Post-migratory body condition and ovarian steroid production predict breeding decisions by female gray-headed albatrosses.

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Running title: Condition dependent carryover effects in female albatrosses

Abstract

Carryover effects have been documented in many migratory bird species, but we know little about the physiological mechanisms that mediate those effects. Here we show that the energetic, endocrine, and aerobic characteristics of post-migratory female gray-headed albatrosses (*Thalassarche chrysostoma*) can affect their decision to breed. All females in this study, whether breeding or not, were secreting ovarian steroids when they arrived at the breeding colony at Bird Island, South Georgia, which suggests that all were responding to seasonal cues. However, deferring, non-breeding birds were characterized by a steroid profile of high progesterone (P4) and low testosterone (T), whereas breeding birds showed the opposite pattern. Deferring birds also had low body mass, hematocrit, and hemoglobin. These results suggest that post-migratory condition can influence patterns of ovarian steroidogensis, and that the maintenance of high P4 without subsequent conversion to T favours breeding deferral. Whereas breeding females normally convert P4 to T, which is a key deterministic step towards 17β-estradiol synthesis, vitellogenesis, and follicle development, deferring females did not make this conversion and instead maintained high levels of P4, perhaps due to inhibition of the hydroxylase-lyase enzyme complex, thus rendering them infertile for the current season. Results are discussed within the context of this species' biennial breeding system, and comparisons with other biennially and annually breeding albatrosses are made.

Keywords: progesterone; testosterone; hematocrit; hemoglobin; aerobic capacity; migration; egg production; carryover effects; trade-offs; deferred reproduction

Introduction

Migratory carryover effects have been documented in several bird species, which can influence many aspects of reproduction including the timing of breeding (Marra et al. 1998; Norris et al. 2004; Descamps et al. 2011; Harrison et al. 2011), breeding decision (Ebbinge and Spaans 1995; Crossin et al. 2012), breeding output (Ebbinge and Spaans 1995; Sorenson et al. 2009), and breeding success (Baker et al. 2004; Inger et al. 2010; Crossin et al. 2012). However, the physiological and endocrine mechanisms that mediate carryover effects are largely unknown. Recent work in birds has shown how variable individuals are in their ability to accrue and store resources in advance of reproduction, which can then have effects that are either positive for breeding (e.g. high somatic fat leading to early timing of breeding; Prop et al. 2003; Smith and Moore 2003), or negative (e.g. low fat or poor condition leading to a trade-off between current reproduction and survival). Recent work has also linked endocrine processes to carryover effects. However, the links between pre-breeding condition and endocrinology remain elusive. Despite the intuitive appeal that individual condition must play a role in hormonally-mediated carryover effects, very few studies that link wintering habitat, migration, breeding activity, and carryover effects include direct measures of body condition (e.g. body mass, fat mass, or mass change), either alone or in combination with endocrine measures (Marra et al. 1998; Ebbinge and Spaans 1995; Baker et al. 2004; Harrison et al. 2011). Although the mechanisms through which condition might influence breeding decisions are not well understood, this must surely involve integration with, and modulation of, the hypothalamic-pituitary-gonadal (HPG) axis (or other hormonal systems that interact with the HPG axis, e.g. [Goutte et al. 2010a]), so that reproductive readiness can be assessed before a commitment to breeding.

For the few studies that have simultaneously examined condition and endocrine effects on breeding decisions, the results have been somewhat equivocal (Harshman and Zera 2007;

72 Williams 2012). A recent study of female black-browed albatrosses (*Thalassarche melanophrys*)
73 for example showed that both condition-dependent and endocrine traits were expressed at low
74 levels in deferring (i.e. non-breeding) birds relative to those that laid (Crossin et al. 2012). In
75 contrast, body condition was not linked to breeding decisions by snow petrels (*Pagodroma*
76 *nivea*), or by black-legged kittiwakes (*Rissa tridactyla*), though clear hormonal differences
77 between deferring and breeding birds were evident (e.g. glucocorticoid levels [Goutte et al.
78 2010a; Goutte et al. 2010b]). Here, we examine the influence of body condition and endocrine
79 state on the breeding behaviour of gray-headed albatrosses (*Thalassarche chrysostoma*), and
80 draw comparisons with other albatrosses, and with birds more generally.
81 The gray-headed albatross usually breeds biennially if successful, and annually if failure
82 occurs in incubation or early chick-rearing. However, in any given year, there are also many
83 deferring individuals that do not follow this pattern and instead skip the opportunity to breed
84 (Ryan et al. 2007). Such decisions obviously have important implications for the trade-off
85 between current reproduction and survival, and affect lifetime fitness (Weimerskirch 1990;
86 Wooller et al. 1990; Chastel et al. 1995). For females, the decision to lay may depend on their
87 capacity to devote adequate resources to egg production without jeopardizing their own energetic
88 needs, as documented in many annually breeding species (Descamps et al. 2011). Body condition
89 upon arrival at the colony, after winter migration, should thus have some bearing on breeding
90 decision, with correlated effects on pre-breeding physiology. Previous studies of gray-headed
91 albatrosses and wandering albatrosses (*Diomedea exulans*) have shown that the HPG axes and
92 ovaries of mature but deferring females were seasonally responsive, but instead of secreting
93 testosterone (T) and 17-β estradiol (E_2), indicative of a commitment to reproduction, the ovaries
94 secreted progesterone (P4) (Hector et al. 1986a; Hector et al. 1986b; Hector et al. 1990). Without

