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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Clinical Pathology of the Critically Endangered Gough Bunting (*Rowettia goughensis*)

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Summary

The Gough bunting (*Rowettia goughensis*) is indigenous to Gough Island and critically endangered due to predation by invasive house mice (*Mus musculus*). A planned ecosystem restoration by eradication of house mice via aerially delivered rodenticide requires a reproductively balanced population of Gough buntings being held in captivity to avoid primary and secondary poisoning. To aid disease detection during the period of captivity, Gough buntings ($n = 25$; five adult females, 15 adult males and five juveniles) were captured, measured and sampled to determine reference ranges for routine haematological and biochemical parameters and to identify any faecal bacterial species and intestinal and haemoparasites. Adult females had significantly higher blood glucose ($P = 0.02$ and 0.05 for different analyzers) and globulins ($P = 0.02$) than adult males or juveniles, and juveniles had consistently higher, although not significant, concentrations of creatine kinase. Juveniles had significantly ($P = 0.007$) more heterophils than adults; eosinophils were rare in adults and absent in juveniles and azurophils were absent from all individuals sampled. No parasite eggs were found in the faeces and no haemoparasites were found in blood smears. Several faecal bacterial species were recorded including *Enterococcus* spp. ($n = 12$), *Klebsiella* spp. ($n = 7$), *Staphylococcus aureus* ($n = 6$), *Staphylococcus intermedius* ($n = 1$), *Escherichia coli* ($n = 1$) and *Pseudomonas* spp. ($n = 1$). No overt clinical or subclinical disease was found in any of the birds examined, which suggests they are suitable for short-term captivity during ecosystem restoration and the data will provide key haematological and biochemical reference ranges for monitoring their health. However, the capture of a reproductively balanced population may require significant effort due to the relative difficulty with which females were caught.

Keywords: Gough bunting; haematology; parasitology; plasma biochemistry

Introduction

The Gough bunting (*Rowettia goughensis*) is endemic to Gough Island (40.3°S, 9.9°W), which is part of the UK Overseas Territory of Tristan de Cunha in the central South Atlantic Ocean and is approximately 65 km^2 (Ryan, 2007; Ryan *et al.*, 2007; Ryan and Cuthbert, 2008). Genetic studies suggest that the Gough bunting is related to the tanager finches (Thraupidae) of South America, having crossed 3,000 km of ocean to colonize the island, probably soon after it became habitable (Ryan *et al.*, 2013). The population has decreased by 50% between the early 1990s and 2007 to approximately 1,000 individuals and continues to decline, apparently due to predation by house mice (*Mus musculus*), which were introduced to the island during the 19th century (Ryan and Cuthbert, 2008; Gray *et al.*, 2014). Due to the precipitous population decline and its

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severely restricted distribution, the Gough bunting was classified as critically endangered in 2008 (Birdlife International, 2016). The bunting is now largely confined to high elevations, where mouse densities are lower than in coastal fern bush vegetation (Cuthbert *et al.*, 2016), but small numbers also persist along precipitous coastal cliffs (Ryan and Cuthbert, 2008).

House mice also threaten other species on Gough Island, including two seabirds that breed principally, if not solely, on this island: the critically endangered Tristan albatross (*Diomedea dabbenena*) and the endangered Atlantic petrel (*Pterodroma incerta*) (Davies *et al.*, 2015; Dilley *et al.*, 2015). Consequently, Gough Island is recognized as the highest priority for island restoration among the UK Overseas Territories (Dawson *et al.*, 2015). Eradication of invasive rodents from islands to restore ecosystems is a common conservation intervention with >700 successful operations worldwide (Howald *et al.*, 2007; Bellingham *et al.*, 2010; Russell and Holmes, 2015). Such operations entail the spreading of cereal pellets laced with rodenticide across the island, often by helicopter (Towns and Broome, 2003; Cuthbert *et al.*, 2014). For endemic obligate terrestrially-foraging species, such as the Gough bunting, there is a risk of primary poisoning (ingesting bait directly; Wanless *et al.*, 2010; Bond *et al.*, 2016) and/or secondary poisoning (ingesting poisoned rodents or other species; Empson and Miskelly, 1999; Eason *et al.*, 2002; Towns and Broome, 2003; Phillips, 2010). To mitigate this, a small, reproductively balanced population can be placed in captivity during the eradication operation until the rodenticide, brodifacoum, is broken down in the environment (Empson and Miskelly, 1999; Phillips, 2010; Rexer-Huber and Parker, 2011; Oppel *et al.*, 2016). Bringing wild free-ranging species into captivity, even for a limited period, carries several risks including increased transmission of infectious diseases, especially as the captive population density inevitably exceeds greatly that of the free-ranging animals (Goodman *et al.*, 2012; Sainsbury and Vaughan-Higgins, 2012).

