NORMAL PHYSICAL PARAMETERS CHARACTERIZING GALAPAGOS MARINE BIRDS

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ABSTRACT

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The Galapagos Penguin *Spheniscus mendiculus* (GAPE), Flightless Cormorant *Phalacrocorax harrisi* (FLCO), and Waved Albatross *Phoebastria irrorata* (WAAL) are marine birds that are endemic to the Galapagos Islands. To provide health information for these three species, baseline data on several physiological parameters were collected non-invasively—from individuals and populations appearing to be healthy—during the course of physical examinations that were conducted from 2011–2019. Heart rate, respiratory rate, body temperature, and body condition (weight vs. morphometric measurements) were analyzed as a function of age and sex. Mean heart rate differed significantly between juveniles and adults in all three species. Respiratory rate and body temperature varied with species, age, and sex; body condition index of GAPE, FLCO, and WAAL showed that males are heavier than females. We briefly speculate on the ecological context of these patterns.

Key words: Flightless Cormorant, Galapagos Penguin, Waved Albatross, heart rate, respiratory rate, temperature, body condition index

INTRODUCTION

The Galapagos Penguin *Spheniscus mendiculus* (GAPE), Flightless Cormorant *Phalacrocorax harrisi* (FLCO), and Waved Albatross *Phoebastria irrorata* (WAAL) are endemic to the Galapagos Islands. These species are threatened by climate change, introduced species and pathogens, human interactions, and contaminants, and they have been categorized as Endangered, Vulnerable, and Critically Endangered, respectively (Freile *et al.* 2019). Information about the baseline physiological parameters of these species, which can assist in assessing their health status, has been acquired during *in situ* ecological monitoring and has been performed since 2010. This long-term monitoring has increased our understanding of natural biology, ecology, pathogens and parasites, and contaminants (Carrera-Játiva *et al*. 2014, Jiménez-Uzcátegui & Vargas 2019, Jiménez-Uzcátegui *et al.* 2015, 2019, Jiménez *et al.* 2020). Physical exams that are conducted on a yearly basis provide an additional layer of health monitoring. In this study, we present baseline physiological parameters of these species measured over a nine-year period. Our objective was to determine baseline physiological values in healthy GAPE, FLCO, and WAAL, which can be used as reference values to assess future changes in the physiological parameters of these species.

METHODS

Parameters measured

Physiological parameters such as heart and respiratory rates, peripheral temperature, blood pressure, and oxygen saturation are essential indices that non-invasively assess the hemodynamic status of an animal (Harrison & Ritchie 1994, Furlan & Macari 2002). Heart rate is the number of times the heart contracts (beats) in a unit of time, and it varies with an individual's size, age, physical condition, sex, position, diet, as well as during pregnancy, exercise, and with environmental conditions such as temperature (Kelly 1972). Pathological conditions may also affect heart rate (Kelly 1972, Fitzgerald & Beaufrère 2016).

Respiratory rate is the number of breathing cycles (inspiration, expiration) in a unit of time (Kelly 1972). Respiratory rate can vary according to the same factors as heart rate (Kelly 1972, Tully & Harrison 1994).

In birds, body temperature is independent of the environment and is measured using a thermometer in the cloacal or tympanic area (Kelly 1972, Furlan & Macari 2002, O'Malley 2007). Body temperature may vary physiologically due to metabolism and physical activity, and it can also vary with sex, breeding time, size, climate, time-of-day, and stress (Kelly 1972, Harrison & Ritchie 1994). In addition to behavioral strategies, birds harness physiological resources—such as the thermal insulation provided by plumage or adipose tissue, and respiratory evaporation when overheating arises—to regulate their body temperature (Kelly 1972, Furlan & Macari 2002, O'Malley 2007).

