



Blood biochemistry reference values for nesting Kemp's ridley turtles (*Lepidochelys kempii*) in Rancho Nuevo Sanctuary, Mexico

Valeria Leal-Sepúlveda^a, Miguel Angel Reyes-López^b, Fátima Yedith Camacho-Sánchez^b, Héctor Hugo Acosta-Sánchez^c, Kevin Alan Zavala-Félix^a, Catherine E. Hart^d, Alan A. Zavala-Norzagaray^a, Renato Leal-Moreno^a, Brenda Aracely Espinoza-Romo^a, A. Alonso Aguirre^e, Juan Carlos Sainz-Henández^a, César P. Ley-Quinióñez^{a,*}

^a Instituto Politécnico Nacional, CIIDIR Sinaloa- Depto. Medio Ambiente, Lab. Vida Silvestre, Guasave, Sinaloa, Mexico

^b Instituto Politécnico Nacional, Centro de Biotecnología Genómica-Conservation Medicine Lab., Reynosa, Tamaulipas, Mexico

^c Programa de Conservación de Tortugas Marinas en el Santuario Playa de Rancho Nuevo, Terra Asesoría Ambiental S.C, Mexico

^d Centro de Investigaciones Oceánicas del Mar de Cortés - Gran Acuario Mazatlán, Av. de los Deportes, 111, Fracc. Tellería, CP 82017, Mazatlán, Sinaloa, Mexico

^e Department of Fish, Wildlife, and Conservation Biology, Warner College of Natural Resources, Colorado State University, Fort Collins, CO, 80533, USA

1. Introduction

Anthropogenic activities, such as coastal development, landfills and oil spills, have resulted in the loss and degradation of habitats that have exposed sea turtles to pathogens such as bacteria, fungi, parasites, and viruses. In turn, sea turtles spread diseases by being in contact with other populations and species (Reséndiz et al., 2019). Likewise, ocean contamination makes sea turtles susceptible to bioaccumulation of environmental pollutants such as heavy metals and organochlorines that are additively affecting sea turtle populations' health (Gámez-Vivaldo et al., 2009). Implementing a health assessment program for sea turtles is a fundamental research and management plan component; such a plan should incorporate specific strategies under the broad heading of health and disease intended to enhance recovery and prevent further decline of a species (Aguirre and Balazs, 2000; Arthur et al., 2008).

Physical examinations are often used to determine a sea turtle's health status. The examination includes the estimation of epibiotic load (Deem et al., 2009) and the body condition index (BCI), which allows to know the health condition related to the nutritional status and estimated stored energy of organisms (Labrada-Martagón et al., 2010). However, the establishment of reference values using biochemical parameters is an essential tool that, when combined with clinical examination, allows for diagnosing the health status of an organism or a population (Ley-Quinióñez et al., 2017; Mejía-Radillo et al., 2019; Whiting et al., 2007). However, the lack of blood reference values and the variability that may exist between geographic areas, ecological habitat, populations (Camacho et al., 2013; Montilla et al., 2008; Whiting et al., 2007), sexual maturity, reproductive status, migration, as well as diet (Anderson et al.,

2011; Flint et al., 2010) limits the use of these tools. Therefore, it is essential to consider population and geography when establishing health parameters (Aguirre and Balazs, 2000; Arthur et al., 2008). This is particularly essential for endangered species since the information must be included in management plans and Campo conservation strategies (Ley-Quinióñez et al., 2017).

Kemp's ridley turtles (*Lepidochelys kempii*) are subjected to risks such as pollution, fishing, and other anthropogenic activities in the Gulf of Mexico and listed as the most endangered sea turtle on the IUCN Red List (Wibbels and Bevan, 2019). Nearly all nests are found on GOM beaches, with 90% of the population nesting in Rancho Nuevo Sanctuary (RNS), Tamaulipas, Mexico (Lara-Uc and Mota-Rodríguez, 2014a; Márquez, 1994). Their distribution coincides with an area of heavy oil exploration and extraction, where recent spills have had high ecological impacts (Campagna et al., 2011), such as the Ixtoc I oil spill, from June 1979 to March 1980 (Jernelöv and Olof, 1981), the Deepwater Horizon oil spill from April 2010 to August 2010 (Gallaway et al., 2016b) and the Taylor oil spill from September 2004 to present (O'Reilly, 2020). At present, the levels at which chemical contaminants can become harmful to the health of sea turtles are unknown since they present a high physiological sensitivity to small concentrations of pollutants (Cortés-Gómez et al., 2017). Therefore, it is a priority to establish blood biochemistry values to establish preventative, control, or treatment programs for sea turtles (Gámez-Vivaldo et al., 2009).

Our study aimed to establish the blood biochemical reference values for the reproductively active adult female Kemp's ridley turtles nesting at RNS. These values provide indicators that allow health to be monitored and evaluated to allow for the assessment of disease patterns in the

* Corresponding author.

E-mail address: cleyq@ipn.mx (C.P. Ley-Quinióñez).

<https://doi.org/10.1016/j.ecss.2023.108488>

Received 14 March 2023; Received in revised form 2 September 2023; Accepted 6 September 2023

Available online 7 September 2023

0272-7714/Published by Elsevier Ltd.

population contributing to species conservation.

2. Materials and methods

2.1. Sample collection

The collection of blood samples from nesting turtles was performed at RNS, Tamaulipas, located in the Gulf of Mexico and Coastal Plain region of the Mexican state of Tamaulipas (23° 18' 10" N – 97° 45' 40" W and 23° 10' 10" N – 97° 45' 30" W), the primary nesting area for the species (Lara-Uc and Mota-Rodríguez, 2014b; Márquez, 1994).

