (1,000,000/mm^3); TH: thrombocytes (1000 /mm^3); LEU: leukocytes (1000/mm^3); LYM: lymphocytes (%); MOs: monocytes (%); EOS: eosinophil (%); BA: basophils (%); Het: heterophil (%); g/dl: gram/dilution; fl: femtoliter; pg: picogram.

Table 2. Complete blood count for sub adult and juvenile EPGT (*C. mydas*).

Variable	Mean ± SD	Range	Mean ± SD	Range
	Subadult (n=59)		**Juvenile (n=29)**	
Urea	31.03 ± 1.32	19–38	24.30 ± 2.13	21–28
BUN	12.69 ± 1	9–17	10.5 ± 1.12	9–12
Creatinine	0.62 ± 0.12	0.5–0.8	0.62 ± 0.13	0.4–0.9
AST	242.14 ± 2.75	202–255	250.15 ± 19.72	212–278
ALT	33.44 ± 0.52	30–39	34.15 ± 2.79	28–39
ALP	56.16 ± 7.59	40–80	—	—
LDH	142.1 ± 40.63	70–284	267.76 ± 32.15	202–299
TP	7.07 ± 0.59	4–8.6	5.03 ± 0.53	4.2–6.1
Albumin	2.67 ± 0.54	1.2–3.7	1.53 ± 0.27	1.2–1.9
Globulin	3.89 ± 0.41	2.9–4.6	3.46 ± 0.31	3.1–3.9
A/G ratio	0.52 ± 0.06	0.2–0.8	0.26 ± 0.07	0.1–0.4

Urea (mg/dl), BUN: blood urea nitrogen (mg/dl); creatinine (mg/dl); AST: aspartate aminotransferase (U/L); ALT: alanine aminotransferase (U/L); ALP: alkaline phosphatase (U/L); LDH: lactate dehydrogenase (U/L); TP: total protein (g/dl); albumin (g/dl); globulin (g/dl).

Table 3. Blood chemistry values for subadult and juvenile EP green turtles (*C. mydas*).

we calculated albumin/globulin (A/G) values according to [9]. Finally, the values obtained from the blood biometrics of the turtles were recorded in **Table 2** and the blood chemistry values are shown in **Table 3**.

3. Results

During 2016, a total of 79 *C. mydas* were captured in two coastal lagoons in BCS. The organisms were classified as subadults (59) and (20) were classified as juveniles according to their size and weight; these results are shown in **Table 1**.

In the case of the blood values, the values obtained in the complete blood count are reported in **Table 2** and the values of blood chemistry are shown in **Table 3**.

4. Discussion

4.1. Height, weight, and sex distribution

According to the average of CCL (75.61 ± 4.48) and weight (40.94 ± 18.92), 59 turtles were classified as subadults and 20 turtles (CCL, 51.37 ± 3.71; weight, 18.39 ± 1.50) were classified as juveniles [29, 30]. The gender of 34 subadult turtles and 19 juvenile turtles could not be determined and were registered as unidentified gender; 23 subadult turtles were classified as female, 2 as males, and finally 1 juvenile as female which were classified based on the criteria proposed by [22].

The physical exams did not show evidence or clinical signs of neoplasms and diseases nor lesions that could compromise organ function or life.

4.1.1. Vital signs

Vital signs results (corporal temperature, heart rate, and pulse rate according to the weight) help us to determine immediate alterations in the basic functions of marine turtles, monitor health problems, and indicate the physiological state of core organs (brain, heart, lungs, etc.) [31]. These values also indicate immediate functional changes in organisms that otherwise could not be qualified nor quantified [32]. The principal advantage of this type of study is that the values can be registered anywhere and do not require complex material to be measured.

