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Research Article

Condition and Haematological Values of Free-Ranging Eastern Pacific Green Turtles (Chelonia Mydas) from Baja California Sur, Mexico

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Abstract

Background: Marine turtles face a wide range of environmental and anthropogenic factors that can cause disease and in severe cases death. Health and diseases of marine turtles are studied more frequently in different parts of the world; however, their prevention, control or treatment has been infrequently addressed. Therefore, the information generated on the health values of marine turtles becomes a useful tool to achieve the relevant conservation strategies. The objective of this study was to assess the health of free-ranging Eastern Pacific green turtles (*Chelonia mydas*) from the neritic foraging developmental habitats in Baja California Sur.

Methods: 29 *C. mydas* were evaluated with clinical exams, then, 29 blood samples were collected and hematic biometrics were performed.

Results: Turtle health panels included in this study are ranked as “good” based on clinical examination and hematologic diagnostics. Marine turtles are sentinels, ecosystem health indicators, therefore, the good health of these organisms, allows us to infer that Ojo de Liebre lagoon is a healthy ecosystem.

Conclusion: Develop site-specific blood values for wild marine turtle populations, helps to create effective management protocols and improves our ability to understand the effects of anthropogenic and environmental changes on the health of marine turtles and the coastal ecosystems that inhabit.

Introduction

There are different environmental factors in each living population that can affect or modify their behaviour and development, known as “biotic potential”. The biotic potential provides the population with the biotic and abiotic resources necessary for its existence, acting as a limiting factor for its growth and sustenance, which is known as “environmental resistance”. Some of the factors that intervene in this environmental resistance are metabolic and infectious diseases, which also depend on environmental and intrinsic phenomena [1]. To understand fluctuations between health and disease, the epidemiological triad is taken as a reference. This ponders the balance between: (a) etiological agents (infectious, physical, chemical, etc.); (b) host or guest (human, animal); and (c) environment (weather, temperature, food); the breakdown of this balance is called disease [2], which indicates that health and disease are highly multifactorial and dynamic [3]. Marine turtles as an important part of diverse ecosystems can face a wide range of environmental and anthropogenic factors that can cause diseases and in severe cases death [4]. There are different diseases that affect marine turtles, fibropapillomatosis (FP) being one of the most common and studied [5], probably due to the severity of its clinical manifestations and the increasing incidence and prevalence throughout the world [6]. However, in marine turtles some diseases can go unnoticed at first sight [7] and one of the main ways to identify them are haematological analyses [8]. Through these methods, various aspects of an individual health status are obtained. Hematic biometric analysis is a tool of diagnostic orientation for various causative agents of the disease when the agent or damage directly affects the blood cell [9]; for example, presence of hem parasites, and cases of lymphocytosis, heterophilia, leukocytosis or intraerythrocytic inclusions [10]. Findings from application of hematic biometrics allow us to determine changes associated with chronic and active infectious processes in an organism [11]. Internationally, there are reports that describe the morphological characteristics of marine turtle blood cells [12,13] and several authors have reported values and reference intervals considered “normal” for healthy green turtles (*Chelonia mydas*) [6,14,15], in

Mexico there are still few studies on this subject [16-18] and even less in Baja California Sur(B.C.S.) [19-21]. Marine turtles are considered "sentinels" or ecosystem health indicators [1], based on their life history, size, longevity, and interaction between the water-air interfaces; these chelonian species can provide useful information on environmental changes at various spatial, temporal and trophic scales since marine turtles are especially vulnerable to anthropogenic degradation of environmental health [4]. Although it is estimated that the populations of Eastern Pacific green sea turtle (EPGT) are recovering [22] there is a need to develop conservation strategies to operate individually and at the scale of the entire ecosystem [23]. Thus, generating haematological values of free-ranging marine turtles helps to establish their health profiles and diagnose the presence of diseases and risks; then, with this information we can generate marine turtle's management protocols. In addition, because this species is considered a good indicator of ecosystem health [4], it increases the efficiency of monitoring at the habitat level and becomes a useful tool that can lead to the best conservation measures for the ecosystem [1]. Based on this, the objective of this research was to evaluate the health of EPGT by means of clinical examination and to generate their haematological values trough hematic biometrics. This information can serve as a reference and provides support information to assess the health status of a *C. mydas* population from B.C.S. In addition, this regional data can be compared with the various hematologic values reported at the national and international levels.

