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Contribution to the Special 'Managing flatback turtles for the future'

Haematologic and plasma biochemical reference intervals for flatback turtles *Natator depressus*

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ABSTRACT: Blood reference intervals (RIs) provide an indication of systemic health and are central to any baseline health survey. RIs are available for all sea turtle species, except for the flatback turtle *Natator depressus*. We developed the first nesting and foraging flatback turtle RIs from a healthy reference population of 211 individuals. We found flatback turtle RIs were generally similar to other published sea turtle RIs. For flatback turtles, we detected significant differences in blood reference values (RVs) by life stage (nesting vs. foraging), sex, and location, as well as by measurement technique (laboratory vs. field). Clinically significant differences justified the establishment of separate RIs for nesting and foraging flatback turtles and for laboratory and field techniques. Clinical application of these blood RIs for individuals in rehabilitation includes disease diagnosis, health monitoring, and the development of prognostic indicators. As sea turtles are regarded as sentinels of environmental health, flatback turtle baseline health data will also be useful for population health monitoring and as a reference for future studies where changes in RIs may indicate an environment in decline.

KEY WORDS: Flatback sea turtle · Blood reference intervals · Wildlife health assessments · Baseline health study · Haematology · Plasma biochemistry · Western Australia

1. INTRODUCTION

The flatback turtle *Natator depressus* is a marine turtle restricted to the continental shelf waters of northern Australia (Walker & Parmenter 1990), with mainland and coastal island rookeries. It is a specially protected threatened species with vulnerable status at both state and federal levels but is classified as 'data deficient' internationally (Commonwealth of Australia 2017, IUCN 2019). Flatback turtles face numerous anthropogenic threats, including large-scale industrial and coastal development and climate change (Commonwealth of Australia 2017, Department of Biodiversity, Conservation and Attractions 2017). Environmental changes arising from these threatening processes, including chemical pollution, habitat degradation, and abrupt change in diet, may be associated with changes in sea turtle haematological and blood chemistry values over time (Deem & Harris 2017). Western Australia (WA) has some of the largest nesting and foraging flatback turtle populations in Australia (Department of the Environment and Energy 2019), with many rookeries and foraging grounds adjacent to large-scale resource development projects. The proximity of flatback turtles to these sites highlights the importance and urgency of research into this species.

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Blood reference values (RVs) are used to calculate blood reference intervals (RIs), which typically encompass the middle 95% (between 2.5th and 97.5th percentiles) of a healthy reference population and are central to baseline health surveys (Deem et al. 2001, Geffré et al. 2009). Once established, these baseline RIs are essential to assess the health status of individuals and populations, identify clinically unhealthy individuals, monitor trends, and serve as prognostic indicators (e.g. for sea turtles in rehabilitation centres). Given that sea turtles are regarded as environmental sentinels, sea turtle RIs may also indicate aquatic ecosystem health (Aguirre & Lutz 2004).

As ectotherms, sea turtles require additional consideration of the potential effects of intrinsic variables (e.g. species, sex, age, size, physiologic status) and extrinsic variables (e.g. season, habitat, epibiota load, capture method, other environmental conditions) when developing RIs (Stacy & Innis 2017). Further challenges associated with development of RIs in sea turtles include potential uncertainties associated with the case definition of a 'clinically healthy' individual, analytical methodologies, and selection of blood analytes. For example, the convenience sampling typically used in wildlife studies, involving selecting individuals of unknown health, can introduce error. Field methods can also introduce error or variation, such as through the use of manual techniques which may lack the precision, quality assurance, and quality control of accredited laboratories (Flatland et al. 2010, Friedrichs et al. 2011). In a study by Flint et al. (2010a), only a small proportion of haematological and biochemical values were significantly different between unhealthy and healthy loggerhead turtles, suggesting that disease may not be reflected in haematological or biochemical changes or that not all unhealthy individuals had been excluded from the reference group. This would not be surprising given the subtlety of clinical signs in reptiles. RIs are typically based on analytes relevant to humans or domestic animals, which may not be applicable to reptiles (Herbst & Jacobson 2003), with many analytes lacking sensitivity and specificity for reptiles as indicators of disease (Stacy & Innis 2017). Additionally, certain analytical methods are not validated in reptiles (Campbell 2014).

While numerous RIs have been developed for sea turtles, statistical deficiencies associated with small sample sizes, unreported confidence intervals (CIs), and out-dated outlier detection methods may result in inaccurate RIs (Flint et al. 2010a). Accuracy of RI estimates can be improved by following International Federation of Clinical Chemistry (IFCC) and Clinical Laboratory and Standards Institute (CLSI) guidelines, adopted by the American Society for Veterinary Clinical Pathology (ASVCP), and using appropriate statistical techniques (Friedrichs et al. 2011).

In Australia, baseline blood RVs and RIs have been developed for green turtles *Chelonia mydas* (Hamann et al. 2006, Whiting et al. 2007, 2014a, Flint et al. 2010b, Kophamel et al. 2022), loggerhead turtles *Caretta caretta* (Flint et al. 2010a, Trocini 2013), and hawksbill turtles *Eretmochelys imbricata* (Whiting et al. 2014a,b). There are several bloodwork studies for flatback turtles; however, RIs have not been reported (Sperling et al. 2007, Pereira et al. 2013, Scheelings et al. 2020). Only 3 studies investigated nesting turtles (Sperling et al. 2007, Trocini 2013, Scheelings et al. 2020), the remaining studies involved foraging turtles. Further, few of the blood RI studies in sea turtles had more than 120 animals (Flint et al. 2010a,b, Trocini 2013), and not all studies followed the CLSI-IFCC recommendations for determination of RIs, making comparisons between studies challenging.

The aim of this study was to develop a health baseline for flatback turtles by establishing haematological and biochemistry RIs, with specific objectives to (1) develop blood RIs for nesting and foraging flatback turtles; (2) investigate associations between blood RVs and selected variables including life stage, sex, rookery location, nesting year, and foraging year; and (3) investigate the level of agreement for blood RVs between laboratory and field techniques.

2. MATERIALS AND METHODS

2.1. Study sites and animals

This health baseline research was conducted between 2016 and 2022 at 3 study sites in WA. The field sites comprised medium density rookeries at Thevenard Island (21.4563° S, 115.0021° E) and Eighty Mile Beach (19.5931° S, 121.2694° E), and resident foraging grounds at Roebuck Bay (18.0585° S, 122.2831° E) and Eighty Mile Beach. These foraging grounds include flatback turtles of mixed genetic stocks, while the rookeries are separate distinct genetic stocks (Fitz-Simmons et al. 2020). Nesting sites were chosen for ease of access and to sample rookeries across a latitudinal range. Foraging sites were selected because they were the only 2 known foraging locations. The study sites included industrialised (Thevenard Island), undeveloped (Eighty Mile Beach), and urbanised (Roebuck Bay) sites. Study animals (n = 211) included flatback turtles nesting at Thevenard Island (n = 67)

and Eighty Mile Beach (n = 69) and foraging in Roebuck Bay (n = 69) and in waters adjacent to Eighty Mile Beach (n = 6). Nesting turtles were sampled during summer in the wet season (November–February), while foraging turtles were sampled during winter in the dry season (May–August), except for one field trip in February.

2.2. Capture and restraint

Nesting flatback turtles selected for sampling were hand-caught returning to the ocean and restrained on a purpose-built turtle restraining device. Foraging turtles were captured either using a scientific sampling technique known as 'rodeo' whereby turtles are hand-caught by jumping from a small vessel (Limpus & Walter 1980), or using a dip net from Department of Biodiversity, Conservation and Attractions (DBCA) vessels during dedicated foraging flatback surveys, and then restrained manually for blood sampling.

2.3. Health assessment and blood sampling

Blood samples were collected directly post-capture and restraint. A basic external physical examination was conducted, including body condition scoring (Flint et al. 2009, Norton & Wyneken 2015) and recording any abnormalities (e.g. flipper amputations, neurological deficits, presence of barnacles). Morphometrics were recorded (to the nearest 0.1 cm), including curved carapace length (CCL) and curved carapace width (CCW), using flexible measuring tape. Foraging turtles were weighed (to nearest 0.5 kg) using the same mechanical scale, and suspected males also had various tail measurements taken (not reported).

The dorsocervical sinus area was prepared with aqueous chlorhexidine gluconate/alcohol 5% (Chlorhex C®, Jurox), and a 3.8 cm 18G needle was used to collect 20–30 ml of blood which was immediately transferred into lithium heparin (Li-Hep) anticoagulant tubes and placed in a cooler. A subset of nesting (n = 38) and foraging (n = 67) blood samples was transferred to fluoride-oxalate (Fl-Ox) tubes for whole blood glucose analysis, as per the recommendations of the laboratory.

The 10 ml vacutainer tubes were centrifuged within 8 h of blood collection at 1534 × *g* for 10 min (E8V LW Scientific Centrifuge) and the plasma pipetted off into aliquots. Multiple blood films were prepared using Li-Hep whole blood. Samples were prepared for submission to Vetpath Laboratory Services. Additional blood samples were banked for future diagnostic testing.

