Baseline haematology, biochemistry, blood gas values and health status of the Galapagos swallow-tailed gull (*Creagrus furcatus*)

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The swallow-tailed gull, *Creagrus furcatus*, is a seabird endemic to the Galápagos archipelago. In general health, blood chemistry and haematology, parameters have not been published for this species. Blood analyses were run on samples drawn from 58 clinically healthy swallow-tailed gulls captured at Islote Pitt on San Cristóbal Island in July 2016 (28) and South Plaza Island in June 2017 (30). A point of care blood analyzer (iSTAT) was used in the field to obtain results for $\text{HCO}_3^-$, pH, $p\text{CO}_2$, $p\text{O}_2$, TCO2, anion gap, chloride, creatinine, glucose, haematocrit, haemoglobin, ionized calcium, potassium, sodium and urea nitrogen. A portable Lactate Plus™ analyzer was used to measure lactate. The baseline data reported is valuable for comparisons amongst different populations in the archipelago and to detect changes in health status of Galápagos swallow-tailed gulls.

Key words: Biochemistry, *Creagrus furcatus*, Galápagos Islands, swallow-tailed gull, health status, haematology

Editor: Steven Cooke

Received 24 December 2019; Revised 1 June 2020; Editorial Decision 5 July 2020; Accepted 5 July 2020

Cite as: Valle CA, Ulloa C, Regalado C, Muñoz-Pérez J-P, Garcia J, Hardesty BD, Skehel A, Deresienski D, Passingham RK, Lewbart GA (2020) Baseline haematology, biochemistry, blood gas values and health status of the Galapagos swallow-tailed gull (*Creagrus furcatus*). *Conserv Physiol* 8(1): coaa064; doi:10.1093/conphys/coaa064.
grown 5-fold, and with this growth comes added risk to the fragile environment (Pizzitutti et al., 2014). This increased human presence poses a threat to wildlife on many levels, and issues related to climate change are also of serious concern to wildlife in this region (Restrepo et al., 2012). The more baseline health information we gather and publish from healthy animals, the better our understanding of changes that occur related to a species’ health status.

The swallow-tailed gull (Creagrus furcatus) is a member of the Laridae family and is considered endemic to the Galápagos archipelago, although there is a population in Malepelo, Colombia (Townsend 1895 in Pitman et al., 1995). The only other permanent resident gull in Galápagos is the lava gull (Leucophaeus fuliginosus). We believe that this is the first general health assessment that includes biochemistry, blood gas and haematology data for C. furcatus, the only member of its genus. There is no evidence that introduced diseases are affecting the swallow-tailed gull, but other Galápagos birds are being affected by introduced pathogens (Parker et al., 2006; Levin et al., 2011).

Here, we report over 30 anatomical, physiological and blood-specific values that are commonly used to evaluate the health of seabirds (Work 1996; Newman et al., 1997; Padilla et al., 2006; Lewbart et al., 2017; Valle et al., 2018). We used a handheld electronic device (iSTAT, Heska Corporation, Loveland, Colorado, USA 80538) to measure a wide variety of blood gas and chemistry parameters with only a small amount (95 μl) of whole, non-coagulated blood obtained from 58 clinically healthy C. furcatus at two locations over two successive years.

The information presented in this paper expands our knowledge of Galápagos wildlife health, in particular seabirds, and shows that distinct populations of the same species can display differences with regards to readily measured blood parameters.

### Materials and methods

This study was performed as part of a population health assessment authorized by the Galápagos National Park Service (permit No. PC-57-16 and No. PC-59-17 to C.A. Valle) and approved by the Universidad San Francisco de Quito ethics and animal handling protocol. Data were collected at Islote Pitt, San Cristóbal Island (0° 42′ 12″ S; 89° 14′ 50″ W), from a nesting colony of swallow-tailed gulls in July 2016 and at South Plaza Island (0° 34′ 55″ S; 90° 9′ 59″ W) in June 2017.

