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Blood gases, biochemistry and haematology of Galápagos marine iguanas (*Amblyrhynchus cristatus*)

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The marine iguana, *Amblyrhynchus cristatus*, is an iconic lizard endemic to the Galápagos Islands of Ecuador, but surprisingly little information exists on baseline health parameters for this species. We analysed blood samples drawn from 35 marine iguanas captured at three locations on San Cristóbal Island. A portable blood analyser (iSTAT) was used to obtain near-immediate field results for pH, lactate, partial pressure of O_2, partial pressure of CO_2, bicarbonate (HCO_3^-), percentage O_2 saturation, haematocrit, haemoglobin, sodium, potassium, ionized calcium and glucose. Parameter values affected by temperature were auto-corrected by the iSTAT. Standard laboratory haematology techniques were employed for differential white blood cell counts and haematocrit determination; resulting values were also compared with the haematocrit values generated by the iSTAT. Body temperature, heart rate, respiratory rate and body measurements were also recorded. Body length was positively correlated with several blood chemistry values (HCO_3^- and glucose) and two haematology parameters (haemoglobin and manually determined haematocrit). A notable finding was the unusually high blood sodium level; the mean value of 178 mg/dl is among the highest known for any reptile. This value is likely to be a conservative estimate because some samples exceeded the maximal value the iSTAT can detect. For haematocrit determination, the iSTAT blood analyser yielded results significantly lower than those obtained with high-speed centrifugation. The values reported in this study provide baseline data that may be useful in comparisons among populations and in detecting changes in health status among marine iguanas affected by natural disturbances or anthropogenic threats. The findings might also be helpful in future efforts to demonstrate associations between specific biochemical parameters and disease.

Key words: *Amblyrhynchus cristatus*, biochemistry, blood gas, health, haematology, marine iguana

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Introduction

The marine iguana (*Amblyrhynchus cristatus*) is the world's only marine lizard and is endemic to the Galápagos Islands of Ecuador. The marine iguana is currently listed as vulnerable and the San Cristóbal subspecies as endangered on the IUCN Red List; no commercial use is permitted under CITES Appendix I (Nelson *et al.*, 2004). Major threats to marine

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iguana populations include habitat destruction, pollution, disease and predation by feral animals (Wikelski *et al*., 2001, 2002; Romero and Wikelski, 2010; French *et al*., 2010). Considerable research on natural history and physiology has been conducted on this species, but studies on health parameters are largely lacking (Morgareidge and White, 1969; Ackerman and White, 1980; Gleeson, 1980; Nagy and Shoemaker, 1984; Shoemaker and Nagy, 1984; Wikelski *et al*., 1996).

Biochemical and haematology parameters, as measured in peripheral blood, are a useful diagnostic tool in lizard health management (Geffré *et al*., 2009; Gibbons *et al*., 2013). To determine the significance of changes in biochemical and haematological values associated with factors such as disease, injury, pollutants or starvation, it is essential to establish species-specific (or at least taxon-specific) normal values for parameters of interest. Reference intervals for certain iguanid and related species have been investigated (Divers *et al*., 1996; Wagner and Wetzel, 1999; Dennis *et al*., 2001; Hernandez-Divers *et al*., 2005; James *et al*., 2006; Harr *et al*., 2001; Maria *et al*., 2007; Dallwig *et al*., 2011). Nevertheless, few studies have combined blood gas, biochemistry and haematology parameters from the same geographical subpopulation. The present study evaluates selected blood gas, blood biochemical and haematology parameters for 35 wild-caught San Cristóbal marine iguanas (*Amblyrhynchus cristatus mertensi*). In addition, body temperature, pulse and respiratory values are reported.

Materials and methods

Ethics statement

This study was conducted on San Cristóbal Island in the Galápagos archipelago of Ecuador as part of a population health assessment authorized by the Galápagos National Park Service (permit no. PC-75-14 to G.A.L. and K.J.L.) and approved by the Universidad San Francisco de Quito ethics and animal handling protocol. All handling and sampling procedures were consistent with standard vertebrate protocols and veterinary practices and were approved by the North Carolina State University and the University of North Carolina Chapel Hill Institutional Animal Care and Use Committees.

Iguana capture and sampling

Iguanas were captured by hand among lava rocks within 100 m of the shore. The animals were then quickly transported to the field laboratory (usually located within 100 m of the capture site), where they were measured, weighed and sampled. The cloacal temperature of each animal was measured, usually within ~2 min of capture. In addition, the respiratory and heart rates were recorded as soon as the animal arrived at the field laboratory and again immediately before release.