2.4. Field-based tests

Packed cell volume (PCV) was determined using duplicate plain glass capillary tubes (Statspin® Microhematocrit 40 mm untreated glass tubes, Iris) filled with Li-Hep whole blood and centrifuged at 6900 × *g* for 3 min using a ZipCombo Centrifuge (LW Scientific). Total plasma solids (TPS) were determined using a refractometer (Brix 0-32% Refractometer, LW Scientific).

An i-STAT portable point-of-care analyser (Abaxis®) was used for a subset of individuals (n = 70) including nesting (n = 63) and foraging (n = 7) turtles using Li-Hep whole blood and a Chem8+ cartridge. Only the most clinically useful Chem8+ analytes (sodium, potassium, chloride, glucose, blood urea nitrogen [BUN]) were reported. The i-STAT haematocrit (HCT) was also reported to facilitate comparison with PCV values determined by other techniques. Cases with an i-STAT BUN reading of '< 1 mmol l^{-1}' (n = 43) were converted to 0.5 mmol l^{-1}.

2.5. Laboratory testing

For haematology, PCV was determined manually using Li-Hep whole blood in 75 mm plain glass capillary tubes (Hurst Scientific) and centrifuging at 5345 × *g* for 3 min in the Haematokrit 20 (Hettich). Estimated total white blood cell (WBC) counts were performed by counting WBCs in 10 representative fields on a blood film under 10 or 40× and multiplying the WBC count by the square of the objective. Differential percentage was performed by counting 100 WBCs (identifying as heterophil, lymphocyte, monocyte/azurophil, eosinophil, or basophil) under 40×, with absolute count calculated from the percentage of the estimated total WBC count (Heatley & Russell 2019). Heterophil to lymphocyte ratio (H:L) was also calculated, as a potential indicator of stress. Remaining haematology, including haemoglobin (Hb) and red blood cell (RBC) count, was performed for nesting turtles on the Cell-Dyn 3700 (Abbott Diagnostics), and for foraging turtles on the Sysmex XN-1000 (Sysmex). Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), and mean cell volume (MCV) values were calculated. Comments regarding cell morphology, haemoparasites, thrombocyte estimation,

and haemolysis were also reported. Generally, when describing numbers of abnormal cells per 100× objective (1000 times magnification) or degree of change (for example polychromasia or anisocytosis), the grading scheme used was mild (1+, 1–5 cells); moderate (2+, 6–10 cells); or severe (3+, >10). All blood film examinations were performed by board-certified clinical pathologists at Vetpath Laboratory Services, a NATA accredited laboratory.

The biochemical panel included creatine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin, BUN, bile acids, uric acid, glucose (Li-Hep), glucose (Fl-Ox), cholesterol, triglyceride, sodium, potassium, chloride, total protein, albumin, globulin, calcium, phosphorus, magnesium, iron, lactate dehydrogenase (LDH), and glutamate dehydrogenase (GLDH). Sodium to chloride ratio (Na:K), albumin to globulin ratio (A:G), and calcium to phosphorus ratio (Ca:P) were also calculated. All biochemistry was performed on the Beckman Coulter AU680 (Beckman Coulter) at Vetpath Laboratory Services, except for LDH (testing unavailable), which was instead tested using an Advia Chemistry XPT (Siemens) at Western Diagnostic Pathology.

2.6. Data analysis

Exclusion criteria for RIs included immature individuals, recaptures within 3 mo, and 'clinically unhealthy' individuals, as determined by diminished body condition and/or clinical abnormalities detected during examination. Also excluded were samples with haemolysis, blood film errors, >60 h to laboratory analysis, >8 h to centrifugation, >8 h to i-STAT analysis, and other miscellaneous laboratory or sample issues, such as autoanalyzer errors, lipaemic, or clotted blood samples. If laboratory and field PCV values differed by >20%, all laboratory RBC parameters and field PCV were excluded.

RIs were calculated separately for nesting and foraging turtles using Reference Value Advisor (RefVal v2.1) (Solberg 1995, National Veterinary School of Toulouse 2012). Regardless of distribution, RIs were computed using 5 different methods: parametric, robust, and non-parametric methods, the former 2 with and without transformation (Box-Cox) (Geffré et al. 2011). CIs were calculated using non-parametric tables (>120) or non-parametric bootstrapping (<120), except for standard parametric CIs, where parametric bootstrapping was used (Friedrichs et al. 2011, Geffré et al. 2011).

Graphical representations and statistical tests were used to assess distribution, check normality (Anderson-Darling test) and calculate outliers (Tukey and Dixon-Reed tests) for the 2 datasets using RefVal and R (R Core Team 2019). If an individual had 3 or more extreme outliers, that individual was excluded from the analyses. All extreme Tukey and Dixon outliers were removed, and suspect outliers were removed if the means with and without outliers differed by more than 20%. Following the removal of outliers, the narrowest RI was selected for each analyte, preferentially selecting robust methodology (Horn et al. 1998, Friedrichs et al. 2012).

Associations between blood analytes and selected variables, specifically life stage, sex, rookery location, nesting year, and foraging year, were investigated for groups with adequate sample sizes (i.e. group sample size $\geqslant 7$). We also investigated associations between blood analytes that may indicate stress (H:L and glucose levels) and potential stressors including nesting time-period, duration of restraint, and number of blood sampling events. Following Anderson-Darling goodness-of-fit test to assess distribution, Student's *t*-test, or Mann-Whitney *U*-test was performed for parametric or non-parametric data, respectively. For variables with more than 2 groups, ANOVA or the Kruskal-Wallis test was run for parametric and non-parametric data, respectively, followed by post-hoc Tukey's HSD or Dunn test with Bonferroni adjustment respectively.

For analytes with one or more methods of measurement (namely PCV, total protein/TPS, sodium, potassium, chloride, glucose, BUN), agreement between methods was investigated using Bland-Altman plots (if assumptions met for parametric tests) or Passing-Bablok regression (if not). Correlations between laboratory and field methods were examined using Pearson's correlation of the coefficient (for linear data) and Spearman's rank correlation coefficient (for non-linear data). Following Bauer & Moritz (2008), correlations were ranked as excellent (0.93 to 0.99), good (0.80 to 0.92), fair (0.59 to 0.79), or poor (<0.59). Statistical significance was set at $p \leqslant 0.05$.

3. RESULTS

3.1. Body condition and health status

Ten individuals, including 5 nesting and 5 foraging individuals, were categorized as clinically unhealthy and excluded from RI development. These included 5 individuals visually identified as clinically unhealthy

Table 1. Descriptive statistics for nesting and mature foraging flatback turtle morphometrics. RV: blood reference value; CCL: curved carapace length; CCW: curved carapace width

Measurement	Life stage	n	Mean or RV	SD	Median	Min.	Max.
CCL (cm)	Nesting	124	89.2	2.92	89.2	76.9	95.0
	Foraging	74	87.2	2.95	87.4	79.8	94.3
CCW (cm)	Nesting	125	74.7	2.39	75.0	67.1	79.5
	Foraging	75	71.3	3.10	71.0	63.4	78.2
Weight (kg)	Nesting	1	84.0[a]				
	Foraging	62	76.9	7.84	76.8	56.5	91.0

[a]Single RV for n = 1

in the field. The other 5 individuals were excluded based on potential blood abnormalities following analysis of blood results (e.g. WBC 38.6 × 10^9 l^{-1}, lymphocytes 17.37 × 10^9 l^{-1}, monocytes 2.75 × 10^9 l^{-1}, eosinophils 8.88 × 10^9 l^{-1}, AST 996 U l^{-1}, ALT 65 U l^{-1}, calcium 9.83 mmol l^{-1} and GLDH 318 U l^{-1}). No cases of fibropapillomatosis were detected.

3.2. Morphometrics

Descriptive statistics for morphometrics are provided for nesting and mature foraging flatback turtles (Table 1). Nesting flatback turtles had significantly longer CCL and wider CCW than mature foraging flatback turtles (W = 2558, p < 0.001 and W = 1764, p < 0.001, respectively). Nesting turtles at Thevenard Island rookery had wider CCW than those at Eighty Mile Beach (W = 1401, p = 0.003). Mature female foraging turtles had significantly longer CCL and wider CCW than mature male foraging turtles, $t(72)$ = 3.0, p = 0.005 and $t(73)$ = 5.0, p < 0.001, respectively, and were also heavier, $t(38)$ = 4.0, p < 0.001.

3.3. Differences in blood analytes by life stage (nesting vs. foraging)

Analysis of the final dataset (post-outlier removal), which comprised 211 turtles, revealed that 36/47 (76.6%) of blood analytes exhibited significant differences between nesting and foraging populations. Accordingly, separate nesting and foraging flatback RIs were developed and are presented along with descriptive statistics (Tables 2–5). Differences of note include higher values among nesting than foraging turtles for Hb, absolute heterophil and eosinophil counts, H:L, glucose (Li-Hep and Fl-Ox), calcium, phosphorus, magnesium, and iron. Foraging turtles had higher PCV, RBC, absolute lymphocyte, monocyte and basophil counts, CK, ALP, BUN, uric acid, chloride, Ca:P, and LDH.

In the female-only analysis, results were similar, except that triglycerides were significantly higher in female foraging compared to nesting turtles. There were no observed differences in Hb, absolute monocyte count, CK, chloride, calcium, iron, and LDH between foraging and nesting females.

