Blood analytes of immature Kemp’s ridley sea turtles (*Lepidochelys kempii*) from Georgia, USA: reference intervals and body size correlations

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Health assessments of wildlife species are becoming increasingly important in an ever-changing environment. Kemp’s ridley sea turtles (*Lepidochelys kempii*; hereafter, Kemp’s ridleys) are critically endangered and incur several on-going threats to their population recovery; therefore, it is imperative to advance the understanding of baseline blood analyte data as a diagnostic and monitoring tool. For in-water, trawl-captured, immature Kemp’s ridleys (minimum $N = 31$) from Georgia, USA, the objectives of this study were to (1) establish reference intervals (RIs) for packed cell volume (PCV) and 27 plasma biochemistry analytes and (2) determine length-specific relationships in blood analytes. We observed significant positive correlations between minimum straight carapace length and PCV, amylase, calcium:phosphorus ratio, cholesterol, magnesium, triglycerides, total solids, total protein and all protein fractions (e.g. alpha-, beta- and gamma-globulins); aspartate aminotransferase and chloride showed significant negative relationships. These results suggest that certain blood analytes in Kemp’s ridleys change as these animals grow, presumptively due to somatic growth and dietary shifts. The information presented herein, in due consideration of capture technique that may have impacted glucose and potassium concentrations, represents the first report of blood analyte RIs for Kemp’s ridley sea turtles established by guidelines of the American Society for Veterinary Clinical Pathology and will have direct applications for stranded individuals in rehabilitative care and for future investigations into the health status of wild individuals from this population.

**Key words:** Health, in-water study, marine turtle, packed cell volume, plasma biochemistry, protein electrophoresis

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Introduction

Blood analytes (e.g., hematology, biochemistry) are commonly investigated in wildlife species in an effort to further understand physiology and disease (Christopher et al., 1999; Bounous et al., 2000; Borjesson et al., 2000; Geffré et al., 2009; Flint et al., 2010b; Kaufman et al., 2018). These measures provide useful information regarding the physiological state and health status of an individual, such as nutritional and hydration status, electrolyte balance, reproductive state and organ system functions (Geffré et al., 2009). Fifty years ago, the concept of reference intervals (RIs) was introduced in human medicine in an effort to understand changes in blood analytes, by defining health and disease through comparisons to ‘normal’ data of known apparently healthy individuals with refined inclusion criteria, a concept that has been adopted by veterinary medicine (Gräsbeck and Saris, 1969; Gräsbeck, 1983, 1990; Walton, 2001; Geffré et al., 2009; Inoue et al., 2012). These RIs are most commonly formulated by calculating the range that represents the central 95% of the reference population, with 90% confidence intervals of the lower and upper limits of the range also reported (Geffré et al., 2009; Friedricks, 2012).

Logistical challenges (e.g. animal capture, sample size, confounding effects of stress and handling) occur with establishing RIs in threatened and endangered wildlife species in various settings (Wobeser, 2007; Ryser-Gégioirgis, 2013; Deem and Harris, 2017; Coogan et al., 2018). Therefore, RI establishment in wildlife populations is a developing field of study. RIs have been established in some sea turtle species and populations (leatherback sea turtles (Dermochelys coriacea); hereafter, leatherbacks; loggerhead sea turtles (Caretta caretta); hereafter, loggerheads; green turtles (Chelonia mydas)) (Aguirre and Balazs, 2000; Flint et al., 2010a, b; Osborne et al., 2010; Kelly et al., 2015; Page-Karjian et al., 2015, 2020; Stacy et al., 2018a, 2019; Fleming et al., 2020), yet no RIs exist for critically endangered Kemp’s ridley sea turtles [Lepidochelys kempii; hereafter, Kemp’s ridleys (Wibbels and Bevan, 2019)] (Zollinger et al., 2019). Understanding baseline health of this sea turtle species is critical, as several on-going threats to their population recovery still exist (Wibbels and Bevan, 2019).

Nesting grounds for Kemp’s ridleys are mostly restricted to the western Gulf of Mexico (Texas, USA, to Veracruz, Mexico), with Rancho Nuevo, Tamaulipas, Mexico, experiencing the majority of nesting. Developmental and foraging habitats for Kemp’s ridleys produced on these beaches are located throughout the Gulf of Mexico and along the Atlantic coast of the United States (Wibbels and Bevan, 2019). Prior to the 2010 ‘Deepwater Horizon’ (DWH) oil spill, the Kemp’s ridley nesting population of Rancho Nuevo, Mexico, was experiencing an exponential increase. Since that disaster, there has been a deviation from this trend. Reasons for the decline remain unknown but could include fisheries interactions, poaching, pollution impacts from the DWH oil spill and/or carrying capacity of this species in the Gulf of Mexico (Gallaway et al., 2016; McDonald et al., 2017; Mitchelmore et al., 2017; Wallace et al., 2017; Caillouet et al., 2018; Wibbels and Bevan, 2019). The DWH oil spill in the Gulf of Mexico provided significant justification for the need to establish RIs in this species, so that potential deviations in blood analytes may be recognized as a result of future environmental or physiological perturbations (e.g. natural disasters, disease, pollution impacts) (Stacy et al., 2017). A number of studies have investigated blood analytes in Kemp’s ridleys (McNally et al., 2020); however, these are usually in relation to some type of stressor including forced submergence (Stabenau et al., 1991; Snoddy et al., 2009), cold stunning (Carminati et al., 1994; Turnbull et al., 2000; Innis et al., 2007, 2009), transport stress (Hunt et al., 2016) or exposure to biotoxins and toxicants (Innis et al., 2008; Perrault et al., 2014, 2017). Additionally, blood analytes established in other Kemp’s ridley studies used captive, rehabilitating individuals exposed to dietary differences and other captivity effects (e.g. stress, water temperature differences, etc.) (Stabenau et al., 1991; Moon et al., 1999; Anderson et al., 2011; Innis et al., 2007, 2008, 2009; Coleman et al., 2016; Hunt et al., 2016, 2019). Therefore, extrapolation to wild populations should be done with appropriate caution (Bolten and Bjorndal, 1992). For in-water, trawl-captured, immature Kemp’s ridleys from Georgia, USA, the objectives of this study were to (1) establish RIs for blood analytes including packed cell volume (PCV) and 27 plasma biochemistry analytes and (2) determine length-specific relationships with blood analytes.