Blood samples were obtained within ~5 min of capture. Seven iguanas were captured, examined and sampled from La Loberia Beach area (0° 55′ 40″ S, 89° 36′ 43″ W) on 25 June 2014; the following day, 18 iguanas were captured and sampled at a second La Loberia Beach area site (0° 55′ 22″ S, 89° 37′ 05″ W). An additional 10 animals were captured and sampled at Punta Carola (0° 53′ 26″ S, 89° 34′ 46″ W) on 28 June 2014. To avoid capturing the same individual more than once, a line of white zinc oxide ointment was applied to each iguana's skin on the dorsal part of the tail base after a blood sample had been obtained and before the animal was released. The mark remained visible for a few days before fading away.

Each iguana was examined for ectoparasitic ticks, with the highest concentrations noted on the head and axillary regions. When ticks were found, several were removed and preserved in 70% ethanol.

Blood sample collection and handling

Each iguana was manually restrained while a blood sample of ~2.5 ml was obtained from the coccygeal haemal arch (mixed venous and arterial) using a heparinized 22 gauge needle attached to a 3.0 ml syringe. The blood was immediately divided into subsamples, used for making blood films on clean glass microscope slides, or loaded into the CG-8+ and CG-4+ iSTAT cartridges within 10 min of sample collection. The remainder of the sample was stored on ice in sterile plastic vials for future analyses.

Blood gas and biochemistry parameters

Blood gas, electrolyte and biochemistry results were obtained using an iSTAT Portable Clinical Analyzer (Heska Corporation, Fort Collins, CO, USA) with CG8+ and CG4+ cartridges. The iSTAT is a portable, hand-held, battery-operated electronic device that measures a wide variety of blood gas, chemistry and haematology parameters using only a few drops (0.095 ml) of whole, non-coagulated blood. The following parameters were measured: pH, lactate, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-), percentage oxygen saturation (sO_2%), haematocrit, haemoglobin, sodium (Na), potassium (K), ionized calcium (iCa) and glucose. The iSTAT analysed the blood at 37°C and then corrected pH, pO_2 and pCO_2 for body temperature once this information was entered.

Haematology

Heparinized whole blood was stored on ice immediately after collection; time-sensitive analyses were done on the same day as each sample was obtained. Haematocrit was determined using high-speed centrifugation of blood-filled microhaematocrit tubes. Differential white blood cell counts were conducted by examining 100 white blood cells on a peripheral smear stained with Diff-Quick stain (Campbell, 1995). Monocytes with azurophilic granules (azurophils) were included in the total monocyte count.

Iguana measurements and body temperature

A flexible measuring tape was used to determine snout–vent length (SVL), total length (TL) and axillary girth (AG) to the

nearest 0.5 cm. Body weight was measured with a digital scale (precision, 0.1 kg). An EBRO® Compact J/K/T/E Thermocouple Thermometer was used to obtain all temperature readings (model EW-91219-40; Cole-Parmer, Vernon Hills, IL, USA). Core body temperatures were recorded from the cloaca using the probe T PVC epoxy tip 24GA.

The sex of adult iguanas was determined on the basis of external sexual dimorphism, primarily by femoral pore size and hemipene swellings. The sex of immature marine iguanas cannot be determined reliably with an external examination.

Statistical analysis

Given that both types of iSTAT cartridges produced nearly identical results (Lewbart *et al*., 2014), we report results only for the first cartridge used (CG8+) for all but the lactate values (the CG8+ does not measure this parameter). Linear regressions were used to examine possible relationships between body size (SVL) and measured blood chemistry and haematology analytes. We also determined the effect of handling time on blood lactate levels through linear regression analysis. Initial heart rate and heart rate after handling (immediately before release) were compared using Student's paired *t*-test. The iSTAT results for haematocrit were compared with the results of manually determined haematocrit using Student's paired *t*-test. Inability to determine the sex of immature individuals resulted in a very small sample size for females, which prevented the comparison of blood parameters between sexes. A standard α level of $P = 0.05$ was used for all statistical tests with R statistical software, version 3.0.2 (R Development Core Team). Finally, for purposes of comparison, selected blood values of marine iguanas and similar published data for green iguanas and basilisk lizards were compiled.

Results

Iguana demographics and health status

A total of 35 marine iguanas (24 males, four females and seven immature) were sampled from three locations on San Cristobal Island. The mean SVL for the entire group was 40.5 cm (28.5–51.0 cm) and the mean TL (tip of nose to tip of tail) was 98.1 cm (72.5–121.0 cm). The mean AG was 36.0 cm (24.0–46.5 cm). The mean body mass was 4.9 kg (1.5–8.3 kg).

