Research Article

Blood Reference Intervals for Antillean Manatees (Trichechus manatus manatus) from Puerto Rico

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Antillean manatees (Trichechus manatus manatus) are endangered throughout the southwestern Gulf of Mexico, the Caribbean coast of Central and South America, the Greater Antilles, and the northeastern coast of South America to Brazil [1, 2]. It is an endangered marine mammal [2] protected by federal and local laws in their respective countries of origin. Its vulnerability is primarily due to anthropogenic causes of direct hunting, habitat degradation, and human encroachment, resulting in a decrease in population and continued threats to the species’ survival [3, 4]. As a result, conservation programs have been established to assist in the recovery of the populations, particularly addressing the rescue, treatment, rehabilitation, and release of orphaned calves and ill or injured manatees [5].

Establishing blood reference intervals is essential as a tool in classifying health status, diagnosing, determining treatment regimens, and monitoring the progress of a disease in rescued manatees [6]. However, blood reference ranges or values can differ among populations of the same species and vary according to age, sex, diet, environment, physiological conditions, and activity level. The hematology and blood chemistry of various Antillean manatee populations have been documented for Guyana [7], Mexico [8], Belize [9, 10], Brazil [11–14], and for the Florida subspecies (T. manatus latirostris) [6, 15–25]. However, this has not
been ascertained for Antillean manatees inhabiting the
Greater Antilles. Therefore, we sampled the population that
inhabits the coastal waters of Puerto Rico, seeking to es-
establish baseline complete blood cell count and serum
chemistry reference intervals for this population of Antillean
manatees.

2. Materials and Methods

2.1. Ethical Statement. The research has complied with all
the relevant national regulations and institutional policies
for the care and use of animals (Inter American University’s
Institutional Animal Care and Use Committee (IACUC)
#6Mar2018).

2.2. Sample Collection. We collected blood samples from 70
wild captured or rescued manatees from Puerto Rico from
1992 to 2020 (Table 1, Figure 1). Of these, 44 manatees were
captured for health assessment and radiotelemetry studies,
and 26 were primarily rescued as orphaned calves. Manatees
were caught in nets deployed either along the shore or from a
specialized net boat in open water [26] or rescued by hand in
the case of orphaned calves. The captured manatees were
immediately transported to a shaded area where they were
kept moist at all times with the use of a water mister, buckets,
and wet towels throughout the health assessment or brought
into the Caribbean Manatee Conservation Center for vet-
ery examination. Data collected included sex, complete
body measurements, tissue samples for genetic analysis, and
blood draw. In addition, an experienced manatee veterinary
and veterinary technician, with the aid of marine
biologists, conducted a complete physical examination in-
cluding, among many others: visual external and behavioral
assessment, heart rate, and respiratory rate [27] and other
vital signs [28]. All manatees were considered healthy at the
time of sampling. Manatees were categorized into 3 age
classes based on total length and ear bone growth layer
groups (GLGs) or known ages: calves (<175 cm, <2 years
old), subadults (176–225 cm, 2–7 years old), and adults
(>225 cm, >7 years old) [3]. Upon data and sample col-
lection, the manatees were immediately returned to the
water at their capture site, most fitted with a radio-trans-
mitter for tracking studies [26], or in the case of orphaned
calves, maintained in rehabilitation until completion of the
time prescribed for their rehabilitation process and then
released [29].

Blood samples were obtained by venipuncture from
the medial interosseous space of the radius and ulna,
which constitutes the brachial vascular bundle (Figure 2).
Before sample collection, the pectoral flipper was sur-
gically scrubbed with povidone-iodine and alcohol or
post-2017 with chlorhexidine scrub, chlorhexidine so-
lution, and isopropyl alcohol. An 18–21-gauge, ¾–1½-
inch needle with an attached “butterfly” BD Vacutainer
blood collection set (Becton, Dickinson and Company,
Franklin Lakes, NJ 07417, USA) or 14-inch extension set
(International WIN, Limited, Kenneth Square, PA 19348,
USA) was used depending on the size of the manatee
(Figures 3 and 4). Because of the unique structure of the
vascular system in this area, it was difficult to assess the
specific vessel site in the vascular bundle (venous or
arterial); thus, the blood collected was either or a com-
bination of venous or arterial blood. Blood was collected
first for serum chemistry analysis directly into 6 ml red
top sterile vacutainer tubes with a silicone-coated interior
(Becton, Dickinson and Company, Franklin Lakes, NJ
07417, USA), allowed to clot in a cool and shaded area,
and then separated by centrifugation. Blood was then
collected for a complete blood cell count (CBC) analysis
into 4 ml lavender top sterile vacutainer tubes, which
contained EDTA (K3EDTA; Becton, Dickinson and
Company, Franklin Lakes, NJ 07417, USA) as the anti-
coagulant, agitated gently and kept cool until analyzed.
Both serum and whole blood were kept refrigerated and
transported to the same clinical reference laboratory for
processing within 24 hours.

2.3. Complete Blood Cell Count. Values for white blood cell
count (WBC), red blood cell count (RBC), hemoglobin
(HBG), hematocrit (HCT), platelet (PLTS), and the red blood
indices of mean cell volume (MCV), mean cell hemoglobin
(MCH), mean cell hemoglobin concentration (MCHC), and
red cell distribution width (RDW) were obtained with a Cell-
Dy�3200 System Automated Hematology Analyzer (Abbott,
Abbott Park, IL 60064 USA). In addition, a manual leukocyte
differential was conducted under the microscope to allow for
the evaluation of different leukocyte cell types, identifying and
enumerating lymphocytes (LYMP), monocytes (MONO),
eosinophils (EOSI), basophils (BASO), and heterophils
(HETE), in percentages values. In automated CBC machines,
heterophils are usually wrongly categorized as eosinophils due
to their similarity in granulation morphology (Figure 5).
Thus, it was essential to run manual leukocyte counts and, in
doing so, train medical technologists and veterinarians in
manatee leukocyte identification for correct categorization
and counting.