95 T and E_2, the downstream activation of E_2-mediated vitellogenic pathways is not possible
96 (Williams et al. 2004b), thus rendering a female functionally or physiologically sterile. Whether
97 these patterns of steroidogenesis are mediated by variation in condition is not known, but in a
98 study of closely related black-browed albatrosses, females that deferred breeding after winter
99 migration had low energetic (body mass) and aerobic (Hct) condition measures, as well as low P4
100 and T levels (Crossin et al. 2012). That P4 levels were low rather than high in the deferring
101 black-browed albatrosses raises the intriguing possibility that the endocrine mechanisms
102 controlling breeding decision may differ between annual (black-browed albatrosses) and biennial
103 species (gray-headed and wandering albatrosses).

104 In response to these studies, we set out to test the hypothesis that body condition affects
105 pre-breeding endocrine physiology and subsequent breeding decision. To do this, we sampled
106 female gray-headed albatrosses as they returned from winter migrations to a large breeding
107 colony at Bird Island, South Georgia, in order to determine a suite of morphological,
108 hematological, and reproductive parameters. Working from a mechanistic perspective in which
109 improved body condition should influence P4 and T levels, and therefore subsequent investment
110 in reproduction, we hypothesized that measures of relative body condition would underlie
111 breeding decisions. We thus predicted that pre-breeding females in poor condition (e.g. low body
112 mass, low hematocrit [Hct] and hemoglobin [Hb]) would defer breeding. We also predicted that
113 body condition would influence which sex steroids were secreted by the ovary: females in poor
114 condition should secrete high levels of P4, and thus defer breeding, whereas those in good
115 condition should secrete T and eventually lay. Collectively, this predicts that a condition-
116 dependent P4 signal is the mechanism that determines breeding deferral in female gray-headed
117 albatrosses. If this prediction is supported, it would suggest that two closely related, sympatric

118 species of albatrosses have evolved very different physiological mechanisms for the control of
119 breeding decisions.
120
121 **Material and methods**
122 *Field collections*
123 Fieldwork was conducted during the austral summer beginning in September 2008/09 at a gray-
124 headed albatross breeding colony (Colony E) on Bird Island, South Georgia (54°01'S, 38°02'W).
125 Research was approved by the Ethics Committee of the British Antarctic Survey and carried out
126 under permits issued by the Government of South Georgia and South Sandwich Islands; the
127 procedures also conformed to guidelines established by the Canadian Committee on Animal Care
128 (Simon Fraser University Animal Care Permit # 897B-8).
129 Female gray-headed albatrosses were sampled upon their return to the colony after long,
130 pelagic migrations lasting 6-16 months (Croxall et al. 2005). Records from a long-term banding
131 program allowed us to generate a list of breeding-age females, which allowed us to identify
132 newly arrived females during daily colony visits beginning in mid September. Between 5-7
133 October, we sampled 15 birds when they were first sighted in the colony, which included
134 deferring breeders (N=9) and birds that went on to lay (N=6). Within this short window of time,
135 there was no difference in the mean arrival date of deferring and breeding birds (t-test, $F_{1,14}$=2.23,
136 P=0.159; mean date of egg laying for the colony was 21 October). Females were captured on
137 their nests, and 1 ml blood samples were taken from tarsal veins using heparinized syringes with
138 25G needles. Due to near freezing temperatures, it was not always possible to obtain a full 1 ml
139 of blood, and some samples were only approximately 0.25 ml, which limited the volume
140 available for some hormone assays. The time that it took to collect these samples, from our first
141 approach to the nest to end of blood sampling, was recorded to the nearest second. Blood was