Study area

The Galapagos Archipelago is ~960 km west of continental Ecuador (Snell *et al.* 1996). GAPE were captured at Caleta Iguana (00°58.6′S, 091°26.7′W) and Puerto Pajas (00°45.3′S, 091°22.5′W) on Isabela Island, and on the Marielas Islets (00°35.8′S, 091°05.4′W). FLCO were captured at Punta Albemarle (00°09.7′N, 091°21.6′W) on Isabela Island, and at Playa Escondida (00°15.7′S, 091°28.1′W) and Carlos Valle (00°15.6′S, 091°27.5′W) on Fernandina Island. WAAL were captured at Punta Suárez (Plot A: 01°22.3′S, 089°44.4′W and Plot B: 01°22.5′S, 089°44.1′W) on Española Island.

Field sampling

Field work was conducted during 2011–2019, including 24 trips to capture GAPE, 21 to capture FLCO, and eight to capture WAAL. GAPE and FLCO have island and habitat overlap, and thus, the two species were sampled together. In contrast, WAAL were located on a different island and were sampled separately. All sampling was conducted during the breeding season. To capture GAPE, we approached shoreline rocks in a small boat and caught the penguins in a long-handled net. Penguins were then placed individual hard plastic containers with breathing holes before transport to the main research boat. FLCO were easily captured on land with a long-handled net, either on their nest or while basking (Travis *et al.* 2006b). WAAL were captured manually on land by at least two people because they tend to run away when scared. The same types of measurements were collected from all birds, and birds were handled for < 10 min (Table 1). Culmen beak (GAPE, FLCO, WAAL) and tarsus (FLCO, WAAL) lengths were measured in mm using calipers. The wings of GAPE were bent to "expose" the bone, and wing length was measured by flattening one wing against a ruler from the proximal humerus (fixed point A) to the distal phalanx (wing tip; fixed point B) (Proctor & Lynch 1993). Individuals were categorized as juvenile or adult according to plumage and size (GAPE, FLCO, WAAL) and/or eye color (FLCO); sex was determined according to measurement differences (Valle 1994, Awkerman *et al*. 2007, Capello & Boersma 2018; Table 1).

TABLE 1
Field trips and data collected for the Galapagos Penguin *Spheniscus mendiculus* (GAPE), Flightless Cormorant *Phalacrocorax harrisi* (FLCO), and Waved Albatross *Phoebastria irrorata* (WAAL) in the Galapagos Archipelago, Ecuador, 2011–2019

Activity		Species		
		GAPE	FLCO	WAAL
Trips		24	21	8
No. of birds tested		1 699	746	1 011
Physical examination		x	x	x
Morphometric	Beak length	x	x	x
	Wing length	x		
	Tarsus length		x	x
Body weight		x	x	x
Physical examination		x	x	x
Identification	PIT-tag	x	x	x
	Ring band			x
Place	Isabela Island	x	x	
	Marielas Islets	x		
	Fernandina Island		x	
	Española Island			x

After gentle handling while measurements were taken, a dark mask was placed over each bird's eyes and a physical examination was conducted. To decrease sampling variance, the same person (GJU) collected all the data points for the three species (Table 1). Rates were determined relative to one minute. For heart rate, auscultation was performed on the right ventral chest with a pediatric stethoscope, and beats were counted for 15 s and multiplied by four (GAPE, FLCO, and WAAL); for respiratory rate, the inspiratory movement of the coelomic cavity was counted for 15 s and multiplied by four. To determine internal temperature, a digital thermometer (°C) was placed in the cloaca for one minute. For WAAL, an additional temperature measurement was taken in the tympanic area with a digital thermometer because the external ear opening in WAAL is bigger than in GAPE and FLCO. Physical examination and palpation of each individual was conducted (see Harrison & Ritchie 1994). Body weight was obtained using a 5-kg hand-held spring scale (Pesola, Switzerland). Each bird was permanently identified (if it had not been identified previously) by placing a Passive Integrated Transponder (PIT) tag (microchip) under the skin on the dorsal tarsometatarsus (GAPE, FLCO) or in the subcutaneous cervical area (WAAL). For WAAL, a size eight numerical metal ring (band) was placed on the left leg, and a plastic ring was place on the right leg (Table 1).

