



Varying aerobic capacity in relation to breeding stage and reproductive success in giant petrels (*Macronectes* spp.)

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ABSTRACT

Reproduction, and parental care in particular, are among the most energy-demanding activities within the annual cycle of adult birds. Parents that cannot meet the metabolic demands and other physiological costs of raising offspring may opt to abandon chicks in favour of self-maintenance and future reproduction. Recent work examining reproductive trade-offs in birds revealed an important role of oxygen carrying capacity in mediating variation in parental effort. This study explores the aerobic factors underlying the success or failure of parental care in two closely-related petrel species during their breeding season on Bird Island, South Georgia: northern giant petrels (*Macronectes halli*) and southern giant petrels (*M. giganteus*). Failed breeders of both sexes and species had significantly lower hematocrit levels (by $5.48 \pm 0.64\%$) than successful breeders, and reticulocyte counts also tended to be lower in failed males, consistent with the hypothesis that parental care and workload depend on aerobic capacity. We discuss these results in relation to differences in the foraging ecology of both species and sexes.

1. Introduction

The idea that raising offspring can reduce the survival of parents is central to life-history theory (Clutton-Brock, 1991). This concept has been formalized as the trade-off between current reproduction and residual fitness (Stearns, 1992). In iteroparous species that provide parental care, parents must often increase their foraging effort to match the demands of growing offspring, but not to the extent of compromising their own health or survival (Daan, Deerenberg, and Dijkstra, 1996). However, when food is scarce and environmental conditions poor, higher effort may not provide the resources that offspring need to sustain their growth and survival (Guinet, Chastel, Koudil, Durbec, and Jouventin, 1998; Hipfner, 2008; Williams, 2018). Similarly, if the demands of offspring push parents beyond a supposed optimal working capacity of $4\times$ basal metabolic rate, parents may preserve residual fitness by abandoning offspring in favour of self-maintenance (the resource allocation hypothesis; Drent and Daan, 1980; Fowler and Williams, 2017). Indeed, unsustainable physiological costs can be the central cause of reproductive failure, manifested through adult mortality, hatching failure, chick starvation or predation (Grant et al., 2001; Roche, Arnold, and Cuthbert, 2010; Hemmings, West, and Birkhead,

2012). Failed breeders tend to be the lowest quality and competitively-inferior individuals within a population (Garant, Kruuk, McCleery, and Sheldon, 2007; Ponchon, Iliszko, Grémillet, and Tveraa, 2017). Although we know little about the metabolic costs of reproduction (Ketterson and Nolan, 1999; Fowler and Williams, 2017), previous studies have found evidence that failed breeders have a physiological profile indicative of high stress (Breuner, 2011), low energy reserves (Wanless, Harris, Redman, and Speakman, 2005), disease or immunosuppression (Norris and Evans, 2000; Capilla-Lasheras et al., 2017).

Reproduction in birds is a discrete life-history stage within the annual cycle, and often timed to avoid overlap with other expensive processes like feather moult (Kjellén, 1994; Dawson, 2007; Catry, Poisbleau, Lecoq, and Phillips, 2013; but see Williams, 2012). However, several bird species have evolved moulting strategies that directly overlap with parental care (Williams, 2012), which can range from only a matter of days in pied flycatchers (*Ficedula hypoleuca*) (Hemborg, Sanz, and Lundberg, 2001) to near-complete overlap in neotropical birds like the lesser ground-cuckoo (*Morococcyx erythropygus*) (Foster, 1974; Echeverry-Galvis and Hau, 2013). A trade-off between somatic maintenance (i.e. feather renewal) and parental care is to be expected (Svensson and Nilsson, 1997). For example, Hemborg (1999) showed

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that female pied flycatchers which moulted during breeding laid smaller clutches and fledged fewer chicks. The physiological costs and trade-offs involved in a breeding-moult overlap remain largely unexplored (Echeverry-Galvis and Córdoba-Córdoba, 2008; Williams, 2012). However, they likely involve sex and stress hormones (Crossin et al., 2012), energy reserves (Hoye and Buttemer, 2011), or aerobic capacity (Klaassen, 1995; Kern, Bacon, Long, and Cowie, 2007; Mazarro et al., 2013; Podlaszczuk et al., 2016; Fowler, Paquet, Legault, Cohen, and Williams, 2018).