Samples were collected when the nesting female was in a trance while laying her eggs (Deem et al., 2006). Blood extraction was performed using the puncture technique of the dorsal cervical sinuses (Owens and Ruiz, 1980). Between 5 and 8 ml of blood were collected using a 10 ml syringe with a 21-gauge needle (Sikes IV and Klaphake, 2008), then transferred to a 10 ml Vacutainer® tube with lithium heparin as an anticoagulant agent for recovery of plasma and white blood cells. The samples were refrigerated at 4 °C for up to 5 h until laboratory processing (Sykes and Klaphake, 2015).

2.2. Morphometric data and physical evaluation

The turtle's morphometric data was taken at the end of oviposition, according to the methodology proposed by Bolten (2000). The biometrics considered were Curved Carapace Length (CCL), Straight Carapace Length (SCL) and Curved Carapace Width (CCW), taken using vernier calipers and fabric measuring tape, respectively. In addition, captured turtles were tagged on the left front flipper with a Monel (National Band and Tag Company) alloy tag for capture, recapture, and sighting reports (Balazs, 2000). In addition, the turtle's physical condition was evaluated according to their plastron, classifying them as Good if the plastron was convex, Acceptable if it was flat, and Poor if it was concave (Thomson et al., 2009). In turn, a physical evaluation was performed, classifying them as healthy, without apparent diseases, injured if they had epibionts, external lesions on the skin, carapace, or both. Not physically evaluated turtles were categorized as "no data" (Labrada-Martagón et al., 2010).

2.3. Blood biochemistry

For the separation of plasma, white blood cells and red blood cells, the samples with anticoagulant were centrifuged at 3200 rpm for 15 min (Ley-Quinónez et al., 2017) in a COMPACT II centrifuge model 420225 (Becton Dickinson).

Plasma was collected in 1.6-ml cryovials and subsequently analyzed 500 µL using an *Alinity ci* integrated clinical biochemistry and immunoassay system (ABBOTT diagnostics), according to the supplier's specifications to analyze the parameters divided into nutrients and metabolites: Total Protein (TP) (g/dL⁻¹), Albumin (ALB) (g/dL⁻¹), Globulin (GLOB) (g/dL⁻¹), Albumin/Globulin (A/G) ratio, Creatinine (mg/dL⁻¹), Total bilirubin (mg/dL⁻¹), Blood urea nitrogen (BUN) (mg/dL⁻¹), Glucose (GLU) (mg/dL⁻¹), Cholesterol (CHOL) (mg/dL⁻¹), and Triglycerides (TRIG) (mg/dL⁻¹), enzymes: Alanine Aminotransferase (ALT) (U/L⁻¹), Aspartate Aminotransferase (ASP) (U/L⁻¹), Alkaline Phosphatase (ALP) (U/L⁻¹), Creatinine Phosphokinase (CK) (U/L⁻¹), Gamma-Glutamyl Transpeptidase (GGT) (U/L⁻¹), Amylase (AMYL) (U/L⁻¹), and Electrolytes: Calcium (Ca) (mg/dL⁻¹), Phosphorus (P) (mg/dL⁻¹), Sodium (Na) (mg/dL⁻¹) analysis was performed using an ARCHITECT ci8200 analyzer (ABBOTT diagnostics).

2.4. Statistical analysis

The results were presented as arithmetic means and standard deviation (Mean ± SD), followed by the minimum and maximum values. The data normality and homoscedasticity for each variable (morphometric

data and blood biochemistry) were evaluated using the Kolmogorov-Smirnov and Levene tests. One-way analysis of variance (ANOVA) ($\alpha = 0.05$) and Tukey's multiple comparison tests were used to determine if there were significant differences in blood parameters between variables (morphometric data and year). Finally, a simple regression model ($R^2 > 50\%$) was used to determine correlations between variables (morphometric data and the measured blood parameters per year). All analyses are performed using Minitab® 18 statistical software with a confidence level of 95% ($\alpha \leq 0.05$).

2.5. Ethics statement

The study was conducted following the guidelines of the Mexican authorities to study and manage wildlife samples or species under SEMARNAT (Secretary on Environment and Natural Resources) permit numbers SGPA/DGVS/04674/10 and SGPA/DGVS/003769/18 and approved by the National Commission of Protected Natural Areas (CONANP).

3. Results

During the 2020 and 2021 nesting seasons, 50 blood samples were collected from nesting turtles in Rancho Nuevo, Tamaulipas, Mexico, 30 of 2020 and 20 of 2021. There were no recaptures during the study. The size and health parameters of nesting turtles *L. kempii* were summarized in Table 1.

The sampled individuals presented good general health, without external fibropapillomas, deformities, or injuries and low or null epibiotic load. Unfortunately, we could not weigh the nesting females due to regulations at RNS; consequently, we could not calculate the turtle's body condition index.

Nesting turtles had a mean SCL of 60.39 ± 2.86 cm (55.74–65.74 cm). It was observed that the average size of turtles in 2021 was greater than that of nesters in 2020 ($p = 0.003$) (Table 1).

Nineteen blood parameters were analyzed, including the concentration of proteins, electrolytes, lipids, excretion products, enzyme activity, glucose, and inorganic phosphorus (Table 1).

Significant differences were observed in four biochemical values by year (Table 1). Nesting turtles from the 2020 season had significantly higher levels of globulin (GLOB), blood urea nitrogen (BUN), and amylase (AMYL) and lower levels of AST compared to nesters from 2021 ($p < 0.05$). In addition, Creatinine, Alanine aminotransferase (ALT), and Gamma-glutamyl transpeptidase (GGT) values were below the detection limits of the assay kit.

No significant associations were observed regarding the relationship between size and biochemical values ($R^2 < 50\%$). In contrast, by season, the nesting turtles of 2020 presented a positive correlation of the CCL vs GLOB ($R^2 = 1$) and vs A/G ratio ($R^2 = 0.9947$) (Fig. 1).