4.1.2. Temperature

Marine turtles are exposed to a wide diversity of environmental changes during their migration and ontogeny, which is why we place such importance on health monitoring. Sea surface temperature (SST) variation can affect immune system function, subsequently influencing threats to infectious disease. Reports suggest a high frequency in fibropapillomas (FPs) with the increase of water temperature in summer [33]; on the other hand, [34] suggests that when

there is a decrease in temperature, marine turtles undergo stress, also leading to an incidence of FP. According to the average SST during sampling and the turtles' average temperature, a phenomenon among green turtle species, cold stunning (a form of hypothermia) [35, 36], and FP were eliminated as threats. On the other hand, when environmental temperature decreases sharply, the speed of the metabolic chemical reactions decreases as well as the quantity of energy that individuals can exert in their activities [31]. If the corporal temperature increases too much, the biochemical reactions are unbalanced and the protein synthesis involved in physiological functions is disrupted or even reduced [32]. The advantage of the regulatory system of marine turtles lies in their ability to save energy by maintaining their corporal temperature. In addition, the low nutritional needs of this species allow them to survive in different environments during migration [37]. The disadvantage is that their core activities depend on environmental temperature, and thus, they cannot stand long periods of time (more than 24 h) in low temperatures (less than 9°C).

Under this temperature, they become lethargic and affected by cold stunning possibly causing its death [35, 38]. Nevertheless, they use strategies as they enter into a turbid state, a kind of hibernation that helps them to tolerate low temperatures [39]. Deep cloacae temperature is representative of the actual environmental temperature [40]. By acquiring a baseline value threshold, this allows wildlife rehabilitators to act quickly to address cold-stunned turtles, which have a recovery chance [36, 41]. Due to a similar inguinal and cloacae temperature, the use of a digital infrared laser thermometer without contact was effective in order to monitor this vital sign. This type of thermometer is recommended instead of the cloacae one because it is less invasive and less stressful for the turtle. When using the laser thermometer, it is recommended to record the temperature as soon as the turtle is captured and before the physical examination in order to collect the most real-time values. If vital signs are recorded long after capture, the environment can affect the temperature of the turtle and the recorded values then will have a large error margin.

4.1.3. Heart rate (HR)

Cardiac auscultation in sea turtles is complicated due to the presence of the carapace, plastron, and muscular structures [41]. However, there are several ways (Doppler probe or ultrasound) to evaluate heart rate. In this study the HR was measured and recorded using a stethoscope and depended based upon the expertise and experience of the examiner. For subadult and juvenile turtles, the resting heart rate matched with valued proposed by [42, 43] who suggest that normal heart rate in green turtles at a temperature of 24°C (75°F) varies from 30 to 60 beats per minute. Due to the fact that the HR obtained from all the turtles analyzed did not present considerable variations, physiological alterations that can lead to tachycardia, such as fatigue, excitation, digestive processes, or gravid females [32] were eliminated. Hyperthermia, hydremias, septicemia, and pericarditis in the case of the pathological disorders [44] were eliminated. HR values did not indicate bradycardia, which may be associated with physiological processes such as starvation or lethargy [31] and pathological processes such as cerebral compression, vagus nerve excitation, intoxication, or others [44].

4.1.4. Pulse rate

The pulse rate of individuals in relation to their body weight was calculated using the equation cited by [21]. During vital signs analysis, the pulse rate should be comparable with the heart rate. In marine turtles, this is complex due to their anatomy and morphology; however, it is known that in certain pathologies, such as hemodynamically inefficient systems of atrial or ventricular premature complexes or atrial fibrillation with high cardiac frequency, etc. [44], a pulse rate frequency lower than the HR (pulse deficit) can be evident. The pulse rate can also decrease (bradisfigmia, bradisphyllia, or *pulsus rarus*) or increase (tachyphygmia, tachyphyxia, or *pulsus frequens*) [32, 45]. In marine turtles, as in other species, the autonomic nervous system is the main determinant of pulse rate; thereby, in response to vagotonic stimuli, a bradysfixia will be presented and to sympatheticotonic stimuli, a tachysphyxia [31].