2.4. Serum Chemistry. We performed a comprehensive
metabolic panel (CMP) on all the serum samples with a
VITROS 5, 1FS Chemistry System Analyzer (Ortho-Clinical
Diagnostics, Rochester, NY 14626 USA). The resulting
chemistry analytes were grouped based on six physiological
processes: (1) liver-associated enzymes and pigments (lactate
derhyrogenase, LDH; total bilirubin, TOT BL); (2) muscle-
associated enzymes (alanine aminotransferase, ALT; as-
partate aminotransferase, AST; alkaline phosphatase, ALP;
creatine phosphokinase, CPK); (3) kidney-associated com-
pounds and products (blood urea nitrogen, BUN; creatinine,
CREA; blood urea nitrogen-creatinine ratio, BUN-CREA;
uric acid, UA); (4) sugars, lipids, and pancreatic-associated
enzymes (glucose, GLU; triglycerides, TRIG; cholesterol,
CHOL; amylase,AMY); (5) proteins (total protein, TOT
PROT; albumin, ALB; globulin, GLOB; albumin-globulin
ratio, ALB:GLOB); and (6) electrolytes (sodium, Na; chlo-
rine,Cl; potassium, K; phosphate, PO4, or sometimes
Table 1: Antillean manatees sampled in Puerto Rico for this study, including those free-ranging and those rescued and in rehabilitation between 1991 and 2020.

| Date       | Field number | Name     | Sex | Length (cm) | Weight (kg) | Age class | Locality |
|------------|--------------|----------|-----|-------------|-------------|-----------|----------|
| 4 Dec 1991 | NEPST175     | Moisés   | M   | 115         | 28.6        | C         | Toa Baja |
| 13 Apr 1992| CPR9201      | CPR9201  | F   | 251         | —           | A         | Ceiba    |
| 16 Apr 1992| TPR01        | Taino    | M   | 276         | 360         | A         | Ceiba    |
| 16 Apr 1992| TPR02        | Caribe   | M   | 276         | 386         | A         | Ceiba    |
| 18 Apr 1992| CPR9202      | CPR9202  | M   | 242         | —           | A         | Ceiba    |
| 18 Apr 1992| CPR9203      | CPR9203  | M   | 294         | —           | A         | Ceiba    |
| 18 Apr 1992| TPR03        | Ceiba    | M   | 275         | —           | A         | Ceiba    |
| 19 May 1993| TPR04        | Simu     | M   | 244         | —           | A         | Ceiba    |
| 20 May 1993| TPR05        | Mac      | M   | 264         | —           | A         | Ceiba    |
| 22 May 1993| CPR9301      | CPR9301  | M   | 230         | —           | A         | Ceiba    |
| 22 May 1993| CPR9302      | CPR9302  | F   | 210         | —           | SA        | Ceiba    |
| 22 May 1993| TPR06        | Eddie T. | M   | 273         | —           | A         | Ceiba    |
| 23 May 1993| NEPST212     | Glauko   | M   | 122         | 31          | C         | San Juan |
| 7 Sep 1994 | TPR08        | Judini   | M   | 276         | —           | A         | Ceiba    |
| 21 Jul 1995| NEPST380     | Marina   | F   | 102         | 17          | C         | Guayama  |
| 28 Aug 1996| NEPST319     | Katsi    | F   | 118         | 30          | C         | Cabo rojo|
| 7 Aug 1997 | CPR9701      | Bea      | F   | 125         | —           | C         | Mayagüez |
| 7 Aug 1997 | TPR09        | Guanajibo| M   | 241         | —           | A         | Mayagüez |
| 7 Aug 1997 | TPR10        | Sara     | F   | 264         | —           | A         | Mayagüez |
| 11 Jun 1999| NEPST548     | Nina     | F   | 131         | 40          | C         | Guayama  |
| 23 Jul 1999| NEPST556     | Yuisa    | F   | 95          | 16          | C         | Loíza    |
| 14 Sep 1999| NEPST574     | Conquistador | M  | 108         | 23          | C         | Fajardo  |
| 11 Nov 2002| NEPST852     | Santa Cruz| F  | 238         | 195         | A         | Ponce    |
| 3 Jun 2003 | NEPST861     | Anani    | F   | 308         | 440         | A         | Arroyo   |
| 17 Jul 2003| TPR13        | Albanai  | F   | 299         | —           | A         | Cabo rojo|
| 18 Jul 2003| TPR14        | Guami    | M   | 250         | 270         | A         | Cabo rojo|
| 20 Jul 2003| CPR0301      | Iro      | M   | 212         | 161         | SA        | Guayanilla|
| 20 Jul 2003| TPR15        | Atabey   | F   | 296         | 416         | A         | Guayanilla|
| 21 Jul 2003| CPR0302      | CPR0302  | F   | 232         | 254         | A         | Guayanilla|
| 23 Jul 2003| NEPST592     | Rafael   | M   | 247         | 289         | A         | Luquillo  |
| 26 Jul 2003| NEPST868     | Camilia  | F   | 99          | 17          | C         | Isabel   |
| 3 Nov 2003 | TPR11        | Joyuda   | F   | 296         | —           | A         | Cabo rojo|
| 4 Nov 2003 | TPR16        | Igor     | M   | 287         | —           | A         | Cabo rojo|
| 5 Nov 2003 | TPR17        | Eco      | M   | 310         | —           | A         | Guayanilla|
| 5 Nov 2003 | TPR18        | Electra  | F   | 267         | —           | A         | Guayanilla|
| 6 Nov 2003 | TPR19        | Esoubi   | M   | 249         | —           | A         | Guayanilla|
| 7 Nov 2003 | CPR0303      | Guanina  | F   | 236         | —           | A         | Guayanilla|
| 7 Jun 2004 | TPR20        | Gazelle  | F   | 288         | 401         | A         | Guayanilla|
| 7 Jun 2004 | TPR21        | Sally    | F   | 297         | 445         | A         | Guayanilla|
| 8 Jun 2004 | TPR22        | Beethoven| M   | 256         | 310         | A         | Guayanilla|
| 10 Jun 2004| CPR0401      | CPR0401  | F   | 193         | —           | SA        | Cabo rojo|
| 10 Jun 2004| TPR23        | Coral    | M   | 261         | —           | A         | Cabo rojo|
| 23 Aug 2004| NEPST892     | Iani     | F   | 111         | 26          | C         | Ponce    |
| 22 Nov 2004| NEPST895     | Siani    | F   | 109         | 23          | C         | Aguadilla|
| 18 Mar 2005| NEPST899     | Guaili   | F   | 115         | 29          | C         | Guayama  |
| 25 Apr 2005| TPR25        | Maritizia| F   | 296         | —           | A         | Ceiba    |
| 26 Apr 2005| TPR26        | Rosa     | F   | 303         | —           | A         | Ceiba    |
| 27 Apr 2005| CPR0501      | CPR0501  | M   | 222         | —           | SA        | Ceiba    |
| 28 Apr 2005| TPR27        | India    | F   | 255         | —           | A         | Ceiba    |
| 29 Apr 2005| TPR28        | Monty    | M   | 273         | 300         | A         | Ceiba    |
| 29 Apr 2005| TPR29        | Marieta  | F   | 264         | —           | A         | Ceiba    |
| 29 Apr 2005| TPR30        | PJ       | M   | 225         | —           | SA        | Ceiba    |
| 30 Apr 2005| TPR31        | Marina   | F   | 270         | —           | A         | Ceiba    |
| 1 May 2005 | TPR32        | Flipa    | F   | 250         | —           | A         | Ceiba    |
| 2 May 2005 | TPR33        | Treso    | M   | 252         | —           | A         | Ceiba    |
| 30 Sep 2005| NEPST910     | El Tuque | M   | 111         | 29          | C         | Ponce    |
| 18 Sep 2006| NEPST927     | Guarionex| M   | 104         | 17          | C         | Fajardo  |
| 18 May 2011| NEPST940     | Aramana  | M   | 107         | 20          | C         | Dorado   |
| 15 May 2013| NEPST948     | Mayagua  | F   | 138         | 40          | C         | Mayagüez |
referred to as phosphorus, P; calcium, Ca; enzymatic carbon dioxide, CO$_2$; anion gap, AG).