4. Discussion

According to Márquez (1996) and Lara-Uc and Mota-Rodríguez (2014b), nesting Kemp's ridley turtles from RNS present sizes of 60–65 cm, and young nesting turtles recently recruited at the area (first nesting) have a mean length of 61.8 ± 1.8 cm (Caillouet et al., 2011). The nesting turtles in the present study mainly comprised smaller nesting females (60.39 ± 2.86 cm). These sizes were smaller than those previously reported by Wang (2005), who observed an SCL of 65 ± 3.3 cm in nesting turtles, corresponding to mature nesting turtles. Galloway et al. (2016a) found that the Deepwater Horizon oil spill affected 34.5% of Kemp's ridley turtles in 2010. Therefore, recruitment occurs in neritic areas and nesting beaches, with these turtles hatched after 2010 (Caillouet Jr, 2019).

Regarding nesting turtle size by season, turtles nesting in 2020 were primarily composed of young, first-time nesting turtles, while in 2021, we saw significantly larger nesting turtles. This difference may result

Table 1Morphometric and blood biochemistry values of Kemp's ridley turtles (*Lepidochelys kempii*) from a nesting area in Rancho Nuevo, Tamaulipas, Mexico, 2020–2021.

Parameter	Total (n = 50)	2020 (n = 30)	2021 (n = 20)	Statistical test
SCL (cm)	60.39 ± 2.86 (55.74–65.74)	59.55 ± 2.98 ^b (55.74–69.74)	62.19 ± 1.47 ^a (59.30–64.50)	<i>p</i> = 0.003
Total protein (g/dL ⁻¹)	3.12 ± 0.76 (2.02–4.48)	3.058 ± 0.810 (2.04–4.48)	3.220 ± 0.700 (2.020–4.480)	<i>p</i> = 0.471
Albumin (g/dL ⁻¹)	1.08 ± 0.34 (0.51–1.80)	1.106 ± 0.400 (0.51–1.80)	1.050 ± 0.243 (0.550–1.380)	<i>p</i> = 0.577
Globulin (g/dL ⁻¹)	1.72 ± 0.81 (0.24–3.90)	1.95 ± 0.93 ^a (0.24–3.90)	1.376 ± 0.430 ^b (0.670–2.290)	<i>p</i> = 0.013
A/G ration	0.93 ± 1.11 (0.13–7.50)	0.95 ± 1.39 (0.13–7.50)	0.928 ± 0.476 (0.210–1.580)	<i>p</i> = 0.935
Total bilirubin (mg/dL ⁻¹)	0.13 ± 0.017 (0.11–0.16)	0.14 ± 0.02 (0.11–0.16)	0.133 ± 0.014 (0.110–0.160)	<i>p</i> = 0.148
Creatinine (mg/dL ⁻¹)	ND	ND	ND	
BUN (mg/dL ⁻¹)	9.23 ± 0.97 (7.45–10.96)	9.53 ± 1.05 ^a (7.45–10.96)	8.777 ± 0.653 ^b (7.560–9.550)	<i>p</i> = 0.006
Glucose (mg/dL ⁻¹)	76.12 ± 6.33 (61.83–87.00)	76.53 ± 6.55 (64.00–87.00)	75.51 ± 6.11 (61.83–85.63)	<i>p</i> = 0.580
Cholesterol (mg/dL ⁻¹)	217.42 ± 49.55 (130.12–299.70)	216.50 ± 51.87 (134.60–299.70)	218.8 ± 47.1 (130.1–286.4)	<i>p</i> = 0.874
Triglycerides (mg/dL ⁻¹)	68.36 ± 16.95 (36.6–93.00)	69.75 ± 16.33 (36.60–93.00)	66.28 ± 18.08 (36.26–88.48)	<i>p</i> = 0.484
ALKP (U/L ⁻¹)	28.57 ± 5.14 (20.14–38.31)	28.24 ± 4.72 (20.26–35.00)	29.07 ± 5.80 (20.14–38.31)	<i>p</i> = 0.578
ALT (U/L ⁻¹)	ND	ND	ND	
AST (U/L ⁻¹)	17.33 ± 1.56 (14.23–19.74)	16.96 ± 1.63 ^b (14.23–19.74)	17.899 ± 1.27 ^a (16.06–19.74)	<i>p</i> = 0.036
GGT (U/L ⁻¹)	ND	ND	ND	
CK (U/L ⁻¹)	187.9 ± 85.4 (29.0–344.1)	172.70 ± 101.00 (29.00–344.10)	210.8 ± 48.3 (132.6–284.9)	<i>p</i> = 0.123
AMYL (U/L ⁻¹)	254.66 ± 52.86 (162.00–345.29)	268.7 ± 56.0 ^a (162.00–345.30)	233.64 ± 40.59 ^b (165.55–287.16)	<i>p</i> = 0.020
Calcium (mg/dL ⁻¹)	7.48 ± 0.87 (6.06–9.00)	7.420 ± 0.901 (6.06–9.00)	7.57 ± 0.85 (6.290–8.860)	<i>p</i> = 0.541
Phosphorus (mg/dL ⁻¹)	7.26 ± 0.45 (6.56–8.00)	7.33 ± 0.46 (6.57–8.00)	7.174 ± 0.453 (6.560–7.820)	<i>p</i> = 0.243
Sodium (mg/dL ⁻¹)	145.44 ± 3.55 (132.83–149.98)	146.13 ± 2.91 (140.20–149.90)	144.40 ± 4.21 (132.83–149.98)	<i>p</i> = 0.090

Statistics data in Mean ± SD (min-max), SCL: Straight caparace length, A/G = Albumin/Globulin, BUN = Blood urea nitrogen, AMYL = Amylase, ALKP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutamyl transpeptidase, CK = Creatine phosphokinase. ND = Not detected. Standard deviation (SD), Minimum (min), Máximo (max). Differing letters indicate significant difference between groups (*p* ≤ 0.05). Statistical test: ANOVA.