Materials and Methods

Ethical procedures

Our study was carried out in accordance with an Endangered Species Act Section 10(a)(1)(A) permit #19621, a Georgia Department of Natural Resources Scientific Collection Permit #CN21303 and an approved Institutional Animal Care and Use Committee protocol (UF IACUC# 201706823).

Trawls

Sea turtle captures and sampling occurred from 31 May–15 Jul 2016 and 5 Jun–19 Jul 2017 and were conducted similar to methods (to include trawl gear) described in Arendt et al., (2012a, b). Trawls occurred at 2.8 knots for ≤30 min between 4.6 m and 17.0 m depth. One Kemp’s ridley sea turtle was captured off Charleston, South Carolina, USA; all others were captured near Brunswick, Georgia, USA.

Sea turtle capture, morphometrics, physical examination and sample collection and processing

Upon capture, Kemp’s ridley sea turtles were given a physical examination that consisted of visual evaluation of body condition, epibiota, external injuries and any other overt visible
abnormalities. Mass (in kg) and minimum straight carapace length (SCLmin, in cm) were recorded using a digital hanging scale (Pesola PSH200) and calipers, respectively. Body condition index (BCI) was calculated (Bjorndal et al., 2000).

All captured Kemp’s ridleys were examined for internal and external tags; if neither of these tags were present, up to two Inconel flipper tags and an internal PIT tag (Biomark, Inc., Boise, Idaho USA) were applied prior to release (Florida Fish and Wildlife Conservation Commission, 2016). The tagging sites were disinfected with povidone iodine and isopropyl alcohol to prevent infection before and after tag application (Florida Fish and Wildlife Conservation Commission, 2016).

Following physical examinations, 10 ml of blood were collected from the dorsal cervical sinus (i.e. external jugular vein) of each turtle using a 21-gauge, 1.5” needle and 10 ml sodium heparin vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA), following federal regulations regarding animal handling and sample collection (National Marine Fisheries Service Southeast Fisheries Science Center, 2008). The sampling site was swabbed with alternating applications of povidone iodine and alcohol prior to blood collection. A subsample of whole blood was aliquoted for PCV analysis (described below) and the remaining blood was centrifuged immediately (within <5 min) on the research vessel at 944 g (3600 rpm) for 5 min in a Clay Adams Sero-fugeTM centrifuge (Becton Dickinson, Sparks, Maryland, USA). Plasma was separated and stored in liquid nitrogen onboard until completion of multi-day overnight research cruises before storage in a shore-based ultralow freezer for up to 3 months prior to sample analysis, which is generally assumed to have no major effects on various analytes (Cray et al., 2009; Townsend et al., 2020); however, potential artifactual changes cannot be completely ruled out (Thoresen et al. 1995).

**Analysis of blood analytes**

Onboard the vessel, PCV was determined using whole blood drawn into microhematocrit tubes followed by centrifugation for 5 min at 13 000 g (11,500 rpm) using a micro-capillary centrifuge (Model MB, International Equipment Company, Needham Heights, Massachusetts USA). A hematocrit micro-capillary tube reader was used to determine PCV as a percentage. Also onboard, total solids in plasma were estimated using a Westover Scientific RHC-200 ATC handheld refractometer (Woodinville, Washington, USA) and plasma color was visually assessed.

Frozen plasma samples were shipped overnight on dry ice to the University of Miami Avian and Wildlife Laboratory for biochemical analyses using an Ortho 250XR (Ortho Clinical Diagnostics, Rochester, New York, USA) dry slide chemistry analyzer. Biochemical analyses included alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), blood urea nitrogen, calcium, chloride, cholesterol, creatine phosphokinase (CPK), gamma-glutamyl transferase (GGT), glucose, lipase, magnesium, phosphorus, potassium, sodium, total protein, triglycerides and uric acid. The calcium:phosphorus ratio was calculated.

Protein fractions were determined using the SPIFE 3000 system (Helena Laboratories Inc., Beaumont, Texas, USA). Fraction delimits were placed using the following conventions in electrophoretograms: pre-albumin, albumin, alpha1-globulins, alpha2-globulins, beta-globulins and gamma-globulins. Total globulins and the albumin:globulin ratio were calculated. For comparison to free-ranging, immature green turtles, loggerheads, leatherbacks and hawksbill sea turtles (Eretmochelys imbricata; hereafter, hawksbills) of the same life-stage class, representative electrophoretograms from each species using identical methodology for protein electrophoresis were chosen from other projects.

**Statistical analyses**

Statistical analyses were performed using Medcalc® statistical software (version 19.1, Ostend, Belgium). Mean, standard error, standard deviation, median and range are reported for PCV and plasma biochemistry in standard international (SI) units. Length–mass relationships were assessed using power regression.