3.4. Differences in blood analytes by sex

Differences in blood analytes by sex were observed for 23/47 (48.9%) of analytes, with foraging females having significantly higher values than males for PCV, Hb, cholesterol, triglyceride, total protein, albumin, calcium, phosphorus, magnesium, and iron. Males had higher values than foraging females for CK, AST, chloride, and LDH (Table 6).

3.5. Differences in blood analytes by rookery, nesting year, and foraging year

We found significant differences between rookeries (Thevenard Island and Eighty Mile Beach) for 26/51 (51.0%) of blood analytes. Differences of note included higher BUN and glucose (Li-Hep) for nesting turtles at Eighty Mile Beach, and higher Hb, absolute heterophil count, triglyceride, total protein, albumin, and iron for nesting turtles at Thevenard Island (Table 7).

Differences by nesting year were examined for each rookery separately, by combining all trips from each rookery for both the 2016/2017 and 2017/2018 nesting years (summer nesting season extends over a calendar year). This revealed differences by nesting year for 26/47 (55.3%) of analytes for Eighty Mile Beach, and 11/42 (26.2%) of analytes for Thevenard Island. Differences of note by nesting year, both between and within (both) rookeries, included differences in levels of potassium, albumin, and phosphorus. Additional differences of note by nesting year for Eighty Mile Beach included differences in PCV, absolute heterophil count, H:L, uric acid, cholesterol, triglyceride, total protein, calcium, magnesium, iron, and LDH. For Thevenard Island, an additional difference observed by nesting year was the eosinophil absolute count.

Table 2. Laboratory haematological and biochemical blood reference intervals (RIs) and descriptive statistics for nesting flatback turtles including automated measurements on Cell-Dyn 3700 (red blood cell [RBC] parameters), Beckman Coulter AU680 (biochemistry), and Advia Chemistry XPT (lactate dehydrogenase [LDH] only). Mean: descriptive statistics for RIs calculated using transformation are back-transformed values where mean for robust data is the untransformed or back-transformed mean for the respective data; Median: the calculated median for back-transformed data. SD is unavailable for back-transformed data. PCV: packed cell volume; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; WBC: white blood cell; H:L: heterophil to lymphocyte ratio; CK: creatine kinase; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; BUN: blood urea nitrogen; Li-Hep: lithium heparin; Fl-Ox: fluoride-oxalate; A:G: albumin to globulin ratio; GLDH: glutamate dehydrogenase

Parameter (SI unit)	n	Mean	Median	SD	(Min.–Max.)	Lower limit (90% CI)		Upper limit (90% CI)	
PCV (%)	63	35.1	34	5.3	(27–50)	27.6	(27.0–28.6)	48.8	(43.6–50.0)
Haemoglobin (g l^{-1})	63	109.9	109	13.6	(82–141)	86.2	(82.0–92.5)	140.4	(130.4–141.0)
RBC (10^{12} l^{-1})	62	0.25	0.2	0.09	(0.1–0.5)	0.10	(0.10–0.11)	0.48	(0.40–0.50)
MCHC (g l^{-1})	61	322.0	324		(218–350)	271.4	(254.9–284.9)	351.8	(347.1–356.3)
MCH (pg)	59	447.0	462		(242–1010)	251.9	(226.4–281.4)	836.6	(742.4–950.5)
MCV (fl)	61	1446.3	1445		(750–3818)	779.5	(715.4–871.5)	3198.2	(2759.1–3669.3)
WBC (10^9 l^{-1})	80	7.43	7.44		(2.56–14.56)	2.86	(2.30–3.44)	14.14	(12.85–15.33)
Heterophils (10^9 l^{-1})	80	4.23	4.40	1.86	(1.30–8.40)	1.43	(1.30–1.69)	8.31	(7.71–8.40)
Heterophils (%)	84	54.6	55		(24–91)	23.8	(19.3–28.3)	90.6	(84.6–96.8)
Lymphocytes (10^9 l^{-1})	80	2.10	2.18	1.37	(0.03–5.10)	0.13	(0.03–0.19)	4.84	(4.61–5.10)
Lymphocytes (%)	84	26.8	27	14.6	(1–57)	2.0	(1.0–3.1)	54.9	(50.6–57.0)
Monocytes (10^9 l^{-1})	80	0.40	0.32	0.32	(0.00–1.50)	0.00	(0.00–0.00)	1.32	(0.88–1.50)
Monocytes (%)	84	5.0	4	3.6	(0–17)	0.0	(0.0–0.1)	13.0	(11.0–17.0)
Eosinophils (10^9 l^{-1})	80	0.96	0.92	0.58	(0.00–2.65)	0.00	(0.00–0.12)	2.47	(2.03–2.65)
Eosinophils (%)	84	13.0	13	6.4	(0–30)	0.0	(0.0–2.0)	27.8	(22.9–30.0)
Basophils (10^9 l^{-1})	78	0.00		0.00	(0.00–0.00)	0.00	(0.00–0.00)	0.00	(0.00–0.00)
Basophils (%)	82	0.0		0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.0	(0.0–0.0)
H:L	72	1.70	1.67		(0.44–8.89)	0.48	(0.42–0.56)	8.03	(6.06–10.78)
CK (U l^{-1})	83	751.3	482	631.8	(121–2580)	160.1	(121.0–175.0)	2380.8	(2012.2–2580.0)
AST (U l^{-1})	84	180.5	180		(80–337)	83.9	(75.0–95.4)	319.8	(296.2–343.1)
ALT (U l^{-1})	82	6.2	6	3.3	(1–19)	1.1	(1.0–2.1)	17.0	(12.9–19.0)
ALP (U l^{-1})	81	51.7	53		(32–132)	34.6	(33.1–36.6)	98.7	(86.1–114.0)
Bilirubin T (µmol l^{-1})	81	2.79	2.3	1.45	(1.0–6.1)	1.00	(1.00–1.00)	6.03	(5.98–6.10)
BUN (mmol l^{-1})	85	1.62	1.8		(0.5–7.6)	0.53	(0.46–0.63)	5.77	(4.68–7.29)
Bile acids (µmol l^{-1})	82	1.4	1	0.6	(0–3)	1.0	(0.0–1.0)	2.9	(2.0–3.0)
Uric acid (mmol l^{-1})	85	0.062	0.063		(0.031–0.112)	0.037	(0.034–0.040)	0.102	(0.094–0.110)
Glucose Li-Hep (mmol l^{-1})	47	4.54	4.6	0.72	(3.1–6.4)	3.08	(2.81–3.37)	5.99	(5.69–6.28)
Glucose Fl-Ox (mmol l^{-1})	38	3.75	3.8	0.91	(2.0–5.4)	1.88	(1.49–2.29)	5.62	(5.19–6.04)
Cholesterol (mmol l^{-1})	85	6.82	6.9		(3.0–17.5)	3.41	(3.11–3.79)	13.54	(12.23–15.10)
Triglyceride (mmol l^{-1})	85	7.48	7.0	3.84	(1.4–18.8)	1.42	(1.40–2.06)	16.05	(13.99–18.80)
Sodium (mmol l^{-1})	85	153.2	153		(145–161)	146.2	(145.0–147.4)	159.3	(158.4–160.2)
Potassium (mmol l^{-1})	85	4.67	4.7	0.46	(3.5–5.8)	3.76	(3.63–3.89)	5.58	(5.44–5.72)
Na:K	85	33.05	32.83	3.05	(26.55–42.29)	26.94	(26.09–27.83)	39.15	(38.22–40.05)
Chloride (mmol l^{-1})	85	111.8	111	5.0	(100–121)	103.0	(100.0–104.0)	121.0	(119.0–121.0)
Total protein (g l^{-1})	85	45.6	47	6.9	(31–62)	31.8	(29.9–33.8)	59.4	(57.3–61.4)
Albumin (g l^{-1})	85	13.9	14	2.5	(9–19)	9.2	(9.0–10.0)	18.0	(18.0–19.0)
Globulin (g l^{-1})	85	31.7	32	4.7	(22–44)	22.2	(20.9–23.6)	41.2	(39.7–42.6)
A:G	85	0.43	0.43		(0.36–0.62)	0.37	(0.36–0.37)	0.56	(0.53–0.59)
Calcium (mmol l^{-1})	85	3.87	3.65	1.04	(2.09–7.33)	2.27	(2.09–2.58)	6.02	(5.90–7.33)
Phosphorus (mmol l^{-1})	85	3.53	3.53		(1.55–5.09)	1.78	(1.49–2.08)	4.99	(4.76–5.19)
Ca:P	85	1.12	1.09	0.23	(0.71–1.76)	0.64	(0.56–0.71)	1.54	(1.45–1.63)
Magnesium (mmol l^{-1})	84	4.20	4.21		(2.80–6.22)	2.97	(2.77–3.19)	5.66	(5.43–5.89)
Iron (µmol l^{-1})	85	7.47	8.0	2.88	(2.0–14.4)	3.00	(2.00–3.15)	13.85	(12.43–14.40)
LDH (U l^{-1})	85	575.7	529	224.2	(259–1214)	283.7	(259.0–329.3)	1204.1	(1075.4–1214.0)
GLDH (U l^{-1})	27	25.79	22.0		(1.3–150.8)	1.45	(0.40–3.96)	145.71	(93.69–206.77)