Statistical analysis

Data were tested for normality using a Shapiro-Wilk test. Sample sizes are provided for each health parameter because not all data points were collected for all birds. The data for each species were compared according to age (adults, juveniles) and sex (female, male) with Student's *t*-test (Table 2). A body condition index was calculated, according to sex, with a regression between body mass against a linear measure of size (wing length [GAPE] and tarsus length [FLCO, WAAL]) (Green 2001). The values of internal temperature were regressed between internal cloacal versus tympanic temperatures (WAAL). Statistical significance was defined as $P < 0.05$ using Minitab Inc. 15.1.20.0 and SigmaPlot 11.0.0.77.

RESULTS

During the study period, 1 699 GAPE and 746 FLCO were sampled from Isabela Island, Fernandina Island, and the Marielas Islets, and 1 011 WAAL were sampled from Española Island (Table 1).

TABLE 2
Physical and morphometric data for the Galapagos Penguin *Spheniscus mendiculus* (GAPE), Flightless Cormorant *Phalacrocorax harrisi* (FLCO), and Waved Albatross *Phoebastria irrorata* (WAAL) in the Galapagos Archipelago, Ecuador, 2011–2019

Species	Physical examinations	Category[a]	Sample size (n)	Mean (± standard deviation)	Standard error	Range	Student's t-test
GAPE	Heart rate (beats/min)	All birds	933	115 ± 18	0.59	84–180	
		Adults	750	115 ± 19	0.68	84–180	$t = 1.27$; $P = 0.206$; $df = 428$
		Juveniles	220	114 ± 15	1.00	84–180	
		Female	290	115 ± 18	1.00	88–180	$t = -0.92$; $P = 0.357$; $df = 665$
		Male	420	116 ± 20	0.96	84–180	
	Respiratory rate (breaths/min)	All birds	945	20 ± 4	0.13	12–40	
		Adults	761	20 ± 4	0.14	12–40	$t = -4.26$; $P = 0.000$; $df = 362$
		Juveniles	224	21 ± 4	0.27	16–36	
		Female	311	20 ± 4	0.23	12–33	$t = -0.81$; $P = 0.420$; $df = 652$
		Male	472	20 ± 4	0.18	12–40	
	Cloacal temperature (°C)	All birds	982	39.3 ± 0.7	0.02	35.3–41.0	
		Adults	781	39.2 ± 0.7	0.02	35.3–41.0	$t = -1.58$; $P = 0.115$; $df = 420$
		Juveniles	247	39.3 ± 0.6	0.04	37.4–40.5	
		Female	314	39.3 ± 0.6	0.04	36.6–41.0	$t = 0.48$; $P = 0.634$; $df = 688$
		Male	479	39.3 ± 0.6	0.03	35.3–40.7	
	Body weight (kg)	All birds	996	1.9 ± 0.4	0.01	0.9–3.8	
		Adults	799	2.0 ± 0.3	0.01	1.1–3.8	$t = 2.27$; $P = 0.024$; $df = 333$
		Juveniles	247	1.9 ± 0.5	0.03	0.9–3.6	
		Female	311	1.8 ± 0.3	0.02	1.2–2.6	$t = -12.32$; $P = 0.000$; $df = 741$
		Male	439	2.1 ± 0.3	0.02	1.1–3.8	
	Wing length (mm)	Female	287	11.9 ± 0.4	0.02	10.8–13.1	$t = -13.24$; $P = 0.000$; $df = 679$
		Male	395	12.4 ± 0.5	0.03	9.0–4.9	
FLCO	Heart rate (beats/min)	All birds	304	110 ± 19	1.07	60–180	
		Adults	279	108 ± 17	1.00	60–160	$t = -5.01$; $P = 0.000$; $df = 37$
		Juveniles	35	130 ± 26	4.40	84–180	
		Female	157	107 ± 17	1.40	60–152	$t = -0.54$; $P = 0.593$; $df = 238$
		Male	112	108 ± 17	1.60	72–160	
	Respiratory rate (breaths/min)	All birds	301	21 ± 5	0.30	12–36	
		Adults	277	21 ± 5	0.31	12–36	$t = -0.13$; $P = 0.895$; $df = 38$
		Juveniles	34	21 ± 7	1.10	8–36	
		Female	156	22 ± 5	0.41	12–36	$t = 1.72$; $P = 0.087$; $df = 243$
		Male	113	21 ± 5	0.48	12–36	
	Cloacal temperature (°C)	All birds	305	39.8 ± 0.7	0.04	37.1–41.8	
		Adults	278	39.7 ± 0.6	0.04	37.1–41.6	$t = -2.88$; $P = 0.006$; $df = 41$
		Juveniles	37	40.2 ± 0.8	0.14	38.0–41.8	
		Female	158	39.6 ± 0.6	0.05	38.1–41.4	$t = -2.95$; $P = 0.004$; $df = 207$
		Male	112	39.9 ± 0.7	0.07	37.1–41.6	
	Body weight (kg)	All birds	309	3.2 ± 0.6	0.03	2.1–4.6	
		Adults	284	3.2 ± 0.6	0.03	2.1–4.6	$t = -0.23$; $P = 0.815$; $df = 42$
		Juveniles	35	3.2 ± 0.6	0.10	2.2–4.6	
		Female	171	2.8 ± 0.3	0.02	2.1–4.1	$t = -24.26$; $P = 0.000$; $df = 266$
		Male	130	3.7 ± 0.3	0.03	2.5–4.6	
	Tarsus length (mm)	Female	145	72.1 ± 3.2	0.27	56.4– 84.0	$t = -16.66$; $P = 0.000$; $df = 208$
		Male	104	79.4 ± 3.6	0.35	66.0–93.0	