142 then transferred to heparinized 2.5 ml Eppendorf vials and centrifuged for 5 min at 10,000 *g*.
143 Plasma was then transferred to labeled 0.6 ml vials for storage at -20 °C. We recorded body mass
144 (± 10 g), and culmen and tarsus lengths (both ± 1 mm). After sampling, we made weekly visits to
145 note breeding decision, and record dates of laying, hatching, failure (loss of an egg or chick), and
146 fledging.
147
148 *Blood and plasma analyses*
149 Hematocrit (Hct) was measured in fresh whole blood by centrifugation in microhematocrit tubes
150 for 5 min at 10,000 *g*, and is reported as packed cell volume (%). Hemoglobin (Hb, g dl^{-1} whole
151 blood) was measured with the cyanomethemoglobin method modified for use with a microplate
152 spectrophotometer, using 5 µl whole blood diluted in 1.25 ml Drabkin's reagent (D5941 Sigma–
153 Aldrich Canada, Oakville, Ontario, Canada). Absorbance was measured at 540 nm. Progesterone
154 (P4) and testosterone (T) were assayed in duplicate by liquid chromatography-tandem mass
155 spectrometry (LC-MS/MS) based on the method of Koren et al. (2012). Both steroids were
156 assayed in a single injection, starting from a sample volume between 50 and 100 ml. All samples
157 received a bio-identical deuterated internal standard representing a final concentration of 5 ng ml^{-1}
158 P4-d9 and 1 ng ml^{-1} T5-d2, and were diluted to 500 ml with water. Sample preparation
159 consisted of solid phase extraction over C18, with elution in 1 ml of ethyl acetate. Samples were
160 dried under nitrogen gas and reconstituted in 50% MeOH. Liquid chromatography (Agilent 1200
161 SL system) used an injection volume of 40 ml, a 100 x 300 mm Kinetex C18 Column
162 (Phenomenex), and water/methanol as mobile phases. Mass spectrometry (AB Sciex Q-trap
163 5500) used APCI +ve mode, with the following MRM transitions: Progesterone 315/97;
164 Progesterone-d9 324/100; Testosterone 289/97; Testosterone-d2 291/99. Quantitation was by

area ratio against the deuterated internal sample that had gone through sample preparation with the serum sample. Simultaneous assay for 17-ß estradiol (E_2) was not possible because the sample concentrations were too low for quantitation by this method, and the available sample volume was insufficient for a second, dedicated, LC-MS/MS run that would have been above the limit of detection.

Statistical analyses

Analyses were run with JMP 9.0 or SAS 9.0 software packages. All variables were tested for normality via plots of residuals against predicted values followed by Shapiro-Wilk tests. Data transformations were applied when residual distributions were non-normal. Correlations among all variables (date of colony arrival, body mass, Hct, Hb, P4, T) were examined through a Pearson's correlation matrix. Bonferroni corrections for multiple comparisons were applied when assessing significance levels (0.05/15=0.0033). Analysis of variance (ANOVA) tests were used to compare Hct between breeding and deferring birds (no covariates were included as Hct did not correlate with any other variable). Using long-term demographic data from our study colony, we also compared Hct in females according to whether they bred in previous year. Because arrival date was correlated with both body mass and P4, we accounted for this co-variation (analysis of covariance, ANCOVA) when comparing body mass and P4 levels between status groups (breeding *vs.* deferring). Because Hb and T were correlated with both arrival date and body mass, ANCOVA models must account for co-variation from both sources when comparing Hb, and when comparing T, between status groups. Ideally both date and mass would be used as covariates, but due to loss of degrees of freedom, we could not do so. We thus used residuals from a regression of body mass on arrival date as a single covariate in the Hb and T models. All ANCOVA models included interaction terms (e.g. main effect*covariate effect), but when these

were non-significant they were removed from final models to preserve degrees of freedom and increase statistical power (see Results). All values presented in figures are untransformed, least-squares means ± SEM.

Results

We sampled 15 female gray-headed albatrosses upon first arrival at nests after winter migration (9 deferring, 6 breeding). Among the variables (date of arrival, arrival body mass, plasma P4, plasma T, Hct, Hb, tarsus length, culmen length), body mass was significantly correlated with date of arrival ($r=0.606$, $P=0.022$), plasma T levels ($r=0.822$, $P=0.002$), and blood Hb levels ($r=0.666$, $P=0.009$). Date of arrival was also significantly correlated with plasma P4 levels ($r=0.632$, $P=0.021$). No other significant correlations were observed.