RIs (95% with associated 90% confidence intervals) were also calculated using parametric methods based on recommendations by Friedrichs et al. (2012) for sample sizes >20, but <40. Normality was assessed using the Shapiro–Wilk test (Shapiro and Wilk, 1965), while outliers were detected using the Dixon–Reed test (Reed et al., 1971). When appropriate, logarithmic transformations were employed and outliers were removed to generate accurate RIs. For CPK, RIs were calculated using the robust method, as data could not be normalized to fit a Gaussian distribution (i.e. parametric method).

Relationships between SCLmin and the measured blood analytes were determined using least-squares linear regressions. Because of the very strong correlation between SCLmin and mass (see Results section below), only SCLmin was used in analyses. Outliers were determined by Tukey’s test and were removed as appropriate. Data were transformed when necessary to meet the assumptions of the tests. The slopes of the lines of best fit between pre-albumin + albumin and globulins in relation to SCLmin were compared using a Student’s t-test (e.g. the slope of the line of best fit between pre-albumin + albumin and SCLmin was compared to the slope of the line of best fit between total globulins and SCLmin).

**Results**

**Physical examination and morphometrics**

A total of 36 individual Kemp’s ridleys were captured from 31 May–15 July 2016 (N = 17) and 5 June–19 July 2017 (N = 19). A summary of mass, SCLmin and BCI are reported in Table 1. Three turtles were excluded from the length–mass
analyses due to one individual having an abnormally shaped carapace (from an old healed shark predation lesion, 31 cm long × 8 cm wide, to the right posterior carapace), one individual missing a measurement for mass and another individual (the turtle caught in South Carolina) having monofilament fishing line wrapped around the neck and front flippers in addition to observed line extruding from the oral cavity, which likely impacted the ability to forage as shown by a comparatively low BCI (≤ 1.40). This animal was taken to the Sea Turtle Care Center™ at the South Carolina Aquarium for treatment and an extra marginal scute. All the described observations in included study animals were considered representative of the population captured by trawling in a group exposed to similar capture and handling conditions, in addition to acceptable variability in free-ranging sea turtles.

Reference intervals

Hemolysis was absent in all samples, while mild (1+) lipemia was detected in one sample, which is considered insufficient to cause interference with dry chemistry analysis (Andreasen et al., 1997; Stacy and Innis, 2017; Stacy et al., 2019). Measures of central tendency, range and RIs in SI units are reported in Table 2. Supplemental Table 1 reports the same values in conventional units. Two turtles were removed from calculation of RIs: (1) the adult female, as this individual was the only mature turtle captured and her blood biochemical analytes skewed much of the health data to the right; (2) the turtle with severe monofilament line ingestion and low BCI showing evidence of hyporexia based on chemistry results.

A representative electrophoreogram for Kemp’s ridleys is presented in Fig. 2 along with representative electrophoreograms from four other immature, foraging sea turtle species for comparison. All individuals included in Fig. 2 were analyzed using the same laboratory and analytical methods as this study.

A very strong positive relationship \( (r^2 = 0.86; P < 0.001; N = 35) \) existed between total solids and total protein (both in g L\(^{-1}\)), with the relationship described by the formula:

\[
\text{Total solids} = (1.032 \times \text{total protein}) - 0.945
\]

Correlations with Size

Several blood analytes showed significant positive or negative relationships with SCL\(_{\text{min}}\) as determined by linear regression.
Table 2: Measures of central tendency, range and reference intervals (with 90% confidence intervals for upper and lower limits) for packed cell volume and plasma biochemical data (including protein electrophoresis) in standard international units for in-water, immature Kemp's ridley sea turtles (Lepidochelys kempii) from Georgia, USA. Parametric methods for sample sizes ≥ 20 but < 40 were used to calculate reference intervals (Friedrichs et al., 2012), unless otherwise indicated in the footnotes. Normality was assessed using the Shapiro–Wilk test (Shapiro and Wilk, 1965), while outliers were detected using the Dixon–Reed test (Reed et al., 1971). All plasma samples were free of hemolysis and lipemia, except for one sample with mild (1+) lipemia, which is not considered to cause interference using dry chemistry analysis (Andreassen et al., 1997; Stacy and Innis, 2017; Stacy et al., 2019). Abbreviations: CI, confidence interval; LRL, lower reference limit; RI, reference interval; SD, standard deviation; URL, upper reference limit.