In this study, we examine the dynamics of aerobic capacity in two long-lived, pelagic seabirds that exhibit substantial breeding-moult overlap: the northern giant petrel (NGP; *Macronectes halli*) and southern giant petrel (SGP; *M. giganteus*). Both are monogamous central-place foragers with circumpolar distributions on island groups around the Southern Ocean and mean laying dates around 6 weeks apart where they breed in sympatry (Brown, Techow, Wood, and Phillips, 2015). They provide bi-parental care to a single chick, alternating shifts at the nest with foraging trips to sea during the incubation and brood-guard periods, after which the chick can be left unguarded and both parents forage at sea, returning at intervals of several hours to days until fledging (Granroth-Wilding and Phillips, 2018). Throughout the period of parental care, primary (flight) feather moult occurs in both males and females (Hunter, 1984a), but the rate of feather replacement accelerates if parents lose their egg or chick, i.e. if they become failed breeders (Crossin et al., 2012). Not surprisingly, moulting while raising offspring means that giant petrels have greater energetic costs while foraging than other seabirds (Obst and Nagy, 1992), which is presumably offset by higher aerobic capacity, i.e. increased oxygen-carrying capacity of blood, to sustain greater flight muscle activity (Butler, 2016). Indeed, aerobic capacity is an important determinant of foraging effort in many bird species (e.g. Leary, Sullivan, and Hillgarth, 1999; Crossin, Takahashi, Sakamoto, Trathan, and Williams, 2015; Malvat, Lynch, Bennison, and Jessopp, 2020). However, its measurement is also affected by the other costs of reproduction (Wagner, Stables, and Williams, 2008; Jubinville, Williams, Trathan, and Crossin, 2020) and moult (Mazarro et al., 2013), and changes in aerobic capacity are rarely linked directly to reproductive success and fitness (Wagner, Stables, and Williams, 2008; Fowler, Paquet, Legault, Cohen, and Williams, 2018; but see Kalmbach, Griffiths, Crane, and Furness, 2004).

To examine the role of aerobic capacity in breeding-moult trade-offs, we sought to resolve how various aerobic indices (hematocrit [Hct], reticulocyte index [RI]) related to reproductive success in giant petrels monitored throughout the summer breeding period at Bird Island, South Georgia. Our aims were twofold. First, we compared aerobic indices and moult scores from successful and failed breeders of each sex and species at the end of the breeding season, just prior to out-migration, to determine how these related to reproductive outcome, while controlling for body mass. Given that aerobic indices reflect individual workload and phenotype, we predicted that successful breeders with a lower degree of feather moult would have higher Hct levels and RIs than failed breeders in a more advanced state of moult. However, given the difficulty in disentangling cause from consequence with regard to relationships among aerobic capacity, reproductive outcome, and moult, we tested two non-exclusive hypotheses: (i) that high aerobic capacity contributes to successful reproduction and therefore delayed moult, and (ii) that after reproductive failure, moulting occurs at the cost of decreased reproductive capacity. Second, we documented the seasonal progression in aerobic indices in giant petrels over the c.6-month period from egg laying to chick fledging. Few studies have examined aerobic indices over so long a breeding season (Wagner, Stables, and Williams, 2008; Fowler, Paquet, Legault, Cohen, and Williams, 2018), and fewer still in large birds like seabirds. The results are discussed in the context of breeding-moult overlap and associated energetic trade-offs in birds.

2. Methods

2.1. Data collection

Fieldwork was carried out on northern and southern giant petrels breeding in sympatry on Bird Island, South Georgia (54°00'S, 38°03'W) during the austral summer, October 2008 to April 2009. All study nests included banded, sexed adults which were part of the long-term monitoring programme (for details, see Brown, Techow, Wood, and Phillips, 2015; Gianuca et al., 2019). Briefly, nests with eggs are marked at laying, and visited at least weekly to record failures (due to nest abandonment, egg infertility or other hatching failure, chick starvation or predation), up until the chick fledges. Sex was determined visually from bill size, as males have a much larger and deeper bill than females (Copello, Quintana, and Somoza, 2006), and the accuracy confirmed by reference to the sex of the partner (Brown, Techow, Wood, and Phillips, 2015; Gianuca et al., 2019). All sampled birds were therefore of known breeding status.

Banded adults (10–23 males and 10–22 females, see Table 1) were sampled at four points in the breeding season: *early incubation*, *late incubation* (~40 days later), *early chick-rearing* (~15 days later), and *late chick-rearing* (~80 days later). Adults were not serially-sampled, i.e. different individuals were captured at each point in time. NGPs lay in late September-early October, with eggs hatching in late November-early December, and chicks fledging in late March, whereas SGPs lay six weeks later, in early-mid November, with eggs hatching in early January, and chicks fledging in May (Brown, Techow, Wood, and Phillips, 2015, British Antarctic Survey unpublished data). The brood-guard period (when the chick is always attended by at least one parent) is considerably longer in NGPs than SGPs (30 days versus 16 days; Hunter, 1984a).