For foraging turtles, when investigating differences in blood analytes by year, we confined analyses to samples of adult females from Roebuck Bay, to mitigate any confounding effects from age, sex, and location. We grouped samples by year for 2018, 2019, 2020 (2022 was excluded due to small sample size). Samples collected during the wet season were excluded because preliminary analysis revealed differences in analytes by season (wet vs. dry) and because water temperature differences between seasons were high (approximately 10°C difference). Male turtles were also excluded due to differences detected in

Table 3. Field blood reference intervals (RIs) and descriptive statistics for nesting flatback turtles (including i-STAT blood analyser results). PCV: packed cell volume; BUN: blood urea nitrogen; HCT: haematocrit. See Table 2 for further details

Parameter (SI unit)	n	Mean	Median	SD	(Min.–Max.)	Lower limit (90% CI)		Upper limit (90% CI)	
PCV (%)	95	35.8	36	6.1	(25–49)	26.0	(25.0–27.0)	48.2	(46.2–49.0)
Total solids (g l^{-1})	121	52.3	51		(31–80)	35.3	(34.0–36.9)	78.2	(74.3–82.2)
Na (mmol l^{-1})	63	146.7	147	2.8	(137–151)	138.2	(137.0–141.2)	151.0	(150.0–151.0)
K (mmol l^{-1})	62	4.49	4.5	0.39	(3.6–5.2)	3.70	(3.57–3.84)	5.27	(5.13–5.41)
Cl (mmol l^{-1})	63	113.0	114	5.1	(98–122)	102.7	(101.0–104.4)	123.4	(121.5–125.1)
Glucose (mmol l^{-1})	63	3.65	3.9	0.95	(1.7–5.7)	1.76	(1.70–2.20)	5.52	(4.90–5.70)
BUN (mmol l^{-1})	63	1.35	0.5	1.72	(0.5–8.8)	0.50	(0.50–0.50)	8.08	(4.21–8.80)
HCT (%)	63	25.8	26.0		(18–42)	19.1	(18.4–20.0)	38.1	(35.4–41.0)

blood analytes by sex. We subsequently found differences by year for 21/47 (44.7%) of analytes, including RBC, WBC, absolute lymphocyte and monocyte counts, H:L, CK, ALP, uric acid, glucose (Fl-Ox), cholesterol, triglyceride, magnesium, and LDH.

3.6. Differences in H:L ratios and glucose in relation to potential stressors

When considering H:L as a possible stress indicator, significantly higher H:L ratios were detected in nesting turtles for several potential stressors, including >60 min nesting time-period compared with ⩽60 min nesting time-period (W = 357, p = 0.026), >2 min restraint prior to blood collection compared with ⩽2 min restraint prior to blood collection (W = 357.5, p = 0.023), >5 min between capture and blood collection compared with ⩽5 min between capture and blood collection (W = 150, p = 0.012), and >3 blood sampling events compared with ⩽3 blood sampling events (W = 28, p = 0.001). For foraging turtles, no differences in H:L ratios were observed for the aforementioned potential stressors relevant to foraging turtles. When considering glucose levels as a potential stress indicator, for nesting turtles, glucose (Fl-Ox) was higher in the >60 min nesting time-period group, $t(36) = -2.144$, p = 0.039, while glucose (Fl-Ox) in foraging turtles was higher in both the >2 min restraint prior to blood collection group, $t(30) = -2.22$, p = 0.034, and the >5 min between capture and blood collection group, $t(64) = -2.39$, p = 0.020.

3.7. Differences in blood analytes by technique (laboratory vs. field)

When comparing results between laboratory and field techniques, the Bland-Altman plots showed small positive proportional and constant biases of 5.49 and 0.41 mmol l^{-1} for sodium and glucose (Li-Hep), respectively, with lower i-STAT values (field technique) than laboratory values (<5% fell outside limits of agreement for both Bland Altman plots). Passing-Bablok linear regressions showed statistically significant constant and/or proportional bias for sodium, chloride, total protein/TPS, and PCV/i-STAT HCT. Laboratory sodium was constantly and proportionally higher than i-STAT sodium. The y-intercept was 57.57 mmol l^{-1}, indicating constant bias (95% CI, 12.39–86.0), and the slope of the regression was 0.59 (95% CI, 0.4–0.88), consistent with proportional bias found with the Bland-Altman plot. For total protein/TPS, field TPS was constantly and proportionally higher than laboratory total protein. The y-intercept of the regression equation was −20.25 g l^{-1} (95% CI, −28.2 to −14.75), indicating constant bias. The slope of the Passing-Bablok was 1.63 (95% CI, 1.5–1.8), indicating significant proportional bias. Moreover, there was increasing disparity at higher concentrations. Laboratory chloride and PCV were constantly higher than their respective i-STAT values, with a y-intercept of −23.8 mmol l^{-1} (95% CI, 51.15 to −1.0) and −8% (95% CI, −24 to −2.31), respectively. All relationships between laboratory and i-STAT methodologies met the assumptions for Pearson's correlation. Following Bauer & Moritz's (2008) rankings, correlations were excellent for potassium, BUN, and glucose (Li-Hep), good for chloride and glucose (Fl-Ox), fair for laboratory or field PCV and i-STAT HCT, and poor for sodium. Laboratory/field PCV and total protein/TPS correlations were good.

4. DISCUSSION

This research has contributed to knowledge of the health of the data deficient flatback turtle, providing insight into flatback blood RVs and completing RIs for the last remaining sea turtle species without RIs

Table 4. Laboratory haematological and biochemical blood reference intervals (RIs) and descriptive statistics for mature foraging flatback turtles. See Table 2 for further details and abbreviations

Parameter (SI unit)	n	Mean	Median	SD	(Min.–Max.)	Lower limit (90% CI)		Upper limit (90% CI)	
PCV (%)	68	38.0	38	2.7	(30–43)	31.5	(30.0–33.0)	43.0	(43.0–43.0)
Haemoglobin (g l^{-1})	67	102.7	103		(79–115)	86.2	(81.7–90.0)	114.2	(112.5–116.0)
RBC (10^{12} l^{-1})	64	0.40	0.40	0.04	(0.33–0.48)	0.32	(0.31–0.34)	0.47	(0.46–0.49)
MCHC (g l^{-1})	67	269.8	270		(242–297)	244.6	(238.9–250.1)	291.5	(288.3–294.7)
MCH (pg)	64	259.9	258	28.7	(206–325)	200.8	(192.0–209.7)	315.7	(303.3–327.1)
MCV (fl)	64	958.3	936	98.5	(833–1246)	834.3	(833.0–848.5)	1216.6	(1163.1–1246.0)
WBC (10^{9} l^{-1})	70	7.62	7.61		(3.30–14.40)	3.48	(3.06–4.02)	13.15	(12.21–14.07)
Heterophils (10^{9} l^{-1})	70	2.60	2.61		(1.22–7.49)	1.35	(1.21–1.51)	5.88	(4.97–7.07)
Heterophils (%)	70	38.1	36	13.3	(14–71)	16.3	(14.0–22.0)	65.6	(62.2–71.0)
Lymphocytes (10^{9} l^{-1})	70	3.36	3.39		(0.63–7.83)	0.50	(0.26–0.82)	8.17	(7.38–9.02)
Lymphocytes (%)	70	44.0	47	15.0	(8–68)	9.6	(8.0–15.0)	66.5	(63.5–68.0)
Monocytes (10^{9} l^{-1})	69	0.61	0.56	0.41	(0.00–1.76)	0.00	(0.00–0.10)	1.66	(1.46–1.76)
Monocytes (%)	70	8.5	8	5.5	(0–23)	0.0	(0.0–1.0)	20.7	(17.2–23.0)
Eosinophils (10^{9} l^{-1})	70	0.57	0.57		(0.09–2.30)	0.12	(0.09–0.16)	2.07	(1.65–2.54)
Eosinophils (%)	70	8.1	9		(1–28)	1.6	(1.2–2.3)	22.3	(19.5–25.7)
Basophils (10^{9} l^{-1})	67	0.02	0.00	0.04	(0.00–0.18)	0.00	(0.00–0.00)	0.15	(0.12–0.18)
Basophils (%)	68	0.3	0	0.6	(0–2)	0.0	(0.0–0.0)	2.0	(1.3–2.0)
H:L	68	0.80	0.77		(0.24–4.85)	0.25	(0.23–0.29)	3.47	(2.60–4.64)
CK (U l^{-1})	68	1003.7	788	688.2	(162–3190)	188.1	(162.0–232.9)	3150.1	(2200.0–3190.0)
AST (U l^{-1})	67	198.1	192		(107–568)	111.8	(101.3–124.8)	368.9	(315.0–449.6)
ALT (U l^{-1})	68	8.4	7	4.1	(2–24)	2.7	(2.0–3.7)	21.1	(18.0–24.0)
ALP (U l^{-1})	68	82.9	80		(27–264)	29.5	(25.8–34.2)	196.0	(166.2–230.7)
Bilirubin T (µmol l^{-1})	68	2.05	2.00	0.69	(0.00–3.00)	0.00	(0.00–0.73)	3.00	(3.00–3.00)
BUN (mmol l^{-1})	68	18.54	18.5		(9.5–31.0)	11.88	(10.83–13.03)	27.11	(25.44–29.02)
Bile acids (µmol l^{-1})	67	2.0	2	1.1	(0–5)	0.7	(0.0–1.0)	4.3	(4.0–5.0)
Uric acid (mmol l^{-1})	68	0.075	0.077		(0.030–0.190)	0.037	(0.033–0.043)	0.167	(0.140–0.203)
Glucose Li-Hep (mmol l^{-1})	29	3.49	3.4	0.48	(2.6–4.7)	2.49	(2.25–2.74)	4.50	(4.23–4.75)
Glucose Fl-Ox (mmol l^{-1})	67	3.01	2.9	0.64	(1.4–4.5)	1.73	(1.52–1.94)	4.30	(4.08–4.51)
Cholesterol (mmol l^{-1})	68	6.90	5.5	3.96	(2.3–18.4)	2.52	(2.30–2.82)	17.39	(13.95–18.40)
Triglyceride (mmol l^{-1})	68	9.35	4.3	9.32	(0.8–30.9)	0.80	(0.80–1.07)	30.83	(27.29–30.90)
Sodium (mmol l^{-1})	68	152.5	152	4.9	(144–167)	142.6	(141.1–144.2)	162.3	(160.6–163.9)
Potassium (mmol l^{-1})	68	3.99	4.0	0.45	(2.4–5.3)	2.76	(2.40–3.40)	5.23	(4.62–5.30)
Na:K	66	38.18	38.00		(29.62–44.86)	31.52	(30.22–32.75)	44.20	(43.19–45.24)
Chloride (mmol l^{-1})	68	116.4	118	8.2	(96–132)	97.5	(96.0–102.0)	130.6	(126.8–132.0)
Total protein (g l^{-1})	68	44.9	45		(32–68)	33.1	(31.5–34.9)	62.5	(58.9–66.6)
Albumin (g l^{-1})	68	14.1	14	2.4	(8–20)	9.3	(8.5–10.1)	18.9	(18.1–19.7)
Globulin (g l^{-1})	68	30.9	31		(22–48)	22.7	(21.6–23.9)	44.0	(41.3–47.1)
A:G	68	0.45	0.44	0.06	(0.31–0.61)	0.32	(0.31–0.37)	0.60	(0.54–0.61)
Calcium (mmol l^{-1})	68	3.06	2.49	1.45	(1.58–7.30)	1.59	(1.58–1.60)	6.67	(5.87–7.30)
Phosphorus (mmol l^{-1})	68	2.32	2.20	0.77	(0.97–3.93)	1.07	(0.97–1.32)	3.93	(3.73–3.93)
Ca:P	67	1.23	1.23		(0.70–2.26)	0.76	(0.72–0.81)	2.20	(1.95–2.47)
Magnesium (mmol l^{-1})	68	3.67	3.67	0.72	(2.55–5.24)	2.63	(2.55–2.73)	5.23	(4.80–5.24)
Iron (µmol l^{-1})	68	6.58	5.4	3.75	(1.5–15.8)	1.50	(1.50–2.70)	15.22	(14.38–15.80)
LDH (U l^{-1})	68	674.1	623	206.4	(260–1269)	360.1	(260.0–426.4)	1200.9	(1011.5–1269.0)
GLDH (U l^{-1})	65	21.54	21.5		(6.9–85.8)	7.02	(5.89–8.38)	76.95	(59.36–98.01)