Table 2 continued on next page

Table 2 continued from previous page

Species	Physical examinations	Category[a]	Sample size (n)	Mean (± standard deviation)	Standard error	Range	Student's t-test
WAAL	Heart rate (beats/min)	All birds	638	118 ± 25	1.00	64–192	
		Adults	569	115 ± 24	0.99	64–192	$t = -9.18$; $P = 0.000$; $df = 92$
		Juveniles	74	142 ± 24	2.80	100–192	
		Female	238	116 ± 26	1.70	64–192	$t = 0.50$; $P = 0.620$; $df = 398$
		Male	182	115 ± 25	1.80	72–184	
	Respiratory rate (breaths/min)	All birds	642	21 ± 4	0.16	12–36	
		Adults	570	20 ± 4	0.16	12–36	$t = -8.12$; $P = 0.000$; $df = 91$
		Juveniles	77	24 ± 4	0.51	18–36	
		Female	237	20 ± 4	0.28	16–36	$t = 0.71$; $P = 0.479$; $df = 405$
		Male	183	20 ± 4	0.29	12–36	
	Cloacal temperature (°C)	All birds	584	39.6 ± 1.0	0.04	36.9–43.0	
		Adults	532	39.6 ± 1.0	0.04	36.9–43.0	$t = 3.27$; $P = 0.002$; $df = 64$
		Juveniles	54	39.1 ± 1.0	0.13	36.9–40.9	
		Female	223	39.8 ± 0.9	0.06	37.1–42.4	$t = 0.15$; $P = 0.879$; $df = 337$
		Male	163	39.7 ± 0.9	0.07	37.5–43.0	
	Tympanic temperature (°C)	All birds	173	39.8 ± 1.1	0.08	36.3–42.5	
	Body weight (kg)	All birds	630	3.7 ± 0.5	0.02	2.2–5.9	
		Adults	608	3.7 ± 0.5	0.02	2.2–5.3	$t = -5.31$; $P = 0.000$; $df = 21$
		Juveniles	22	4.5 ± 0.7	0.15	3.0–5.9	
		Female	253	3.4 ± 0.4	0.03	2.2–4.9	$t = -12.25$; $P = 0.000$; $df = 392$
		Male	186	3.9 ± 0.4	0.03	3.1–5.3	
	Tarsus length (mm)	Female	161	98.4 ± 3.9	0.30	82.3–109.0	$t = -15.93$; $P = 0.000$; $df = 260$
		Male	108	105 ± 3.0	0.29	96.5–111.8	

[a] In the category "All birds", data from all age classes and sexes are combined.