The mean internal body temperature was 31.2°C (26.8–35.4°C). No statistical difference existed between the mean heart rate at capture (73 beats/min, with a range of 30–108 beats/min) and the mean heart rate at release (71 beats/min, with a range of 42–96 beats/min). The mean respiratory rate was 14 breaths/min (range 8–22 breaths/min) and was negatively correlated with body length ($r^2 = -0.46$, $P < 0.01$).

Blood analysis

The biochemistry, blood gas and haematology results for the 35 iguanas are summarized in Table 1. An unexpected difficulty was that, for 15 individuals, the iSTAT indicated Na values that exceeded the maximal value the iSTAT can detect (180 mmol/l). For the purpose of calculating a mean group value for Na, we used a value of 180 mmol/l for the 15 samples that exceeded the upper limit of the reportable range, but it should be noted that this procedure probably yielded a

Table 1: Mean, standard deviation and range for blood gas and blood biochemical values of wild marine iguanas (*Amblyrhynchus cristatus*)

Analyte	*n*	Mean	SD	Minimum	Maximum
HCO_3^- (mmol/l)	35	23.9	4.7	15.9	36.8
sO2%	35	80	20	35	99
pH	35	7.365	0.153	6.964	7.646
pCO_2 (mmHg)	35	41	15	19	90
pO_2 (mmHg)	35	45	18	22	86
Na (mmol/l)	20	178	3	169	180
K (mmol/l)	20	4.3	0.5	3.3	5.1
iCa (mmol/l)	35	1.51	0.10	1.20	1.68
Glucose (mg/dl)	35	114	12	94	140
Lactate (mmol/l)	34	6.63	4.01	1.67	19.43
$Haematocrit_I$ (%)*	20	27.05	5.67	17.00	37.00
$Haematocrit_M$ (%)*	25	37.96	4.61	28.30	44.90
Haemoglobin (g/l)	20	92	19	58	126

Subscript 'I' denotes values obtained through the instant iSTAT analysis; subscript 'M' indicates values manually obtained by high-speed centrifugation. *Significant difference between iSTAT and manually obtained values. Different sample sizes (*n*) resulted from the iSTAT being unable to determine potassium (K), ionized (iCa), $haematocrit_I$ and haemoglobin whenever sodium (Na) values were >180 mmol/l. For one individual, the iSTAT lactate value extended over the range of 20 mmol/l and was therefore not included.

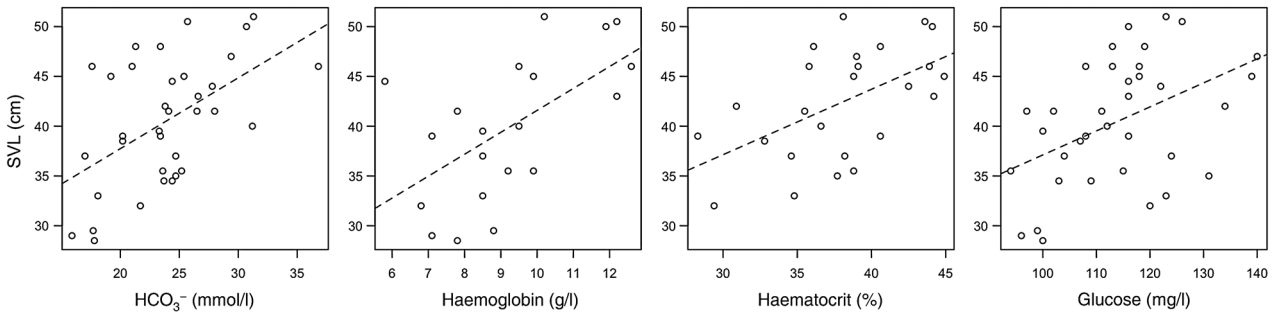

Figure 1: Linear correlations of several blood chemistry and haematology analytes and the marine iguana (*Amblyrhynchus cristatus*) body size (SVL, snout–vent length).

Table 2: Mean, standard deviation and range for differential white blood cell counts for wild marine iguanas (*A. cristatus*)

Cell type	*n*	Mean	SD	Minimum	Maximum
Lymphocyte (%)	34	83.36	11.64	44.50	98.00
Heterophil (%)	34	10.09	8.29	0.50	37.50
Monocyte (%)	34	6.05	3.92	0.50	17.00
Eosinophil (%)	34	0.34	0.68	0.00	3.00
Basophil (%)	34	0.16	0.37	0.00	1.03

lower estimate of mean Na level for the sampled population than genuinely exists.