(Stacy & Innis 2017). Our findings report moderate variability in flatback turtle RIs, and after acknowledging methodological differences, overall results were comparable to other flatback turtle and sea turtle species results, with differences observed between demographic and geographic groups. In agreement with the literature, we found significant differences for blood analytes by life stage (nesting vs. foraging) (Deem et al. 2009, Sözbilen & Kaska 2018), sex (Hasbún et al. 1998, Samour et al. 1998, Santoro & Meneses 2007), rookery location (Harris et al. 2011, Perrault et al. 2012), nesting year, and foraging year (Labrada-Martagón et al. 2010, Kelly et al. 2015).

Similar to previous findings, nesting turtles had significantly higher calcium, phosphorus, and magnesium than foraging turtles, likely related to vitellogenesis and folliculogenesis, and other breeding-related physiological changes (Deem et al. 2006, Harris et al. 2011, Sözbilen & Kaska 2018). While Harris et al. (2011) reported no difference in glucose between

Table 5. Field blood reference intervals (RIs) and descriptive statistics for mature foraging flatback turtles. PCV: packed cell volume. See Table 2 for further details

Parameter (SI unit)	n	Mean	Median	SD	(Min.–Max.)	Lower limit (90% CI)		Upper limit (90% CI)	
PCV (%)	71	38.4	38		(32–45)	33.4	(32.5–34.2)	44.1	(43.1–45.1)
Total solids (g l^{-1})	69	55.3	49	15.8	(31–102)	33.3	(31.0–38.0)	94.5	(82.0–102.0)

nesting and foraging life stages, our study showed the median glucose (Li-Hep) was 35.3% higher in nesting than foraging turtles (4.6 vs. 3.4 mmol l^{-1}). Higher glucose in nesting turtles may be related to temporary glucose spikes associated with fasting or strenuous nesting activity, stress, individual variation, or warmer water temperatures (Goldberg et al. 2013). Although triglyceride and cholesterol are frequently reported to be higher in nesting than foraging turtles, we found that the median triglyceride level was 80.3% higher in foraging than nesting females (13.39 vs. 7.48 mmol l^{-1}), supporting the findings of Harris et al. (2011). Lower triglyceride in the nesting turtles may be related to the timing of the sampling, which usually occurred mid-nesting season when fat reserves are lower (Hamann et al. 2002, Goldberg et al. 2013, Perrault & Stacy 2018).

Adult foraging flatback turtles had statistically higher PCV (38 vs. 34%) and RBC (0.4 vs. 0.2 × 10^{12} l^{-1}) median values compared to nesting turtles, similar to differences found in other species (Innis et al. 2010, Harris et al. 2011). Even though erythropoiesis decreases in winter, when most foraging turtles were sampled, these values are likely to be higher in foraging than nesting turtles given that foraging often confers better nutritional status, and nesting may be associated with increased stress and physiological effects on bone marrow (Kelly et al. 2015, Perrault et al. 2016, Perrault & Stacy 2018).

Blood urea nitrogen and uric acid median values were higher for foraging than nesting turtles (18.5 vs. 1.8 mmol l^{-1} and 0.077 vs. 0.063 mmol l^{-1} for BUN and uric acid, respectively), likely related to fasting during nesting (Casal et al. 2009, Deem et al. 2009, Perrault et al. 2012). Other reptilian species also show a temperature-dependent increase in uric acid in winter due to reduced tubular function at low temperatures (Dessauer 1970). Levels of most enzymes were higher in foraging than nesting turtles, noting that the former were sampled during autumn–winter seasons and the latter were sampled in summer. In contrast, for reptiles which hibernate, many enzyme levels decrease during winter (Christopher et al. 1999). The higher levels of enzymes in foraging than nesting turtles found in our study may be related to higher activity levels when foraging (Campbell 2012), given that some enzymes (CK, AST) are found primarily in muscle, and most other enzymes (e.g. ALP, ALT) have a wide tissue distribution, including in muscle (Anderson et al. 2013, Petrosky et al. 2015).

Observed differences in blood analytes by sex included higher AST and sodium in male than female foraging turtles, supporting previous findings (Bolten & Bjorndal 1992, Hasbún et al. 1998, Innis et al. 2010). Males also had higher CK, chloride, and LDH (Table 6). The higher enzyme levels in males compared to females could relate to increased muscle catabolism associated with capture and restraint. Some analytes were higher in both foraging and nesting females than males, including Hb, calcium, phosphorus, magnesium, and iron. Foraging females also had higher cholesterol, triglyceride, and total protein (and fractions) than males (Table 6). Higher PCV and Hb (and associated iron) in foraging females than males may be related to higher oxygen-carrying capacity in females, while higher iron (measured as protein-bound iron) may also reflect the higher protein in females than males (Hasbún et al. 1998, Harr et al. 2001). As foraging females were significantly larger than foraging males (a sexually dimorphic trait of sea turtles), differences in certain analytes (e.g. PCV, total protein, cholesterol, iron) may be related to size (Hasbún et al. 1998, Godley et al. 2002, Casal et al. 2009). Alternatively, as some of these differences are generally related to reproductive status rather than sex (Casal et al. 2009), this could indicate that some foraging females were previtellogenic and preparing for breeding.