2.5. Statistical Analysis. We used Microsoft Excel for Mac (Microsoft Corp. Version 12.2.8) for statistical analyses. Summary statistics (sample size, mean, maximum value, minimum value, and standard deviation) of hematology and serum chemistry values were calculated on all the samples for every parameter. Minimum and maximum intervals using ±2 standard deviation around the mean were calculated, and values outside this range were considered outliers and eliminated. Means and standard deviations were then recalculated, and new reference ranges were determined as ±1 standard deviation around the mean. An unpaired two-sample $T$-test was done comparing males versus females and adults versus calves to see if there were any significant differences ($p \leq 0.05$) between these. Subadults were not included in the latter due to small sample size.

### 3. Results

3.1. Sample Description. Seventy captured or rescued manatees from Puerto Rico were included in the study (Table 1). Of these, 33 were males, and 37 were females; 23 were calves, 6 were subadults, and 41 were adult individuals. Sex by age group was evenly distributed, with 9 male and 14 female calves, 3 male and 3 female subadults, and 21 males and 20 female adults. The distribution of manatees sampled around the island was also evenly distributed (Figure 1). However, directed telemetry and health assessment captures were conducted in Ceiba, Salinas, Guayama (Jobos Bay), Guayanilla, Cabo Rojo, and Mayagüez.

3.2. Complete Blood Cell Count. Hematological parameters were obtained for the entire population sampled, separately for calves, subadults, adults (Table 2), males, and females (Table 3). Leukocytes in the Puerto Rico manatee population were composed primarily of heterophils (52.3%, range of 38–67%) and lymphocytes (42.8%, range of 28–57%), with few monocytes, and rare eosinophils and basophils. We found significant differences between calf and adult manatees in red blood cell count, hemoglobin, hematocrit, mean cell volume, white blood cell count, and basophils. All were found to be higher in calves, except for mean cell volume and basophilcs, which were lower than in adults (Table 2). Manatee neonates and calves had noticeably increased red blood cell count (3.0–3.8 $10^6$/mm$^3$), decreasing after 2–3 months of age (data not shown). White blood cell count was also found to be higher in calves, leveling to normal values after 3 months of age (data not shown). There were no observed significant differences between males and females regarding hematocrit values (Table 3), except for red cell distribution width ($p = 0.007$) and platelets ($p = 0.047$), which were slightly higher in females.

3.3. Serum Chemistry. Serum chemistry parameters were also obtained for the entire population sampled and separately for calves, subadults, adults (Table 4), males, and females (Table 5). Significant differences ($p \leq 0.05$) were found in all chemistry analytes when comparing adults versus calves, except aspartate aminotransferase, alkaline phosphatase, creatine phosphokinase, lactic dehydrogenase, blood urea nitrogen, blood urea nitrogen-creatinine ratio, triglycerides, cholesterol, amylase, globulin, and potassium (Table 4). Adult manatees had a higher mean value for alanine aminotransferase, creatinine, uric acid, glucose, total protein, albumin, albumin-globulin ratio, sodium, chloride, potassium, and anion gap. Calves had a lower mean value for total bilirubin, calcium, and enzymatic carbon dioxide. When comparing males versus females, no significant differences were found in all chemistry analytes (Table 5).

### 4. Discussion

We establish the reference intervals of hematology and blood chemistry for the population of Antillean manatees in Puerto Rico and compare them with those established for manatees from Belize, Brazil, Florida, Guyana, and Mexico. Blood reference intervals can differ among populations of the same species and vary according to age, sex, physiological condition, degree of physical activity, and environment [30]. They can also be altered by collecting and testing methods. They may also vary within individuals of the same population, suggesting the clinical need for veterinarians to compare and contrast hematology and blood chemistry results on a particular manatee patient as it develops.
medically during treatment and in long-term care. While differences in immunoglobulin G (IgG) reference values for manatees from Florida, Colombia, and Puerto Rico were found [31] and comparisons were made on hematological and serum chemistry reference ranges between Antillean manatees in Guyana and Florida manatees [7], these studies found that the majority of hematological and serum chemistry results were similar to those reported for the Florida manatee. Blood parameters of Antillean manatees in Brazil were also evaluated, and differences between sexes and environments were discussed [14]. Tabulated reference ranges for both subspecies of West Indian manatees (Antillean and Floridian) from Guyana, Mexico, Belize, Brazil, and Florida (Table 6 and 7) were used to discuss similarities or differences between these populations in comparison to hematology and blood chemistry reference ranges found for Puerto Rico.

4.1. Complete Blood Cell Count. Erythrocyte and total leukocyte counts measured in this study were similar to those previously reported for manatees in Guyana, Mexico, Belize, Brazil, and Florida [6–9, 14, 25] (Table 6). Red blood cell count was highest in neonates (>3.0 × 10⁶/mm³) and decreased as manatees grew and learned to dive [6, 32], as evident in significant variation among age classes observed in the study (Table 2). This is opposite to erythrocyte count in common bottlenose dolphins (Tursiops truncatus), pinnipeds, and terrestrial mammals as they mature in age [33].