Physiological data

Heart rate

For GAPE, when age classes and sexes were combined, the average heart rate was 115 ± 18 beats/min (Table 2); average heart rate was not significantly different in adults compared to juveniles (t-test; $P = 0.206$, $df = 428$), and average heart rate was not significantly different in females compared to males (t-test; $P = 0.357$, $df = 665$). For FLCO, when age classes and sexes were combined, the average heart rate was 110 ± 19 beats/min; average heart rate was significantly different in adults compared to juveniles (t-test; $P = 0.000$, $df = 37$), whereas average heart rate did not differ between females and males (t-test; $P = 0.593$, $df = 238$). For WAAL, when age classes and sexes were combined, the average heart rate was 118 ± 25 beats/min; average heart rate was significantly different in adults compared to juveniles (t-test; $P = 0.000$, $df = 92$), whereas average heart rate did not differ between females and males (t-test; $P = 0.620$, $df = 398$; Table 2).

Respiratory rate

For GAPE, when age classes and sexes were combined, the average respiratory rate was 20 ± 4 breaths/min; average respiratory rate was significantly different in adults compared to juveniles (t-test; $P = 0.000$, $df = 362$), whereas average respiratory rate did not differ significantly between females and males (t-test; $P = 0.420$, $df = 652$). For FLCO, when age classes and sexes were combined, the average respiratory rate was 21 ± 5 breaths/min; average respiratory rate did not differ significantly between adults and juveniles (t-test; $P = 0.895$, $df = 38$), nor did it differ significantly between females and males (t-test; $P = 0.087$, $df = 243$). For WAAL, when age classes and sexes were combined, the average respiratory rate was 21 ± 4 breaths/min; average respiratory rate was significantly different in adults compared to juveniles (t-test; $P = 0.000$, $df = 91$), whereas average respiratory rate did not differ significantly between females and males (t-test; $P = 0.479$, $df = 405$; Table 2).

Cloacal temperature

For GAPE, when age classes and sexes were combined, the average cloacal temperature was 39.3 ± 0.7 °C; average temperature did not differ significantly between adults and juveniles (t-test; $P = 0.115$, $df = 420$), nor did it differ significantly between females and males (t-test; $P = 0.634$, $df = 688$). For FLCO, when age classes and sexes were combined, the average cloacal temperature was 39.8 ± 0.7 °C; average temperature was significantly different in adults compared to juveniles (t-test; $P = 0.006$, $df = 41$), and it also differed in females compared to males (t-test; $P = 0.004$, $df = 207$). For WAAL, when age classes and sexes were combined, the average cloacal temperature was 39.6 ± 1.0 °C; average temperature was significantly different in adults compared to juveniles (t-test; $P = 0.002$, $df = 64$), whereas average temperate did not differ between females and males (t-test; $P = 0.879$, $df = 337$; Table 2).

Tympanic temperature

The average tympanic temperature of WAAL when age classes and sexes were combined was 39.8 ± 1.1 °C. Cloacal versus tympanic temperature was compared in WAAL, and regression analysis indicated a positive association, but it was not significant ($R^2 = 0.272$; $y = 0.356 + 25.75$; $df = 171$).

Morphometric data

Average body weight was 1.9 ± 0.4 kg in GAPE when all age classes and sexes were combined. Adults were heavier than juveniles (t-test; $P = 0.024$, $df = 333$). Comparison between sexes showed that males were significantly heavier (t-test; $P = 0.000$, $df = 741$) and had significantly longer wing length (t-test; $P = 0.000$, $df = 679$; Table 2) than females. Body condition index for GAPE based on sex, after adjusting for overall wing size, showed that males were heavier than females (Fig. 1).

Average body weight was 3.2 ± 0.6 kg in FLCO when all age classes and sexes were combined; there was no significant difference in the body weight of adults and juveniles (t-test; $P = 0.815$, $df = 42$). Comparison between sexes showed that males were heavier (t-test; $P = 0.000$, $df = 266$) and had a longer tarsus length (t-test; $P = 0.000$, $df = 208$; Table 2) than females. Body condition index for FLCO based on sex, after adjusting for overall tarsus size, showed that males were heavier than females (Fig. 2).