| Analyte                                      | Mean ± SD | Median | Range           | N  | RI           | LRL 90% CI | URL 90% CI |
|----------------------------------------------|-----------|--------|-----------------|----|--------------|------------|------------|
| **Hematology**                               |           |        |                 |    |              |            |            |
| Packed cell volume [L L⁻¹]                   | 0.32 ± 0.05 | 0.31   | 0.20–0.44       | 33 | 0.23–0.41    | 0.21–0.25  | 0.39–0.43  |
| **Biochemistry**                             |           |        |                 |    |              |            |            |
| Alkaline phosphatase [U L⁻¹]                 | 119 ± 54  | 108    | 40–344          | 34 | 52–232*      | 44–63*     | 193–280*   |
| Amylase [U L⁻¹]                              | 461 ± 86  | 473    | 229–622         | 34 | 293–629      | 250–335    | 587–671    |
| Aspartate aminotransferase [U L⁻¹]           | 185 ± 51  | 175    | 130–427*        | 34 | 122–233b     | 108–130b   | 219–247b   |
| Blood urea nitrogen [mmol L⁻¹]               | 25.5 ± 4.8| 24.8   | 17.5–38.6       | 34 | 16.1–34.9    | 13.7–18.5  | 32.5–37.3  |
| Calcium [mmol L⁻¹]                           | 2.4 ± 0.2 | 2.4    | 2.0–2.9         | 34 | 1.9–2.8      | 1.8–2.1    | 2.7–3.0    |
| Calcium:phosphorus ratio                     | 0.88 ± 0.13 | 0.86  | 0.65–1.19       | 34 | 0.63–1.13    | 0.57–0.69  | 1.07–1.19  |
| Chloride [mmol L⁻¹]                          | 124 ± 5   | 124    | 115–139         | 34 | 115–134      | 112–117    | 132–136    |
| Cholesterol [mmol L⁻¹]                       | 2.7 ± 0.5 | 2.6    | 1.6–4.2         | 34 | 1.6–3.7      | 1.4–1.9    | 3.4–4.0    |
| Creatine phosphokinase [U L⁻¹]               | 1513 ± 865| 1239   | 784–4513        | 34 | 510–2955c    | 395–693c   | 2187–3812c |
| Gamma glutamyl transferase [U L⁻¹]           | –         | <5     | <5–7            | 34 | –            | –          | –          |
| Glucose (plasma) [mmol L⁻¹]                  | 6.8 ± 1.1 | 6.7    | 4.7–9.2         | 34 | 4.7–8.9      | 4.1–5.2    | 8.4–9.4    |
| Lipase [U L⁻¹]                               | 22 ± 18   | 14     | 1–65d           | 32 | 4–714d       | 3–64d      | 49–1044d   |
| Magnesium [mmol L⁻¹]                         | 2.3 ± 0.2 | 2.3    | 1.9–2.8         | 34 | 1.9–2.8      | 1.7–2.0    | 2.7–2.9    |
| Phosphorus [mmol L⁻¹]                        | 2.8 ± 0.4 | 2.7    | 1.9–3.7         | 34 | 2.0–3.5      | 1.8–2.2    | 3.3–3.7    |
| Potassium [mmol L⁻¹]                         | 4.9 ± 0.3 | 5.0    | 4.3–5.5         | 34 | 4.3–4.6      | 4.1–4.5    | 5.4–5.7    |
| Sodium [mmol L⁻¹]                            | 164 ± 4   | 164    | 154–175         | 34 | 155–172      | 153–157    | 170–174    |
| Triglycerides [mmol L⁻¹]                     | 1.1 ± 0.5 | 1.1    | 0.4–2.7         | 34 | 0.4–2.5*     | 0.3–0.5*   | 2.0–3.1*   |
| Uric acid [μmol L⁻¹]                         | 103.0 ± 31.2 | 107.6 | 41.6–178.4      | 34 | 41.9–164.2   | 26.5–57.3  | 148.8–178.0|
| **Total solids and protein electrophoresis** |           |        |                 |    |              |            |            |
| Total protein [g L⁻¹]                        | 38 ± 6    | 38     | 24–52           | 34 | 26–50        | 23–29      | 47–53      |
| Total solids [g L⁻¹]                         | 37 ± 6    | 38     | 24–50           | 34 | 25–49        | 22–28      | 46–52      |
| Pre-albumin [g L⁻¹]                          | 2.2 ± 1.5 | 1.7    | 0.7–6.4         | 34 | 0.6–5.7*     | 0.5–0.8*   | 4.3–7.5*   |
| Albumin [g L⁻¹]                              | 7.8 ± 1.7 | 7.6    | 4.8–12.2        | 34 | 4.5–11.0     | 3.7–5.4    | 10.2–11.8  |
| Alpha1-globulins [g L⁻¹]                     | 3.3 ± 1.1 | 3.5    | 1.3–5.2         | 34 | 1.2–5.5      | 0.6–1.7    | 4.9–6.0    |
| Alpha2-globulins [g L⁻¹]                     | 4.3 ± 1.1 | 4.2    | 2.4–7.7         | 34 | 2.1–6.4      | 1.6–2.6    | 5.9–7.0    |
| Beta-globulins [g L⁻¹]                       | 9.3 ± 2.6 | 8.9    | 5.0–19.4        | 34 | 5.5–14.8*    | 4.9–6.2*   | 13.0–16.7* |
| Gamma-globulins [g L⁻¹]                      | 10.7 ± 2.8| 10.3   | 5.3–17.5        | 34 | 5.4–16.1     | 4.0–6.7    | 14.8–17.4  |
| Total globulins [g L⁻¹]                      | 27.6 ± 5.0| 28.0   | 17.4–39.8       | 34 | 17.9–37.4    | 15.5–20.4  | 34.9–40.0  |
| Albumin:globulin ratio                       | 0.37 ± 0.07 | 0.37  | 0.21–0.53       | 34 | 0.22–0.51    | 0.19–0.26  | 0.48–0.55  |

*Reference intervals were calculated using logarithmic transformations, as original data were non-normal.

1437 U L⁻¹ was an outlier; this value was removed from reference interval calculations. The second highest value was 246 U L⁻¹.

4Reference intervals were calculated using the robust method with a logarithmic transformation, as data could not be transformed to meet the assumptions of normality for parametric methods.

