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Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses

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ABSTRACT

Physiological mechanisms mediating carryover effects, wherein events or activities occurring in one season, habitat, or life-history stage affect important processes in subsequent life-history stages, are largely unknown. The mechanism most commonly invoked to explain carryover effects from migration centres on the acquisition and utilization of resources (e.g. body mass, or individual 'condition'). However, other mechanisms are plausible, e.g. trade-offs reflecting conflict or incompatibility between physiological regulatory systems required for different activities or life-history stages (migration vs. reproduction). Here we show that in female black-browed albatrosses (*Thalassarche melanophris*) the decision to reproduce or to defer reproduction, made prior to their arrival at breeding colonies after long-distance migration, is associated with condition-related (body mass, hematocrit, hemoglobin concentrations) and hormonal (progesterone, testosterone, estrogen-dependent yolk precursors) traits. In contrast, reproductive success showed little association with condition but showed significant associations with the steroidogenic processes underlying follicle development. Specifically, success was determined by reproductive readiness via differences in steroid hormones and hormone-dependent traits. Successful albatrosses were characterized by high progesterone and high estradiol-dependent yolk precursor levels, whereas failed albatrosses had high testosterone and low yolk precursor levels. Results are discussed with reference to migratory carryover effects and how these can differentially affect the physiologies influencing reproductive decisions and reproductive success.

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1. Introduction

An ever-increasing number of studies are documenting the existence of carryover effects in animal populations, wherein events or activities occurring in one season, habitat, or life-history stage affect important processes at subsequent stages (see reviews by [16,25]). The majority of studies examining carryover effects have examined the influence of over-wintering and migratory experiences on aspects of reproduction including breeding decisions, timing of reproduction, reproductive output, and in some cases reproductive success [16]. However, despite the growing number of studies examining these phenomena, our understanding of the physiological and hormonal mechanisms driving carryover effects remains rudimentary.

In migratory animals, the mechanism most commonly invoked to explain carryover effects on reproduction centres on the acquisition and utilization of resources, i.e. differences in individual condition. Under such a model, the events and activities occurring at the transition from the migratory non-breeding period in winter to spring breeding can "carry over" to affect patterns of resource acquisition and utilization which directly determine reproductive success [2,16,20,23,24,28]. Some studies show links between individual condition at arrival and aspects of reproduction (laying date and egg size), but individual variation in these traits tends to be high and their direct effects on reproductive success are currently largely speculative (but see [11]). As an alternative hypothesis, there is growing evidence that endocrine processes themselves might mediate carryover effects to influence reproductive decisions. For example, Goutte et al. [13] showed that in an annually-breeding seabird, elevated baseline corticosterone levels during the pre-laying period after migration were associated with a higher probability of deferred breeding, and speculated that elevated corticosterone might inhibit luteinizing hormone (LH) and the

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downstream secretion of sex-steroids necessary for reproduction. Other studies have shown that non-breeding or deferring birds are fully capable of elevating LH, when presented with a luteinizing hormone releasing hormone (LHRH) challenge, but they do not or cannot sustain gonadal steroid production [5,14,15]. These studies suggest that the metabolic demands of migration might down-regulate the reproductive axis in terms of gonadal steroid production, an effect that might be mediated by, or simply correlated with, elevated corticosterone levels and/or inhibition of LH. Few studies have considered individual variation in circulating gonadal steroids at arrival in migratory birds in relation to subsequent reproduction. Furthermore, the studies cited above only considered breeding decisions as breeding/not breeding (all-or-none decisions), and did not extend the analysis of hormonal patterns at arrival to consider the consequence of the decision to breed in terms of success or failure. Recently, Crossin et al. [8] showed that migratory carryover effects can place constraints on the estrogen-dependent production of yolk precursors by egg-producing females, with negative effects on egg sizes, and that these effects are unrelated to variation in individual condition. However, the functional significance of reduced or constrained sex-steroid production, during the migration-reproduction transition, to reproductive success remains largely unknown. Nevertheless, what is emerging is the recognition that condition (or resource state) at arrival need not be the only mechanism affecting reproductive success. The endocrine processes controlling female reproduction may be directly affected by migratory carryover effects, via constraints on, or incompatibilities between, competing physiological regulatory systems [8,17,37].