During *early incubation*, *late incubation* and *early chick-rearing*, active breeders were sampled at their nests, whereas in the *late chick-rearing* period, both successful and failed breeders were captured either near their nests or in open areas. At capture, mass (± 50 g) of adults was measured with a 10 kg Pesola spring scale, and tarsus length, bill length and minimum bill depth measured with calipers to the nearest mm. Blood samples of 2.0 ml were taken from the tarsal vein using pre-heparinized syringes fitted with 25-G needles, and transferred to 2.5-mL Eppendorf vials. Almost all samples (299 of 302) were collected within 3 min of capture to minimise the effect of the stress response (Romero and Reed, 2005). Small portions of these samples were removed using micro-hematocrit tubes, and both the 2.5-mL Eppendorf vials and micro-hematocrit tubes centrifuged for 5 min at 10,000g to separate plasma and determine Hct (packed cell volume %) respectively. Plasma was then transferred to 0.6-mL cryovials for storage at -20°C until analysis. Moult was quantified by scoring 10 primary (flight) feathers on a scale of 0–4, following Dawson and Newton (2004) and Crossin, Phillips, Lattin, Romero, and Williams (2013).

Table 1

Sample sizes of giant petrels (*Macronectes* spp.) studied in each period of their breeding season, from October 2008 to April of 2009 on Bird Island, South Georgia.

		Early incubation	Late incubation	Early chick-rearing	Late chick-rearing
Northern giant petrels (<i>M. halli</i>)					
Males	N	13	10	17	18
Females	N	14	9	18	9
Southern giant petrels (<i>M. giganteus</i>)					
Males	N	20	21	19	14
Females	N	19	19	18	16

2.2. Blood processing and smear analysis

Hct, or the volume of erythrocytes per unit plasma volume, provides an indication of relative aerobic capacity and overall health in birds (Richner et al. 1993; Piersma, Everaarts, and Jukema, 1996; Wagner, Stables, and Williams, 2008). In addition, the RI, or the number of reticulocytes (juvenile erythrocytes) in circulation is used to gauge regenerative erythropoiesis (Cooper, Sears, and Bienzle, 2005). Reticulocytosis, or the enhanced production of new red blood cells due to the effects of the cytokine erythropoietin, is considered to be the best indicator of rebound from depleted Hct (Fernandez and Grindem, 2006).

Five microlitre (5 µl) subsamples of whole blood were applied to glass slides. These smears were stained with new methylene blue (Reticulocyte stain R4132, Sigma-Aldrich Canada, Oakville, Ontario) to identify reticulocytes. Glass cover slips were then affixed to smears using Permount (SP15–500, Fisher Scientific Canada, Ottawa, Ontario, Canada) to prevent damage and displacement during oil immersion. Stained smears were analyzed under 1000 × oil immersion microscopy, and the number of reticulocytes per 1000 erythrocytes was recorded by evaluating 100 cells from 10 different regions of each smear. RI was calculated using the Hct correction formula outlined by Riley, Ben-Ezra, Goel, and Tidwell (2001), with 'normal Hct' set to the sample mean (see Results).

$$RI = \%reticulocytes * \left(\frac{\text{observed Hct}}{\text{normal Hct}} \right)$$

Reticulocytes were distinguished from mature erythrocytes according to Campbell (1988), Fernandez and Grindem (2006), and Wagner, Stables, and Williams (2008). Accordingly, reticulocyte types were differentiated by their interspersed chromatin and relatively large size (Campbell, 1988). Reticulocytes were identified as cells with a ring of reticulum surrounding the nucleus, or at least five reticulum aggregates in the cytoplasm (Fernandez and Grindem, 2006; Wagner, Stables, and Williams, 2008; see Fig. S1 in Supplementary material). All smears were counted by the same individual, who was blind to the bird sex, sampling period and status.