41 U L⁻¹ was an outlier; this value was removed from reference interval calculations. The second lowest value was 5 U L⁻¹.
Figure 2: Representative plasma protein electrophoretograms of five immature, free-ranging sea turtle species based on identical laboratory methodology: (a) Kemp’s ridley sea turtle, Lepidochelys kempii (SCLmin: 45.8 cm; this study), (b) green turtle, Chelonia mydas (SCLmin: 25.9 cm; captured in Florida’s Big Bend, USA; Perrault, unpublished data), (c) loggerhead sea turtle, Caretta caretta (SCLmin: 33.1 cm; captured in Florida’s Big Bend, USA; Perrault, unpublished data), (d) leatherback sea turtle, Dermochelys coriacea (curved carapace length: 134.3 cm; captured off of Shackleford Banks, NC, USA; Harms, unpublished data) and (e) hawksbill sea turtle, Eretmochelys imbricata (SCLmin: 36.0 cm; captured off of Key West, FL, USA; Wood, unpublished data), showing the fractions of interest: pre-albumin, albumin, alpha_1-globulins, alpha_2-globulins, beta-globulins and gamma-globulins. By convention, no units are reported on the y-axis (Gicking et al., 2004).

PCV ($r^2 = 0.69; P < 0.001; N = 32$), amylase ($r^2 = 0.55; P < 0.001; N = 34$), calcium:phosphorus ratio ($r^2 = 0.19; P = 0.011; N = 33$), cholesterol ($r^2 = 0.17; P = 0.017; N = 33$), magnesium ($r^2 = 0.16; P = 0.020; N = 33$), triglycerides ($r^2 = 0.14; P = 0.030; N = 33$), total solids ($r^2 = 0.47; P < 0.001; N = 34$), total protein ($r^2 = 0.48; P < 0.001; N = 34$), pre-albumin ($r^2 = 0.13; P = 0.039; N = 34$), albumin ($r^2 = 0.28; P = 0.001; N = 34$), alpha_1-globulins ($r^2 = 0.29; P = 0.001; N = 34$), alpha_2-globulins ($r^2 = 0.16; P = 0.021; N = 34$), beta-globulins ($r^2 = 0.15; P = 0.022; N = 34$), gamma-globulins ($r^2 = 0.23; P = 0.005; N = 34$) and total globulins ($r^2 = 0.34; P < 0.001; N = 34$) showed a significant positive relationship with SCL_{min}, while AST ($r^2 = 0.15; P = 0.025; N = 33$) and chloride ($r^2 = 0.23; P = 0.005; N = 33$) showed a significant negative relationship with SCL_{min} (see Supplemental Table 2 for complete statistical results).

The slopes of the lines of best fit comparing pre-albumin + albumin (m = 0.16) and total globulins (m = 0.35) in relation to SCL_{min} were significantly different ($t(64) = -2.11; P = 0.008$) (Fig. 3).
were not observed 2 h after a meal (Anderson Carolina, USA, significant changes in glucose concentrations in rehabilitating Kemp’s ridleys and green turtles in North (Turnbull et al., 2000; Innis et al., 2007, 2009). Additionally, previous investigations of clinically convalescent Kemp’s ridleys (14.7%) were capture stress, as glucose concentrations in only 5/34 turtles Stabenau and Vietti, 2003). We observed minimal evidence of Kemp’s ridleys from this study. Despite this finding, feeding immediately before capture may also have contributed to slight plasma glucose variations in additional effects on blood analytes associated with the time frame to reduce the impacts of forced submergence; however, possible reducing blood analyte changes in association with presumptive somatic growth and dietary shifts, which will be useful for interpretations on an individual and population level. Trawl times in this study were kept to a minimum (≤30 mins) to Turtles were clinically healthy and representative of a free-ranging, in-water study in Georgia, USA. In addition to filling a knowledge gap for basic clinicopathological data for this life stage, geographical region and season (spring/summer) of this species, the data herein contribute to understanding blood analyte changes in association with presumptive somatic growth and dietary shifts, which will be useful for interpretations on an individual and population level. Trawl times in this study were kept to a minimum (≤30 mins) to reduce the impacts of forced submergence; however, possible effects on blood analytes associated with the time frame and method of animal capture utilized in this study cannot be ruled out (Stabenau et al. 1991; Gregory et al., 1996; Stabenau and Vietti, 2003). We observed minimal evidence of capture stress, as glucose concentrations in only 5/34 turtles (14.7%) were >8.3 mmol/L (>150 mg/dL), a cut-off value considered suggestive of hyperglycemia as compared to previous investigations of clinically convalescent Kemp’s ridleys (Turnbull et al., 2000; Innis et al., 2007, 2009). Additionally, in rehabilitating Kemp’s ridleys and green turtles in North Carolina, USA, significant changes in glucose concentrations were not observed 2 h after a meal (Anderson et al., 2011). Despite this finding, feeding immediately before capture may also have contributed to slight plasma glucose variations in Kemp’s ridleys from this study.

**Discussion**

This study reports blood analyte RIs and length-specific correlations for immature Kemp’s ridley sea turtles from an in-water study in Georgia, USA. In addition to filling a knowledge gap for basic clinicopathological data for this life stage, geographical region and season (spring/summer) of this species, the data herein contribute to understanding blood analyte changes in association with presumptive somatic growth and dietary shifts, which will be useful for interpretations on an individual and population level. Trawl times in this study were kept to a minimum (≤30 mins) to reduce the impacts of forced submergence; however, possible effects on blood analytes associated with the time frame and method of animal capture utilized in this study cannot be ruled out (Stabenau et al. 1991; Gregory et al., 1996; Stabenau and Vietti, 2003). We observed minimal evidence of capture stress, as glucose concentrations in only 5/34 turtles (14.7%) were >8.3 mmol/L (>150 mg/dL), a cut-off value considered suggestive of hyperglycemia as compared to previous investigations of clinically convalescent Kemp’s ridleys (Turnbull et al., 2000; Innis et al., 2007, 2009). Additionally, in rehabilitating Kemp’s ridleys and green turtles in North Carolina, USA, significant changes in glucose concentrations were not observed 2 h after a meal (Anderson et al., 2011). Despite this finding, feeding immediately before capture may also have contributed to slight plasma glucose variations in Kemp’s ridleys from this study.