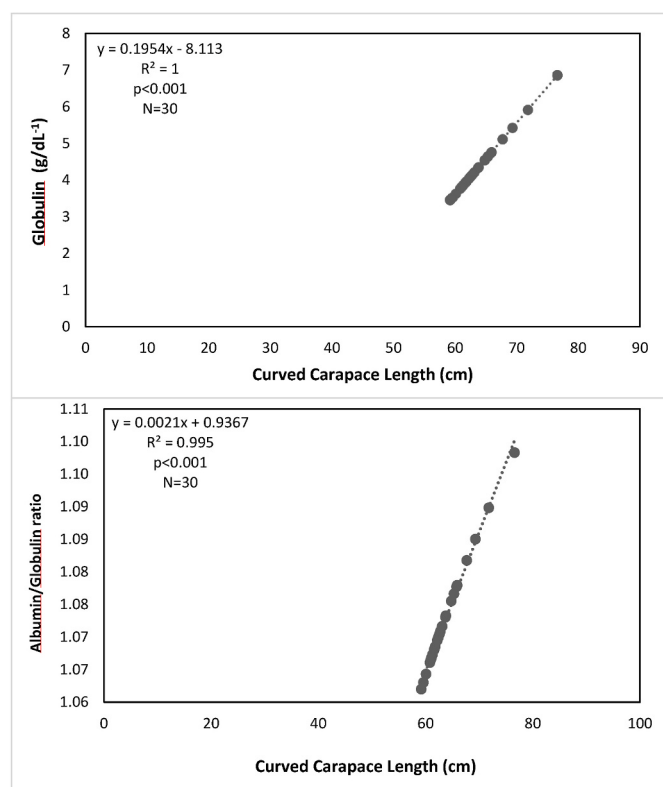


Fig. 1. Relationship between Curved Carapace Length (cm) vs Globulin (g/dL⁻¹) and Albumin/Globulin ratio for the nesting Kemp's ridley sea turtles (*Lepidochelys kempii*) from Rancho Nuevo, Mexico, 2020.

from different population cohorts nesting each season since the mean remigration interval of the species' nesting is two years. However, it also occurs at one- and three-year intervals (US Fish and Wildlife Service, 2018).

Sea turtle health parameters are population-specific and can vary between populations and by a turtle's life stage and sex. Therefore, reference values must be generated for each geographic area,

considering the turtles habitat type, diet, individual condition, size, sex, and health status. In addition, sample handling, species, reproductive status, and contamination can cause variations in parameters (Camacho et al., 2013; Flint et al., 2010; Labrada-Martagón et al., 2010; Montilla et al., 2008; Whiting et al., 2007).

No blood biochemical reference values exist for nesting turtles in RNS, even though 90% of Kemp's ridley clutches occur on these beaches (Lara-Uc and Mota-Rodríguez, 2014b; Márquez, 1994; Polidoro et al., 2008; SEMARNAT, 2011). Despite Kemp's ridley being the sea turtle species at greatest risk of extinction (Polidoro et al., 2008; SEMARNAT, 2011), blood biochemistry is regarded as an essential tool for assessing the health status of individuals. Despite this, the existing biochemistry studies for the species have focused on turtles from foraging areas (Anderson et al., 2011; Innis et al., 2008; Snoddy et al., 2009).

The blood biochemical values of nesting Kemp's ridley turtles obtained in this study were similar to those reported for the species in other life stages and males studied in foraging areas (Table 2). Although other species of sea turtles, such as green turtles (*Chelonia mydas*), present different blood biochemistry values depending on their age and diet (Anderson et al., 2011; Labrada-Martagón et al., 2010), this appears not to be the case for Kemp's ridleys. The difference observed in some blood parameters between the foraging and nesting areas, such as GLU, CHOL, TRIG, ALB, GLOB, BUN, AST, CK and AMYL, could be the result of diet and capture method, as well as the nesting process (Anderson et al., 2011; Perrault et al., 2020; Snoddy et al., 2009).

We found low levels of BUN and GLU compared to those reported for turtles from different life stages captured in foraging areas (Perrault et al., 2020; Snoddy et al., 2009). However, blood biochemistry values decrease during the sea turtle nesting process due to stress and loss of energy, as well as the fasting state (Honarvar et al., 2011) present in females as they reduce foraging during mating and nesting (Ehsanpour et al., 2015; Santillana-Segovia, 2013).

Similarly, the low levels of total protein (TP) and ALB found in nesting turtles when compared to turtles on foraging grounds were previously described in loggerhead turtles (*Caretta caretta*) by Kakizoe et al. (2013), who found that the concentrations of TP and ALB in nesting loggerheads increased between winter and spring but decreased during the nesting period; likewise, lipids presented seasonal changes as did TP. In addition, TRIG increased from winter until the nesting season, which is related to using these nutrients during folliculogenesis.

Table 2

Reference values for biochemistry for different populations of sea turtles *Lepidochelys kempii* clinically healthy.