The haematocrit figures generated by the iSTAT were significantly lower than the manually determined values in the 20 samples in which these results could be compared ($t = -23.75$, $P < 0.001$; Table 1). We therefore consider the manually determined values to be more reliable. The iSTAT determines haematocrit via conductometry, which is dependent on both Na and K (Ng *et al.*, 2014); thus, for the 15 animals with Na values exceeding the iSTAT maximum, the iSTAT was unable to measure the haematocrit or to calculate the haemoglobin concentration (the latter depends on the haematocrit value).

The SVL of iguanas was positively correlated with several blood chemistry measures (HCO_3^-, $r^2 = 0.53$, $P = 0.001$; and glucose, $r = 0.45$, $P < 0.01$) and two haematology parameters (haemoglobin, $r^2 = 0.59$, $P < 0.01$; and manually determined haematocrit, $r^2 = 0.55$, $P < 0.01$; see Fig. 1). Furthermore, handling time before obtaining a blood sample was significantly correlated with blood lactate levels ($r^2 = 0.45$, $P < 0.01$). White cell differential counts are summarized in Table 2, and examples of different marine iguana white blood cell types are shown in Fig. 2.

For purposes of comparison, selected blood values of marine iguanas (from this study) and those of two related species (obtained in other studies) are listed in Table 3. All haematocrit values shown in this table were derived manually using high-speed centrifugation.

Discussion

When assessing the health of animals, clinicians desire species-specific baseline values for parameters that can easily be measured with commercial blood gas and chemistry analysers. In reptiles, species specificity of health data is especially important, due to the diverse environmental conditions that exist in different habitats. Our study provides the first blood gas, biochemistry and haematology measures in marine iguanas. Although the relatively small sample size ($n = 35$) precludes the calculation of formal reference intervals (Geffré *et al.*, 2009), these results provide a useful starting point for clinicians and researchers. All of the iguanas we examined were judged to be clinically healthy, and their blood parameters support this assessment.

Most of the blood parameters we recorded for marine iguanas were similar to those reported previously for other iguanids (Divers *et al.*, 1996; Harr *et al.*, 2001; Maria *et al.*, 2007; Dallwig *et al.*, 2011; Gibbons *et al.*, 2013; Table 3). An exception was Na (mean 178+ mmol/l), which was present at concentrations that are among the highest ever reported in reptiles (Dessauer, 1970).

Marine iguanas feed primarily on marine algae, resulting in a high intake of sodium and chloride (Dunson, 1969; Shoemaker and Nagy, 1984; Wikelski *et al.*, 1993). Potent salt glands excrete most of the ingested salts (Shoemaker and Nagy, 1984; Hazard *et al.*, 1998). Interestingly, highly variable blood concentrations of Na have been reported in snakes and lizards, some of which are tolerant of hypernatraemia. An intriguing possibility is that tolerance of high Na concentrations may have been an important factor that enabled ancestral marine iguanas to begin to exploit marine algae as a food source (Dessauer, 1970; Shoemaker and Nagy, 1984).

The measured heart rates of specimens within 5 min of capture on lava rocks for this study were comparable to those of marine iguanas during periods of treadmill activity (Butler *et al.*, 2002), but relatively low when compared with captive basilisk lizards (Dallwig *et al.*, 2011). Likewise, respiration was much lower compared with the basilisk lizards and might be related to the adaptation of marine iguanas to hold their