In this case, it was not possible to evaluate if the pulse rate presented irregularities in a practical way nor the succession of pauses that separate the pulsating waves (in a physical way). However, it was considered that subadult and juvenile turtle pulse rates were regular. The data suggest that the succession of diastolic pauses was stable and continuous (the duration between them is similar). Therefore, pathological causes such as arrhythmias due to variable blocks, polyextrasyses, or atrial fibrillation were dismissed. It is also true that intermittent pulse rate due to sinoatrial or atrioventricular blockages, false intermittent pulse rate by premature ventricular systoles, and hemodynamically inefficient extrasystoles [44] were eliminated.

4.2. Hematological values

Hematological values for subadult and juvenile organisms are similar to the previous ones reported internationally for healthy green turtles [11] and probably are associated to age, reproductive stage, and food availability as [25] suggested. These values can be related to sea turtle's home range; therefore, there are no "optimal" values. When integrating the physical examination results and vital signs, no clinical symptoms were observed (anemia, dehydration, bleeding, and malnutrition) [46], nor were the presence of possible respiratory, renal, gastrointestinal, inflammatory, and infectious chronic diseases or neoplasms [10].

4.3. Complete blood count (CBC)

CBC is a diagnostic orientation tool for diverse causal agents of diseases. It is only diagnostic when the agent or damage directly affects the blood cells [47], for example: the presence of hemoparasites and cases of lymphocytosis, heterophilia, leukocytosis, red blood cells with intracytoplasmic inclusions, remains of organelles, precipitated hemoglobin, and others [48]. Those mentioned above are reported in the literature as changes associated with chronic and active infectious processes [7, 49]. Morphology of blood cells in this study was similar to the previously reported cases by [26, 28, 50]; thus, the presence of chronic active infectious processes and the presence of hemoparasites [7] were dismissed.

4.3.1. Hematocrit (HCT)

HCT values were similar to those reported for healthy *C. mydas* in Hawaii [9] and Peru [51] and those reported by [13] from the same region in BCS. Then, anemia, dehydration,

hemorrhages, destruction of red blood cells, and malnutrition (iron deficiency and vitamins) were dismissed. Cardiac diseases, dehydration, hypoxia, pulmonary fibrosis, erythrocytosis, etc. were also excluded because there were not increased values [52, 53, 54].

4.3.2. Total proteins (TP)

The values for TP obtained in this work were found within normal ranges for *C. mydas* [12, 13, 25], which show that there is no hyperproteinemia, hypoproteinemia, or chronic inflammatory diseases, malabsorption, protein-losing enteropathies (e.g., by parasites), renal failures, or hepatic problems [54].

4.3.3. Erythrocytes

There were no erythrocytosis observed according to Reséndiz [13] criteria and the values reported by [55], so we confirm that there were no dehydration, renal diseases, oxygenation problems or drowning threats, heart disease or neuropathies, and polycythemia [56, 57]. Low values were not found either and autoimmune diseases, blood loss, bone marrow insufficiency, infections or neoplasms, hemolysis, prolonged infections, and nutritional deficiencies were dismissed [56, 58].

4.3.4. Leukocytes

Leukocyte values obtained were similar to those reported by [10] and were within the ranges indicated by [51] for healthy *C. mydas*. Our values suggested that there was no leukocytosis; therefore, there was no presence of infectious diseases, inflammatory diseases, severe physical stress, or tissue damage [59]. Likewise, leukopenia was dismissed due to the absence of autoimmune diseases, bone marrow failure due to neoplasms, fibrosis and renal diseases [60], as well as pathologies in the liver and spleen [49].

4.3.5. Hemoglobin (HGB)

HGB values were within the ranges reported for healthy *C. mydas* by [9, 51]; therefore, hemolytic anemia, bone marrow deficiency, renal diseases, poor nutrition, and bleeding in the digestive tract [56] were eliminated. No elevated levels were found so there is no presence of hypoxia, dehydration, or pulmonary lesions and diseases [61].

4.3.6. Mean corpuscular volume (MCV)

MCV values were normal according to what was reported by [55, 51], for which microcytic anemia caused by low values and macrocytic anemia caused by increased values [52, 56] were dismissed.