When comparing breeding and deferring birds, significant differences were observed in all endocrine and condition-related traits. Deferring females had significantly higher P4 levels (1.24 ng ml^{-1} ± 0.19) relative to breeding females (0.44 ng ml^{-1} ± 0.31) (ANCOVA, whole model $F_{2,13}=8.811$, $P=0.006$; main effect $F=6.591$, $P=0.028$, date of arrival covariate $F=15.156$, $P=0.003$) (Fig. 1a). Conversely, plasma T was higher in breeding females (6.52 ng ml^{-1} ± 1.05) than in deferring females (1.11 ng ml^{-1} ± 0.66) (ANCOVA, whole model $F_{2,13}=19.671$, $P<0.001$; main effect $F=17.866$, $P=0.002$, residual body mass covariate $F=1.573$, $P=0.241$) (Fig. 1b). There was a negative relationship between P4 and T ($P=0.013$, $N=15$): T decreased exponentially as P4 increased (Fig. 2).

Regarding condition traits, deferring females were significantly lighter than breeding females (3.44 kg ± 0.06 vs. 3.72 kg ± 0.09; ANCOVA, $F_{2,13}=9.708$, $P=0.003$; main effect $F=6.398$, $P=0.026$, date of arrival covariate $F=8.763$, $P=0.012$) (Fig. 3a). Deferring females also had lower Hct (38.3% ± 1.3 vs. 45.0% ± 1.7; ANOVA, $F_{1,14}=6.700$, $P=0.027$), and lower Hb

213 levels (14.7 g dl^{-1} whole blood ± 0.5 vs. 17.5 g dl^{-1} whole blood ± 0.9; ANCOVA, $F_{2,13}$=6.796,
214 P=0.012; main effect F=5.920, P=0.033, residual body mass covariate F=0.010, P=0.922) (Fig.
215 3b-c).

216 Whether a bird bred in the present year depended on its breeding status in the previous
217 year. Birds breeding in year x were significantly more likely to defer in year x+1, whereas birds
218 deferring in year x were more likely to breed in year x+1 (contingency analysis, χ^2 likelihood
219 ratio = 4.58, P = 0.032, N=15). Those same breeding birds in year x had significantly lower Hct
220 levels upon their arrival at the breeding colony in year x+1, relative to those that deferred in year
221 x but bred in year x+1 (ANOVA, $F_{1,12}$=5.486, P=0.037; Fig. 4).

222

223 **Discussion**

224 In this study we assessed the effects of body condition on patterns of ovarian steroidogenesis and
225 subsequent breeding decisions by post-migratory female gray-headed albatrosses. Our results
226 suggest that after pelagic a migration lasting 6-16 months (Croxall et al. 2005), breeding decision
227 is the cumulative effect of individual variation in pre-breeding state, which reflects variation in
228 both body condition and hormonal status at time of arrival at the breeding colony (e.g. circulating
229 sex steroid levels). Specifically, the condition measures of low body mass, Hct, and Hb
230 concentrations were associated with high ovarian P4 secretion and with breeding deferral. By
231 favouring the secretion of P4 instead of T at this early stage of the breeding season, deferring
232 females had presumably decided, long before their arrival at the breeding colony and reunion
233 with their mates, to physiologically preempted E_2 synthesis and vitellogenesis and thus their
234 commitment to reproduction. These results support the idea that a migratory carryover effect on
235 pre-breeding body condition constrained the allocation of resources to egg production via a

236 condition-dependent modulation of ovarian steroidogenesis. We also identified significant links
237 between aerobic capacity and breeding decision, which we discuss in the context of costs of
238 reproduction and migratory carryover effects. Collectively, we provide new physiological
239 insights to the mechanisms through which migratory carryover effects might constrain breeding
240 activity (reviewed by Harrison et al. 2011).
241
242 *Seasonality, ovarian steroidogenesis, and breeding decisions*
243 Breeding and deferring female gray-headed albatrosses had elevated levels of ovarian sex-
244 steroids upon arrival at the colony, which contrasts with the basal levels that occur towards the
245 end of the breeding season (Hector et al. 1986a). This suggests that all the individual birds in our
246 study were responding to seasonal cues via HPG up-regulation, which presumably involved the
247 secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, and of
248 gonadotropins from the pituitary (luteinizing hormone, LH; follicle stimulating hormone, FSH)
249 (Dawson 2008). Our results are supported by an earlier study of gray-headed albatrosses in which
250 breeding and deferring birds exhibited hormonal profiles indicative of an active HPG axis, and
251 that despite great inter-individual variation, all birds had higher LH levels when they arrived at
252 the colony than later in the breeding season (Hector et al. 1986b). Together with our data, this is
253 consistent with the hypothesis that day length or photoperiod is the principal *zeitgeber* of the
254 annual cycle, controlling seasonal events like the timing of the spring migrations back to the
255 breeding areas and regulation of the initial recrudescence and development of the reproductive
256 axis prior to breeding (Dawson 2008).
257 Despite their apparent seasonal responsiveness in terms of gonadotropin release, breeding
258 and deferring females in our study differed markedly at the downstream end of the HPG axis, at
259 the level of the ovary. Patterns of hormonal secretion differed between birds according to their