2.3. Statistics

Generalized linear models were used to assess relationships between physiological and life-history parameters towards the end of the season (in the *late chick-rearing* period), and to analyze seasonal progressions in Hct and RI. Analysis for the *late chick-rearing* period included four models with reproductive outcome, moult-score, Hct, and RI as response variables, and mass, sex, species, and various interaction terms as factors. Models were dredged from a comprehensive global model and fit using Gaussian or binomial distributions with identity or logit link functions, respectively. All variables were included as fixed factors rather than random effects, as there were ≤ 5 values for all. To select the best-fitting model (with consideration for goodness of fit and simplicity) Akaike Information Criterion [AIC], ΔAIC, and adjusted R² values were generated. All analyses were performed using R (version 4.0.2; 2020-09-3). Unless indicated otherwise, differences were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Aerobic capacity and reproductive success

Hct and RI data were obtained from 78 giant petrels (55 successful, 23 failed) sampled in the *late chick-rearing* period, including 39 NGPs (27 successful, 12 failed), and 39 SGPs (28 successful, 11 failed). Mean Hct was significantly higher in successful than failed breeders in all four species-sex groups (Table 2). Similarly, mean RI of successful birds was significantly higher than that of failed breeders in males of both species,

Table 2

Hematocrit (Hct), body mass, moult-score, and reticulocyte indices (RI) of northern and southern giant petrels (*Macronectes* spp.) measured at the end of the season in birds that failed or were successful in fledging their chick. Data were collected from October 2008 to April 2009 on Bird Island, South Georgia. Values are least square means ± SEM. Bold text indicates statistically significant contrasts ($p < 0.05$).

		Successful breeders	Failed breeders
Northern giant petrels (<i>M. halli</i>)			
Males	Hct (%)	47.56 ± 0.73	41.40 ± 1.03
	Mass (kg)	4.67 ± 0.06	4.54 ± 0.18
	Moult-score	0.21 ± 0.02	0.66 ± 0.14
	RI	5.58 ± 0.45	3.93 ± 0.85
	N	18	5
Females	Hct (%)	48.22 ± 0.80	42.38 ± 0.53
	Mass (kg)	3.59 ± 0.07	3.49 ± 0.09
	Moult-score	0.06 ± 0.03	0.52 ± 0.07
	RI	6.51 ± 0.91	4.44 ± 0.65
	N	9	8
Southern giant petrels (<i>M. giganteus</i>)			
Males	Hct (%)	48.57 ± 0.86	43.00 ± 1.08
	Mass (kg)	4.59 ± 0.07	4.73 ± 0.12
	Moult-score	0.19 ± 0.03	0.69 ± 0.00
	RI	6.40 ± 0.77	2.68 ± 0.85
	N	15	4
Females	Hct (%)	47.07 ± 1.00	42.50 ± 1.36
	Mass (kg)	3.51 ± 0.06	3.70 ± 0.11
	Moult-score	0.08 ± 0.02	0.37 ± 0.04
	RI	5.44 ± 0.71	3.90 ± 1.89
	N	15	7

but not in females. In addition, mean moult-score was significantly higher in failed than successful breeders.

In generalized linear models used to assess relationships among physiological and life-history parameters in the *late chick-rearing* period, Hct was the variable with the strongest effect on reproductive success or failure (*fate*) and moult-score (Table 4a; $t = -3.50$, $p < 0.001$ and Table 4b; $t = -6.58$, $p < 0.001$, respectively). Moreover, in analyses of *fate*, sex and an interaction between mass and sex had significant effects (Table 4a; $t = 2.07$, $p = 0.038$ and $t = -2.04$, $p = 0.041$, respectively). Additionally, moult-score (*moult*) and reproductive outcome (*fate*) had the strongest effects on Hct and RI, respectively (Table 4c; $t = -6.58$, $p < 0.001$ and Table 4d; $t = 3.76$, $p < 0.001$), and mass also had a significant effect on RI (Table 4d; $t = -2.35$, $p = 0.021$). The correlation between Hct and RI was weak but significant ($r = 0.13$, $F(1, 249) = 4.31$, $p = 0.04$), which prevented the coincident use of both aerobic indices in the same generalized linear model (Table 3).

3.2. Seasonal changes in aerobic capacity

These analyses were of 252 giant petrels sampled over the c.6-month breeding season that successfully fledged their chick, including 108 NGPs (58 males, 50 females), and 146 SGPs (74 males, 72 females; Table 1). Mean Hct and RI across the season were $45.77 \pm 0.24\%$ and $6.50 \pm 0.23\%$ respectively (Fig. 1b; Fig. 1c). There were significant changes in mean mass, Hct, and RI within each of the four species-sex groups over the course of the breeding season, but without clear trends (Table 3; Fig. 1). However, both sexes in SGPs exhibited higher Hct in the *late chick-rearing* period than in *early incubation* (Fig. 1b), and in all species-sex groups except male NGPs, RI values in *early chick-rearing* and *late chick-rearing* periods were significantly lower than in *early incubation* (Fig. 1c).