From 20 to 26 June 2016 (Islote Pitt), 28 adult swallow-tailed gulls were captured by hand, or with a hand net, one at a time from or near their nests, closely examined by a board-certified (American Board of Veterinary Practitioners) veterinarian (DD) and sampled before the next bird was captured. This same procedure was repeated from 29 to 30 June 2017 on South Plaza Island. Standard measurements were taken utilizing calipers, and the animals were weighed with a Pesola scale (Pesola Präzisionswaagen AG, Schindellegi, Switzerland 8834). The body temperature was obtained from the cloaca using an EBRO® Compact J/K/T/E thermocouple thermometer (model EW-91219-40; Cole-Parmer, Vernon Hills, IL, USA 60061) inserted ~5 cm into the vent. The heart rates were measured with a stethoscope and respiratory rates by observing chest movements.

Table 1 contains all vital signs and measurements. Samples were obtained as soon as safely possible to minimize the potential effects of handling on blood parameters; in most cases, this was within 10 min of capture. Based on their behaviour, response to handling and physical examination by the veterinarian, all the birds in this study were deemed clinically healthy.

Brachial vein venepuncture was performed using a heparinized 25-gauge needle and 1.0 ml of syringe to collect up to 1.0 ml of blood per bird. The blood was then used for instant analysis with portable blood chemistry and lactate analyzers or stored on ice in sterile plastic vials within 10 min of sample collection for haematology and archiving. Blood films were made within a few minutes of blood collection and fixed and stained with Dip Quik (Jorgenson Laboratories, Loveland, CO, 80538 USA) 2 weeks after sampling. The stained blood films were used for the 100-cell white blood cell count (WBC) differentials and for estimated WBC counts. A single technician performed the counts by counting 10 × 40 fields, calculating the average and multiplying by 2000 (Work 1996).

The blood gas, electrolyte and biochemistry results were obtained using an iSTAT Portable Clinical Analyzer (Heska Corporation, Loveland, Colorado, USA 80538) with CG8+ cartridges in 2016 and Chem8 cartridges in 2017. The following parameters were measured and recorded at Islote Pitt: base excess, pH, partial pressure of oxygen (pO2), oxygen saturation (sO2), partial pressure of carbon dioxide (pCO2), total carbon dioxide (TCO2), bicarbonate (HCO3−), haematocrit (Hct), haemoglobin (Hb), sodium (Na), potassium (K), ionized calcium (iCa) and glucose. The following parameters

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**Table 1**: Summary of morphological and physiological measurements with time to obtain blood samples, reported in mean ± standard deviation, median, and range for 28 swallow-tailed gulls (Creagrus furcatus) at Punta Pitt, San Cristóbal

| Parameter                  | Value                                      |
|----------------------------|--------------------------------------------|
| Wing length (cm) (n = 28)  | 41.49 ± 1.3741.35 (40.0–45.73)            |
| Tarsus length (cm) (n = 28) | 5.04 ± 0.33560.64 (5.14–5.55)             |
| Weight (kg) (n = 28)       | 0.67 ± 0.040657 (0.6–0.76)                |
| Body mass index (n = 28)   | 671.42 ± 0.826740.00 (588.9–771.9)        |
| Body temperature (°C) (n = 20) | 40.25 ± 0.8240.35 (38.7–41.9)           |
| Heart rate (beats/min−1) (n = 28) | 257.5 ± 63.86250.00 (110–400)       |
| Respiratory rate (b/min) (n = 28) | 38.75 ± 7.6540.00 (20–50)              |
| Sample time (min) (n = 28) | 6.35 ± 2.5611 (11)                        |
| Handling time (min) (n = 28) | 9.29 ± 2.4895 (5–13)                      |
were measured and recorded at South Plazas: pH, total carbon dioxide (TCO₂), haematocrit (Hct), haemoglobin (Hb), sodium (Na), potassium (K), chloride (Cl), ionized calcium (iCa), creatinine, urea nitrogen, anion gap and glucose. At both sites, blood lactate was determined using a portable Lactate Plus™ analyzer (Nova Biomedical, Waltham, Massachusetts, 02454 USA).