Differences in blood analytes detected in this study by rookery, nesting year, and foraging year, could potentially be associated with spatiotemporal differences in environmental conditions, or rookery-specific differences in migration distances (Labrada-Martagón et al. 2010). When considering differences by year, higher median PCV (38 vs. 30%), mean cholesterol (7.52 vs. 5.88 mmol l^{-1}), and median triglyceride (7.00 vs. 2.65 mmol l^{-1}) for the first compared to the second nesting year at Eighty Mile Beach could potentially be related to between-year differences in environmental conditions and subsequent food avail-

Table 6. Descriptive statistics of laboratory and field blood analytes for female and male mature foraging flatback turtles and statistical test results of the differences in analytes by sex using the appropriate parametric (Student's *t*-test) or non-parametric (Mann-Whitney *U*-test) method. No i-STAT comparison between sexes is available due to only one male foraging turtle with i-STAT values. See Table 2 for abbreviations

Parameter (SI unit)	Female					Male					p	df	*t*	*W*
	n	Mean	SD	Median	(Min.–Max.)	n	Mean	SD	Median	(Min.–Max.)				
PCV (%)	43	38.7	2.6	38	(33–43)	25	36.8	2.7	37	(30–42)	0.007			746
Haemoglobin (g l^{-1})	42	104.2	5.6	103	(91–115)	25	98.6	7.6	100	(79–114)	0.003			757
RBC (10^{12} l^{-1})	40	0.40	0.04	0.41	(0.33–0.48)	24	0.39	0.03	0.39	(0.34–0.47)	0.288	62	1.07	
MCHC (g l^{-1})	42	270.0	11.4	269	(249–297)	25	268.2	12.2	271	(242–286)	0.563	65	0.58	
MCH (pg)	40	262.5	31.5	255	(206–325)	24	255.6	22.3	258	(206–295)	0.351	62	0.94	
MCV (fl)	40	969.6	112.7	936	(833–1246)	24	939.5	66.9	934	(835–1079)	0.677			510.5
WBC (10^9 l^{-1})	44	8.03	2.28	8.05	(4.20–12.24)	26	7.38	2.71	6.90	(3.30–14.40)	0.281	68	1.09	
Heterophils (10^9 l^{-1})	44	2.86	1.04	2.7	(1.3–6.2)	26	2.80	1.31	2.6	(1.2–7.5)	0.64			611
Heterophils (%)	44	37.59	13.82	35.00	(14.00–64.00)	26	39.00	12.55	37.00	(22.00–71.00)	0.572			525
Lymphocytes (10^9 l^{-1})	44	3.92	1.84	4.2	(0.6–7.8)	26	3.04	1.90	2.6	(0.7–7.3)	0.06	68	1.92	
Lymphocytes (%)	44	46.82	14.62	50.50	(13.00–68.00)	26	39.31	14.79	39.50	(8.00–62.00)	0.034			747
Monocytes (10^9 l^{-1})	43	0.55	0.40	0.5	(0.0–1.6)	26	0.70	0.41	0.7	(0.1–1.8)	0.063			408.5
Monocytes (%)	44	7.50	5.25	6.00	(0.00–23.00)	26	10.15	5.72	10.00	(1.00–20.00)	0.054			413.5
Eosinophils (10^9 l^{-1})	44	0.62	0.43	0.5	(0.1–1.9)	26	0.82	0.55	0.6	(0.2–2.3)	0.101			436.5
Eosinophils (%)	44	7.68	4.33	8.00	(1.00–21.00)	26	11.35	6.15	10.00	(4.00–28.00)	0.009			357.5
Basophils (10^9 l^{-1})	41	0.02	0.05	0.0	(0.0–0.2)	26	0.02	0.04	0.0	(0.0–0.1)	0.64			560
Basophils (%)	42	0.33	0.61	0.00	(0.00–2.00)	26	0.23	0.51	0.00	(0.00–2.00)	0.5			586
H:L	44	1.09	1.05	0.70	(0.24–4.85)	24	1.04	0.65	0.96	(0.37–3.05)	0.32			450
CK (U l^{-1})	43	770.8	618.4	568	(162–3190)	25	1404.1	623.4	1495	(411–3135)	<0.001			203
AST (U l^{-1})	43	198.1	63.5	188	(107–415)	24	240.2	105.9	210	(148–568)	0.035			354.5
ALT (U l^{-1})	43	8.0	3.4	7	(2–18)	25	9.2	5.2	7	(3–24)	0.744			511.5
ALP (U l^{-1})	43	87.7	42.5	77	(27–217)	25	96.8	52.6	80	(35–264)	0.464			479.5
Bilirubin T (µmol l^{-1})	43	2.01	0.75	2.0	(0.0–3.0)	25	2.12	0.56	2.0	(1.0–3.0)	0.954			532.5
BUN (mmol l^{-1})	43	18.17	3.49	18.1	(12.0–25.7)	25	19.81	4.14	19.2	(9.5–31.0)	0.085	66	−1.75	
Bile acids (µmol l^{-1})	43	2.2	1.1	2	(0–5)	24	1.8	0.8	2	(1–4)	0.107			633.5
Uric acid (mmol l^{-1})	43	0.080	0.039	0.071	(0.030–0.190)	25	0.084	0.018	0.080	(0.051–0.129)	0.029			366
Glucose Li-Hep (mmol l^{-1})	20	3.45	0.51	3.3	(2.6–4.7)	9	3.60	0.42	3.5	(3.0–4.3)	0.434	27	−0.79	
Glucose Fl-Ox (mmol l^{-1})	43	2.93	0.63	2.9	(1.4–4.5)	24	3.16	0.64	3.0	(2.0–4.4)	0.168	65	−1.4	
Cholesterol (mmol l^{-1})	43	8.53	4.08	8.2	(2.6–18.4)	25	4.10	1.26	3.7	(2.3–7.1)	<0.001			899
Triglyceride (mmol l^{-1})	43	13.39	9.59	14.0	(1.1–30.9)	25	2.40	1.53	2.0	(0.8–7.8)	<0.001			934.5
Sodium (mmol l^{-1})	43	150.6	3.9	151	(144–160)	25	155.6	4.8	156	(149–167)	<0.001	66	−4.67	
Potassium (mmol l^{-1})	43	3.97	0.42	4.0	(2.4–5.3)	25	4.03	0.51	3.9	(2.9–5.2)	0.903			527.5
Na:K	42	37.78	2.78	37.53	(29.62–43.14)	24	38.66	3.71	39.22	(31.35–44.86)	0.275	64	−1.1	
Chloride (mmol l^{-1})	43	113.3	8.1	113	(96–126)	25	121.6	5.5	123	(108–132)	<0.001			235.5
Total protein (g l^{-1})	43	47.6	7.8	49	(32–68)	25	42.2	4.7	41	(34–50)	<0.001	66	3.56	
Albumin (g l^{-1})	43	15.0	2.3	15	(10–20)	25	12.6	1.8	13	(8–15)	<0.001	66	4.4	
Globulin (g l^{-1})	43	32.6	5.81	33	(22–48)	25	29.6	3.5	30	(24–35)	0.009	66	2.7	

Table continues on next page

Table 6 (continued)

Parameter (SI unit)	Female					Male					p	df	t	W
	n	Mean	SD	Median	(Min. –Max.)	n	Mean	SD	Median	(Min. –Max.)				
A:G	43	0.46	0.05	0.45	(0.38–0.61)	25	0.43	0.06	0.42	(0.3–0.56)	0.012			734
Calcium (mmol l^{-1})	43	3.69	1.48	3.46	(1.58–7.30)	25	1.96	0.30	1.86	(1.59–2.51)	<0.001			914
Phosphorus (mmol l^{-1})	43	2.62	0.77	2.68	(1.11–3.93)	25	1.79	0.38	1.73	(0.97–2.50)	<0.001			879.5
Ca:P	42	1.39	0.39	1.38	(0.70–2.26)	25	1.13	0.23	1.14	(0.81–1.65)	0.007			733.5
Magnesium (mmol l^{-1})	43	3.91	0.72	4.00	(2.66–5.24)	25	3.27	0.53	3.13	(2.55–4.57)	0.001			797.5
Iron (µmol l^{-1})	43	7.99	4.01	7.6	(1.5–15.8)	25	4.16	1.18	4.2	(1.5–6.6)	<0.001			862.5
LDH (U l^{-1})	43	601.6	196.6	554	(260–1269)	25	798.6	160.4	839	(525–1029)	<0.001			201
GLDH (U l^{-1})	42	27.32	16.28	22.7	(6.9–80.1)	23	24.06	17.88	18.4	(8.6–85.8)	0.214			574
Field PCV (%)	45	38.9	2.7	39	(32–45)	26	37.7	2.4	38	(33–43)	0.043			753
Total solids (g l^{-1})	43	61.3	16.9	62	(34–102)	26	45.3	6.3	47	(31–56)	<0.001			861.5
i-STAT Na (mmol l^{-1})	6	146.5	4.0	146	(143–154)									
i-STAT K (mmol l^{-1})	6	4.02	0.29	4.1	(3.6–4.4)									
i-STAT Cl (mmol l^{-1})	6	118.5	2.7	118	(117–124)									
i-STAT glucose (mmol l^{-1})	6	2.40	0.15	2.5	(2.1–2.5)									
i-STAT BUN (mmol l^{-1})	6	19.65	2.73	19.1	(16.6–23.8)									
i-STAT HCT (%)	6	28.5	2.5	29	(25–31)									

ability in the lead up to breeding. The impact of such interannual environmental differences on sea turtle health has been documented in marine megafauna at Shark Bay, WA. Here, a marine heatwave in 2010/2011 caused mass dieback of seagrasses, the main food source for green turtles. This event continued to affect green turtle health parameters for 2 yr, evidenced by lower body condition scores after the heatwave (Thomson et al. 2015).

Our study found differences in protein levels between rookeries, with higher median values for turtles from Thevenard Island than Eighty Mile Beach (47.5 vs. 41.0 g l^{-1}). While these turtles are different genetic stocks, Thevenard Island turtles were also larger on average than Eighty Mile Beach turtles, and protein is reported to correlate positively with body size (Hasbún et al. 1998, Whiting et al. 2007, Delgado et al. 2011).