3.3. Seasonal differences in Hct between species-sex groups

There were significant differences in Hct among the four species-sex groups in all breeding periods except in *late chick-rearing* (Fig. 1b). Hct

Table 3

Generalized linear models evaluating variation in (a) reproductive success or failure (*fate*), (b) moult-score (*moult*), (c) hematocrit (Hct), and (d) reticulocyte index (RI) ($A, N = 77$) in relation to mass, sex, species, and other explanatory variables measured in giant petrels (*Macronectes* spp.) at the end of the breeding season. Models were also run to evaluate variation in (e) Hct and (f) RI ($A, N = 252$) in relation to mass, reproductive period (*period*), sex, and species measured at four distinct periods during the reproductive season. Data were collected from October 2008 to April 2009 on Bird Island, South Georgia. The best-fitting models (indicated by the lowest AIC value) and null models are signified by bold text, and the global models (from which the stronger models were dredged) are indicated by italics.

(a)			
Response = fate	AIC	Δ AIC	Adj R^2
<i>Fate ~ Hct + mass*sex + sex*species</i>	84.2	0	0.26
Fate ~ Hct + mass + sex + species	88.1	3.9	0.17
(null) ~ 1	96.6	12.4	0
(b)			
Response = moult	AIC	Δ AIC	Adj R^2
Moult ~ Hct + mass + sex + species	-39.2	0	0.41
<i>Moult ~ Hct + mass*sex + sex*species</i>	-38.6	0.6	0.43
(Null) ~ 1	-6.6	32.6	0
(c)			
Response = Hct	AIC	Δ AIC	Adj R^2
Hct ~ mass + moult + sex + species	405.7	0	0.39
<i>Hct ~ mass*sex + mass*species + moult</i>	408.6	2.9	0.40
(null) ~ 1	436.6	30.9	0.00
(d)			
Response = RI	AIC	Δ AIC	Adj R^2
RI ~ fate + mass + sex + species	369.5	0	0.21
<i>RI ~ fate + mass*sex + sex*species</i>	371.5	2.0	0.23
(Null) ~ 1	379.5	10.0	0.00
(e)			
Response = Hct	AIC	Δ AIC	Adj R^2
Hct ~ mass + moult + period + sex + species	1363.3	0	0.16
<i>Hct ~ mass*period + mass*sex + moult*sex + sex*species + moult*species</i>	1369.8	6.5	0.17
(null) ~ 1	1388.5	25.2	0.00
(f)			
Response = RI	AIC	Δ AIC	Adj R^2
RI ~ mass + moult + period + sex + species	1310.6	0	0.21
<i>RI ~ mass*period + mass*sex + moult*sex + sex*species + moult*species</i>	1310.7	0.1	0.24
(null) ~ 1	1350.5	39.9	0.00

was significantly higher in female NGPs than female SGPs in the *early incubation* and *late incubation* periods (Fig. 1b), but the reverse was found in *early chick-rearing*, i.e., Hct was higher in female SGPs (Fig. 1b). In generalized linear models the principal effects on Hct were mass, moult-score (*moult*), breeding period (*period*), and sex (Table 4; $t = 25.7, p < 0.001$).

3.4. Seasonal differences in RI between species-sex groups

RI varied significantly among the four species-sex groups in all breeding periods with the exception of the *late chick-rearing* (Fig. 1c). In all other periods, the RIs of NGPs were significantly higher than those of