This study reports the health status of free-ranging Gough buntings by evaluating a range of standard haematological and plasma biochemical parameters used in clinical pathology, and by examining faecal samples for gastrointestinal parasite burden and resident bacterial species, together with the presence of any potential pathogens. The data provide reference ranges to aid health monitoring and identify potential aetiologies should birds show clinical signs of disease in the period of captivity during the planned mouse eradication operation.

Materials and Methods

Capture, Measuring and Sampling Procedures

Gough buntings were captured at two locations on Gough island: (1) nine birds from *Spartina arundinacea* tussock grass and scattered island trees (*Phylica arborea*) along the sea cliffs around the weather station (40.349°S, 9.880°W) ≤40 m above sea level, and (2) 16 birds from wet heath at 400–700 m above sea level from Gonydale to Low Hump (40.332–40.346°S, 9.911–9.939°W), a transitional vegetation type comprising a variety of grasses, sedges and other flowering plants as well as mosses and ferns (Ryan, 2007). Birds were captured with a long-handled net, sometimes with the assistance of audio playback and imitation of conspecific calls.

All birds were held in cloth bags until they were examined visually for subjective assessment of body condition and sexing based on plumage differences (adults only as juveniles of this species cannot be sexed by morphometric measurements; Ryan and Cuthbert, 2008), weighed with a spring balance (±1 g), measured (flattened straightened wing chord ± 1 mm using stopped ruler, culmen ± 0.1 mm, bill depth at base ± 0.1 mm, tarsus ± 0.1 mm and head + bill ± 0.1 mm, all using Vernier callipers) and marked with a uniquely numbered metal ring and a unique combination of three coloured rings if not already so identified. Subsequent to this, 0.35 ml of blood was sampled via the right jugular vein using a 1.0 ml syringe with a swaged on 29 gauge × 12.7 mm needle (BD Medical, Oxford, UK), placed immediately into 0.5 ml tubes containing lithium citrate anticoagulant (0011-25, Vetlab Supplies, Pulborough, West Sussex, UK) and mixed gently. If birds defecated at any point from capture until release the sample was collected and placed into 0.5 ml sterile plastic capped tubes and processed for faecal parasite egg content and bacteriological examination either within 30 min or stored chilled and processed within 6 h for bacteriology or 12 h for parasite egg examination (depending on capture location). Birds that did not defecate were subjected to cloacal swabbing (Flocked swab, SLIN502CS01, VWR International Ltd., Lutterworth, UK), which was suitable for bacteriological examination only.

Haematological and Plasma Biochemical Analyses

Blood samples were processed immediately for determination of haematocrit, haemoglobin concentration, pH, pCO_2, pO_2, base excess, bicarbonate, total CO_2, sO_2, sodium, potassium, ionized calcium and glucose with a portable VetScan i-Stat 1 handheld analyzer using i-Stat CG8+ cartridges (Abaxis Europe GmbH, Griesheim, Germany) according to the manufacturer's

instructions. Blood samples were also analyzed for total protein, albumin, globulin, aspartate aminotransferase, bile acids, creatine kinase, uric acid, glucose, calcium, phosphate, potassium and sodium with a laboratory based VetScan VS2 using the Avian/Reptile Profile Plus cartridges (Abaxis Europe). Parameters examined by the VetScan VS2 were performed either within 10 min of blood sampling (for individuals caught near the weather station and blood sampled in the laboratory) or between 6 and 8 h after sampling (for individuals caught in Gonydale to Low Hump, blood sampled in the field and the samples transported to the laboratory in a single batch).

Thin blood smears were prepared by placing 2 μl of anticoagulated blood at one end of a glass microscope slide and smearing with a clean slide. Slides were air dried, fixed for 1 min in methanol, air dried, stained with May–Grunwald/Giemsa (Kayla Africa Suppliers and Distributors, Johannesburg, South Africa) and mounted to allow estimation of leucocyte number and white blood cell differential counts, red and white blood cell morphology and presence of blood parasites by light microscopy.

Faecal Bacteriology and Parasite Evaluation

Identification of faecal bacterial species was performed using the MultiChrome™ Veterinary Microbiology Culture System (Kacey™ Diagnostics, Ashville, North Carolina, USA), which isolates gram-positive from gram-negative bacteria using selective media and further differentiates bacteria by inciting genus- or species-specific colour reactions. Faecal samples were streaked out onto microbiology plates using a sterile loop or, if no faecal sample was available, using the cloacal swab. The plates were incubated at 38°C in air and examined after 24 and 48 h by two observers (MPD and ALB).