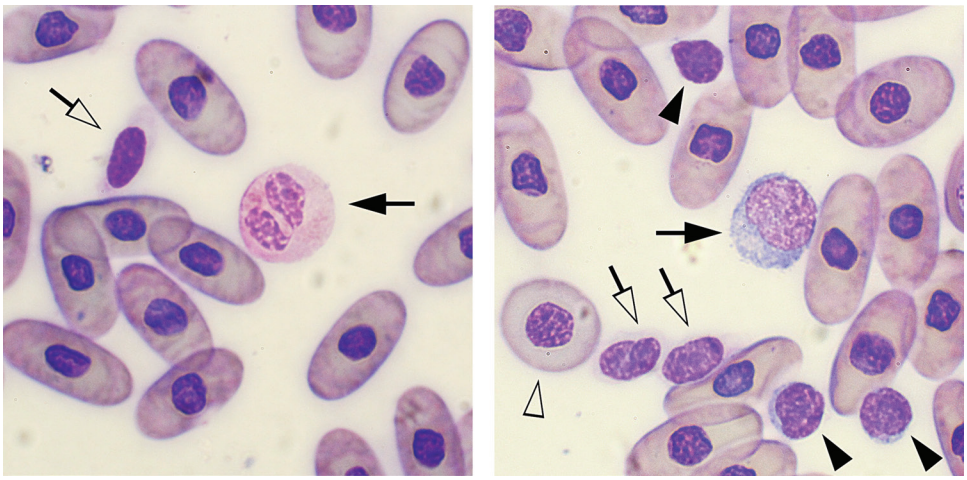

Figure 2: Reference photomicrographs of selected marine iguana (*A. cristatus*) blood cells stained with Diff-Quick stain. In the left panel, filled arrow indicates a heterophil, and open arrow indicates a thrombocyte. In the right panel, filled arrow indicates a monocyte, filled arrowheads indicate lymphocytes, open arrows indicate thrombocytes, and open arrowhead indicates an immature erythrocyte.

Table 3: Comparison of selected haematology and blood chemistry values between three iguanid species

Analyte	*A. cristatus*			*Iguana iguana* (Harr *et al.*, 2001)			*Basilicus plumifrons* (Dallwig *et al.*, 2011)		
	n	Mean	±SD	*n*	Mean	±SD	*n*	Mean	±SD
Haematocrit (%)	35	37.96	4.61	36	36.67	4.48	19	31.4	8
Haemoglobin (g/l)	20	92	19	36	96	11.96	n.a.	n.a.	n.a.
Na (mmol/l)	20	178	3	48	160	6.37	19	153.5	7
K (mmol/l)	20	4.3	0.5	48	3.8	1.13	16	5.4	1.7
Ca (mg/dl)	20	6.05	0.40	48	12.7	2.03	19	10.6	1.3
Glucose (mg/dl)	35	114	12	48	203	64.86	19	193	48.1

Abbreviation: n.a., not assessed.

breath for an extended time during foraging dives (Vitousek *et al.*, 1997; Dallwig *et al.*, 2011). Faster heart rates of iguanas with smaller body sizes were reported by Bartholomew and Lasiewski (1965), which might explain the increase in respiratory rate with smaller body size in this study.

Handling of the animals was kept to a minimum during this study to avoid affecting the measured blood chemistry results. Blood pH levels and, subsequently, lactate concentrations can increase rapidly due to excitement and activity in reptiles (Dessauer, 1970). This may explain the increase in blood lactate during increased handling time in marine iguanas and emphasizes the need for efficient field sampling procedures when the goal is to evaluate normal blood ranges of wild iguanids.

The reason for the low glucose levels of marine iguanas relative to their terrestrial counterparts (Table 3) is not known. One possibility is that this reflects differences in the nutritional quality of food available to each group. It might also reflect less predictable access to food in the marine environment; for example, foraging of marine iguanas is strongly influenced by tidal cycles (algae in the intertidal zone is more easily reached at low tide). Feeding might also be influenced by the availability of sunlight for thermoregulation before and after heat loss during underwater foraging bouts (Wikelski *et al.*, 1993; Vitousek *et al.*, 1997).

The finding that larger marine iguanas had higher glucose levels than smaller iguanas is consistent with reports that larger individuals make longer foraging excursions and dives (Wikelski and Trillmich, 1994; Wikelski and Wrege, 2000). In addition, differences in diet among iguanas of different sizes might contribute to the observed pattern. The algal composition in the intertidal zone, where smaller individuals feed, differs from that in the subtidal zone (Wikelski *et al.*, 1993; Wikelski and Trillmich, 1994; Vitousek *et al.*, 1997; Wikelski and Wrege, 2000).

Our results show that ratios of white blood cell counts in marine iguanas are similar to those of green iguanas.

Lymphocytes are the most abundant in both species, followed by heterophils and monocytes, while eosinophils and basophils are nearly absent (Harr *et al*., 2001). Basilisk lizards, in turn, have much lower lymphocyte counts, higher heterophil concentrations and few, if any, eosinophils (Dallwig *et al*., 2011).

In summary, the data reported in this study represent an important step towards determining the normal range of values for blood gas, biochemistry, haematology and other health parameters in marine iguanas. Differences in a variety of blood chemistry and haematology values between marine iguanas and related iguanids emphasize the need for species-specific data in order to provide a reliable baseline for health monitoring and disease diagnostics. Given that marine iguanas are a protected species with importance in the wildlife biology research community, health assessments are important from the standpoint of wildlife conservation and management. These results add to a growing database of knowledge about health management in wild reptiles. Future research should establish formal reference intervals for this species and facilitate comparisons of blood values across age groups, geographical localities and subspecies.

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