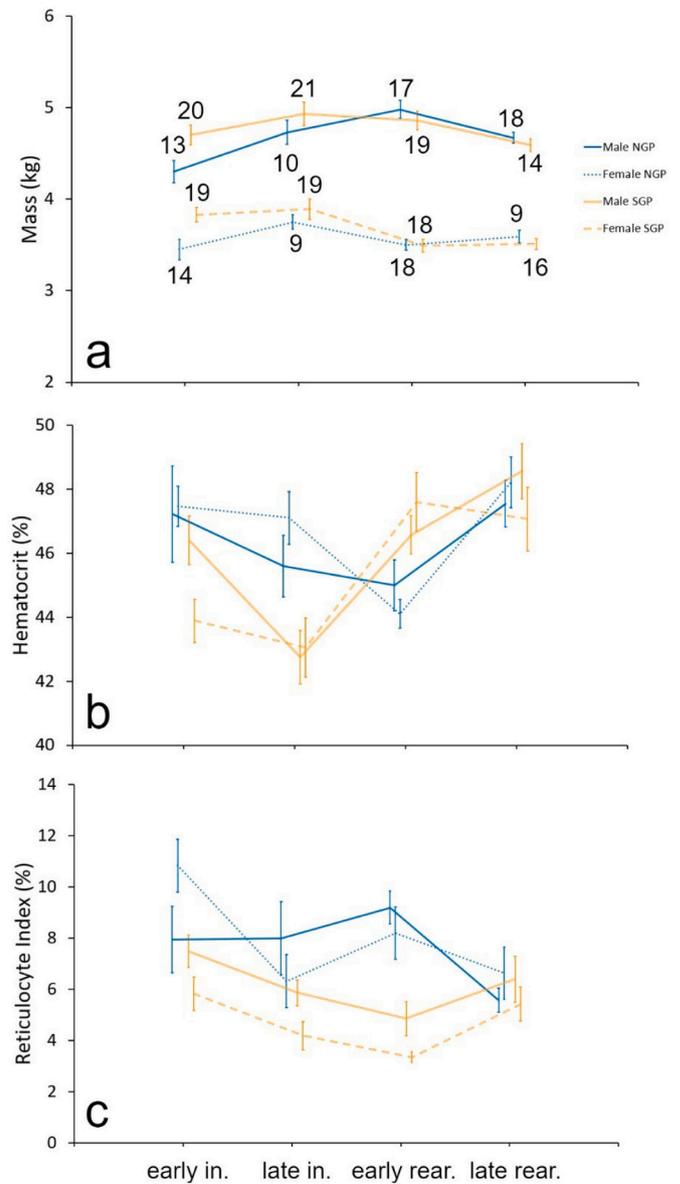


Fig. 1. (a) Mass (kg), (b) Hct (%), and (c) RI (%) of successful breeding giant petrels (*Macronectes* spp.) over the breeding season. Samples were collected from October 2008 to April 2009 on Bird Island, South Georgia. To allow for more easily readable axis labels, “incubation” was shortened to “in.”, and “rearing” to “rear.”. Values are least square means \pm SEM. Data was jittered along the x-axis for visual clarity.

SGPs. In addition, in the *early incubation* and *late incubation* periods, the RIs of female SGPs were significantly lower than those of male SGPs (and every other species-sex group). In generalized linear models breeding period (*period*) and species were the strongest predictors of RI (Table 4; $t = 5.71, p < 0.001$).

4. Discussion

This study shows that hematological indices of aerobic capacity were higher in giant petrels that breed successfully, compared with those that failed. The strongest support came from analyses of Hct, which was higher in successful than failed breeders in all species-sex groups (Table 2). RIs showed similar relationships in males of both species, but differences in females were not significant (although the direction of the difference was the same in female southern giant petrels; Table 2). These findings are consistent with our two hypotheses, and illustrate the

Table 4

Significant parameter estimates ($p < 0.05$) of the best-fitting generalized linear models (indicated by the lowest AIC value) presented in Table 3. Data were collected from October 2008 to April 2009 on Bird Island, South Georgia.

(a) fate ~ Hct + mass*sex + sex*species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	9.38	7.04	1.33	0.183
Hct	-0.36	0.10	-3.50	< 0.001
Sex	20.4	9.86	2.07	0.038
Mass*sex	-4.91	2.40	-2.04	0.041
(b) moult ~ Hct + mass + sex + species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	1.69	0.38	4.45	< 0.001
Hct	-0.04	0.01	-6.58	< 0.001
(c) Hct ~ mass + moult + sex + species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	47.1	4.94	9.53	< 0.001
Moult	-10.6	1.61	-6.58	< 0.001
(d) RI ~ fate + mass + sex + species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	13.1	3.92	3.35	0.001
Fate	2.32	0.62	3.76	< 0.001
Mass	-2.60	1.10	-2.35	0.021
(e) Hct ~ mass + moult + period + sex + species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	51.1	1.99	25.7	< 0.001
Mass	-1.48	0.55	-2.70	0.007
Moult	-6.76	2.44	-2.77	0.006
Period late incubation	-1.67	0.65	-2.56	0.011
Period late chick-rearing	2.47	0.80	3.09	0.002
Sex	2.31	0.76	3.03	0.003
(f) RI ~ mass + moult + period + sex + species				
Parameter	Estimate	Standard error	t value	p-value
Intercept	10.2	1.79	5.71	< 0.001
Period early chick-rearing	-1.60	0.55	-2.90	0.004
Period late incubation	-1.77	0.59	-3.01	0.003
Period late chick-rearing	-1.85	0.72	-2.58	0.011
Species	-2.51	0.41	-6.08	< 0.001