Materials and Methods

Study site

Ojo de Liebre Lagoon (LOL) is a coastal lagoon located in the northern Pacific of B.C.S. (Latitude 27° 35' and 28° 15' N, longitude 113° 50' and 114° 20' W), and is part of the "El Vizcaíno" Biosphere Reserve. The habitat in LOL has extensive areas of sea grass and macro algae mainly [24]; this coastal lagoon is inhabited by EPGT in their juvenile, sub-adult and adult stages [25]. During the monitoring of 31 August 2010, we captured EPGT by setting "Castillo" monofilament nets (100 meters in length, five meters deep and 60 cm of mesh size) following the methodology of the Export adora de Sal Company of Guerrero Negro (ESSA) and "El Vizcaíno" Biosphere Reserve of the National Commission of Natural Protected Areas (REBIVI-CONANP).We deployed the net from a boat in the channels of the lagoon where turtles were known to transit. The net remained fixed for six to eight hours during day periods, and was checked in periods of an hour and a half to two hours. Then, we pulled entangled turtles into the boat for further examination and sample collection.

Clinical exam

Once we loaded the turtles onto the boat, they rested for a few minutes alone and in a clear space on the boat; during this time, we observed the turtles in detail, paying special attention to their behaviour and movements. Next, two people conducted the physical evaluation. One examiner held the animal in dorsal recumbence and the other performed a detailed systematic inspection with cranial-caudal and dorsal-ventral orientation as described in Reséndiz et al. [21]. Next, we recorded their morphometric data following the methodology proposed by Bolten [26]. Immediately after, we tagged the turtles with metallic monel 400 / inconel 625 tags in the rear flippers, following the methodology described by Balazs [27], and finally we released the turtles.

Blood sample collection

At the end of the physical examination, we collected approximately 5 ml of blood from the dorsal cervical sinuses as described in Owens and Ruiz [28]. We then transported blood samples in a cooler at 4°C to the Marine Botany Laboratory at the Autonomous University of Baja California Sur (UABCS) for immediate processing.

Hematic biometrics

We measured and evaluated the following hematologic values: haematocrit (HCT), total proteins (TP), erythrocytes (E), leukocytes (Leu), lymphocytes (Lym), monocytes (MOs), eosinophils (EOS), basophils (Ba), and heterophils (Het), according to Aguirre et al. [14]. For the total red blood cell count (erythrocytes) and white blood cell count (leukocytes), we used the Natt and Herrick methodology [29], and reviewed them using an optical microscope (Olympus® CX31) under a minor objective (10x). We carried out the leukocyte count through the blood smears made at the time of blood sample collection. After that, we stained the blood smears with a rapid blood staining kit (Hemacolor®, Merck-Millipore®) at the Marine Botany Laboratory in the UABCS and reviewed them for total leukocyte counts and differentials based on the morphological features and criteria established by Casal and Orós [12] and Sykes and Klaphake [13], using a microscope Olympus® CX31 with 40× and 100× objectives. We calculated mean, standard deviation (SD) and the reference intervals for each of the haematological values, and for the curved carapace length (CCL), straight carapace length (SCL), weight, and body condition index (BCI=mass (kg) * 10000 / SCL^3) [30].

Results

Turtle biometrics, body condition index and sex

We captured and released in the LOL a total of 29 EPGT. All turtles were classified as sub-adults. The Biometrics: Straight carapace length (SCL), Curved carapace length (CCL), weight, and Body condition index (BCI) are shown in Table 1. The gender of the turtles could not be determined and all were registered as unidentified gender.

Clinical exam

During clinical examination we did not observe any injuries, dermal lesions, neoplasms or fractures that could compromise organ function or life, so the turtles were deemed to be "clinically healthy".