PARAMETER	Tamaulipas, Mexico Nesting Area (<i>This Study</i>)	USA Adults Foraging Area (Snoddy et al., 2009)	USA Juveniles Foraging Area (Anderson et al., 2011)	USA Juveniles Foraging Area (Perrault et al., 2020)
Sample (n)	50	4	10	34
Total protein (g/dL ⁻¹)	3.12 ± 0.76	3.1	3.7	38 ± 6
Albumin (g/dL ⁻¹)	1.08 ± 0.34	1.3	6.1	7.8 ± 1.7
Globulin (g/dL ⁻¹)*	1.72 ± 0.813	1.8	4.3	27.6 ± 5.0
A/G ration	0.93 ± 1.108	NA	NA	0.37 ± 0.07
Total bilirubin (mg/dL ⁻¹)	0.13 ± 0.017	NA	NA	NA
Creatinine (mg/dL ⁻¹)	ND	NA	NA	NA
BUN (mg/dL ⁻¹)*	9.23 ± 0.97	73.7	NA	71.6 ± 4.8
Glucose (mg/dL ⁻¹)	76.12 ± 6.33	115.2	-1.8	122.51 ± 1.1
Cholesterol (mg/dL ⁻¹)	217.42 ± 49.55	NA	5.3	1027.78 ± 0.5
Triglycerides (mg/dL ⁻¹)	68.36 ± 16.95	NA	8.2	96.25 ± 0.5
ALKP (U/L ⁻¹)	28.57 ± 5.14	NA	4.5	119 ± 54
ALT (U/L ⁻¹)	ND	NA	5.0	NA
AST (U/L ⁻¹)*	17.33 ± 1.56	144.7	5.5	185 ± 51
GGT (U/L ⁻¹)	ND	NA	NA	NA
CK (U/L ⁻¹)	187.9 ± 85.4	NA	6.2	1513 ± 865
AMYL (U/L ⁻¹)*	254.66 ± 52.86	NA		461 ± 86
Calcium (mg/dL ⁻¹)	7.48 ± 0.87	7.4	-2.6	9.62 ± 0.2
Phosphorus (mg/dL ⁻¹)	7.26 ± 0.45	6.8	2.3	8.62 ± 0.4

A/G = Albumin/Globulin, BUN = Blood urea nitrogen, AMYL = Amylase, ALKP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma-glutamyl transpeptidase, CK = Creatine phosphokinase. Standard deviation (SD). ND = Not detected. NA= Not Analyzed.

In the present study, the levels of electrolytes observed in nesting turtles, including Ca, K, P and Na, are similar to those previously reported for adult Kemp's ridley turtles in foraging areas (Snoddy et al., 2009). The levels of Ca and P are elevated during the reproductive period in nesting turtles since Ca is mobilized for the formation of eggs (Deem et al., 2006; Goldberg et al., 2011) and tends to decrease with each nest-laid during the season, which ranges between two and three nests per female (Shaver et al., 2016a; US Fish and Wildlife Service, 2018). Blood samples were taken during the first arribada mass nesting event of the season, which may account for the turtles not presenting a significant depletion in the levels of Ca and P.

Lipids TRIG and CHOL concentrations were higher in nesting Kemp's ridleys than for individuals in foraging areas. This coincides with Espinoza-Romo et al. (2018), who found lower concentrations of TRIG and CHOL in foraging areas than at nesting sites. Increases in these parameters in *L. kempii* during the reproductive season are due to vitellogenesis and folliculogenesis (Casal et al., 2009; Hamann et al., 2003; Santoro and Meneses, 2007) and are consistent with the observed in other species of sea turtles (Deem et al., 2009; Prieto-Torres et al., 2013; Santoro and Meneses, 2007).

Enzymes such as ALP, AST, GGT, CK, and AMYL are good indicators of health because an increase in these enzymes in the blood indicates liver or muscle damage in sea turtles (Anderson et al., 2013; Martínez-Silvestre et al., 2013). However, when interpreting the results, it is essential to consider factors such as the location of turtles nesting and

foraging areas. For example, in the present study, CK values were lower than those of Innis et al. (2008) and Anderson et al. (2011) in *L. kempii* in foraging areas. Such differences could result from stress during capture on foraging grounds. At the same time, in nesting areas, a cavity was made in the sand below the individual's head to increase blood flow through the dorsocervical sinuses, facilitating the sample collection and avoiding undue stress to the turtle (Montilla et al., 2008).

Our study found lower concentrations of ALT, AST, ALP and CK than those reported previously in the species in foraging areas (Anderson et al., 2011; Perrault et al., 2020; Snoddy et al., 2009). Ehsanpour et al. (2015) mentions that in nesting sea turtles, the values of ALP, AST, TP, CK and AMYL increase due to vitellogenesis, while in foraging areas, there is an increase in the levels of ALT and AST enzymes due to their metabolic role during the transformation of ingested food into energy. Therefore, the high levels of these enzymes observed in previous studies result from turtles in foraging areas. It should be noted that in the case of CK, Snoddy et al. (2009) reported that the levels of this enzyme can increase in turtles depending on the time of capture and the method used.

In 2020 a more heterogeneous population was composed of a larger number of small, unmarked females, which suggests that they were first-time nesters, while in 2021, fewer of these smaller unmarked individuals were observed. Therefore, the differences in the blood parameters per year are related to the difference in sizes between nesting years, which is consistent with the positive correlations observed between carapace length (SCL) and GLOB levels and the A/G ratio for the 2020 females. The furthermore, Goldberg et al. (2011) observed a positive relationship between SCL and SCW with enzymes such as ALP and AST, attributing it to larger turtles having greater enzymatic activity. Likewise, Santillana-Segovia (2013) mentions that parameters such as GLU, CHOL, BUN, Creatinine, AST, ALT, and TBIL positively correlated with the CCL. This was also observed by Whiting et al. (2014) and Ley-Quinóñez et al. (2017), who reported that the values of Creatinine, TBIL, ALP, CK, ALB, GLOB, Ca, P and Fe correlate positively with CCL.

Although the present study was carried out exclusively on nesting female Kemp's ridley turtles, Pinto et al. (2015) reported that there were no significant differences by sex for blood parameters, and this is consistent with what was observed in other sea turtle species, populations and foraging and nesting areas (Casal et al., 2009; Ehsanpour et al., 2015; Prieto-Torres et al., 2013; Santoro and Meneses, 2007; Wrobel et al., 2011). That said, CHOL, TRIG, and Ca parameters tend to be more prevalent in nesting females compared to males as these are related to the vitellogenesis during nesting ((Deem et al., 2009; Espinoza-Romo et al., 2018; Hamann et al., 2003; Hamann et al., 2002).

5. Conclusions

The nesting population of Kemp's ridley turtles observed during this study presented both first-time nesters and more mature females. This is encouraging as the recruits correspond to turtles born after the 2010 Deepwater Horizon oil spill, which caused the loss of large numbers of turtles.