**Physical examination and morphometrics**

For the establishment of clinically relevant RIs, the population of interest must be apparently healthy (Walton, 2001; Osborne et al., 2010). The animals included in this study were assumed to be in ‘good health’ at time of capture and representative of a free-ranging, in-water caught group based on external physical examination as the turtles (1) had few, mostly minor, external abnormalities, (2) were free of excessive epibiota and (3) all had good BCI. It is possible that some stress effects occurred due to trawling capture methods, as several blood analytes (e.g. CPK, glucose, lactate, LDH, phosphorus) in sea turtles reportedly change significantly in response to increased entanglement times in gillnets (Snoddy et al., 2009); however, trawl times in our study were limited to a maximum of 30 min, which was lower than the study conducted by Snoddy et al., (2009) with soak times ranging from 20–240 min.

The BCI of all turtles in this study ranged from 1.45 to 1.90 (mean ± SE: 1.62 ± 0.02), which overlaps with apparently healthy Kemp’s ridleys of similar size (mean ± SE: 1.56 ± 0.02) captured in Florida’s Big Bend (Perrault et al., 2017). In immature and adult loggerhead turtles that were stranded along the southeastern coast of the United States, BCIs were, on average, 1.09, 1.27 and 1.49 for individuals that stranded dead with chronic debilitation (CD), stranded alive with CD and then began feeding for up to 10 weeks in rehabilitation and for those that recovered from CD (pre-release data, respectively. In that same study, healthy control loggerheads had a mean BCI of 1.56 (Stacy et al., 2018b). Therefore, it can be assumed that the turtles in this study were of good body condition and that our blood analytes and subsequent BCIs are likely representative of an apparently healthy population that can serve as a reference for future health-based investigations of Kemp’s ridleys. Subsequent studies should provide descriptions of BCI by species, life-stage class and health status (i.e. emaciated, normal, robust), so that these scores may be used to interpret the results of physical examinations and morphometric measurements (Flint et al., 2010a; Stacy et al., 2018b). For example, one of the excluded turtles of this study with monofilament line ingestion was removed due to a number of measured blood analytes falling on the extreme low or high end of the range in comparison to other study animals.

**Packed cell volume**

PCV is an important indicator of health as it provides information on hydration status and anemia (Stamper et al., 2005). The PCV of study turtles ranged from 0.20 to 0.44 L L⁻¹ (mean ± SE: 0.32 ± 0.01 L L⁻¹), which is similar to mean values reported in other immature Kemp’s ridley studies (Stabenau et al., 1991: 0.31 L L⁻¹; Carminati et al., 1994: 0.31 L L⁻¹; Innis et al., 2009: 0.30 L L⁻¹). We observed a positive relationship between PCV and SCLmin, a finding that has been documented in six of seven sea turtle species including green, hawksbill, Kemp’s ridley, leatherback, loggerhead and olive ridley sea turtles (Lepidochelys olivacea) (Frair, 1977; Wood and Ebanks, 1984; Casal et al., 2009; Rousselet et al., 2013; Perrault et al., 2016b; Stacy et al., 2018a). Red blood cells (RBCs) are known to increase in diameter, number and volume as turtles grow and age (Frair, 1977). It is also presumed that an increased number of circulating RBCs is beneficial for meeting oxygen demands associated with longer dive times in larger turtles, hence the higher PCV in mature turtles.
Life stages (Stamper et al., 2005; Perrault et al., 2016b; Stacy et al., 2018a).

Electrolytes and minerals

Plasma electrolytes (e.g., sodium, chloride, potassium) and minerals (e.g., calcium, phosphorus, magnesium) of study turtles fell within the normal ranges for other sea turtle species (Stacy and Innis, 2017). Negative size-related changes in electrolytes in sea turtles have been previously observed (Hasbún et al., 1998; Stacy et al., 2018a). The negative association between SCL\textsubscript{min} and plasma chloride may be associated with changes in diet as these organisms transition from oceanic to neritic habitats. Kemp’s ridleys are described as having an oceanic-neritic developmental pattern (Snover et al., 2007), where they undergo early development in the pelagic environment followed by recruitment back to neritic zones for foraging and reproduction (Collard and Ogren, 1990; Bolten, 2003). This habitat shift likely leads to a dietary shift, whereby oceanic Kemp’s ridleys forage on epipelagic organisms (e.g., gastropods, malacostracans, algae) and then change to a diet of benthic crabs and mollusks in the neritic environment (Shaver, 1991; Jones and Seminoff, 2013). Other potential explanations for this association include osmoregulatory differences that may occur as these animals transition in diets and change their diving behavior or as they experience somatic growth, including that of the salt gland (Stacy et al., 2018a). The observed positive correlations of SCL\textsubscript{min} with calcium/phosphorus ratio and plasma magnesium are presumptively associated with dietary shifts in addition to phases of somatic growth of this life stage in this region, as lower concentrations of minerals reportedly are typical for immature life-stage classes in comparison to adults (Delgado et al., 2011).