Presence, number and morphology of parasite eggs within faeces were assessed by the modified McMaster's method (Whitlock, 1948). Faecal samples were added to a saturated solution of NaCl at a ratio of 1 g:14 ml (w/v) in a stomacher bag and mixed thoroughly to emulsify, prior to passing through a 0.15 mm wire mesh sieve. The filtrate was stirred thoroughly, drawn up in a Pasteur pipette and run carefully into either side of a McMaster counting chamber (Vetlab Supplies). All eggs within both grids were counted under light microscopy and the total number multiplied by 50 to derive the number of eggs per gram of faeces.

Statistical Analyses

Differences in haematological and plasma biochemical parameters between the three groups (i.e. adult females, adult males and juveniles) were examined using a series of general linear models in R 3.3.1 (R Core Team, 2016). We examined models that included differences between adult males captured at the two sites, but as none found any difference in any parameter, we grouped all adult males for subsequent analyses. We examined differences in blood sodium, potassium and glucose measured on both instruments using paired t-tests. Differences were considered significant when $P \leq 0.05$.

Results

Bird Numbers and Morphometric Data

A total of 25 Gough buntings were captured; nine (two adult females, six adult males and one juvenile) from coastal cliffs around the weather station and 16 (three adult females, nine adult males and four juveniles) in Gonydale–Low Hump. Mass and linear measurements (Table 1) were representative of previous studies of the population (Abbott, 1978; Ryan and Cuthbert, 2008; PGR, unpublished data).

Haematology and Plasma Biochemistry

Results from both blood analyzers were obtained from 20 birds (four adult females, 13 adult males and three juveniles). Blood was not sampled from two birds (one adult male and one juvenile) and occasional individual results are missing due to haemolysis, icterus or other unknown reasons affecting the samples, but the number is stated from which the mean and range is derived for each parameter (Tables 2 and 3). For two birds (one adult female and one adult male) results were available from the VetScan VS2 only due to the VetScan i-Stat 1 handheld analyzer malfunctioning at low ambient temperature in Gonydale, and for one juvenile bird, results were available from the VetScan i-Stat 1 handheld analyzer only due to a small blood sample.

There were few significant differences between groups (Tables 2 and 3). Adult females had significantly higher concentrations of glucose irrespective of which blood analyzer was used (iStat $P = 0.02$, VetScan $P = 0.05$) and these values were consistently greater with the iStat compared with the VetScan ($t_{19} = 8.37$, $P < 0.001$). Globulin concentrations were significantly ($P = 0.02$) higher in adult females compared with adult males or juveniles and juveniles had increased, although not significant, concentrations of creatine kinase compared with adult females and males. Results for mean blood sodium concentrations were slightly greater and more consistent across adult female, adult male and juvenile birds when analyzed with

Table 1
Morphometric measurements of Gough buntings

Parameter	*Adult female*	*Adult male*	*Juvenile*	*Overall*	*Statistical test*
Mass (g)	57 ± 4 (52–61) (5)	60 ± 3 (55–68) (15)	58 ± 2 (56–60) (5)	59 ± 4 (52–68) (25)	$F_{2,22} = 2.81$ $P = 0.08$
Head + bill (mm)	42.4 ± 0.9 (40.9–43.1) (5)$^{a, b}$	43.0 ± 0.5 (42–44.2) (12)^{a}	42.2 ± 0.7 (41.6–43.3) (5)^{b}	42.7 ± 0.7 (40.9–44.2) (22)	$F_{2,19} = 3.57$ $P = 0.05$
Culmen (mm)	17.9 ± 0.7^{a} (17–18.8) (5)	18.8 ± 0.6^{b} (17.9–19.9) (15)	18.3 ± 0.6$^{a, b}$ (17.9–19.2) (5)	18.5 ± 0.7 (17–19.9) (25)	$F_{2,22} = 3.83$ $P = 0.04$
Bill depth (mm)	8.7 ± 0.2 (8.4–9) (5)	8.8 ± 0.2 (8.4–9.1) (15)	8.6 ± 0.3 (8.2–9.1) (5)	8.8 ± 0.2 (8.2–9.1) (25)	$F_{2,22} = 1.80$ $P = 0.19$
Tarsus (mm)	31.5 ± 0.9 (31–33) (5)	31.9 ± 0.6 (30.6–32.8) (15)	31.7 ± 1.5 (30–33.6) (5)	31.8 ± 0.9 (30–33.6) (25)	$F_{2,22} = 0.52$ $P = 0.60$
Wing chord (mm)	101 ± 2^{a} (97–103) (5)	106 ± 2^{b} (102–111) (15)	103 ± 2$^{a, b}$ (101–105) (5)	104 ± 3 (97–111) (25)	$F_{2,22} = 8.75$ $P = 0.002$

Values are presented as the mean ± SD (range) (n). Groups with different superscript letters are significantly different ($P \leq 0.05$).

the iStat machine compared with the VetScan ($t_{19} = 3.27$, $P = 0.004$), but no such pattern was present for blood potassium concentrations ($t_{19} = 1.28$, $P = 0.22$).