4.3.7. Mean corpuscular hemoglobin (MCH)

There were no values below those proposed by [9] so that the evaluated turtles did not present hypochromic anemia. There were no higher values than the ranges proposed by [51], dismissing hyperchromic anemia [62].

4.3.8. Mean corpuscular hemoglobin concentration (MCHC)

MCHC values obtained were similar to those reported by [9], suggesting that there were no iron anemia deficiency, blood loss, neoplasms in the digestive tract, macrocytic anemia, or hepatic problems [54, 63].

4.3.9. Thrombocytes

Thrombocyte counts were similar to those reported by [55], for which thrombocytopenia, thrombocytosis, autoimmune disorders, and anemia [64] were dismissed.

4.4. Lymphocytes

The number of lymphocytes that were obtained was similar to those reported by [10, 25] for healthy *C. mydas* and does not indicate lymphocytosis; therefore, struggle and lymphocytosis in young animals by physiological reasons were eliminated [63]; lymphopenia was not observed as there are no severe stress, hyperadenocorticism (endocrine diseases), viral infections [60], lymphangiectasia, and quilotorax [54].

4.4.1. Eosinophils

Eosinophilia was not observed, indicating no stress and all possible cases of parasitosis, hypersensitivity, tissue degradation, hypoadrenocorticism, hypereosinophilic syndrome, and leukemia [60] were dismissed.

4.4.2. Basophils

Basophilia was not identified; thus, there was no response to disease- or antigen-specific production; therefore, hypersensitivity and mastocytemia were dismissed [54].

4.4.3. Heterophiles

The values for heterophiles obtained were similar to those reported by [9] and by [10] for which heterofilia and any pathological process caused by bacteria and fungi were dismissed, as well as severe stress, hyperadrenocorticism, inflammation, and leukemia [54, 63]. Heterophilia was not observed and there were no severe inflammations, excessive food intake, bacteriological infection (gram negative), and myeloid hypoplasia [60].

4.5. Blood chemistry (BC)

Measurement of the chemical elements that compound the blood with other lab procedures and clinical exams help to diagnose, emit a prognosis, and if appropriate, evaluate the efficiency of a treatment [65]. BC is a diagnostic tool, so the metabolites that are tested do not specify tissue or organ damage [59]; therefore, it is not an effective method to correctly or completely diagnose the health status of any species [7, 53].

Collectively, plasma proteins perform a nutritive function, exert colloid osmotic pressure, and help maintain acid–base balance. Individually they work as enzymes, coagulation factors, hormones,

and transport substances [66]. Hyperproteinemia was not observed, eliminating the possible cases for dehydration, hyperglobulinemias associated with chronic inflammatory diseases, hyperalbuminemias, or hemolysis [61]. Hypoproteinemia was also not observed; thus, possible cases of chronic malnutrition, protein malabsorption, poor digestion, protein-losing enteropathies (parasitism), blood loss, chronic hepatitis, or renal diseases were all dismissed [65].

4.5.1. Albumin

Albumin values were in the normal range according to [67, 18], eliminating the possible cases of dehydration. No alterations in protein synthesis (starvation, malabsorption of the small intestine, hepatic processes, and severe trauma) were observed, nor a decrease in protein synthesis from kidney, intestine, hemorrhage, and sepsis [65].

4.5.2. Globulins

Globulin values were within the normal range indicated by [3, 11, 18]; in BCS, therefore, possible cases of hyperglobulinemia and hypoglobulinemia were dismissed [65].

4.5.3. Albumin/globulin (A/G)

Values obtained were similar to [18] in a nearby area, whereby possible cases of renal proteinuria or increased production of immunoglobulins by antigenic stimulation were dismissed. Likewise, there were no increase in A/G values, so possible cases of immunoglobulin production were dismissed too. Hemorrhages and dehydration were also dismissed as possible cases [65].