eventual breeding fate, with the ovaries of deferring females favouring P4 secretion (as previously observed, [Hector et al. 1986a]; [Hector et al. 1986b]; [Hector et al. 1990]). For example, Hector et al. (1986b) suggested that ovarian P4 secretion by mature females represents a functional block to vitellogenesis and follicle development. By secreting P4 instead of converting this to T and then to E_2, the female is left functionally sterile for the current annual cycle (Hector et al. 1986a; Hector et al. 1990). This occurs in the ovarian theca cells surrounding the developing follicles, where P4 is synthesized. Although we did not measure E_2 in this study (due to a lack of plasma), our interpretation of the condition and hormonal data provide a logical explanation for the breeding patterns that we observed. Having said that, estrogen synthesis can also occur via the adrostenedione (A4) pathway, which involves neither P4 nor T, and hence measurement of A4, and indeed E_2, would be worthwhile in future studies.

Working through the P4 pathway of ovarian development places this all in context. P4 is the first sex-steroid produced by the ovary in response to luteinizing hormone (LH), and is itself a precursor for other steroids including T and E_2. Under normal circumstances for birds about to breed, P4 is then converted to the androgens in the granulosa cells, which are then converted to the estrogens. As our data show, an absence of T when P4 is high suggests a low activity of 17α-hydroxylase and 17,20 lyase (known collectively as hydroxylase-lyase) in the theca. Because LH is generally accepted as the activating agent for the enzymes controlling P4 synthesis, and follicle stimulating hormone (FSH) as the activating agent for enzymes controlling androgen synthesis, we postulate a potential inhibition at the level of FSH, although this would need to be confirmed with controlled experimental studies. Whatever the mechanism, this endocrine tactic, where P4 secretion by deferring females favours self-maintenance at the expense of reproductive investment, has been previously observed in biennially breeding albatrosses, notably in the wandering albatross (Hector et al. 1986a; Hector et al. 1990), and previously in the gray-headed

albatross (Hector et al. 1986b). What is intriguing is that this pattern differs markedly from a closely-related but annual breeder, the black-browed albatross, in which both deferring and breeding females arrive at the colony with up-regulated T levels (Crossin et al. 2012). This suggests that all female black-browed albatrosses arrive at the colony physiologically ready to breed, but that the decision to defer is then made subsequently. This raises the possibility that two very different regulatory mechanisms have evolved to control breeding in sister species of the genus *Thalassarche*.

Role of body condition

Although the relationship is not universal, many studies show that low body condition tends to be associated with deferred breeding activity, which suggests an energetic threshold to breeding (e.g. the 'prudent parent' hypothesis (Drent and Daan 1980; Descamps et al. 2011). Consistent with several (but not all) seabird studies, our data show clearly that low body mass characterized deferred breeding, with deferring females weighing nearly 500g less than breeding females. However, unlike Hector et al. (1986a, 1986b), we also provide a link between condition and steroidogenesis in gray-headed albatrosses, which suggest a condition dependent mechanism of breeding decision. How condition might influence patterns of ovarian steroidogenesis is not presently known. One possibility is that increased glucocorticoid secretion (e.g. corticosterone), due to pre-breeding nutritional and/or other stressors encountered before the return to the breeding colony, could suppress ovarian function as observed in other seabirds (Goutte et al. 2010a). From this perspective, P4 secretion could be viewed as an indirect signal of a stress response. By forgoing conversion to T and a commitment to reproduction, P4 is made available as a substrate for glucocorticoid synthesis, an important component of the acute stress response and emergency energy mobilization (via 21-hydroxylase and 11β-hydroxylase activity). P4 is

thus an important precursor at a crossroad between the androgenic and glucocorticoid biosynthetic pathways, and represents a key deterministic step in the regulation of breeding decisions in female gray-headed albatrosses. Whether this process is mediated by or simply correlated with elevated plasma glucocorticoid levels requires further study (Goutte et al. 2010a; Goutte et al. 2010b).