Blood smears from 23 birds (five adult females, 14 adult males and four juveniles) showed erythrocyte morphology to be normal in all samples except for one juvenile, which had an anisocytosis score of 1+. Leucocyte morphology was normal in all samples and no blood parasites were found (Fig. 1). Juveniles had significantly ($P = 0.007$) more heterophils than adults of either sex; eosinophils were rarely seen in adults, were absent in juveniles and no azurophils were found in any sample (Table 4).

Faecal Bacteriology and Parasite Evaluation

Samples for faecal bacterial culture were available from 23 birds (five adult females, 14 adult males and four juveniles), but two of these (two adult males) were from cloacal swabs rather than voided faecal material and neither yielded any bacterial growth. For the 21 faecal samples, various bacteria were present (n = number of birds positive after 24 and 48 h of culture, respectively); *Enterococcus* spp. ($n = 7$ and 12), *Klebsiella* spp. ($n = 7$ and 7), *Staphylococcus aureus* ($n = 4$ and 6), *Staphylococcus intermedius* ($n = 1$ and 1), *Escherichia coli* ($n = 1$ and 1) and *Pseudomonas* spp. ($n = 0$ and 1). Eight birds had a single genus/species of bacteria present, six birds had two different bacterial genera/species, one bird had three and one bird had four different genera/species. Five birds (one adult female, three adult males and one juvenile) had no bacterial growth after 48 h incubation and no fungal organisms were visible on any of the 23 culture plates.

No parasites eggs were found in the faecal samples ($n = 21$). In light of this finding, samples of fresh subantarctic fur seal (*Arctocephalus tropicalis*) faeces were analyzed identically as a positive control, and numerous fur seal parasite eggs were detected, thereby validating the results in the bird faeces.

Discussion

This is the first study to examine the clinical pathology of Gough buntings and to assess the presence of potential pathogens in this species. The results provide reference ranges, which will be essential for monitoring the health of birds that will be held in captivity, at unavoidably greater densities compared with natural conditions, during the planned eradication of mice from Gough Island.

Gough buntings appear to be free from both gastrointestinal helminths and haemoparasites unless there is a marked seasonal variation in parasite load or

Table 2
Blood and biochemistry parameters of Gough buntings measured using the Abaxis i-STAT

Parameter	*Adult female*	*Adult male*	*Juvenile*	*Overall*	*Statistical test*
pH	7.599 ± 0.074 (7.513–7.693) (4)	7.592 ± 0.069 (7.476–7.711) (13)	7.628 ± 0.108 (7.495–7.735) (4)	7.600 ± 0.075 (7.476–7.735) (21)	$F_{2,18} = 0.34$ $P = 0.72$
pCO_2 (mm Hg)	26.7 ± 3.6 (21.5–29.6) (4)	26.1 ± 4.2 (17.9–31.1) (13)	27.2 ± 5.5 (19.2–31.9) (4)	26.4 ± 4.2 (17.9–31.9) (21)	$F_{2,18} = 0.10$ $P = 0.91$
pO_2 (mm Hg)	54 ± 19 (42–82) (4)	45 ± 7 (38–60) (12)	53 ± 12 (42–67) (4)	48 ± 11 (38–82) (20)	$F_{2,17} = 1.69$ $P = 0.21$
Base excess (mmol/l)	5 ± 2 (1–6) (4)	3 ± 2 (−2 to 6) (13)	3 ± 4 (−1 to 6) (4)	3 ± 2 (−2 to 6) (21)	$F_{2,18} = 0.50$ $P = 0.62$
HCO_3 (mmol/l)	26.0 ± 1.6 (23.8–27.5) (4)	24.9 ± 1.8 (21.3–27.2) (13)	25.0 ± 2.3 (22.1–27.7) (4)	25.1 ± 1.8 (21.3–27.7) (21)	$F_{2,18} = 0.53$ $P = 0.60$
TCO_2 (mmol/l)	27 ± 1 (25–28) (4)	26 ± 2 (22–28) (13)	26 ± 2 (23–29) (4)	26 ± 2 (22–29) (21)	$F_{2,18} = 0.53$ $P = 0.60$
sO_2 (%)	91 ± 5 (87–98) (4)	87 ± 5 (83–95) (12)	91 ± 6 (82–95) (4)	89 ± 5 (82–98) (20)	$F_{2,17} = 1.12$ $P = 0.35$
Sodium (mmol/l)	149 ± 3 (146–152) (4)	149 ± 3 (145–156) (13)	149 ± 4 (144–154) (4)	149 ± 3 (144–156) (21)	$F_{2,18} = 0.11$ $P = 0.89$
Potassium (mmol/l)	4.0 ± 0.3 (3.6–4.2) (4)	3.7 ± 0.6 (2.5–4.9) (13)	4.3 ± 0.1 (4.2–4.5) (4)	3.8 ± 0.6 (2.5–4.9) (21)	$F_{2,18} = 2.74$ $P = 0.11$
Calcium (ionized) (mmol/l)	1.00 ± 0.07 (0.91–1.09) (4)	0.95 ± 0.09 (0.82–1.11) (12)	1.09 ± 0.12 (0.92–1.19) (4)	0.99 ± 0.1 (0.82–1.19) (20)	$F_{2,17} = 2.99$ $P = 0.08$
Glucose (mmol/l)	21.5 ± 1.5 (20.2–23.7) (4)[a]	18.2 ± 1.9 (14.1–20.6) (13)[b]	18.1 ± 2.1 (15.4–20.4) (4)[b]	18.8 ± 2.2 (14.1–23.7) (21)	$F_{2,18} = 5.08$ $P = 0.02$
Haematocrit (%)	41 ± 3 (39–45) (4)	43 ± 4 (35–50) (13)	40 ± 3 (37–44) (4)	42 ± 4 (35–50) (21)	$F_{2,18} = 0.70$ $P = 0.51$
Haemoglobin (mmol/l)	8.6 ± 0.6 (8.2–9.5) (4)	9.0 ± 0.9 (7.4–10.6) (13)	8.5 ± 0.6 (7.8–9.3) (4)	8.8 ± 0.8 (7.4–10.6) (21)	$F_{2,18} = 0.76$ $P = 0.48$