physiological underpinnings of two major, overlapping life-history processes in giant petrels. First, the relatively high Hct and RIs in successful breeders reinforce that aerobic capacity is critical for supporting the demands of parental care, which involves long pelagic foraging trips, particularly in females, and high levels of intra- and inter-specific competition for carrion on beaches, particularly by males (Granroth-Wilding and Phillips, 2018; Mills, Morley, Votier, and Phillips, 2021). Second, the relatively low aerobic indices of failed breeders may also reflect the potential costs or physiological adjustments (such as increased plasma volume) associated with feather replacement (Table 2). Lacking a chick for which to care, failed breeders have reduced workloads (as there is no requirement for provisioning), and hence low (but not necessarily anemic) levels of Hct are probably sufficient to support foraging for self-maintenance. Additionally, unlike most other seabirds, giant petrels moult during early breeding, but suspend this during periods of higher demand, i.e., once chicks hatch; however, feather replacement is resumed after reproductive failure, and this cost seems to be reflected in the hematological variables (Ots, Murumägi, and Hörak, 1998; Crossin et al., 2012; Mazarro et al., 2013; Table 2). We discuss these findings in greater detail below.

4.1. Aerobic capacity and reproductive success

The average Hct and RIs of successful breeders were higher than

those of failed breeders by $5.48 \pm 0.64\%$ and $2.05 \pm 0.63\%$, respectively. Hct peaked at $48.6 \pm 0.86\%$ in successful male SGPs, and was lowest in failed male NGPs, at $41.40 \pm 1.03\%$ (Table 2). The latter represents a significant decline in Hct over the breeding season from an early incubation average Hct of 46.40 ± 3.82 , which may represent 'reproductive anemia' (a decline in Hct of greater than 2 standard deviations (often $\sim 10\%$) from pre-laying to post-laying; Williams et al., 2004; Crossin et al., 2010).

Aerobic upregulation leads to several fitness advantages in breeding birds, as it supports higher oxygenation of tissues for sustaining intense workloads while foraging or in nest defence (Williams, 2012). For example, previous work has shown that relatively high aerobic capacity improves diving ability in sea birds (Mill and Baldwin, 1983; Crossin, Takahashi, Sakamoto, Trathan, and Williams, 2015), minimizes heat loss (Guard and Murrish, 1975; Hayes and Garland, 1995), and increases flight performance at high altitudes (Clemens, 1990; Fair, Whitaker, and Pearson, 2007; Borras and Cabrera, 2010). In this study, elevated aerobic capacity presumably improves competitive ability when fighting for seal and penguin carrion at South Georgia, which comprises the majority of the diets of males of both NGP and SGP during the breeding season (Mills, Morley, Votier, and Phillips, 2021). These food sources are critical to successful reproduction, and giant petrels exhibit high levels of intra- and inter-specific aggression for access (Hunter, 1983). Aggressive behaviour in birds, like the battles for dominance ('cock-fights') seen in male red junglefowl (*Gallus gallus*), has been linked to testosterone levels (Wingfield, Ball, Dufty Jr., Hegner, and Ramenofsky, 1987; Soma, 2006), and also elevated aerobic capacity in dominant compared with subordinate males (Hammond, Chappell, Cardullo, Lin, and Johnsen, 2000). Increased aerobic capacity is presumably also an advantage to giant petrels when commuting long distances to pelagic waters, which is the main habitat utilised by the females, which are considerably smaller and consume a much greater proportion of marine prey (Granroth-Wilding and Phillips, 2018; Mills, Morley, Votier, and Phillips, 2021).

The low aerobic indices in failed breeders may indicate an increased investment in moult (Ots, Murumägi, and Hörak, 1998; Mazarro et al., 2013), which in terms of primary (flight) feathers, was much more advanced in failed breeders by the late season when sampling took place (Table 2) (Crossin et al., 2012). Increased moult investment (stemming from decreased prolactin, see Crossin et al., 2012) could improve flight performance (Echeverry-Galvis and Hau, 2013) and at least partly explain why foraging ranges are longer in failed than successful (González-Solís, Croxall, and Afanasyev, 2008). By extension, advanced moult may theoretically result in better preparedness for out-migration, though this is yet to be tested. Moreover, the decrease in Hct during moult is widely thought to be a result of a substantial increase in blood plasma volume (hemodilution) in order to fill the extensive vascular networks of growing quills (Chilgren and DeGraw, 1977; Mazarro et al., 2013), particularly as there is no evidence of reduced erythrocyte production (DeGraw and Kern, 1985; Minias, Kaczmarek, Włodarczyk, and Janiszewski, 2013). This could, in turn, explain the more consistent reduction in Hct than in RIs, which was recorded in failed breeders across all species-sex groups (Table 2). In particular, that Hct decreased by $\sim 6\%$ and RI only by $\sim 0.05\%$ in failed female NGPs, which invested more heavily in moult (Crossin et al., 2012), suggests considerable hemodilution, but not reduced reticulocytosis (see Table 2).