Our study is the first to establish blood biochemical parameters of nesting kemp's ridley turtles from RNS, the main nesting area for this critically endangered species. These values can be used as blood reference intervals for a healthy population, and this information will serve as a reference for the health and monitoring of nesting Kemp's ridley turtles.

CRedit authorship contribution statement

Valeria Leal-Sepúlveda: Writing – original draft, Investigation, Conceptualization. **Miguel Angel Reyes-López:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Fátima Yedith Camacho-Sánchez:** Methodology, Investigation. **Héctor Hugo Acosta-Sánchez:**

Methodology, Investigation. **Kevin Alan Zavala-Félix:** Methodology, Investigation. **Catherine E. Hart:** Writing – review & editing. **Alan A. Zavala-Norzagaray:** Methodology, Investigation. **Renato Leal-Moreno:** Methodology, Investigation. **Brenda Aracely Espinoza-Romo:** Methodology, Investigation. **A. Alonso Aguirre:** Writing – review & editing, Supervision, Investigation. **Juan Carlos Sainz-Henández:** Writing – review & editing, Supervision, Project administration. **César P. Ley-Quinóñez:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank the community of Rancho Nuevo Tamaulipas, Mexico and especially J. Martínez and M. López, director of CONANP Tamaulipas, for their support. We want to acknowledge The State of the World's Sea Turtles Program (SWOT), in partnership with the Association of Zoos & Aquariums and its Sea Turtle SAFE (Save Animals from Extinction) Program to target priority sea turtle populations, for supporting this study (SWOT Grants 2022).