Values are presented as the mean ± SD (range) (*n*). Groups with different superscript letters are significantly different ($P \leq 0.05$).

Table 3
Blood and biochemistry parameters of Gough buntings as measured using the Abaxis VetScan

Parameter	*Adult female*	*Adult male*	*Juvenile*	*Overall*	*Statistical test*
Aspartate aminotransferase (IU/l)	879 ± 570 (290–1,581) (4)	787 ± 513 (255–2,007) (14)	1,616 ± 1,220 (753–2,478) (2)	894 ± 615 (255–2,478) (20)	$F_{2,16} = 1.69$ $P = 0.22$
Creatine kinase (IU/l)	1,587 ± 607 (971–2,396) (5)	1,859 ± 1,022 (772–4,396) (14)	2,558 ± 2,145 (851–4,966) (3)	1,892 ± 1,114 (772–4,966) (22)	$F_{2,19} = 0.71$ $P = 0.51$
Uric acid (µmol/l)	438 ± 78 (372–550) (4)	541 ± 264 (203–989) (9)	972 ± 695 (480–1,463) (2)	571 ± 323 (203–1,463) (15)	$F_{2,12} = 2.26$ $P = 0.15$
Glucose (mmol/l)	20.2 ± 2 (17.7–23.2) (5)[a]	17.6 ± 1.9 (13.7–20) (14)[b]	17.5 ± 2.6 (14.6–19.7) (3)[a, b]	18.2 ± 2.2 (13.7–23.2) (22)	$F_{2,19} = 3.42$ $P = 0.05$
Calcium (mmol/l)	1.99 ± 0.16 (1.77–2.18) (5)	1.91 ± 0.14 (1.74–2.27) (14)	2.04 ± 0.02 (2.02–2.06) (3)	1.95 ± 0.14 (1.74–2.27) (22)	$F_{2,19} = 1.42$ $P = 0.27$
Phosphorus (mmol/l)	0.51 ± 0.35 (0.19–1.1) (5)	0.49 ± 0.23 (0.16–0.89) (14)	0.70 ± 0.09 (0.59–0.77) (3)	0.52 ± 0.25 (0.16–1.1) (22)	$F_{2,19} = 0.85$ $P = 0.45$
Albumin (g/l)	35 ± 2 (33–36) (5)	35 ± 3 (30–40) (12)	35 ± 2 (33–37) (3)	35 ± 2 (30–40) (20)	$F_{2,17} = 0.04$ $P = 0.96$
Globulin (g/l)	10 ± 8 (3–20) (5)[a]	3 ± 4 (0–11) (14)[b]	5 ± 2 (4–7) (3)[a, b]	5 ± 5 (0–20) (22)	$F_{2,19} = 4.89$ $P = 0.02$
Total protein (g/l)	45 ± 9 (37–55) (5)	38 ± 5 (32–47) (12)	40 ± 3 (37–42) (3)	40 ± 6 (32–55) (20)	$F_{2,17} = 2.86$ $P = 0.08$
Potassium (mmol/l)	4.0 ± 0.8 (2.9–4.7) (4)	3.4 ± 1.1 (1.9–5.3) (13)	3.8 ± 1.2 (2.9–5.1) (3)	3.6 ± 1.0 (1.9–5.3) (20)	$F_{2,17} = 0.67$ $P = 0.53$
Sodium (mmol/l)	148 ± 2 (145–150) (5)	148 ± 2 (146–152) (14)	146 ± 1 (145–147) (3)	147 ± 2 (145–152) (22)	$F_{2,19} = 1.19$ $P = 0.33$