4.2. Seasonal changes in aerobic capacity

A second aim of this study was to document the seasonal changes in aerobic indices across the breeding season, from early incubation to late chick-rearing, with the analyses restricted to successful breeders to avoid confounding effects of breeding status. Hct and RI varied considerably throughout breeding in both species and sexes, but the most striking feature was a convergence of values to high Hct and low RI levels in late chick-rearing, shortly before both chicks and adults depart the colony

(Fig. 1b; Fig. 1c). This upregulation of Hct through chick rearing may be related to the increasing demands of provisioning, particularly given that the mean meal mass is higher in the latter half of rearing (Hunter, 1983), and foraging trips are longer both in terms of flight distance and duration in both species (Granroth-Wilding and Phillips, 20189). In a study by Love, Breuner, Vézina, and Williams (2004), glucocorticoid secretion in European starlings (*Sturnus vulgaris*) increased through the stages of chick development, supporting higher foraging and locomotor activity (Wingfield, Schwabl, and Mattocks Jr, 1990). To aid this increased workload, increases in aerobic indices have also been documented (Davey, Lill, and Baldwin, 2000; Navarro et al., 2007). For example, in the short-tailed shearwater (*Ardenna tenuirostris*), Davey, Lill, and Baldwin (2000) found hemoglobin to increase at a constant rate throughout the breeding season, which was attributed to the demands of increasingly long foraging trips. Studies have shown Hct to increase with flight costs (Hörak, Ots, and Murumägi, 1998) and fluctuate with seasonal changes in energy demand (Butler, 2016), often peaking immediately prior to or during long-distance migration (Wingfield, Schwabl, and Mattocks Jr, 1990; Krause et al., 2016). However, proximity to migration is clearly not as important as demands associated with chick provisioning in determining aerobic capacity of giant petrels, otherwise we would expect similar levels of Hct in failed and successful breeders. That was not the case, even though failed and successful birds would have departed on migration not long after sampling. Finally, neither mean mass nor RI showed a consistent seasonal pattern, and values were within levels measured in previous studies (Uhart, Quintana, Karesh, and Braselton, 2003; Fig. 1a; Fig. 1c). This was unusual, as we expected evidence of reproductive anemia in successful females across the season (Williams et al., 2004; Crossin et al., 2010). Instead, the strongest indication of anemia was in failed females, who, despite having low Hct by comparison with their successful counterparts (Table 2), had comparable RIs (Table 2), suggesting an attempt to upregulate erythropoiesis.

4.3. Conclusion

These findings suggest aerobic capacity is a critical determinant of the success of parental care in migratory seabirds. As workload increases during the breeding season, so too must the aerobic measures that in part determine the ability of an individual to provision its growing offspring. This was illustrated most clearly by changes in Hct, which was depleted in failed breeders and up-regulated over the breeding season, suggesting it is a suitable metric for studies relating aerobic capacity to workload and fitness. Changes in RI complemented the trends in Hct, but less reliably, which may reflect sample-size limitations or the physiology of moult and species-sex differences therein. Generally, these results suggest that the ability to accommodate the stress associated with prolonged flight partly determine reproductive success. A previous study suggested that in terms of adult survival, giant petrels would benefit in the short term from warming in the southwest Atlantic (Gianuca et al., 2019). However, the range of Antarctic krill has contracted southwards, and its abundance has reduced sharply at the northern limit, which is around South Georgia (Atkinson et al., 2019). As krill and krill-dependent predators (penguins and Antarctic fur seals) form the bulk of the diet of breeding giant petrels (Mills, Morley, Votier, and Phillips, 2021), it is possible that adults may in the future be forced to forage further from the colony, raising flight costs and aerobic demands, and amplifying competition, potentially leading to higher rates of reproductive failure. Future research should (i) evaluate relationships among a wider suite of hematological and physiological metrics to establish more comprehensive aerobic profiles, (ii) examine the relationship between aerobic measures in the pre-laying and early breeding periods, and the decision to breed and eventual outcome, and (iii) determine carry-over effects by establishing relationships between end-of-season physiological profiles, migratory performance and fitness in subsequent breeding seasons.

Ethical approval

All sampling protocols were covered by with permits issued by the Canadian Council for Animal Care (Simon Fraser University Animal Care Permit 897B-8) and approved by the British Antarctic Survey Ethics Committee and carried under permit by the Government of South Georgia and the South Sandwich Islands.

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Credit author statement

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Jubenville I: Methodology, Software, Formal analysis, Writing – Review & Editing.

Williams TD: Writing – Review & Editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2022.111155>.

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