References

- Aguirre, A.A., Balazs, G.H., 2000. Blood biochemistry values of Green Turtle, *Chelonia mydas*, with and without fibropapillomatosis. *Comp. Hematol. Int.* 10, 132–137.
- Anderson, E.T., Minter, L.J., Clarke 3rd, E.O., Mroch 3rd, R.M., Beasley, J.F., Harms, C. A., 2011. The Effects of Feeding on Hematological and Plasma Biochemical Profiles in Green (*Chelonia mydas*) and Kemp's Ridley (*Lepidochelys Kempii*) Sea Turtles. *Veterinary Medicine International*, 890829.
- Anderson, E.T., Socha, V.L., Gardner, J., Byrd, L., Manire, C.A., 2013. Tissue enzyme activities in the loggerhead sea turtle (*Caretta caretta*). *J. Zoo Wildl. Med. : off. publ. Am. Assoc. Zoo Vet. Annu.* 44, 62–69.
- Arthur, K., Limpus, C., Whittier, J., 2008. Baseline blood biochemistry of Australian green turtles (*Chelonia mydas*) and effects of exposure to the toxic cyanobacterium *Lyngbya majuscula*. *Aust. J. Zool.* 56.
- Balazs, G.H., 2000. Factores a considerar en el marcado de tortugas marinas. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas Grupo Especialista en Tortugas Marinas*. UICN/CSE D.C., pp. 116–125.
- Bolten, A.B., 2000. Técnicas para la medición de tortugas marinas. In: Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., Donnelly, M. (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas. Grupo Especialista en Tortugas Marinas*. UICN/CSE, D.C., pp. 126–131.
- Caillouet Jr., 2019. Excessive annual numbers of neritic immature Kemp's ridleys may prevent population recovery. *Mar. Turt. Newsl.* 158, 2.
- Caillouet Jr., C.W., Shaver, D.J., Landry, A.M., Owens, D.W., Pritchard, P.C.H., 2011. Kemp's ridley Sea Turtle (*Lepidochelys kempii*) age at first nesting. *Chelonian Conserv. Biol.* 10, 288–293.
- Camacho, M., Oros, J., Boada, L.D., Zaccaroni, A., Silvi, M., Formigaro, C., Lopez, P., Zumbado, M., Luzardo, O.P., 2013. Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): results from a nesting colony from Cape Verde, West Africa. *Mar. Environ. Res.* 92, 15–22.
- Campagna, C., Short, F.T., Polidoro, B.A., McManus, R., Collette, B.B., Pilcher, N.J., Sadovy de Mitcheson, Y., Stuart, S.N., Carpenter, K.E., 2011. Gulf of Mexico oil blowout increases risks to globally threatened species. *Bioscience* 61, 393–397.
- Casal, A.B., Camacho, M., Lopez-Jurado, L.F., Juste, C., Oros, J., 2009. Comparative study of hematologic and plasma biochemical variables in Eastern Atlantic juvenile and adult nesting loggerhead sea turtles (*Caretta caretta*). *Vet. Clin. Pathol./Am. Soc. Vet. Clin. Pathol.* 38, 213–218.
- Cortés-Gómez, A.A., Romero, D., Girondot, M., 2017. The current situation of inorganic elements in marine turtles: a general review and meta-analysis. *Environ. Pollut.* 229, 567–585.
- Deem, S.L., Dierenfeld, E.S., Sounguet, G.P., Alleman, A.R., Cray, C., Poppenga, R.H., Norton, T.M., Karesh, W.B., 2006. Blood values in free-ranging nesting leatherback sea turtles (*Dermochelys coriacea*) on the coast of the Republic of Gabon. *J. Zoo Wildl. Med. : off. publ. Am. Assoc. Zoo Vet. Annu.* 37, 464–471.
- Deem, S.L., Norton, T.M., Mitchell, M., Segars, A., Alleman, A.R., Cray, C., Poppenga, R. H., Dodd, M., Karesh, W.B., 2009. Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (*Caretta caretta*) along the coast of Georgia, USA. *J. Wildl. Dis.* 45, 41–56.
- Ehsanpour, M., Ahmadi, M.R., Bahri, A.H., Afkhami, M., Reich, K.J., 2015. Plasma biochemistry values in wild female hawksbill turtles (*Eretmochelys imbricata*), during nesting and foraging seasons in Qeshm Island, Persian Gulf. *Comp. Clin. Pathol.* 24, 561–566.
- Espinoza-Romo, B.A., Sainz-Hernandez, J.C., Ley-Quinonez, C.P., Hart, C.E., Leal-Moreno, R., Aguirre, A.A., Zavala-Norzagaray, A.A., 2018. Blood biochemistry of olive ridley (*Lepidochelys olivacea*) sea turtles foraging in northern Sinaloa, Mexico. *PLoS One* 13, e0199825.
- Fish, US, Wildlife Service, 2018. Species Status Assessment Report, pp. 1–104.
- Flint, M., Morton, J.M., Limpus, C.J., Patterson-Kane, J.C., Mills, P.C., 2010. Reference intervals for plasma biochemical and hematologic measures in loggerhead sea turtles (*Caretta caretta*) from Moreton Bay, Australia. *J. Wildl. Dis.* 46, 731–741.
- Gallaway, B.J., Gazey, W.J., Wibbels, T., Bevan, E., Shaver, D.J., George, J., 2016a. Evaluation of the status of the Kemp's ridley Sea Turtle after the 2010 deepwater Horizon oil spill. *Gulf Mex. Sci.* 33, 192–205.
- Gallaway, B.J., Gazey, W.J., Wibbels, T., Bevan, E., Shaver, D.J., George, J., 2016b. Evaluation of the status of the Kemp's ridley sea turtle after the 2010 Deepwater Horizon oil spill. *Gulf Mex. Sci.* 33.
- Gómez-Vivaldo, S., García-Márquez, L.J., Osorio-Sarabia, D., Vázquez-García, J.L., Constantino-Casas, F., 2009. Patología de las tortugas marinas (*Lepidochelys olivacea*) que arribaron a las playas de Cuyutlán, Colima, México. *Vet. Acta* 40, 69–78.
- Goldberg, D.W., Wanderlinde, J., Freire, I.M.A., da Silva, L.C.P., Almosny, N.R.P., 2011. Serum biochemistry profile determination for wild loggerhead sea turtles nesting in Campos dos Goytacazes, Rio de Janeiro, Brazil. *Ciência Rural* 41, 143–148.
- Hamann, M., Limpus, C.J., Whittier, J.M., 2002. Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). *J. Comp. Physiol. B* 172, 485–493.
- Hamann, M., Limpus, C.J., Owens, D.W., 2003. Reproductive cycles of males and females. In: Lutz, P.L., Musick, J.A., Wyneken, J. (Eds.), *The Biology of Sea Turtles*. CRC Press, Boca Raton, pp. 135–198.
- Honarvar, S., Brodsky, M., Fitzgerald, D., Rosenthal, K., Hearn, G., 2011. Changes in plasma chemistry and reproductive Output of nesting leatherbacks. *Herpetologica* 67, 222–235.
- Innis, C., Thurst, M., Perkins, C., Holladay, S., Merigo, C., Weber, E.S., 2008. Trace metal and organochlorine pesticide concentrations in cold-stunned juvenile Kemp's ridley turtles (*Lepidochelys kempii*) from cape cod, Massachusetts. *Chelonian Conserv. Biol.* 7, 230–239.
- Jernelöv, A., Olof, L., 1981. Ixtoc I: a case study of the World's largest oil spill. *Ambio* 10, 299–306.
- Kakizoe, Y., Sakaoka, K., Akune, Y., Kanou, Y., Saito, T., Uchida, I., 2013. Change of plasma chemistry values in captive breeding loggerhead turtles (*Caretta caretta*). *ISRN Zool.* 2013, 1–7.
- Labrada-Martagón, V., Méndez-Rodríguez, L.C., Gardner, S.C., Cruz-Escalona, V.H., Zenteno-Savín, T., 2010. Health indices of the green turtle (*Chelonia mydas*) along the pacific coast of Baja California Sur, Mexico. II. Body condition index. *Chelonian Conserv. Biol.* 9, 173–183.
- Lara-Uc, M., Mota-Rodríguez, C., 2014a. Conociendo a la Tortuga lora (*Lepidochelys kempii*) (Garman, 1880). *BIOMA, La naturaleza en tus manos* 39–46.
- Lara-Uc, M.M., Mota-Rodríguez, C., 2014b. Conociendo a la Tortuga Lora (*Lepidochelys kempii*) (Garman, 1880). *BIOMA, La naturaleza en tus manos*.
- Ley-Quinóñez, C.P., Rossi-Lafferriere, N.A., Espinosa-Carreón, T.L., Hart, C.E., Peckham, S.H., Aguirre, A.A., Zavala-Norzagaray, A.A., 2017. Associations between trace elements and clinical health parameters in the North Pacific loggerhead sea turtle (*Caretta caretta*) from Baja California Sur, Mexico. *Environ. Sci. Pollut. Res. Int.*
- Márquez, R., 1994. Synopsis of Biological Data on the Kemp's Ridley Turtle, *Lepidochelys Kempii* (Garman, 1880). *NOAH Technical Memorandum NMFS-SEFSC-343*, 91–91.
- Márquez, M.R., 1996. Las Tortugas Marinas Y Nuestro Tiempo. Fondo de Cultura Económica, México D.F.
- Martínez-Silvestre, A., Lavin, S., Cuenca, R., 2013. La bioquímica sanguínea en clínica de reptiles. *Consult. Difusión Vet.* 200, 31–40.
- Mejía-Radillo, R.Y., Zavala-Norzagaray, A.A., Chavez-Medina, J.A., Aguirre, A.A., Escobedo-Bonilla, C.M., 2019. Presence of chelonid herpesvirus 5 (ChHV5) in sea turtles in northern Sinaloa, Mexico. *Dis. Aquat. Org.* 132, 99–108.
- Montilla, A.J., Hernandez, J.L., Bravo, A.R., Vera, V.J., Mesa, J.F., 2008. Blood biochemistry values of green turtle (*Chelonia mydas*) present in the Alta Guajira. *Venezuela Rev. Cient. Fac. Cien. V* 18, 351–357.
- O'Reilly, C.L., 2020. The taylor energy oil spill: implications for marine oil spill science and policy. In: Department of Earth, Ocean and Atmospheric Science. FLORIDA STATE UNIVERSITY, p. 106.
- Owens, D.W., Ruiz, G.J., 1980. New methods of obtaining blood and cerebrospinal-fluid from marine turtles. *Herpetologica* 36, 17–20.
- Perrault, J., Arendt, M., Schwenter, J., Byrd, J., Harms, C., Cray, C., Tuxbury, K., Wood, L., Stacy, N., 2020. Blood analytes of immature Kemp's ridley sea turtles (*Lepidochelys kempii*) from Georgia, USA: reference intervals and body size correlations. *Conserv. Physiol.* 8 coaa091.
- Pinto, F.E., Buzin, A.R., Neto, E.P., Ferreira, G.B., Castheloge, V.D., Ferreira, P.D., Endringer, D.C., de Andrade, T.U., Lenz, D., 2015. A hematologic and biochemical profile on 3-month-old hatchlings of *Lepidochelys olivacea*. *Comp. Clin. Pathol.* 24, 1333–1337.