Although Flint et al. (2010a) reported that established RIs for green and loggerhead turtles were generally similar within a region irrespective of age and sex and suggested that they may be comparable between regions (Flint et al. 2010b), Whiting et al. (2007) recommended separate RVs in different geographic regions where diet and habitat may differ between foraging areas. In this study, we found both differences and similarities between the RVs of the study populations and those of other flatback turtle populations. Investigation of geographical differences remains difficult, particularly when studies use different methodologies (Stacy & Innis 2017); however, blood values are known to be affected by environmental and biological factors including temperature, salinity, and body size (Dessauer 1970, Lutz & Dunbarcooper 1987, Young 2022), and where these vary by geographic region, region-specific RIs may be useful.

PCV, Hb, and RBC values were higher in flatbacks than other sea turtle species, the only exception were leatherback turtles, likely related to the latter's higher oxygen carrying capacity (Lutcavage & Lutz 1997, Sperling et al. 2007, Stacy et al. 2019). Our study found higher H:L values in nesting compared to foraging flatback turtles, while for leatherback turtles, Harris et al. (2011) reported higher H:L ratios in foraging than nesting turtles. H:L ratio has been used as a proxy for stress in other species, including reptiles (Davis et al. 2008), and also as a disease indicator in sea turtles (Aguirre et al. 1995, Work et al. 2001, 2003). Our study supports findings by Stamper et al. (2005), Innis et al. (2014), and Flower et al. (2018) of positive correlations between H:L (and glucose) and potential stressors such as longer nesting time-period, longer duration of restraint, and higher number of blood sampling events. The H:L relationships were found in

Table 7. Descriptive statistics of laboratory and field blood analytes for nesting flatback turtles at Thevenard Island and Eighty Mile Beach rookeries and statistical test results of the differences in analytes by rookery using the appropriate parametric (Student's *t*-test) or non-parametric (Mann-Whitney *U* test) method. No i-STAT comparison between sexes available due to only one male foraging turtle with i-STAT values. See Table 2 for abbreviations

Parameter (SI unit)	Eighty Mile Beach					Thevenard Island					p	df	*t*	*W*
	n	Mean	SD	Median	(Min.–Max.)	n	Mean	SD	Median	(Min.–Max.)				
PCV (%)	30	34.8	6.1	34	(27–50)	33	35.5	4.5	35	(29–46)	0.287			417.5
Haemoglobin (g l^{-1})	30	106.4	13.4	103	(82–141)	33	113.1	13.2	113	(90–140)	0.038			343.5
RBC (10^{12} l^{-1})	29	0.24	0.08	0.20	(0.10–0.47)	33	0.25	0.09	0.22	(0.10–0.50)	0.772			458
MCHC (g l^{-1})	28	318.3	30.1	328	(218–346)	33	319.7	15.1	318	(291–350)	0.297			534.5
MCH (pg)	28	469.3	150.8	473	(248–1010)	31	471.1	150.0	450	(242–950)	0.897			443
MCV (fl)	29	1617.6	652.3	1500	(750–3700)	32	1540.8	606.6	1446	(760–3818)	0.696			491.5
WBC (10^{9} l^{-1})	37	7.38	2.74	6.88	(2.56–14.56)	43	7.97	2.90	7.68	(2.56–14.10)	0.356	78	−0.93	
Heterophils (10^{9} l^{-1})	37	3.79	1.77	3.3	(1.4–8.3)	43	4.61	1.87	4.6	(1.3–8.4)	0.04			582.5
Heterophils (%)	40	51.13	13.05	53.00	(31.00–79.00)	44	58.98	18.92	58.00	(24.00–91.00)	0.029	77	−2.23	
Lymphocytes (10^{9} l^{-1})	37	2.15	1.17	2.1	(0.1–4.8)	43	2.06	1.54	2.2	(0.0–5.1)	0.65			843
Lymphocytes (%)	40	29.00	11.82	28.00	(2.00–53.00)	44	24.75	16.54	25.50	(1.00–57.00)	0.177	78	1.36	
Monocytes (10^{9} l^{-1})	37	0.38	0.26	0.3	(0.0–0.9)	43	0.43	0.37	0.3	(0.0–1.5)	0.908			783
Monocytes (%)	40	5.03	3.53	4.50	(0.00–17.00)	44	4.95	3.62	4.00	(0.00–13.00)	0.829			904.5
Eosinophils (10^{9} l^{-1})	37	1.05	0.57	1.0	(0.2–2.5)	43	0.87	0.58	0.8	(0.0–2.6)	0.171	78	1.38	
Eosinophils (%)	40	14.80	5.69	14.00	(2.00–28.00)	44	11.32	6.55	11.00	(0.00–30.00)	0.011	82	2.59	
Basophils (10^{9} l^{-1})	35	0.00	0.00	0.0	(0.0–0.0)	43	0.00	0.00	0.0	(0.0–0.0)	na			752.5
Basophils (%)	38	0.00	0.00	0.00	(0.00–0.00)	44	0.00	0.00	0.00	(0.00–0.00)	na			836
H:L	38	2.07	1.48	1.68	(0.62–8.63)	34	2.57	2.33	1.70	(0.44–8.89)	0.901			634.5
CK (U l^{-1})	41	708.6	664.3	374	(121–2580)	42	792.9	603.5	592	(170–2181)	0.224			727
AST (U l^{-1})	42	195.8	55.5	191	(106–330)	42	176.0	63.7	164	(80–337)	0.133	82	1.52	
ALT (U l^{-1})	43	7.0	3.3	6	(3–19)	39	5.2	3.0	5	(1–17)	0.001			1189.5
ALP (U l^{-1})	39	57.3	19.5	54	(36–132)	42	53.3	13.3	53	(32–93)	0.461			897.5
Bilirubin T (µmol l^{-1})	42	2.10	1.06	2.0	(1.0–6.0)	39	3.55	1.44	3.0	(1.0–6.1)	<0.001			307
BUN (mmol l^{-1})	43	2.64	1.53	2.0	(0.5–7.6)	42	1.30	0.53	1.3	(0.5–2.3)	<0.001			1467
Bile acids (µmol l^{-1})	42	1.3	0.5	1	(0–2)	40	1.5	0.6	1	(1–3)	0.080			677.5
Uric acid (mmol l^{-1})	43	0.061	0.017	0.056	(0.038–0.112)	42	0.067	0.015	0.068	(0.031–0.106)	0.029			653.5
Glucose Li-Hep (mmol l^{-1})	27	4.77	0.67	4.8	(3.2–6.4)	20	4.23	0.66	4.2	(3.1–5.4)	0.008	45	2.76	
Glucose Fl-Ox (mmol l^{-1})	16	3.44	1.04	3.2	(2.0–5.4)	22	3.98	0.75	4.0	(2.1–5.2)	0.075	36	−1.84	
Cholesterol (mmol l^{-1})	43	6.91	2.30	6.9	(3.0–12.5)	42	7.56	2.85	6.8	(4.1–17.5)	0.479			822
Triglyceride (mmol l^{-1})	43	6.28	4.31	5.1	(1.4–18.8)	42	8.70	2.86	7.9	(4.5–16.2)	<0.001			504.5
Sodium (mmol l^{-1})	43	153.4	3.6	154	(145–161)	42	152.8	2.9	153	(145–159)	0.337			1012
Potassium (mmol l^{-1})	43	4.88	0.42	4.9	(4.0–5.8)	42	4.46	0.39	4.5	(3.5–5.3)	<0.001	83	4.86	
Na:K	43	31.63	2.77	31.60	(26.55–38.75)	42	34.50	2.64	34.27	(30.00–42.29)	<0.001	83	−4.88	
Chloride (mmol l^{-1})	43	115.3	3.8	117	(105–121)	42	108.2	3.2	108	(100–114)	<0.001			1654
Total protein (g l^{-1})	43	42.8	7.2	41	(31–60)	42	48.4	5.3	48	(39–62)	<0.001	77	−4.09	
Albumin (g l^{-1})	43	13.0	2.6	13	(9–18)	42	14.8	2.0	14	(11–19)	<0.001			519
Globulin (g l^{-1})	43	29.8	4.82	29	(22–42)	42	33.6	3.8	33	(27–44)	<0.001	83	−4.05	
A:G	43	0.43	0.04	0.43	(0.36–0.62)	42	0.44	0.05	0.44	(0.36–0.61)	0.582			840

Table continues on next page

Table 7 (continued)