Values are presented as the mean ± SD (range) (*n*). Groups with different superscript letters are significantly different ($P \leq 0.05$).

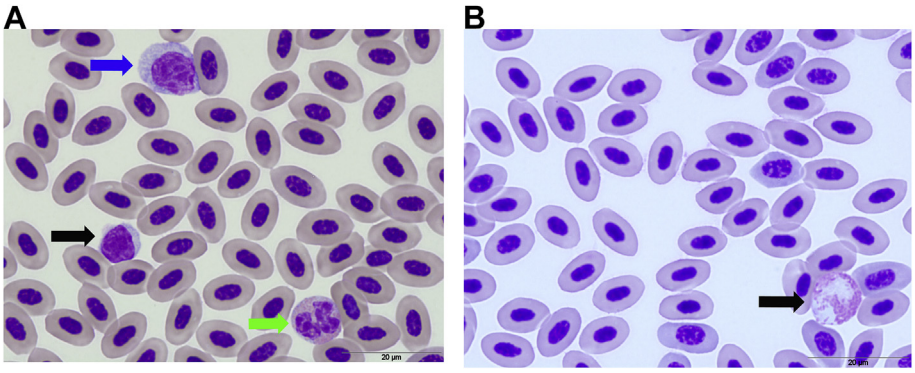

Fig. 1. Thin blood smears from Gough buntings showing typical morphology of nucleated erythrocytes and selected leucocytes. (A) Note the lymphocyte (black arrow), monocyte (blue arrow) and eosinophil (green arrow). (B) Note the heterophil (black arrow). May–Grunwald/Giemsa stain. Bar, 20 μm.

helminth fecundity. Such seasonal variation is unlikely, as is evolution of a bird species totally resistant to parasites. This suggests that any helminths or haemoparasites present when the Gough bunting evolved in its original habitat either required a vector to complete their life cycle, which is not present on Gough Island, or if they had a direct life cycle, the parasites were unable to maintain this in the different environmental conditions these birds now inhabit. The phenomenon of parasite-free wild birds has been reported in other displaced species that have survived and flourished in a foreign environment (Little and Earlé, 1994). This is supported further by the very low to absent levels of circulating eosinophils found in the Gough buntings, which could be explained by a lack of exposure to endoparasites (Deem *et al.*, 2011). This finding is significant because if parasites are present, even at a very low density in the free-