Leukocyte counts in manatees tend to be slightly lower than in most domestic mammals but in general similar to several whale and dolphin species [6, 25]. For example, the range for Antillean manatees in Puerto Rico reported here is between 4.0 and 8.0 × 10⁹/L, with calves having a slightly higher reference range (5.3 and 9.3 × 10⁹/L) (Table 2). However, it is advised that a manatee with a white blood cell count above 8.0 × 10⁹/L should be closely monitored, and anything above 10 × 10⁹/L be considered abnormal. It is of extreme importance that white blood cell types be ascertained using a manual method, rather than by machine, and the technician reading the slide be forewarned, as heterophils may be mistaken for eosinophils given their granulocyte nature (Figure 5). Eosinophils in manatees are rarely found, usually 0–4%. Eosinophilia reported for manatees was usually performed by untrained laboratory technicians or veterinarians, which mistakenly read granular heterophils as eosinophils (AAMG personal observation).
4.2. Serum Chemistry

4.2.1. Liver-Associated Enzymes and Pigments. Wider ranges of lactic dehydrogenase observed in our marine-dwelling manatees as well as in other marine mammals may be due to muscle exertion associated with diving. Although manatees in Puerto Rico are not usually deep or long divers (<10 m, <5–10 min), differences in reported lactic dehydrogenase ranges may be due also to the administration of intramuscular injections and the manipulation prior to or during sampling [34]. Artificial changes in lactic dehydrogenase levels were reported in cetaceans due to severe hemolysis similar to those observed in individuals that received intramuscular injections but of a lesser magnitude [35]. Total bilirubin values in Puerto Rico were significantly lower in adults than in calf manatees, as calves have a higher metabolic demand for protein absorption from their mother’s milk and complementary vegetable diet (Table 4). However, overall total bilirubin values were similar to those found in other studies in Guyana, Mexico, Belize, Brazil, and Florida [6–9, 14, 24] (Table 7).

4.2.2. Muscle-Associated Enzymes. While Bossart et al. [6] included alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the liver-associated enzymes, to date, these analytes are considered more indicative of the muscular system [22]. Alanine aminotransferase values for Puerto Rico were significantly higher in adults compared to calves (Table 4) and were similar to Mexico, Belize, and Florida [6, 8, 9, 24] but higher than those found in Brazil [14]. Aspartate aminotransferase appears to be of little or no clinical value in manatees [6]. However, values for Puerto Rico were comparable to those described for Florida [6, 24], Guyana [7], and Brazil [14] but lower than in Mexico and Belize [8, 9]. Increased alkaline phosphatase may be present in growing, young mammals in some terrestrial species associated with osteoblastic activity [36], as seen in our reference ranges of Puerto Rican manatee calves versus adults. Although no detailed studies have been conducted in marine mammals, similar trends for alkaline phosphatase activity may also exist [6], and decreased alkaline phosphatase values appear...
to indicate older individuals, pernicious anemia, hypothyroidism, inanition, or decreased osteoblastic activity [6]. In manatees from Puerto Rico, alkaline phosphatase values for calves were clearly higher than in subadult and adult manatees.

Creatine phosphokinase values for Puerto Rico were similar to those found for Guyana [7], Mexico [8], and Florida [6]. However, they were notably different from those reported by Florida’s later study [24]. Creatine phosphokinase is an important parameter to measure in manatees in rehabilitation, as it is indicative of handling stress [6] and intestinal tissue remodeling due to colitis and enteritis. In our experience, in digestively compromised Antillean manatees, creatine phosphokinase values rapidly surpass the 500–2,000 U/L levels, and intestinal inflammation is not resolved until the values return to normal ranges (68–132 U/L). Therefore, rapid multifaceted treatment is warranted in these cases, or the chances of the manatee’s demise increase as time passes from necrotizing enteritis and pneumatosis intestinalis.

Figure 5: White blood cell types from an Antillean manatee from Puerto Rico: (a) lymphocytes, (b) monocyte, (c) eosinophil, (d) basophil, (e) heterophil, and (f) comparison between a heterophil (E) and two eosinophils (C). Wright-Giemsa, x100 objective.
4.2.3. Kidney-Associated Compounds and Products.
Reference intervals for blood urea nitrogen for Puerto Rican manatees were slightly lower than those initially established for the Florida manatee [6] but similar to those in Guyana [7], Mexico [8], Belize [9], and Florida’s later study [24]. Blood urea nitrogen reacts to a complex combination of several variables such as nutritional age, and metabolic and physiologic conditions during sampling and restraint. Decreased blood urea nitrogen may reflect their herbivorous diet [34]. Although Florida and the Antillean manatee diet are similar, the difference in blood urea nitrogen ranges may reflect different feeding behaviors during the changing seasons in Florida. Starvation and liver failure cause blood urea nitrogen levels to decrease [6].

Creatinine reference ranges in Puerto Rico were significantly different between calves and adults, being higher in the latter (Table 4). However, creatinine values were

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**Table 2:** Mean and standard deviation hematology values for Antillean manatees from Puerto Rico for all age classes (calves, subadults, and adults; n = 70): calves only, n = 23; subadults only, n = 6; and adults only, n = 41, with ±1 standard deviation ranges in parentheses. Significant differences using p values between calves and adults are indicated with an asterisk (*).