Parameter (SI unit)	Eighty Mile Beach				Thevenard Island				p	df	*t*	*W*
	n	Mean	SD	Median	(Min.–Max.)	n	Mean	SD	Median	(Min.–Max.)				
Calcium (mmol l^{-1})	43	3.88	1.18	3.62	(2.09–7.33)	42	3.86	0.88	3.87	(2.49–6.03)	0.748			866
Phosphorus (mmol l^{-1})	43	3.69	0.72	3.62	(2.18–4.91)	42	3.30	0.83	3.43	(1.55–5.09)	0.023	83	2.31	
Ca:P	43	1.05	0.19	1.03	(0.71–1.60)	42	1.20	0.23	1.19	(0.79–1.76)	0.001			528.5
Magnesium (mmol l^{-1})	42	4.39	0.66	4.37	(3.13–6.22)	42	4.06	0.65	4.09	(2.80–5.58)	0.02	82	2.37	
Iron (µmol l^{-1})	43	5.74	2.46	5.0	(2.0–14.0)	42	9.25	2.09	9.0	(6.0–14.4)	<0.001			237
LDH (U l^{-1})	43	561.5	245.0	483	(259–1208)	42	590.3	202.6	551	(282–1214)	0.156			741
GLDH (U l^{-1})	6	44.00	43.24	27.0	(12.5–129.3)	21	33.91	35.07	21.5	(1.3–150.8)	0.441			77
Field PCV (%)	56	36.0	6.8	35	(25–49)	39	35.5	5.1	36	(26–45)	0.958			1099.5
Total solids (g l^{-1})	64	49.9	10.0	48	(31–75)	57	57.5	10.1	56	(35–80)	<0.001			1032
i-STAT Na (mmol l^{-1})	39	147.3	2.6	148	(139–151)	24	145.7	2.8	147	(137–150)	0.009			651.5
i-STAT K (mmol l^{-1})	39	4.68	0.37	4.7	(3.8–5.7)	24	4.22	0.32	4.2	(3.6–4.8)	<0.001	61	5.09	
i-STAT Cl (mmol l^{-1})	39	116.1	3.1	116	(110–122)	24	108.1	3.8	108	(98–114)	<0.001	61	9.03	
i-STAT glucose (mmol l^{-1})	39	3.70	1.04	3.9	(1.8–5.7)	24	3.58	0.80	3.8	(1.7–4.8)	0.532			512.5
i-STAT BUN (mmol l^{-1})	39	1.86	2.03	0.5	(0.5–8.8)	24	0.52	0.10	0.5	(0.5–1.0)	<0.001			686
i-STAT HCT (%)	39	24.9	3.5	25	(18–33)	24	29.0	5.2	29	(20–42)	0.002	36	−3.38	

nesting turtles but not foraging turtles. It is possible that the stress heterophilia in foraging turtles sampled during the winter months may not be apparent because of temperature-related immune suppression (Zapata et al. 1992) and lower relative heterophil counts in foraging than nesting turtles.

When comparing our flatback turtle biochemical values with those for other sea turtle species, the flatback turtle values generally fell within blood reference ranges for the appropriate life stage, i.e. nesting or adult foraging turtles (when available) for green (Samour et al. 1998, Hamann et al. 2006, Page-Karjian et al. 2020), loggerhead (Casal et al. 2009, Deem et al. 2009, Sözbilen & Kaska 2018), hawksbill (Caliendo et al. 2010, Goldberg et al. 2013, Whiting et al. 2014b), olive ridley (Santoro & Meneses 2007, Espinoza-Romo et al. 2018, Reséndiz et al. 2019), leatherback (Innis et al. 2010, Harris et al. 2011, Perrault et al. 2012), and flatback (Scheelings et al. 2020) turtles. However, for flatback turtles, the upper limits for nesting and foraging turtle cholesterol (17.5 and 18.4 mmol l^{-1}, respectively) and triglyceride (18.8 and 30.9 mmol l^{-1}, respectively) were higher than most other reported ranges, such as 16.6 mmol l^{-1} cholesterol in a nesting loggerhead turtle (Trocini 2013) and 12.5 mmol l^{-1} triglyceride in a foraging leatherback (Innis et al. 2010). Blooms of tomato jellyfish were reported anecdotally in Roebuck Bay at the time of sampling, and many turtles were seen consuming jellyfish, which is a source of lipid, albeit low (Machovsky-Capuska & Raubenheimer 2020). Video footage has also revealed benthic feeding by flatback turtles (Hounslow et al. 2022), which could include dietary items with higher fat content such as scallops and bivalves. Interestingly, high cholesterol in nesting leatherback turtles was related to low hatching and emergence success rates (Perrault et al. 2012). Additional comparisons of blood analytes between flatback turtles from this study and other sea turtle species are reported in Young (2022).

Although most results for the laboratory and field-testing methods were correlated, significant proportional and constant biases were detected. However, not all differences were clinically significant. The clearest clinically significant difference related to i-STAT was a median i-STAT HCT value of 26%, which was 24 and 26% lower than laboratory PCV (34%) and field PCV (35%), respectively. The reliability of haematological parameters measured on i-STAT blood analysers has been questioned previously in sea turtle research (Muñoz-Pérez et al. 2017, Stacy & Innis 2017). While the need for practical clinical testing in the field remains important, ideally, separate RIs should be developed for different analysers

(McCain et al. 2010). In certain situations, an i-STAT or similar portable blood machine may be the preferred option, such as for remote fieldwork and for monitoring populations with previously established i-STAT blood RIs, as well as for analytes requiring immediate analysis (e.g. lactate). There is also less potential for changes to analytes over time with field analytical methods, compared to laboratory methods with delayed processing times (Eatwell 2007).

Field work in remote locations presents challenges, including the logistics of sample collection, processing and transportation, and the risk of temporal and temperature effects on samples causing artefact. Some limitations for our study include lack of control for inter-observer variation at the laboratory (e.g. haemolysis grading). Furthermore, all LDH samples were transferred from the first laboratory to a second laboratory (Western Diagnostics) for LDH analysis, where there was variation in handling protocols between samples, including freezing of samples that arrived on weekends, and the potential that a small number of samples were analysed outside the 60 h time frame.

Use of different methodologies presents a major challenge when comparing results between studies. Use of different analysers (McCain et al. 2010), anticoagulants (Hrubec et al. 2002, Phillips et al. 2017), and plasma storage conditions, such as fresh versus frozen (Ramer et al. 1995, Hawkins et al. 2006), can potentially alter blood values. In our study, use of different haematological analysers between nesting turtles (Cell-Dyn 3700) and foraging turtles (Sysmex XN-1000) limits our capacity to compare haematological parameters (Hb, RBC, and their calculated values) between the different life stages. Further, the reliability of automated haematological methods for analytes such as Hb and RBC in chelonians is uncertain, given the machines are not calibrated for animals with nucleated RBCs (Heatley & Russell 2019). Artefacts, such as mild haemolysis not grossly observable can also skew results, and this is frequently unreported (Stacy et al. 2019). Analytes including phosphorus, AST, CK, LDH, and especially potassium may be affected by haemolysis and other pre-analytical factors (Thoresen et al. 1992, Benson et al. 1999, Eisenhawer et al. 2008, Eshar et al. 2018). For example, potassium levels may increase with increased time to plasma separation and elevated storage temperatures, as intracellular potassium is released from damaged erythrocytes (Abou-Madi & Jacobson 2003, Asirvatham et al. 2013), or when serum tubes are used, related to unpredictable clot formation and release of potassium from thrombocytes in the clot (Bolten et al. 1992, Raskin 2000, Stacy et al. 2019).

Our remote field locations and other logistical challenges resulted in longer processing times than are generally accepted, including 60 h (instead of the generally accepted 48 h) to laboratory analysis, and 8 h (instead of 6 h) to centrifugation. While this represents the reality of sea turtle research in remote locations, further investigation is warranted to evaluate whether these extended timeframes significantly affect haematology and biochemistry results (Young 2022). In an anticoagulant trial in a subset of animals tested in duplicate (n = 28), although glucose values fell within the normal range of 3.33–5.55 mmol l^{-1} (Stacy et al. 2017), Fl-Ox whole blood mean was significantly lower (12.3%) than Li-Hep plasma (3.51 vs. 3.08 mmol l^{-1}; $t(27) = 5.8$, $p < 0.001$), further supporting the use of standard Li-Hep anticoagulant tubes for sea turtle haematology and biochemistry analyses (Stacy et al. 2019). A larger sample size is also recommended to reduce risk of type II error (false negatives). Finally, important research into toxicology, heavy metal and electrophoresis baselines, as well as novel biomarkers, metabolomics, and proteomics, is ongoing using banked samples (Melvin et al. 2021).

In a changing world, establishment of wildlife health baseline data are of paramount importance, acting as a safeguard against shifting baselines — where gradual changes in environmental and health conditions become accepted as normal over time. Our baseline RIs comprised an extensive panel of analytes which included those not validated in reptiles (e.g. bromocresol green method of albumin measurement is considered inferior to electrophoresis) to facilitate comparison with similar studies (Macrelli et al. 2013). By focusing on the most useful analytes for assessing health in reptilian diagnostics, including PCV, WBC parameters, CK, AST, BUN, uric acid, glucose, sodium, potassium, chloride, protein, calcium, and phosphorus, the most appropriate RIs can be used for the group under investigation in comparative studies (Wilkinson 2004, Campbell 2014, Eatwell et al. 2014, Stacy & Innis 2017). In the absence of other RIs, and until further sampling can be performed for the various groups (e.g. juveniles and breeding males), these nesting and foraging flatback turtle RIs have filled a major knowledge gap, providing good representation of the species, covering different life stages, multiple locations, multiple nesting years, and multiple years of mixed genetic stocks (Rees et al. 2016). These RIs and insights from this study will be of value for managing flatback turtle clinical cases in rehabilitation, monitoring trends in wild populations, and guiding future health-related research and conservation.

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