We found no significant trends with turtle size and plasma potassium concentrations. However, potassium should be interpreted in context of possible spurious increases, especially if hemolysis is observed in plasma (Stacy et al., 2019) or based on methodology of turtle capture since trawling can result in rapidly increased potassium due to metabolic acidosis in response to short-term forced submergence (Stabenau et al., 1991). Mean potassium concentrations of Kemp’s ridleys in this study (mean ± SD = 4.9 ± 0.3 mmol L\textsuperscript{−1}; range 4.3–5.5 mmol L\textsuperscript{−1}) were within previously reported normal limits for this species (Innis et al., 2009; Coleman et al., 2016; Stacy and Innis, 2017; McNally et al., 2020); however, 17/34 (50%) of turtles had values >5.0 mmol L\textsuperscript{−1}, with two turtles having potassium concentrations of 5.5 mmol L\textsuperscript{−1}. In cold-stunned Kemp’s ridleys, potassium concentrations >5.5 mmol L\textsuperscript{−1} were associated with mortality (Innis et al., 2009; Keller et al., 2012), with concentrations ranging from 5.0 to 5.4 mmol L\textsuperscript{−1} also considered to be abnormally high (Stacy et al., 2013). For healthy, foraging Kemp’s ridleys captured by trawl, underlying mechanisms for hyperkalemia include metabolic acidosis and/or muscle damage from exertion during trawling, and thus slightly elevated potassium concentrations may be less concerning here than with cold-stunned individuals; yet, hyperkalemia should be considered due to potential impacts on cardiac function (Innis et al., 2014).

Tissue enzyme activities

Generally, plasma enzyme activities (e.g. ALP, AST, GGT) are highly variable in sea turtles and not as tissue specific as in mammals, making interpretation difficult and thereby limiting their diagnostic value (Anderson et al., 2013; Petrosky et al., 2015). Conversely, amylase and lipase are two digestive enzymes with reported high activities in pancreatic tissue; however, the clinical significance of these enzymes in sea turtles remains unknown (Anderson et al., 2013; Petrosky et al., 2015). A moderate positive correlation between SCL\textsubscript{min} and plasma amylase was observed in Kemp’s ridleys from this study, similar to captive, immature loggerheads from Japan (Kakizoe et al., 2007). Juvenile green turtles reportedly showed significantly higher plasma activities of amylase post-prandially in comparison to baseline values established pre-prandially (Anderson et al., 2011), suggesting that food intake increases amylase activity. The positive size-related trend observed between SCL\textsubscript{min} and amylase in this study could be attributed to post-prandial increases as the most recent food consumption in study turtles is unknown. Additionally, general dietary and/or water salinity shifts that occur as younger Kemp’s ridleys transition from their oceanic phase to their neritic phase could be at play, as these animals are known to forage on dissimilar food items during these different stages of development (Shaver, 1991; Jones and Seminoff, 2013).

We also observed a weak negative correlation between SCL\textsubscript{min} and plasma AST activity, a trend that has been documented in immature and mature green turtles from Taiwan (Fong et al., 2010). This enzyme has low organ specificity and showed activities in 19/30 (63.3%) and 9/9 (100%) different tissues of loggerheads and Kemp’s ridleys, respectively, with highest tissue activities observed in liver, kidney, pancreas and cardiac/skeletal muscle (Anderson et al., 2013; Petrosky et al., 2015). Normal plasma AST activity for reptiles is <250 U L\textsuperscript{−1}, and all Kemp’s ridleys in this study, except one (427 U L\textsuperscript{−1}, an outlier), had values below this threshold (Campbell, 2006). Increased AST activities in clinical settings can suggest hepatic or muscular injury, but due to its low organ specificity, other conditions must be considered (Campbell, 2006; Anderson et al., 2013). A possible explanation for the negative correlation between AST activity and SCL\textsubscript{min} includes faster tissue growth at smaller body sizes (Stacy and Innis, 2017; Stacy et al., 2018a), with a gradual slowing of growth rate as turtles continue to mature (Chaloupka and Zug, 1997; Bolten, 2003).

Lipids

The observed positive size-related correlations in Kemp’s ridleys between cholesterol and triglycerides and SCL\textsubscript{min} were
expected (Day and Alexander, 1974; Morpurgo and Gelman, 1991; Hasbún et al., 1998; Labrada-Martagón et al., 2010; Delgado et al., 2011; Rousselet et al., 2013). Similar to other analytes, these size-related changes are presumably associated with dietary shifts (Shaver, 1991; Jones and Seminoff, 2013; Stacy et al., 2018a). Changes in plasma lipids as a result of nutritional alterations occurring during seasonal changes have also been documented in other reptiles (Morpurgo and Gelman, 1991; Labrada-Martagón et al., 2010).

**Protein electrophoresis and total solids**

Plasma protein fractionation has been evaluated in just two studies of ridley turtles to date, both in Kemp’s ridleys from the eastern Gulf of Mexico in Florida’s Big Bend; however, RIs were not established due to low sample sizes and the presence of a harmful algal bloom (HAB) at the time of sampling (Perrault et al., 2014, 2017). Perrault et al. (2017) used the same laboratory and analytical methodology as this study to analyze for plasma proteins in Kemp’s ridleys from the Big Bend. Results for total protein and all protein fractions were similar between the two populations with the exception of the gamma-globulins, which were 5 g/L lower in turtles from this study. This can be explained by the red tide (HAB) bloom and its associated brevetoxins that were present near the time of sampling in the Big Bend, as a number of protein fractions, including gamma-globulins, have been shown to be positively correlated with brevetoxin exposure in manatees (Trichechus manatus), loggerheads and green turtles (Walsh et al., 2015, 2019; Perrault et al., 2016a, 2017). Other potential explanations for this dissimilarity include additional differences in antigenic stimulation (e.g. parasite burden) between the two populations (Osborne et al., 2010).