| Parameter           | All manatee samples | Calves | Subadults | Adults | p value | Calves vs. adults |
|---------------------|---------------------|--------|-----------|--------|---------|------------------|
| WBC (10^3/L)        | Mean±SD             | Range  | Mean±SD   | Range  | Mean±SD | Range            |
| RBC (10^6/mm³)      | 6.0±2.0             | (4.0–8.0) | 7.3±2.0   | (5.3–9.3) | 4.9±1.8 | (3.1–6.7) | 5.4±1.7 | (3.8–7.1) | 0.004* |
| HGB (g/dL)          | 2.7±0.6             | (2.1–3.3) | 3.4±0.4   | (3.0–3.8) | 2.3±0.1 | (2.2–2.4) | 2.3±0.02 | (2.1–2.6) | ≤0.001* |
| HCT (%)             | 11.2±2.3            | (9.0–14) | 13.8±1.5  | (12–15) | 9.4±0.5 | (8.9–9.8) | 9.8±0.8 | (8.9–11) | ≤0.001* |
| PLTS (10^3/mm³)     | 3.6±4.6             | (28–41) | 41.6±4.1  | (38–46) | 30.3±4.0 | (26–34) | 30.7±3.5 | (27–34) | ≤0.001* |
| Red blood cell indices |                     |        |           |        |         |                  |
| MCV (FL)            | 128.6±8.2           | (120–137) | 124.5±7.2 | (117–132) | 124.9±8.1 | (117–133) | 131.7±7.7 | (124–140) | 0.001* |
| MCH (pg)            | 41.1±1.4            | (40–43) | 40.9±1.7  | (39–43) | 40.6±0.7 | (40–41) | 41.3±1.2 | (40–43) | 0.424 |
| MCHC (g/dL)         | 32.2±1.2            | (31–34) | 32.5±1.2  | (31–34) | 32.5±1.5 | (31–34) | 32.0±1.3 | (31–33) | 0.124 |
| RDW (%)             | 18.0±2.8            | (15–21) | 17.9±2.8  | (15–21) | 17.2±2.1 | (15–19) | 18.2±2.9 | (15–21) | 0.681 |
| White blood cell differential |             |        |           |        |         |                  |
| LYMP (%)            | 42.8±14.5           | (28–57) | 38.2±16.5 | (22–55) | 54.0±8.0 | (46–62) | 44.2±13.0 | (31–57) | 0.165 |
| MONO (%)            | 3.6±2.3             | (1–6) | 4.1±2.7   | (1–7) | 2.5±1.7 | (1–4) | 3.5±2.2 | (1–6) | 0.414 |
| EOSI (%)            | 1.0±1.4             | (0–3) | 0.6±1.1   | (0–2) | 2.3±2.1 | (0–4) | 1.1±1.5 | (0–3) | 0.118 |
| BASO (%)            | 0.3±0.5             | (0–1) | 0.0±0.2   | (0–0) | 0.5±0.6 | (0–1) | 0.5±0.6 | (0–1) | ≤0.001* |
| HETE (%)            | 52.3±14.7           | (38–67) | 51.7±16.9 | (40–74) | 40.8±7.5 | (33–48) | 50.7±13.0 | (38–64) | 0.145 |

**Note:** WBC = white blood cell count, RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, PLTS = platelet count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, LYMP = lymphocytes, MONO = monocytes, EOSI = eosinophils, BASO = basophils, and HETE = heterophils.

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**Table 3:** Mean and standard deviation hematology values for Antillean manatees from Puerto Rico for all sex classes (males and females, n = 70): males only, n = 33, and females only, n = 37, with ±1 standard deviation ranges in parentheses. Significant differences using p values are indicated with an asterisk (*).

| Parameter           | All manatee samples | Males | Females | p values |
|---------------------|---------------------|-------|---------|----------|
| WBC (10^3/L)        | Mean±SD             | Range | Mean±SD | Range    |          |
| RBC (10^6/mm³)      | 6.0±2.0             | (4.0–8.0) | 5.8±1.9   | (3.9–7.7) | 6.2±2.1 | (4.1–8.2) | 0.539 |
| HGB (g/dL)          | 2.7±0.6             | (2.1–3.3) | 2.6±0.6   | (2.1–3.2) | 2.7±0.6 | (2.1–3.4) | 0.522 |
| HCT (%)             | 11.2±2.3            | (9.0–14) | 10.9±2.0  | (8.9–13) | 11.5±2.4 | (9.0–14) | 0.322 |
| PLTS (10^3/mm³)     | 298.3±91.3          | (207–390) | 269.9±81.9 | (188–352) | 323.2±93.3 | (230–417) | 0.047* |
| Red blood cell indices |                     |       |         |          |         |
| MCV (FL)            | 128.6±8.2           | (120–137) | 128.3±7.9 | (120–136) | 128.8±8.6 | (120–137) | 0.824 |
| MCH (pg)            | 41.1±1.4            | (40–43) | 40.8±1.3  | (40–42) | 41.4±1.4 | (40–43) | 0.168 |
| MCHC (g/dL)         | 32.2±1.2            | (31–34) | 32.2±1.1  | (31–33) | 32.2±1.4 | (31–34) | 0.941 |
| RDW (%)             | 18.0±2.8            | (15–21) | 16.9±2.2  | (15–19) | 18.9±2.9 | (16–22) | 0.007* |
| White blood cell differential |       |       |         |          |         |
| LYMP (%)            | 42.8±14.5           | (28–57) | 42.2±10.5 | (32–53) | 43.2±17.2 | (26–60) | 0.776 |
| MONO (%)            | 3.6±2.3             | (1–6) | 3.4±2.3   | (1–6) | 3.8±2.4 | (1–6) | 0.584 |
| EOSI (%)            | 1.0±1.4             | (0–3) | 1.1±1.5   | (0–3) | 0.9±1.5 | (0–2) | 0.618 |
| BASO (%)            | 0.3±0.5             | (0–1) | 0.4±0.6   | (0–1) | 0.3±0.5 | (0–1) | 0.304 |
| HETE (%)            | 52.3±14.7           | (38–67) | 52.8±10.7 | (42–64) | 51.8±17.4 | (34–69) | 0.783 |

**Note:** WBC = white blood cell count, RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, PLTS = platelet count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, LYMP = lymphocytes, MONO = monocytes, EOSI = eosinophils, BASO = basophils, and HETE = heterophils.
Table 4: Mean serum chemistry values for manatees from Puerto Rico for all samples, (subadults, and adults, n = 70): calves only, n = 23; subadults only, n = 6; and adults only, n = 41, with ±1 standard deviation range in parentheses. Abbreviations for parameters are detailed in the Materials and Methods section. Significant differences using p values between calves and adults are indicated with an asterisk (*)