Aerobic condition and implications for carryover effects breeding decisions

In addition to poor body condition (e.g. low body mass), deferred breeding by female gray-headed albatrosses was also associated with low Hct and Hb concentrations, two traits that reflect aerobic performance and oxygen transport capacity (Wagner 1996) and which are key for sustaining the high energetic costs of flight. An interesting avenue for future study is the role that phenotypic variation in the aerobic capacity and oxygen transport capacities of blood plays in breeding decisions (Calbet et al. 2006; Williams 2012). We have shown a similar pattern previously in black-browed albatrosses, where breeding deferral was also associated with reduced Hct and Hb concentration (Crossin et al. 2012). We suggest two possible explanations for reduced hematocrit at arrival: it either reflects foraging conditions and success during the latter part of the non-breeding period, and in particular, potentially the short-term cost of the final few days spent in flight back to the colony, or it indicates a longer-term "cost of reproduction" in the form of reproductive anemia stemming from the previous breeding season. Although there is an intuitive appeal to the idea that long-distance flight could exact a cost in the form of reduced hematocrit, this is not consistent with studies that identify Hct up-regulation as an adaptation to increase oxygen-carrying capacity in migrating birds (Bairlein and Totzke 1992; Piersma et al. 1996; Landys-Ciannelli et al. 2002).

331 Conversely, there is evidence for long-term costs of reproduction via E_2-mediated
332 reproductive anemia (Williams et al. 2004a). By experimentally increasing the cost of current
333 reproduction in great skuas (*Stercorarius skua*), Kalmbach et al. (2004) show that increased
334 levels of egg production via egg removals increased E_2-mediated vitellogenesis, which had the
335 consequence of reduced hematocrit and red blood cell numbers that persisted for more than a
336 year, spanning winter migration and parts of the next breeding season. This suggests that
337 hematocrit reductions and reproductive anemia might be proportional to reproductive effort, such
338 that females laying more (or any) eggs may incur higher costs of reproduction relative to those
339 laying fewer (or none). Although we did not measure pre-migratory Hct levels in the albatrosses
340 in this study, we do know the breeding histories for each individual female that we sampled. As
341 we have shown in Fig. 3, deferring females had significantly lower post-migratory (pre-laying)
342 Hct levels than breeding females (Fig. 3b). But when we group females according to their
343 previous year's breeding outcome, current post-migratory Hct levels were significantly lower in
344 those females who bred in the previous year than those which did not breed (Fig. 4). This
345 suggests a potential cost of reproduction on Hct levels (i.e. reproductive anemia) which carried
346 over to the next breeding season and led in part to deferred breeding, but to confirm this we
347 would need systematic measurements of Hct levels in individuals before and after migration
348 (repeated measures). Nevertheless, this seems an intriguing possibility that could explain the low
349 body mass of deferring females in this study. If females did suffer from reproductive anemia in
350 the previous year, then this cost of reproduction could have persisted through the winter to
351 influence future breeding activity, as observed by Kalmbach et al. (2004). If this is the case, then
352 reduced Hct would limit aerobic capacity, and thus migratory efficiency in terms of energy use
353 and foraging efficiency, resulting in the lower pre-breeding body masses that we measured in
354 deferring birds upon arrival at the breeding colony. Certainly though, reductions in Hct could

occur due to events experienced during winter migrations independently from reproductive anemia. Albatrosses migrating for 6-16 months throughout the Southern Ocean experience frequent storms and other challenges, so it is feasible that anemia could develop in some individuals who have difficulty coping, or that such environmental conditions could exacerbate a pre-existing reproductive anemia. Whatever the case, the important point is that previous studies have linked Hct to aerobic capacity and flight performance (Hammond et al. 2000). Some suggest that Hct can be adaptively regulated to match the aerobic demands associated with specific life-history events like migration (Bairlein and Totzke 1992; Piersma et al. 1996; Landys-Ciannelli et al. 2002), which could form the basis for potential trade-offs. Our results are consistent with this idea, but experimental work is still needed to firmly establish the relationships between haematological status, aerobic capacity, workload, individual quality, and trade-offs, including costs of reproduction and carryover effects.

Acknowledgements

We extend thanks to Derren Fox for field support, to Lea Bond for laboratory support, and to Andy Wood for data support. Financial support was provided by the British Antarctic Survey through an Antarctic Funding Initiative Collaborative Gearing Scheme. Additional support was provided by a National Science and Engineering Research Council of Canada (NSERC) Post-doctoral Fellowship and NSERC E-BIRD funding to GTC, and by NSERC Discovery Grants to TDW and KEWE. This study represents a contribution to the British Antarctic Survey Ecosystem Programme.