Hematic biometric analysis

Morphologically, the blood cells were "normal" in size and shape compared with Casal and Orós [12] and Sykes and Klaphake [13].

Table 1: Mean and SD of the SCL, CCL, weight and BCI of sub-adult EPGT.

Variable	Units	Mean	SD	Reference interval
SCL	cm	67.59	10	60.2 - 90.5
CCL	cm	73.12	10.82	62.3 - 96.5
Weight	Kg	45.44	20.1	32 - 92
BCI		1.47	0.17	1.3 - 1.6

Eastern Pacific Green Turtles (*Chelonia mydas*) Sub-adults (n= 29).

Table 2: Mean and SD of the hematic biometrics of sub-adult EPGT.

Variable	Units	Mean	SD	Reference interval
Haematocrit	%	32.76	2.11	29.4 - 39.5
Total Protein	g/dL	6.41	0.43	5.6 - 7.2
Erythrocytes	103/µL	3.28	1.6	2.19 - 6.22
Leukocytes	103/µL	8.58	2.99	6.92 - 10.10
Lymphocytes	103/µL	6.29	2.17	2.31 - 8.99
Monocytes	103/µL	1.34	0.55	1-3
Eosinophils	103/µL	1.17	0.38	1-2
Heterophils	103/µL	6.7	4.75	6.11 - 7.90
Basophils	103/µL	0	0	0

Haematological profile of Eastern Pacific Green Turtles (*Chelonia mydas*) Sub adults (n= 29).

The analysis of the hematic biometrics values, when related to the clinical examinations, indicated an absence of systemic, autoimmune and metabolic diseases; nutritional problems, neoplasms and traumatisms; therefore these values are considered "normal" for "healthy" EPGT (Table 2).

Discussion

According to the average of their size and weight, we classify the EPGT described here as sub-adults, following the proposal by Seminoff et al. [31], who indicate that the minimum size for adults in B.C.S. is 77.3 cm of CCL. Likewise, the presence of sub-adults in this area coincides with what was previously proposed by Reséndiz et al. [26], who established LOL as an area inhabited mainly by juvenile, sub-adult and adult EPGT. Turtle BCI was similar to the values reported for previous studies in B.C.S. by Reséndiz et al. [26] and López-Castro et al. [30], indicating indirectly that the quality of the environment and forage availability support this turtle aggregation's capacity for future favourable reproductive performance. The turtles were registered as unidentified gender based on the criteria of sexual dimorphism proposed by Wyneken [32].

The hematologic values reported here are similar to and fit within the reference intervals of those previously reported for *C. mydas* stocks with similar characteristics and considered "healthy" in other parts of the world, such as Hawaii [6,14], Australia [33], Puerto Rico [15], Peru [34], Brazil [35], Chile [36] and in the same place in B.C.S. [21].

Blood cell morphology was similar to previously reported cases by Casal and Orós [12], Sykes and Klaphake [13] and Prieto-Torres et al. [37], and no hem parasites were noted [20]. It is worth noting that blood values can vary according to the season of the year, age class, sex, species, area, or if they are animals in migration or residents [15,23,33]. In this study, with the integration of the results obtained, and based on the Eco health model of conservation medicine [1], we could infer that the ecosystems of the Ojo de Liebre Lagoon provide the adequate environmental conditions for this type of organisms to develop the activities corresponding to their juvenile, sub-adult and adult stage of their life cycle.

Conclusion

It is necessary to know in detail the anatomy and basic morphology of marine turtles in order to identify clinical signs during the clinical exams.

Obtained results should not be taken as parameters or as prevalence or incidence data, since they do not calculate the disease rate.

We proposed to continue this kind of study with complementary analysis of blood chemistry, bacteriology, toxicology, parasitology, etc. in order to determine more completely the health status of the EPGT.

Regular health monitoring allows estimating future spread of transmissible diseases.

These studies also help us to infer the health of the ecosystem in the marine environment and leading to the development of strategically the conservation programs.

The good health of marine turtles, allows us to infer that the health status of Laguna Ojo de Liebre is suitable.

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