| Parameter                              | All manatee samples | Calves | Subadults | Adults | p value | Calves vs. Adults |
|----------------------------------------|---------------------|--------|------------|--------|---------|-----------------|
| **Liver-associated enzymes and pigments** |                     |        |            |        |         |                 |
| LDH (U/L)                              | 425.3 ± 164.6       | 394.6 ± 174.1 | 538.2 ± 144.6 | 420.2 ± 159.7 | 0.620   |                 |
| TOT BILI (mg/dL)                       | 0.2 ± 0.1           | 0.3 ± 0.2 | 0.1 ± 0.04 | 0.2 ± 0.1 | 0.009*  |                 |
| **Muscle-associated enzymes**          |                     |        |            |        |         |                 |
| ALT (U/L)                              | 15.4 ± 7.1          | 12.0 ± 6.5 | 18.5 ± 5.5  | 16.8 ± 7.1 | 0.011*  |                 |
| AST (U/L)                              | 11.4 ± 5.2          | 10.3 ± 4.9 | 13.5 ± 6.4  | 11.9 ± 5.5 | 0.316   |                 |
| ALP (U/L)                              | 78.2 ± 20.4         | 86.9 ± 20.6 | 70.2 ± 20.7 | 76.2 ± 19.8 | 0.096   |                 |
| CREA (mg/dL)                           | 99.8 ± 32.3         | 85.8 ± 24.1 | 119.4 ± 20.9 | 101.4 ± 34.9 | 0.136   |                 |
| **Kidney-associated compounds and products** |                     |        |            |        |         |                 |
| BUN (mg/dL)                            | 4.2 ± 2.1           | 4.1 ± 2.5 | 5.2 ± 2.2  | 4.1 ± 2.0  | 0.896    |                 |
| CRE (mg/dL)                            | 1.5 ± 0.4           | 1.3 ± 0.4 | 1.5 ± 0.5  | 1.6 ± 0.4  | 0.014*   |                 |
| BUN:CREA                               | 21.0 ± 7.1          | 17.0 ± 0.8 | 23.0 ± 0.4  | 22.0 ± 0.7  | 0.078    |                 |
| UA (mg/dL)                             | 0.9 ± 0.5           | 1.2 ± 0.4 | 0.8 ± 0.6  | 0.8 ± 0.5  | 0.009*   |                 |
| **Sugars, lipids, and pancreatic-associated enzymes** |         |        |            |        |         |                 |
| Glucose (mg/dL)                        | 91.2 ± 18.5         | 78.5 ± 14.6 | 86.7 ± 21.5 | 97.0 ± 16.9 | 0.001*   |                 |
| Triglycerides (mg/dL)                  | 109.3 ± 25.2        | 110.7 ± 28.0 | 88.4 ± 17.5 | 112.4 ± 24.4 | 0.867    |                 |
| Cholesterol (mg/dL)                    | 117.5 ± 25.7        | 130.8 ± 14.9 | 115 ± 19.8  | 116.2 ± 26.7 | 0.105    |                 |
| Amylase (U/L)                          | 599.7 ± 149.1       | 667.0 ± 179.3 | 573.5 ± 145.3 | 573.5 ± 145.3 | 0.399    |                 |
| **Proteins**                           |                     |        |            |        |         |                 |
| TOT PROT (g/dL)                        | 7.0 ± 0.5           | 6.7 ± 0.6 | 6.8 ± 0.4  | 7.1 ± 0.4  | 0.004*   |                 |
| ALB (g/dL)                             | 3.9 ± 0.5           | 3.7 ± 0.5 | 3.9 ± 0.4  | 4.1 ± 0.4  | 0.012*   |                 |
| GLOB (g/dL)                            | 2.9 ± 0.4           | 3.0 ± 0.4 | 2.8 ± 0.5  | 2.8 ± 0.4  | 0.143    |                 |
| ALB:GLOB                               | 1.4 ± 0.3           | 1.2 ± 0.3 | 1.5 ± 0.3  | 1.4 ± 0.2  | 0.032*   |                 |
| **Electrolytes**                       |                     |        |            |        |         |                 |
| Na (mmol/L)                            | 151.0 ± 5.5         | 147.8 ± 5.1 | 151.0 ± 6.2 | 152.6 ± 5.0  | 0.001*   |                 |
| Cl (mmol/L)                            | 99.6 ± 6.3          | 97.3 ± 6.5 | 99.9 ± 6.1  | 101.0 ± 5.9  | 0.036*   |                 |
| K (mmol/L)                             | 5.3 ± 0.7           | 4.8 ± 0.5 | 5.5 ± 0.5  | 5.5 ± 0.6  | 0.001*   |                 |
| PO4 (mg/dL)                            | 5.9 ± 1.1           | 6.2 ± 1.1 | 6.3 ± 1.3  | 5.7 ± 1.1  | 0.110    |                 |
| Ca (mg/dL)                             | 9.8 ± 0.7           | 10.1 ± 0.6 | 9.5 ± 0.5  | 9.7 ± 0.7  | 0.020*   |                 |
| CO2 (mmol/L)                           | 26.7 ± 9.5          | 33.7 ± 4.5 | 23.9 ± 11.7 | 23.0 ± 9.3  | 0.001*   |                 |
| AG (mmol/L)                            | 27.9 ± 10.6         | 19.9 ± 6.5 | 32.4 ± 11.7 | 32.2 ± 9.7  | 0.001*   |                 |

Similar to those established for Antillean manatees in Guyana [7], Belize [9], Brazil [14], and Florida [6], but not when compared to later study of Mexico [8] or Florida [24]. Variations associated with food intake, type of food, and salinity of environment in Florida manatees were suggested to possibly be affecting serum creatinine levels [20]. Rehabilitated Antillean manatees from Puerto Rico exhibited an increased serum creatinine following release back to the wild; while blood urea nitrogen and other blood parameters remained within their normal baseline ranges. An increase in creatinine was documented on all rehabilitated and released Antillean manatees in Puerto Rico immediately after being transferred to a sea pen during a soft release back into the wild. After the manatees acclimated to the new environment and learned to find a freshwater source, creatinine levels decreased in subsequent veterinary examinations.

Uric acid parameters were significantly higher in manatee calves from Puerto Rico when compared to adult manatees (Table 4), most probably due to the higher protein content in the calves' diet. However, uric acid values were not available from Guyana, Mexico, Belize, and Florida to further understand any differences between populations.

4.2.4. Sugars, Lipids, and Pancreatic-Associated Enzymes. Adult manatees in Puerto Rico showed significantly higher values for glucose in comparison to calves (Table 4). Hypoglycemia in rescued calves or debilitated manatees is a significant problem that must be addressed urgently upon admittance to a critical care facility. Glucose and triglyceride levels were similar among Puerto Rico, Guyana, Mexico, Belize, Brazil, and Florida populations. However, cholesterol levels from all Antillean manatees throughout the countries were similar but appreciably lower than those found in Florida manatees [6, 24], given that Florida manatees are larger in size and
TABLE 5: Mean and standard deviation hematology values for Antillean manatees from Puerto Rico for all sex classes (males and females, \( n = 70 \)): males only, \( n = 33 \), and females only, \( n = 37 \), with \( \pm 1 \) standard deviation ranges in parentheses. Significant differences using \( p \) values are indicated with an asterisk (*).