**Statistics**

We used the Welch’s two-sample t-test for comparison between groups because sample sizes were close to 30 for each group for all variables investigated. The relationship between body condition and each biochemistry measurement was explored using Pearson’s correlation coefficients. Body condition, an indicator of an individual physiological condition (Peig and Green 2009), was calculated as the ‘scaled mass index’ (for more details see Peig and Green 2009, 2010) that produces a standardized body mass. The R package (standardized) major axis estimation and testing routines ‘smatr’ 3.4-8 (Warren et al., 2012) was used for computing the scaling exponent using standardized major axis (SMA) regression. The potential effect of handling time on each blood biochemistry variable measured was assessed using Pearson’s correlation coefficients. All statistical analyses were conducted in R version 3.6.1 (R Core Team 2019), and significance was set to an alpha level of 0.05.

**Results**

This study reports an in-depth array of morphometric, vital, blood biochemical and haematological parameters from 58
Figure 1: Standard boxplots showing the inter-colony comparison between swallow-tailed gulls from Islote Pitt (PITT) and South Plaza Island (SPLA). The two studied colonies showed large and highly significant differences (see the Discussion for statistical information) in total protein, lactate, sodium (Na), packed cell volume (PCV) measured with the iSTAT (Pavitt) and manual PCV (PCVmanual). Graphs represent standard boxplots.

Figure 2: Standard boxplots showing significant differences in monocyte (MONOC) counts and white blood cell (WBC) counts between swallow-tailed gulls from Islote Pitt (PITT) and South Plaza (SPLA). See text for detailed statistical test information.

Discussion

The most interesting finding in this study was that the two populations of gulls, separated by ~50 km, were for the most part very similar with regards to size, vital signs and blood values. There were some significant differences between the adult swallow-tailed gulls. Adult birds are not sexually dimorphic; thus, sex could not be determined in the field, although DNA sexing could be used on feathers in future studies. Different iSTAT cartridges were used in the two locations so the reported parameters differ slightly.

Table 1 summarizes the blood sample time from capture, total handling time and the morphological and physiological measurements, and Table 2 contains the blood chemistry and haematology values on Islote Pitt. Table 3 summarizes the blood sample time from capture and the morphological and physiological measurements, and Table 4 contains the blood chemistry and haematology values on Plaza South. Averages of the various blood biochemistry and blood gas parameters are shown in Tables 2 and 4. When combined, this presents more pertinent data but limits comparisons between the two populations.
populations with regards to selected blood parameters, but these were few and are discussed in the following paragraphs.

Several works focus on Laridae blood values (Work 1996; Newman et al., 1997, Garcia et al., 2019), but just one that we are aware of on C. furcatus (Padilla et al., 2006). The latter only addresses PCV and the WBC from up to 20 birds on the island of Genovesa. The Islote Pitt birds from this study and the Genovesa birds had very similar WBC values (both total count and percentages). A significantly different finding was the PCV mean of 54% for the Genovesa birds (Padilla et al., 2006). This might be explained by the difference in geography but not by the time of year, as the Genovesa birds were sampled in July.

Values for haematocrit, total protein and glucose are fairly consistent between C. furcatus and the sooty tern, Sterna fuscata (Work 1996); black-legged kittiwake, Rissa tridactyla (Newman et al., 1997); glaucous-winged gull, Larus glaucescens (Newman et al., 1997); and Olrog’s gull, Larus atlanticus (Garcia et al., 2019). The potassium values for the sooty tern (Work 1996) and Olrog’s gull (Garcia et al., 2019) are also in the same ranges as C. furcatus. Aside from calcium, there were no other overlapping blood chemistry values.

When comparing the Islote Pitt and Plaza South birds, most of the overlapping blood chemistry values are consistent. Noted differences are the higher total protein ($t = 31.36$, $df = 14.53$, $P < 0.001$) for the Islote Pitt birds, and higher lactate values ($t = 3.81$, $df = 43.98$, $P < 0.001$) for the Plaza South birds (Fig. 1). Highly significant differences between the two colonies were also found for sodium ($t = 7.70$, $df = 54.85$, $P < 0.001$) and PCV iSTAT values ($t = 3.66$, $df = 51.54$, $P < 0.001$) (Fig. 1). Lower values were found in Islote Pitt for monocytes ($t = 6.52$, $df = 37.03$, $P < 0.001$) and WBC ($t = 18.05$, $df = 33.19$, $P < 0.001$) (Fig. 2).