- Polidoro, B.A., Livingstone, S.R., Carpenter, K.E., Hutchinson, B., Mast, R.B., Pilcher, N., Sadovy de Mitcheson, Y., Valenti, S., 2008. Status of the World's Marine Species. IUCN, Gland, Switzerland.
- Prieto-Torres, D.A., Hernández, J.L., Henríquez, A.R.B., Alvarado, M.C., Dávila, M.J., 2013. Blood biochemistry of the breeding population of green turtles (*Chelonia mydas*) in the Aves Island wildlife refuge. Venezuela S Am. J. Herpetol. 8, 147–154.
- Reséndiz, E., Fernández-Sanz, H., Barrientos-Torres, D.S., Lara-Uc, M.M., 2019. Clinical pathology and health reference values for loggerhead sea turtles (*Caretta caretta*) and olive ridley turtles (*Lepidochelys olivacea*) in the Gulf of Ulloa, Baja California Sur, Mexico. Comp. Clin. Pathol. 28, 1637–1650.
- Santillana-Segovia, P.R., 2013. Valores hematológicos y bioquímicos sanguíneos de tortugas anidantes de Golfina (*Lepidochelys olivacea*) en El Salvador. Bioma, pp. 11–16.
- Santoro, M., Meneses, A., 2007. Haematology and plasma chemistry of breeding olive ridley sea turtles (*Lepidochelys olivacea*). Vet. Rec. 161, 818–819.
- SEMARNAT, 2011. Plan Binacional de Recuperación de la Tortuga Marina Lora (*Lepidochelys kempii*), p. 194.
- Shaver, D.J., Rubio, C., Shelby Walker, J., George, J., Amos, A.F., Reich, K., Jones, C., Shearer, T., 2016a. Kemp's ridley sea turtle (*Lepidochelys kempii*) nesting on the Texas coast: geographic, temporal, and demographic trends through 2014. Gulf Mex. Sci. 33, 4.
- Sikes IV, J.M., Klaphake, E., 2008. Reptile hematology. Vet. Clin. Exot. Anim. 11, 481–500.
- Snoddy, J.E., Landon, M., Blanvillain, G., Southwood, A., 2009. Blood biochemistry of Sea Turtles captured in gillnets in the lower cape fear river, North Carolina, USA. J. Wildl. Manag. 73, 1394–1401.
- Sykes, J.M.t., Klaphake, E., 2015. Reptile hematology. Clin. Lab. Med. 35, 661–680.
- Thomson, J.A., Burkholder, D., Heithaus, M.R., Dill, L.M., 2009. Validation of a rapid visual-assessment technique for categorizing the body condition of green turtles (*Chelonia mydas*) in the field. Copeia 2009 251–255.
- Wang, H-C., 2005 [Doctor of Philosophy thesis] Trace metal uptake and accumulation pathways in kemp's ridley sea turtles (*Lepidochelys kempii*), 2005. Texas A&M University, p. 63.
- Whiting, S.D., Guinea, M.L., Limpus, C.J., Fomiatti, K., 2007. Blood chemistry reference values for two ecologically distinct populations of foraging green turtles, eastern Indian Ocean. Comp. Clin. Pathol. 16, 109–118.
- Whiting, S.D., Guinea, M.L., Fomiatti, K., Flint, M., Limpus, C.J., 2014. Plasma biochemical and PCV ranges for healthy, wild, immature hawksbill (*Eretmochelys imbricata*) sea turtles. Vet. Rec. 174, 608.
- Wibbels, T., Bevan, E., 2019. *Lepidochelys Kempii*. The IUCN Red List of Threatened Species 2019 e.T11533A142050590.
- Wrobel, D., Wanderlinde, J., Alexandre, I., Pereira da Silva, L.C., Pereira, N.R., 2011. Serum Biochemistry Profile Determination for Wild Loggerhead Sea Turtles Nesting in Campos Dos Goytacazes, Rio de Janeiro, Brazil, vol. 41. Ciencia Rural, Santa Maria, pp. 143–148.

Further reading

- Shaver, D.J., Hart, K.M., Fujisaki, I., Rubio, C., Sartain-Iverson, A.R., Peña, J., Gamez, D. G., de Jesus Gonzales Diaz Miron, R., Burchfield, P.M., Martinez, H.J., Ortiz, J., 2016b. Migratory corridors of adult female Kemp's ridley turtles in the Gulf of Mexico. Biol. Conserv. 194, 158–167.
- Witzell, W.N., Schmid, J.R., 2005. Diet of immature Kemp's ridley turtles (*Lepidochelys kempi*) from Gullivan bay, ten thousand Islands, Southwest Florida. Bull. Mar. Sci. 77, 191–199.