Table 4
White blood cell composition of Gough buntings

Parameter	*Adult female*	*Adult male*	*Juvenile*	*Overall*	*Statistical test*
Total white blood cells (10^9/l)	5.9 ± 3.9 (1.5–11.7) (5)	4.0 ± 1.3 (2.9–7.8) (14)	6.1 ± 2.3 (3.4–9.0) (4)	4.8 ± 2.3 (1.5–11.7) (23)	$F_{2,20} = 2.19$ $P = 0.14$
Heterophils (%)	15 ± 10 (9–33) (5)	19 ± 8 (7–31) (14)	38 ± 20 (9–55) (4)	22 ± 13 (7–55) (23)	–
Heterophils (10^9/l)	0.97 ± 0.85 (0.14–1.88) (5)[a]	0.76 ± 0.36 (0.21–1.44) (14)[a]	2.04 ± 1.06 (0.81–3.3) (4)[b]	1.03 ± 0.77 (0.14–3.3) (23)	$F_{2,20} = 6.36$ $P = 0.007$
Lymphocytes (%)	81 ± 11 (63–89) (5)	76 ± 9 (65–92) (14)	58 ± 20 (40–86) (4)	74 ± 14 (40–92) (23)	–
Lymphocytes (10^9/l)	4.62 ± 3.07 (1.34–9.13) (5)	3.04 ± 1.14 (2.04–6.71) (14)	3.79 ± 2.74 (1.6–7.74) (4)	3.52 ± 1.99 (1.34–9.13) (23)	$F_{2,20} = 1.24$ $P = 0.31$
Eosinophils (%)	0 ± 1 (0–1) (5)	0 ± 0 (0–1) (14)	0 ± 0 (0–0) (4)	0 ± 0 (0–1) (23)	–
Eosinophils (10^9/l)	0.00 ± 0.00 (0–0.12) (5)	0.00 ± 0.00 (0–0.05) (14)	0.00 ± 0.00 (0.00–0.00) (4)	0.00 ± 0.00 (0–0.12) (23)	$F_{2,20} = 3.15$ $P = 0.06$
Monocytes (%)	4 ± 1 (2–5) (5)	4 ± 3 (0–10) (14)	4 ± 1 (3–5) (4)	4 ± 2 (0–10) (23)	–
Monocytes (10^9/l)	0.24 ± 0.22 (0.03–0.59) (5)	0.16 ± 0.12 (0–0.45) (14)	0.22 ± 0.09 (0.17–0.36) (4)	0.19 ± 0.14 (0–0.59) (23)	$F_{2,20} = 0.82$ $P = 0.46$
Azurophils (%)	0 ± 0 (0–0) (5)	0 ± 0 (0–0) (14)	0 ± 0 (0–0) (4)	0 ± 0 (0–0) (23)	–
Azurophils (10^9/l)	0.00 ± 0.00 (0.00–0.00) (5)	0.00 ± 0.00 (0.00–0.00) (14)	0.00 ± 0.00 (0.00–0.00) (4)	0.00 ± 0.00 (0.00–0.00) (23)	–
Basophils (%)	0 ± 0 (0–1) (5)	1 ± 1 (0–5) (14)	1 ± 1 (0–2) (4)	1 ± 1 (0–5) (23)	–
Basophils (10^9/l)	0.01 ± 0.03 (0.00–0.07) (5)	0.02 ± 0.06 (0.00–0.21) (14)	0.05 ± 0.06 (0–0.12) (4)	0.03 ± 0.05 (0–0.21) (23)	$F_{2,20} = 0.55$ $P = 0.59$

Values are presented as the mean ± SD (range) (n). Groups with different superscript letters are significantly different ($P \leq 0.05$).

ranging birds, captivity will inevitably increase endoparasite exposure with potentially detrimental consequences (Sainsbury and Vaughan-Higgins, 2012).

The significantly higher blood glucose concentrations present in the adult female buntings is similar to that reported in other avian species, and can be attributed to glucocorticoids controlling the timing of reproduction in sexually mature females, and consequently elevating blood glucose (Schoech *et al.*, 2009). Similarly, the significantly higher blood globulin concentrations in adult female buntings may be associated with reproduction, due to the formation of increased maternal antibodies for the creation of the egg yolk (Okuliarova *et al.*, 2014). Gough buntings breed from October to November (Voisin, 1979; Ryan, 2007), shortly after the birds were sampled in this study, and so the females would be coming into breeding condition.

Circulating creatine kinase levels were elevated in comparison with captive birds (Hochleithner, 1994); however, this is a skeletal muscle-associated enzyme, which is released on physical restraint and/or extreme exertion, either of which may have occurred during the capture process and would account for the mild elevation (Hochleithner, 1994). Calcium and particularly phosphorus concentrations are generally higher (although not clinically significantly) in juvenile animals compared with adults, and this is likely to be associated with skeletal development and remodelling typical of growth (Rayhel *et al.*, 2015). A similar pattern with respect to creatine kinase levels was present in the juvenile Gough buntings. However, the numbers of this age group captured were small and this may account for the lack of statistical significance compared with the adults. The higher blood sodium concentrations given by the iStat compared with the VetScan analyzer, although significant, were very small. The different times between blood sampling to analysis would explain this discrepancy, as sodium would diffuse from the plasma and into blood cells without opposition if the cellular transmembrane sodium pump mechanism was compromised in any way (Alberts *et al.*, 1994), as would be the case when blood is removed from the normal homeostasis mechanism of the body.

Heterophils are primarily associated with the innate immune response and a greater heterophil:lymphocyte ratio is encountered frequently with the relatively more naïve immune system associated typically with juvenile animals (Kamau *et al.*, 2002). This would explain the significantly higher number of circulating heterophils and lower, but not significant, number of lymphocytes present in the juvenile Gough buntings, as they would have had been exposed to less antigenic stimulation compared with the adults, which can live for up to at least 18 years (Ryan and Cuthbert, 2008; PGR, unpublished data).