On Islote Pitt (Fig. 3), body condition correlated positively with lactate ($r = 0.465$, $t = 2.62$, $df = 25$, $P = 0.015$) but negatively with base excess of extracellular fluid (BEefc) ($r = -0.429$, $t = 2.33$, $df = 24$, $P = 0.029$), HCO3 ($r = -0.495$, $t = 2.98$, $df = 23$, $P = 0.008$) and WBC ($r = -0.532$, $t = 3.13$, $df = 22$, $P = 0.005$).
body temperature and heart rate (mean = 61.7%, P = 0.010), 
TCO2 (r = −0.478, t = −2.67, df = 24, P = 0.014) and sodium (r = −0.392, t = 2.13, df = 25, P = 0.043). On South Plaza (Fig. 4), body condition was found to negatively correlate only with sodium (r = −0.497, t = 3.03, df = 28, P = 0.0052).

On Islote Pitt, neither correlation was found between body temperature and heart rate (r = −0.370, t = 2.03, df = 26, P = 0.053), nor with respiratory rate (r = 0.265, t = 1.40, df = 26, P = 0.173). Similarly, on South Plaza, neither correlation was found between body temperature and heart rate (r = 0.129, t = 0.69, df = 28, P-value = 0.497), nor with respiratory rate (r = 0.087, t = 0.45977, df = 28, P = 0.6492). No effect of handling time was found for any measured parameter (all P ≥ 0.1611) on both Islote Pitt and South Plaza Island.

Since different iSTAT cartridges were used on the two populations not all values overlap. The iSTAT machine measures haematocrit electronically versus the more traditional and reliable high-speed centrifugation. It has been well documented that the iSTAT technology routinely underestimates haematocrit (Harter et al., 2015), and this held true for the Punta Pitt gulls. Unfortunately, we did not have a reliable way to assay manual haematocrit of the South Plaza gulls.

For the Islote Pitt birds, heterophils were the most predominant granulocyte (mean = 52%) followed by eosinophils (mean = 6%), monocytes (mean = 1.4%) and basophils (mean = 0.7%). Lymphocytes made up 40% (mean) of the leukocytes. For the Plaza South birds, heterophils were also the most predominant granulocyte (mean = 48.8%) followed by eosinophils (mean = 7.2%) and monocytes (mean = 4.9%). No basophils were observed in this population. Lymphocytes made up 40% (mean) of the leukocytes for the Islote Pitt birds, and 41% for the Plaza South birds. The erythrocyte, thrombocyte and leukocyte morphology appeared normal compared to those in other birds and the packed cell volume (PCV), and total solids were robust and consistent with good avian health.

Climate change, diseases, pollution and other anthropogenic impacts threaten many seabird populations (Barraclough and Davies 2005; Edgar et al., 2008; Levin et al., 2012; Altizer et al., 2013; Wilcox et al., 2016). Population growth, tourism and increased human mobility between the archipelago and continental Ecuador make the Galápagos area particularly vulnerable (Walsh & Mena 2016). Increasing human presence and pressure on attractive wildlife domains, including seabird rookeries, places these localities at increased risk. The more we know, understand and monitor vital diagnostic parameters of healthy animals, the better our ability to modify human behaviour and actions as necessary. This swallow-tailed gull health assessment reports on a little-studied species with regards to health and adds to the foundation of veterinary knowledge for this species and seabirds in general.

Acknowledgements

This research was authorized by the Galápagos National Park Service and was conducted with support of the Heska Corporation, the Galápagos Academic Institute for the Arts and Sciences (GAIAS)-Universidad San Francisco de Quito (USFQ) and the Galápagos Science Center-USFQ/University of North Carolina-Chapel Hill. We thank Galo Quezada, Maryuri Yepez, Diego Quiroga, Carlos Mena, Stephen Walsh, Philip Page, Tillie Laws and Michael Levy for their support and assistance with this project.

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