| Parameter | All manatee samples | Males | Females | \( p \) values |
|-----------|---------------------|-------|---------|---------------|
|           | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | Range | |
| Liver-associated enzymes and pigments | | | | | | | |
| LDH (U/L) | 425.3 ± 164.6 | (261–590) | 392.3 ± 155.0 | (237–547) | 458.4 ± 170.0 | (288–628) | 0.127 |
| TOT BILI (mg/dL) | 0.2 ± 0.1 | (0.1–0.3) | 0.2 ± 0.1 | (0.1–0.3) | 0.2 ± 0.1 | (0.1–0.3) | 0.573 |
| Muscle-associated enzymes | | | | | | | |
| ALT (U/L) | 15.4 ± 7.1 | (8.3–23) | 14.6 ± 8.1 | (6.5–23) | 16.0 ± 6.3 | (9.8–22) | 0.435 |
| AST (U/L) | 11.4 ± 5.2 | (6.1–17) | 9.9 ± 4.9 | (5.0–15) | 12.8 ± 5.3 | (7.5–18) | 0.053 |
| ALP (U/L) | 78.2 ± 20.4 | (58–99) | 75.1 ± 20.3 | (55–95) | 81.0 ± 20.4 | (61–101) | 0.271 |
| CK-PK (U/L) | 99.8 ± 32.3 | (68–132) | 101.2 ± 32.1 | (69–103) | 98.5 ± 33.2 | (65–132) | 0.785 |
| Kidney-associated compounds and products | | | | | | | |
| BUN (mg/dL) | 4.2 ± 2.1 | (2.1–6.3) | 4.6 ± 2.2 | (2.4–6.8) | 3.9 ± 2.1 | (1.9–6.0) | 0.222 |
| CREA (mg/dL) | 1.5 ± 0.4 | (1.1–1.9) | 1.5 ± 0.4 | (1.1–2.0) | 1.5 ± 0.4 | (1.1–1.9) | 0.771 |
| BUN-CREA | 2.1 ± 0.7 | (1.4–2.8) | 2.3 ± 0.7 | (1.5–3.0) | 2.0 ± 0.7 | (1.2–2.7) | 0.234 |
| UA (mg/dL) | 0.9 ± 0.5 | (0.4–1.4) | 0.9 ± 0.5 | (0.4–1.5) | 0.9 ± 0.5 | (0.4–1.4) | 0.723 |
| Sugars, lipids, and pancreatic-associated enzymes | | | | | | | |
| GLU (mg/dL) | 91.2 ± 18.5 | (73–110) | 90.4 ± 18.7 | (72–109) | 91.8 ± 18.6 | (73–110) | 0.771 |
| TRIG (mg/dL) | 109.3 ± 25.2 | (84–135) | 109.8 ± 24.2 | (86–134) | 108.8 ± 26.6 | (82–135) | 0.900 |
| CHOL (mg/dL) | 117.5 ± 25.7 | (92–143) | 121.0 ± 23.5 | (97–144) | 114.3 ± 27.7 | (87–142) | 0.380 |
| AMY (U/L) | 599.7 ± 149.1 | (451–749) | 596.4 ± 134.0 | (462–730) | 609.0 ± 209.3 | (400–818) | 0.916 |
| Proteins | | | | | | | |
| TOT PROT (g/dL) | 7.0 ± 0.5 | (6.4–7.5) | 7.0 ± 0.5 | (6.5–7.5) | 6.9 ± 0.5 | (6.4–7.5) | 0.772 |
| ALB (g/dL) | 3.9 ± 0.5 | (3.5–4.4) | 4.0 ± 0.4 | (3.5–4.4) | 3.9 ± 0.5 | (3.4–4.4) | 0.746 |
| GLOB (g/dL) | 2.9 ± 0.4 | (2.5–3.3) | 2.9 ± 0.4 | (2.4–3.3) | 2.9 ± 0.4 | (2.4–3.3) | 0.998 |
| ALB:GLOB | 1.4 ± 0.3 | (1.1–1.7) | 1.4 ± 0.3 | (1.1–1.7) | 1.4 ± 0.3 | (1.1–1.7) | 0.682 |
| Electrolytes | | | | | | | |
| NA (mmol/L) | 151.0 ± 5.5 | (146–157) | 151.0 ± 5.7 | (145–157) | 151.0 ± 5.4 | (146–156) | 0.956 |
| K (mmol/L) | 99.6 ± 6.3 | (93–106) | 99.2 ± 5.5 | (94–105) | 99.9 ± 6.9 | (93–107) | 0.669 |
| PO4 (mg/dL) | 5.3 ± 0.7 | (4.4–5.9) | 5.3 ± 0.6 | (4.8–5.9) | 5.3 ± 0.7 | (4.5–6.0) | 0.713 |
| Ca (mg/dL) | 9.8 ± 0.7 | (9.2–11) | 9.9 ± 0.6 | (9.3–10) | 9.8 ± 0.7 | (9.1–11) | 0.676 |
| CO3 (mg/dL) | 26.7 ± 9.5 | (17–36) | 26.9 ± 8.4 | (19–35) | 26.5 ± 10.5 | (16–37) | 0.863 |
| AG (mmol/L) | 27.9 ± 10.6 | (17–39) | 27.8 ± 9.2 | (19–37) | 28.0 ± 11.7 | (16–40) | 0.997 |

Table 6: Hematology reference intervals for West Indian manatees from Puerto Rico, Guyana, Mexico, Belize, Brazil, and Florida. Puerto Rico values included all samples (calves, subadults, and adults). Abbreviations for parameters are detailed in the materials and methods section. Columns with an asterisk (*) signify that the range values are minimum and maximum.

| Parameter | Antillean manatees | Florida manatees |
|-----------|---------------------|------------------|
|           | Puerto Rico | Guyana [7] | Mexico [8] | Belize [9] | Brazil [14]* | Florida [6] | Florida [25]* |
| WBC (10^3/L) | 4.0–8.0 | 4.6–8.6 | 3.9–9.1 | 3.4–7.9 | 4.4–11 | 4–12 | 2.8–14 |
| RBC (10^6/mm³) | 2.1–3.3 | 2.2–2.8 | 2.3–3.3 | 2.2–3.0 | 2.5–3.0 | 2.4–3.4 | 2.2–3.4 |
| Hb (g/dL) | 9.0–14 | 8.9–11 | 9.8–13 | 9.5–12 | 9.1–11 | 9.8–13 | 9.4–14 |
| HCT (%) | 28–41 | 17–24 | 31–42 | 30–37 | 29–34 | 30–40 | 29–44 |
| PLTS (10^3/mm³) | 207–390 | — | 138–266 | 156–384 | — | 195–412 | 111–424 |
| MCV (fl) | 120–137 | — | — | 100–146 | 109–116 | 122–149 | 114–140 |
| MCH (pg) | 40–43 | — | — | 36–44 | 33–38 | 38–46 | 37–45 |
| MCHC (g/dL) | 31–34 | — | 30–33 | 29–35 | 29–33 | 30–33 | 28–35 |
| RDW (%) | 15–21 | — | — | — | — | — | 14–23 |
| White blood cell differential | | | | | | | |
| LYMPH (%) | 28–57 | — | — | — | — | — | — |
| MONO (%) | 1–6 | — | — | — | — | — | — |
| EOSIN (%) | 0–3 | — | — | — | — | — | — |
| BASO (%) | 0–1 | 0–0 | — | 0–0 | 0–0 | 0–0 | — |
| HET (%) | 38–67 | — | — | — | — | — | — |
have higher fat reserves from being a northern and subtropical subspecies.