The gastrointestinal tract of birds will always have a population of bacteria and often other microorganisms as well. Many gut bacteria are essential commensals that play a role in protecting the host from pathogenic bacterial species, yet under certain conditions these bacteria may become pathogenic (McKenney and Kendall, 2016). Any resultant disease will require specific pharmaceutical treatment and so it is important to know which bacteria are present prior to captivity. *Enterococcus* spp. was the most common genus of bacteria isolated in the sampled Gough buntings (57%). This genus has been isolated from a number of species of captive and wild birds and *Enterococcus hirae* has been reported as a cause of septicaemia (Devriese *et al.*, 1991, 1992, 1995). While the species of *Enterococcus* was not identified in this study, the possibility that enteropathogenic forms are present in the Gough bunting cannot be ruled out. Conversely, *Klebsiella* spp., which were present in one third of the birds sampled, are reported as common avian pathogens both of the respiratory and gastrointestinal tract (Bangert *et al.*, 1988; Fudge, 2001; Hernandez *et al.*, 2003). This organism has the potential to cause significant morbidity and possible mortality which, in addition to its frequent multidrug resistant nature (Giacopello *et al.*, 2016), makes it a potential threat to any survival plan for the Gough bunting. Reports of *Staphylococcus* spp. are uncommon in domestic birds, but when infection occurs, disease tends to be associated with joint, tendon and bone infections with occasional septicaemia (Gross, 1978). However, *S. aureus* was present in the faeces of six (29%) Gough buntings. This bacterium has been isolated previously in cases of septicaemia and associated with mortalities in birds that were immunocompromised or suffered immune impairment due to concurrent disease or iatrogenic medication (i.e. glucocorticoids) (Wobeser and Kost, 1992). This may be highly significant when the Gough buntings are brought into captivity for the duration of action of the rodenticide, as captivity will invariably be stressful and so potentially immunosuppressive. *Escherichia coli* has been isolated from a range of avian species, including apparently healthy passerines and waterfowl (Damare *et al.*, 1979; Brittingham *et al.*, 1988; Foster *et al.*, 1998). Avian strains of *E. coli* can be pathogenic in birds and have been reported as such in domestic poultry and wild finches where cytolethal distending toxin has been isolated (Foster *et al.*, 1998). In this study, the strain of *E. coli* was not identified, but only one bird was positive. *Pseudomonas aeruginosa* is another common avian pathogen,

chiefly of the upper and lower respiratory tract, but cases of septicaemia and enteritis have been reported in psittacine birds (Brittingham *et al.*, 1988; Walker *et al.*, 2002; Rich, 2003). Only one Gough bunting had faeces positive for this bacteria and, as it is a significant pathogen, which, like *Klebsiella*, often demonstrates multidrug resistance, it may be significant during any period of captivity where stocking densities will be increased and cross contamination between enclosures is a possibility.

It is noteworthy that no evidence of several significant and well-documented avian enteropathogens such as *Salmonella* spp., *Campylobacter* spp. or *Yersinia* spp. were isolated from these birds (Pennycott *et al.*, 1988; Benskin *et al.*, 2009), despite *Salmonella* spp. and *Campylobacter* spp. having both been recovered from Tristan skuas (*Catharacter antarctica hamiltoni*) on Gough Island (M. Cuella-Cerda, personal communication). This was probably due to the Multi-Chrome™ Veterinary Microbiology Culture System, as *Salmonella* spp. would appear as very small, translucent colonies, which would be difficult to see and easily be masked by other coloured bacterial species. Furthermore, *Campylobacter* spp. would not grow in this system as they require selective media and microaerophilic conditions, which were beyond the scope of this study.

In conclusion, the Gough buntings sampled in this study were in good body condition with no obvious signs of clinical or subclinical disease and there were no concurrent disease concerns with respect to bringing them into short-term captivity. However, the greater frequency with which males were captured compared with females, possibly due to males being more territorial in the approach to the breeding season, may make establishing a reproductively-balanced captive population difficult. The lack of gastrointestinal helminths and haemoparasites suggests this is a relatively 'clean' population with respect to parasites. However, it may also make them highly susceptible to disease, as they could have lost any natural or age-related immunity (Love and Duncan, 1992; De Coster *et al.*, 2010) to such parasites, especially if subjected to a relatively high and/or sustained pathogen challenge such as can occur during captivity. A high captive population density will be exacerbated by the logistical constraints on Gough Island, which dictate that all aviaries will need to be in a single, easily accessible location (Rexer-Huber and Parker, 2011). Additionally, as commensal bacteria can rapidly become pathogenic under circumstances of overcrowding or chronic stress (Wobeser and Kost, 1992; Pennycott *et al.*, 1998), it is recommended that a wide range of therapeutic preparations, including anthelmintics, antiprotozoals and antibiotics, known to be safe for use in similar bird species, are present in sufficient quantities immediately before, throughout and for some time after captivity during the mouse eradication operation planned for Gough Island.

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Conflict of Interest Statement

The authors declare no conflict of interest with respect to publication of this manuscript.

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