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467

468 **Figure Legends**

469 Fig. 1: Endocrine differences (progesterone [P4], panel A; testosterone [T], panel B) between
470 breeding and deferring gray-headed albatrosses, measured upon their return to a breeding colony
471 at Bird Island, South Georgia, after winter migration. Columns represent least-squares means
472 (ANCOVA) +SEM. Fifteen females were sampled (9 deferring, 6 breeding).

473

474 Fig. 2: The relationship between pre-breeding progesterone (P4) and testosterone (T)
475 concentrations is negatively exponential in female gray-headed albatrosses. White circles indicate
476 breeding individuals, black circles are deferring individuals. Albatrosses were sampled at the end
477 of migration upon first arrival at the colony.

478

479 Fig. 3: Energetic (panel A) and aerobic (panels B and C) traits differ significantly between
480 breeding and deferring gray-headed albatrosses. Individual females were sampled on arrival at
481 the breeding colony. Columns represent least-squares means (ANCOVA) +SEM.

482

483 Fig. 4: Hematocrit levels in post-migratory female gray-headed albatrosses were significantly
484 influenced by their breeding status in the previous year. Females that bred in year x had
485 significantly reduced Hct before breeding in year x+1. Columns represent least-squares means
486 (ANCOVA) +SEM.

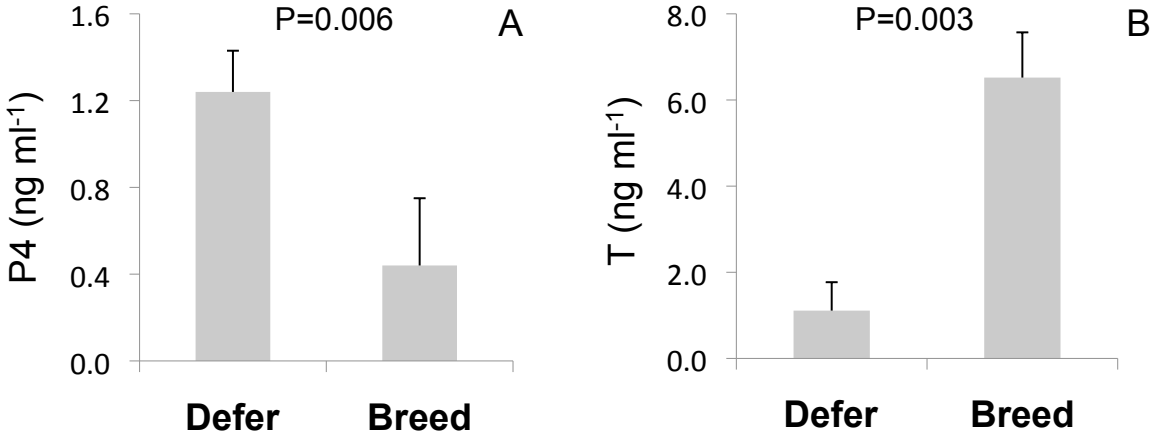
P=0.006
A
1.6
1.2
0.8
0.4
0.0
P4 (ng ml-1)
Defer
Breed
P=0.003
B
8.0
6.0
4.0
2.0
0.0
T (ng ml-1)
Defer
Breed

Fig. 1

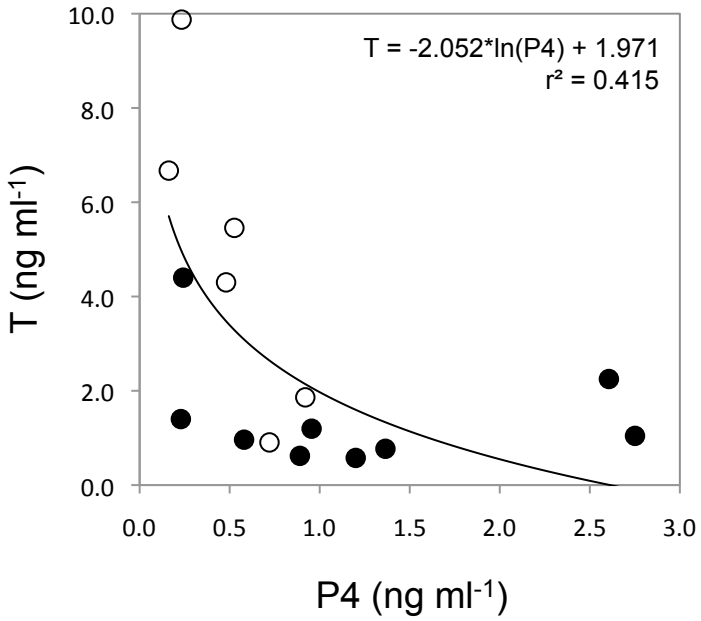
T = -2.052*ln(P4) + 1.971
r² = 0.415
T (ng ml-1)
P4 (ng ml-1)
10.0
8.0
6.0
4.0
2.0
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 2