As expected, all plasma proteins showed significant positive correlations with SCLmin. Other sea turtle studies have noted similar size-related increases and suggest that these changes are related to dietary shifts, immune stimulation, physiological changes associated with vitellogenesis and/or somatic growth (Frair and Shah, 1982; Kakizoe et al., 2007; Casal et al., 2009; Osborne et al., 2010; Rousselet et al., 2013; Espinoza-Romo et al., 2018; Stacy et al., 2018a).

Globulins (alpha-, beta- and gamma-globulins) show variations more frequently than albumin (often with or without pre-albumin included) in sea turtles (Stacy et al., 2018a). Size-related increases in plasma globulin concentrations are presumably related to nutritional alterations and/or increased exposure to antigens as turtles grow and age, especially as they recruit back to neritic habitats where more frequent antigen exposure occurs (Innis et al., 2010; Stacy et al., 2018a). This is further confirmed by the increasing trend of beta- and gamma-globulins with SCLmin as both of these protein fractions are known to contain immunoglobulins and various acute phase proteins (Osborne et al., 2010; Evans, 2011). Globulins in Kemp’s ridleys from this study showed a rate of increase 2.2 times that of pre-albumin + albumin. Plasma globulins in juvenile Azorean loggerheads showed a rate of increase 3.6 times that of albumin, further indicating that globulins increase faster than albumin in juvenile turtles undergoing somatic growth (Stacy et al., 2018a); however, pre-albumin was not included in those calculations as protein electrophoresis was not conducted in that study. Interestingly, the albumin:globulin ratio did not decrease with increasing SCLmin in turtles from this study, as it did in Azorean loggerheads (Stacy et al., 2018a). This is possibly due to the slower rate of increase of globulins versus pre-albumin + albumin seen here in comparison to Azorean loggerheads, although differences in analytical methodology must be considered (Stacy et al., 2018a).

The comparison of immature Kemp’s ridleys electrophoretograms to those from representatives of four other immature sea turtle species in Fig. 2 provides a unique opportunity for visual comparison and shows some similarities, including the presence of a pre-albumin fraction in all species. Some minor variations are observed in alpha-globulins, with more obvious differences in beta- and gamma-globulins. Considerations for these species-specific differences include variations in diet, fibrinogen concentration and antigenic exposure in various habitats.

The gold standard for determination of total plasma protein in sea turtles is the Biuret method, while refractometry provides the ability to rapidly and easily estimate total solids, which correlate with total plasma protein in normal colored samples (Macrelli et al., 2013, Stacy and Innis, 2017). Potential overestimation errors in total solid estimates can arise due to confounding factors including hemolysis, lipemia or elevated concentrations of various biochemical analytes (e.g. glucose, urea, sodium, chloride) (Stacy and Innis, 2017). Previous studies of loggerhead and green turtles have documented strong to very strong correlations between total solids and total protein (Bolten and Bjorndal, 1992; Rousselet et al., 2013), similar to our findings. Additionally, our results showed overlapping measures of central tendencies and reference ranges for total solids and total protein (Table 2), similar to leatherback and loggerhead turtles (Deem et al., 2006, 2009). Mammalian conversion equations from total solids to total protein exist; yet, these have not been proven useful in reptiles to date, and species-specific total solid to total protein conversion equations should be developed for sea turtles (Bolten and Bjorndal, 1992).

**Conclusions**

Here, we provide morphometric and blood analyte data from immature, actively foraging Kemp’s ridleys from the northwest Atlantic Ocean that were considered representative of the population and that were captured by trawling. Conservation physiology projects like these seek to explore wildlife populations and provide a baseline for future studies investigating their responses to physiological changes, environmental disturbances or disease, so that we may better understand reasons for population declines or changes in population...
dynamics (Wikelski and Cooke, 2006, Cooke and O’Connor, 2010). The RIs established here, in due consideration of capture technique in this study, provide valuable information for individuals that strand and enter rehabilitation facilities, in addition to future health assessment studies that may be conducted for population monitoring or in response to environmental changes or disturbances (Deem et al., 2001; Aguirre and Lutz, 2004; Osborne et al., 2010; Flint et al., 2015; Kelly et al., 2015; Page-Karjian et al., 2015, 2020; Villa et al., 2017; Stacy et al., 2017, 2018a). We identified a number of physiological changes in these organisms considered in ‘good health’ that are likely associated with dietary/habitat shifts, somatic growth and/or other physiological alterations. The information herein provides a better understanding of physiological changes associated with growth in immature Kemp’s ridley sea turtles, which can provide clinical utility of physiological changes in these organisms considered in ‘good health’ that are likely associated with dietary/habitat shifts, somatic growth and/or other physiological alterations. The information herein provides a better understanding of physiological changes associated with growth in immature Kemp’s ridley sea turtles, which can provide clinical utility for individuals during rehabilitation and in interpretations of blood data in population-level health assessments to help guide conservation and management decisions.

Supplementary material
Supplementary material is available at Conservation Physiology online.

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J.R.P., M.D.A. and N.I.S. conceptualized the study, M.D.A., J.A.S. and J.L.B. led all fieldwork and sample collection, with data contributions from C.A.H., C.C., K.A.T. and L.D.W.; J.R.P. analyzed the data, with contributions from all authors. J.R.P., M.D.A. and N.I.S. wrote the original manuscript, while all authors reviewed and edited the final draft. All co-authors reviewed, edited and approved the final version of this manuscript.

Competing interests
The authors declare no competing interests.

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