4.5.4. Urea

Urea values rise due to increased protein degradation, which is caused by intestinal hemorrhage, necrosis, hyperthyroidism, and others. This can also be increased by a reduction of renal perfusion (dehydration, shock, and hypoalbuminemia), for acute or chronic renal insufficiency, and for obstruction of urinary flow. While values decrease if there is abnormal hepatic function or by reduced protein intake [61, 68], in this study, urea values were similar to those reported by [3, 51], therefore suggesting normal, healthy ranges when compared to the above situations.

4.5.5. Creatinine

Creatinine values were similar to the ones proposed by [3, 69] for healthy *C. mydas*; therefore, dehydration, acute and chronic renal failure, and obstruction of the primary urinary flow and rupture of the bladder were dismissed [61, 68].

4.5.6. Aspartate aminotransferase (AST)

AST values are within the limits reported by [11] for healthy *C. mydas* and were similar to those reported by [18]. Thus, these levels were considered normal for the region and this species. Therefore, liver damage, skeletal and cardiac muscle damage (ischemia), septicemia, toxemia, and hemolysis [70] were dismissed.

4.5.7. Alanine aminotransferase (ALT)

ALT values were similar to those proposed by [12] for healthy *C. mydas*; therefore, hepatic problems such as damage, failure, etc., were dismissed.

4.5.8. Alkaline phosphatase (ALP)

ALP values were similar to those obtained by [11] in Puerto Rico and to those described by [18] in BCS. Therefore we concluded that there were no biliary obstruction, hepatic damage, extensive or generalized bone disease, neoplasms, septicemia, starvation, and hepatic regeneration [61, 70].

4.5.9. Blood urea nitrogen (BUN)

BUN levels obtained were within the ranges indicated by [51, 11] for healthy *C. mydas*. Thus, heart failure, excessive levels of protein in the digestive tract, dehydration or renal diseases, as well as malnutrition and hepatic failure [68] were dismissed as possible cases.

4.6. Lactate dehydrogenase (LDH)

Similar values of LDH were observed to those reported by [3, 51]. Thus, any blood flow deficiency, hemolytic anemia, hepatic diseases, muscle injury or muscle weakness [70], and abnormal formation of new tissues (neoplasms) [9] were dismissed as possible cases.

5. Conclusion

A baseline of the health assessments and vital signs for turtles in BCS was established, which can be used as a reference for future research; also, hematological values of these organisms were generated. These results indicate that sampled turtles were healthy.

CBC and BC values in sea turtles may vary with species, sex, age, weight, diet, and stage of development, reproductive phase, whether they are in migration or whether they are resident, and dependence on environment. CBC levels allow researchers to diagnose diseases when the presence of a causative agent or damage directly affects the blood cells. BC analysis is a diagnostic tool; however, the metabolites do not specify tissue or organ damage. The hematological results obtained should not be taken as parameters or as prevalence or incidence data since they do not calculate the disease rate because the statistical characteristics of spatial epidemiology were not achieved. Rather, these values work as reference indicators of health status for Eastern Pacific green sea turtles in BCS.

The establishment of a baseline of health assessments and hematology profiles for healthy wild sea turtles is a priority for their conservation and management. Since normal hematology values have not been established for most free-ranging marine turtle populations, this type of research could be included as part of the monitoring protocol for sea turtles in foraging areas. Such information can provide a foundation for conservation strategies such as the early detection of threats, risks, and diseases. Sea turtle health studies should be integral and sequential, as they must follow a rigorous medical order. Regular health monitoring allows

the diagnosis of infectious disease threats, prevents diseases, and can estimate future spreads leading to the development of strategically relevant conservation programs. These studies act as indicators for sea turtle health status at the ecosystem and population levels.

It is necessary to continue collecting data surrounding vital signs and hematological values, as well as develop a diagnostic interpretation to generate health parameters for comparison with other sea turtle populations. By improving the health status monitoring of sea turtles in BCS, researchers can effectively prevent population species declines, potentially related to diseases.

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Conflict of interest

The authors declare that there is no conflict of interest; this manuscript is original, and it has not been published or submitted to another journal or editorial.

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