Average body weight was 3.7 ± 0.5 kg in WAAL when all age classes and sexes were combined; juveniles were heavier than the adults (t-test; $P = 0.000$, $df = 21$). Comparison between sexes showed that males were significantly heavier (t-test; $P = 0.000$, $df = 392$) and had a longer tarsus length (t-test; $P = 0.000$, $df = 260$; Table 2) than females. Body condition index for WAAL based on sex, after adjusting for overall tarsus size, showed that males were heavier than females (Fig. 3, Table 2).

DISCUSSION

These data provide baseline indices of heart rate, respiratory rate, and body temperature for GAPE, FLCO, and WAAL monitored during a nine-year period, 2011–2019. These physiologic parameters had not been previously acquired despite extensive infectious and non-infectious disease monitoring (see Padilla *et al*. 2003; Travis *et al.* 2006a, 2006b; Merkel *et al.* 2007; Levin *et al*. 2013). GAPE, FLCO, and WAAL appeared healthy, and no dead individuals were found in the study areas. GAPE, FLCO, and WAAL are indicators,, or sentinel species of their ecosystem (see Jiménez-Uzcátegui *et al*. 2019); therefore, we propose that the populations that share these habitats were likewise healthy.

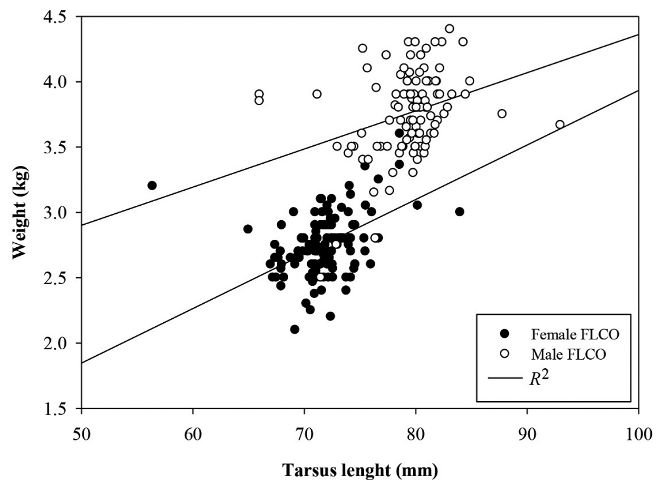

Fig. 2. Body condition index for Flightless Cormorant *Phalacrocorax harrisi* (FLCO) by sex based on body weight and tarsus length. $R^2 = 0.242$ and $y = -0.239 + 0.0417x$ in females, and $R^2 = 0.0991$ and $y = 1.443 + 0.0292x$ in males.

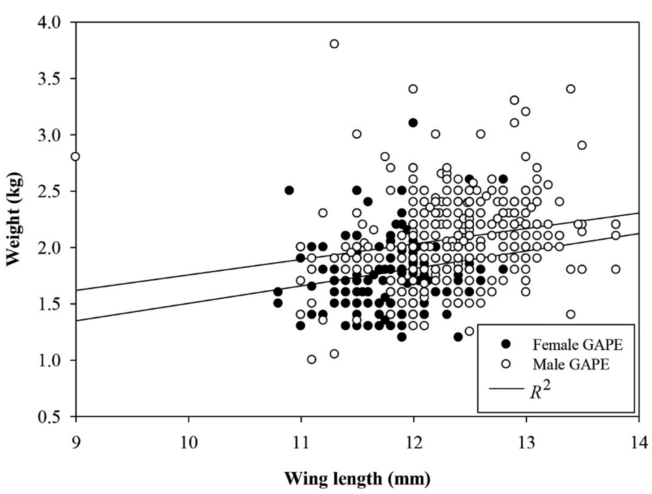

Fig. 1. Body condition index for the Galapagos Penguin *Spheniscus mendiculus* (GAPE) by sex based on body weight and wing length. $R^2 = 0.0336$ and $y = 0.284 + 0.127x$ in females, and $R^2 = 0.0439$ and $y = 0.472 + 0.130x$ in males.