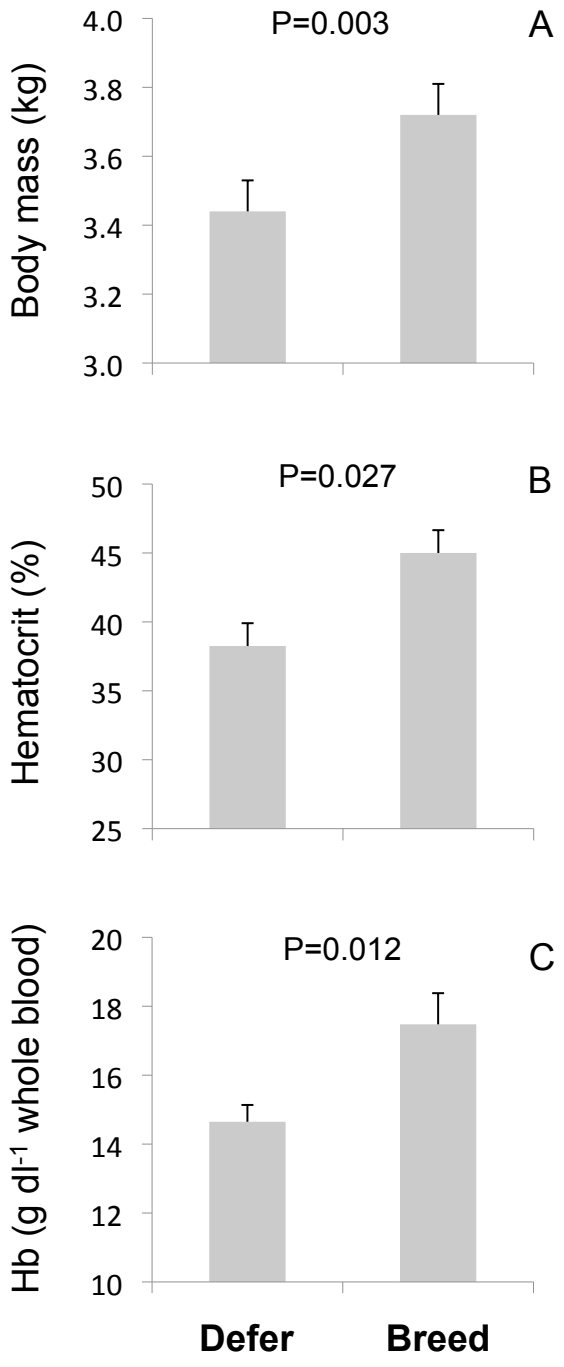
P=0.003
A
Body mass (kg)
4.0
3.8
3.6
3.4
3.2
3.0
P=0.027
B
Hematocrit (%)
50
45
40
35
30
25
P=0.012
C
Hb (g dl-1 whole blood)
20
18
16
14
12
10
Defer
Breed

Fig. 3

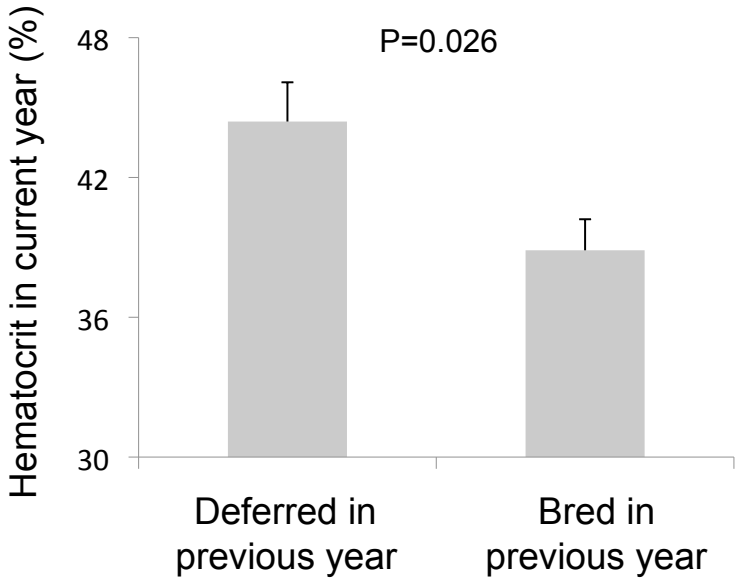
Hematocrit in current year (%)
48
42
36
30
P=0.026
Deferred in previous year
Bred in previous year

Fig. 4