4.2.5. Proteins. Values for total protein, albumin, and albumin-globulin ratio were significantly higher in adult manatees in Puerto Rico than in calf manatees (Table 4). Plasma or serum proteins move between the blood and other fluids. The total concentration of all proteins in the blood may vary depending on changes in the volume of water or the amount of the individuals' proteins. Reference intervals for total protein established for the Puerto Rico population were slightly lower than those reported for Mexico [8] and Florida [6, 24] but similar to those in Guyana, Belize, and Brazil [7, 9, 14]. An increase in total proteins, indicative of dehydration, was observed, while manatees were in the more severe diet reduction phase of a simulated release [20]. Variations between freshwater and marine diets may explain the difference in total protein values between captive calves and free-ranging adults. Additionally, blood sample quality should be considered when evaluating total protein values due to an artificial increase caused by lipemia, icterus, and hemolysis. Albumin serves as a protein carrier, and given that it is typically higher in most marine mammals, the use of automated analyzers using human standards may give erroneous results [37]. As an early indicator of hepatic disease in marine mammals, the use of albumin is limited since it appears that these have a tremendous reserve capacity for hepatic albumin production [6]. Albumin reference intervals for manatees in Puerto Rico were slightly lower than those reported for both Antillean and Floridamanatees in other compared countries. These values were confirmed by protein electrophoresis, the preferred and recommended detection method [6].

### Table 7: Serum chemistry reference intervals for West Indian manatees from Puerto Rico, Florida, Guyana, Mexico, Belize, and Brazil.

| Parameter                  | Puerto Rico | Antillean manatees | Florida manatees |
|----------------------------|-------------|--------------------|-----------------|
|                            | *n* = 70    | *n* = 11           | *n* = 18        |
|                            | *n* = 82    | *n* = 30           | *n* = 23        |
|                            | *n* = 55    |                    |                 |
| Liver-associated enzymes and pigments |             |                    |                 |
| LDH (U/L)                  | 261–590     | —                  | —               |
| T BILI (mg/dL)             | 0.1–0.3     | 0.2–0.4            | 0.0–0.4         |
| Muscle-associated enzymes  |             |                    |                 |
| ALT (U/L)                  | 8.3–23      | 14–24              | 4.4–33          |
| AST (U/L)                  | 6.1–17      | 18–19              | 19–52           |
| ALP (U/L)                  | 58–99       | 45–80              | 52–106          |
| CPK (U/L)                  | 68–132      | 75–228             | 78–191          |
| Kidney-associated compounds and products |             |                    |                 |
| BUN (mg/dL)                | 2.1–6.3     | 1.6–6.4            | 3.1–13          |
| CREA (mg/dL)               | 1.1–1.9     | 1.0–1.4            | 0.0–4.4         |
| BUN:CREA                   | 1.4–2.8     |                    | 1.0–2.4         |
| UA (mg/dL)                 | 0.4–1.4     |                    | 1.6–2.2         |
| Sugars, lipids, and pancreatic-associated enzymes |             |                    |                 |
| GLU (mg/dL)                | 73–110      | 70–97              | 67–101          |
| TRIG (mg/dL)               | 84–135      |                    | 82–134          |
| CHOL (mg/dL)               | 92–143      |                    | 88–170          |
| AMY (U/L)                  | 451–749     |                    | 134–210         |
| Proteins                   |             |                    |                 |
| T PROT (g/dL)              | 6.4–7.5     | 6.5–7.3            | 6.9–8.3         |
| ALB (g/dL)                 | 3.5–4.4     | 4.1–5.1            | 4.0–5.6         |
| GLOB (g/dL)                | 2.5–3.3     |                    | 3.3–5.1         |
| ALB:GLOB                   | 1.1–1.7     |                    | 1.9–3.7         |
| Electrolytes               |             |                    |                 |
| NA (mmol/L)                | 146–157     | 138–149            | 142–160         |
| CL (mmol/L)                | 93–106      | 92–105             | 87–105          |
| K (mmol/L)                 | 4.6–5.9     | 4.2–5.0            | 4.6–6.0         |
| PO₄ (mg/dL)                | 4.8–7.0     | 4.2–5.6            | 3.7–7.0         |
| CA (mg/dL)                 | 9.2–11      | 9.6–11             | 9.1–12          |
| CO₂ (mmol/L)               | 17–36       | 13–18              |                |
| AG (mmol/L)                | 17–39       | 30–37              |                |
released subadults in the process of acclimating to the saline environment, and in rescued calves that are typically brought in with colic and other gastrointestinal complications due to malnourishment.

4.2.6. Electrolytes. Electrolytes analytes were significantly different between adults and calves, except for phosphate. Sodium, chloride, potassium, and anion gap were higher in adults, while calcium and enzymatic carbon dioxide were higher in calves. However, electrolyte values across countries of the Antillean subspecies and the Florida subspecies were similar.

5. Conclusions

Here, we establish the normal reference intervals for the population of Antillean manatees in the waters around the island of Puerto Rico (Tables 6 and 7). Most findings in this study were similar to those previously reported for other West Indian manatee populations of both subspecies from Guyana, Mexico, Belize, Brazil, and Florida. Factors to be considered when evaluating and explaining these similarities or differences include diet, time from last feeding, water composition (salinity, temperature, pH, etc.), body condition, capture stress, and health status. This study’s interpretation of hematology and blood chemistry data was complicated since most adult samples were collected from targeted free-ranging manatees for radiotelemetry studies, and most calves were in a captive environment for medium-term care after rescue and health stabilization. Therefore, blood reference ranges provided herein should be considered complementary guidelines for veterinary examinations and health assessments. If possible, baseline blood parameters should be established for each individual manatee as a patient or during long-term care before declaring any value as truly abnormal.

Data Availability

The datasets used and/or analyzed during the current study can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

AAMG and MMAG contributed equally to conceptualization, data curation, formal analysis, investigation, methodology, writing, review, and editing. AAMG was responsible for funding acquisition, project administration, and resources.

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