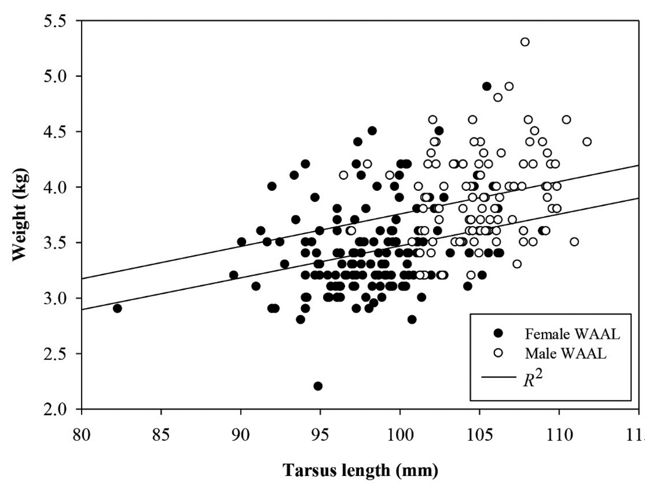

Fig. 3. Body condition index for Waved Albatross *Phoebastria irrorata* (WAAL) by sex based on body weight and tarsus length. $R^2 = 0.0789$ and $y = 0.602 + 0.0287x$ in females, and $R^2 = 0.0491$ and $y = 0.837 + 0.0292x$ in males.

The baseline values for the parameters measured in this study are similar to those of other avian studies (Harrison & Ritchie 1994, Fitzgerald & Beaufrère 2016, Pollock & Carpenter 2016). High coefficients of variation (SD) suggest that the measured values for the parameters studied may have been affected by external or environmental factors such as the hour of monitoring, physical activity before the capture, or handling (see Kelly 1972).

The cloacal temperature of GAPE fell over a wider range and had a higher mean compared to other penguin species. Rectal temperature in the Adelie Penguin *Pygoscelis adeliae* was 37.8 °C with a rise of 0.4 °C, and it ranged from 37.3 ± 0.3 °C to 38.9 ± 0.1 °C in the Emperor Penguin *Aptenodytes forsteri*. Temperature in the Gentoo Penguin *P. papua* was recorded at 38.8 °C, although the source of the temperature measurement was not identified (Boyd & Sladen 1971, Groscolas 1985, Clarke & Kerry 2000; Table 2). This could be due to more variation in environmental temperatures in Galapagos compared to the Antarctic. The cloacal temperature of FLCO was very similar to that of the Neotropic Cormorant *P. brasilianus*, the latter having a body temperature measured with a gas analyzer of 39.7 ± 0.1–41.6 ± 0.9 °C (Alzola-Chiramo *et al*. 2009). Because FLCO and GAPE share the same islands and environmental conditions, they are subject to the same temperature variables (Snow 1965, Valle 1994). Cloacal temperature differed from tympanic temperature in WAAL, with a slight positive association between the two. Therefore, we hypothesize that, because the tympanic membranes are superficial, tympanic temperature is more affected by environmental temperature than cloacal temperature (see Harrison & Ritchie 1994), and likewise, cloacal temperature is more accurate and less variable than tympanic temperature (Merck 2000). We suggest that cloacal temperature is less invasive and easier to collect than tympanic temperature; we have not found other studies that permit comparison of cloacal and tympanic temperatures in WAAL.

The higher body condition indices for GAPE, FLCO, and WAAL (Figs. 1, 2, 3) were not statistically significant between sexes, though males showed a higher index score. This may be an advantage to males, as a heavier body mass may allow birds to go longer intervals without food, allowing males more time to protect the nest (see Snow 1965, Padilla *et al*. 2003, Travis *et al*. 2006a, 2006b). In FLCO, the male covers the nest until the chicks are independent, while the female continues to reproduce with another male (a sequential polyandrous system); in this case, a higher body condition index in males is advantageous in that it protects the juveniles (Snow 1965, Valle 1994). Future studies could include monitoring water temperature and environmental temperature (according to season), and correlating these results to body temperature, heart rate, and respiratory rate. For now, the baseline values for the physiological parameters presented here for GAPE, FLCO, and WAAL provides new and robust information that can be used in future health assessments of these species.

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