Procellariiforme seabirds (the albatrosses and petrels) are pelagic species which also make good models for exploring the effects of migratory activity on the hormonal processes underlying reproduction. Like many seabirds, the Procellariiformes have a slow life-history characterized by delayed age of reproduction, a single-egg clutch, prolonged parental care, and longevity, and they make very long, pelagic migrations during a non-breeding period which lasts 6–16 months in *Thalassarche* spp. [9,26]. Among breeding-age albatrosses and petrels, there is typically a high proportion of non-breeding individuals, and among even the most experienced breeders, breeding deferral for one or more years is a common tactic underlying lifetime fitness [27,36]. The annual breeding pattern of black-browed albatrosses (*Thalassarche melanophris*) make this species a particularly tractable model for the study of carryover effects. Each year, black-browed albatrosses return to breeding colonies from distant foraging areas occupied during the non-breeding period [26], and in the weeks preceding arrival, birds are presumably balancing their own physiological requirements against those needed for the initiation of reproduction. Upon arrival at breeding colonies, they then experience one of three inevitable outcomes: they will defer reproduction entirely until the next season, or they will lay and then either fail or succeed at fledging a chick [26].

In this study, we investigate the physiological mechanisms that mediate reproductive decisions (breeding or deferral) and reproductive outcomes (success or failure) in female black-browed albatrosses during the transition between winter migration and spring breeding. Specifically we consider condition or resource-related traits (mass, hematology), as well as the steroidogenic hormones and estrogen-dependent yolk precursor pathways underlying reproductive readiness (*sensu* [8]), in mediating decisions and outcomes. We predicted that the decision to lay or to defer would be condition-dependent, and that females deferring reproduction would be in poorer condition (low body mass, low hematocrit [Hct] and hemoglobin [Hb] concentrations) relative to breeding females. However, we also predicted that reproductive success (e.g. fledging of chicks) might reflect differences in “reproductive readiness” upon arrival at the colony, as indicated by the estrogen-

dependent production of the yolk precursors vitellogenin [VTG] and yolk-targeted very low density lipoprotein [VLDL], as well as by patterns of production of the reproductive steroid hormones progesterone [P4] and testosterone [T].

2. Materials and methods

2.1. Study site and field sampling protocol

Fieldwork was conducted during the austral summer beginning in September 2008 at a large long-term demographic study-colony of black-browed albatrosses (Colony J) breeding on Bird Island, South Georgia (54°01'S, 38°02'W). Research was conducted through permits issued by the British Antarctic Survey, and conformed to guidelines established by the Canadian Council on Animal Care (Simon Fraser University Animal Care Permit # 897B-8).

Beginning in late September when black-browed albatrosses return from sea, we made daily visits to the colony in order to identify newly arrived females with previous breeding experience. All females had first bred at least six years previously, and were identifiable by unique leg rings. We captured newly arrived females ($N = 33$) at nest sites and collected 2 ml blood samples from tarsal veins using heparinized syringes fitted with 25G needles. We recorded the time (± 1 s) from initial approach until the end of blood sampling for each bird. Blood was then transferred to heparinized 2.5 ml Eppendorf vials, centrifuged for 5 min at 10,000g, and the isolated plasma was transferred to labelled 0.6 ml vials for storage at -20°C . We recorded body mass (± 10 g), and measured bill length and tarsus length (both ± 1 mm). After sampling, we continued daily (later reduced to weekly) visits to monitor breeding fate, noting dates of laying, hatching, failure (loss of an egg or chick), and fledging.

2.2. Blood and plasma analysis

Blood samples were assayed in duplicate for hematocrit and hemoglobin concentrations. Hematocrit (Hct) was measured on fresh whole blood at the time of blood sampling as reported as packed cell volume (%) following centrifugation of whole blood in microhematocrit tubes for 5 min at 10,000g. Hemoglobin (Hb, g dl^{-1} whole blood) was measured with the cyanomethemoglobin method modified for use with a microplate spectrophotometer, using 5 μl whole blood diluted in 1.25 ml Drabkin's reagent (D5941 Sigma–Aldrich Canada, Oakville, Ontario, Canada). Absorbance was measured at 540 nm.

Progesterone (P4) and testosterone (T) were assayed in duplicate from each of 33 samples by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Both steroids were assayed in a single injection, starting from a sample volume between 50 and 100 μl . All samples received a deuterated internal standard representing a final concentration of 5 ng ml^{-1} P4-d9 and 1 ng ml^{-1} T5-d2, and were diluted to 500 μl with water. Sample preparation consisted of solid phase extraction over C18, with elution in 1 ml of ethyl acetate. Samples were dried under nitrogen gas and reconstituted in 50% MeOH. Liquid chromatography (Agilent 1200 SL system) used an injection volume of 40 μl , a 100 \times 300 mm Kinetex C18 Column (Phenomenex), and water/methanol as mobile phases. Mass spectrometry (AB Sciex Q-trap 5500) used APCI +ve mode, with the following MRM transitions: Progesterone 315/97; Progesterone-d9 324/100; Testosterone 289/97; Testosterone-d2 291/99.

Quantification of E_2 was not possible at these volumes as sample preparation and optimal LC–MS conditions at sample concentrations (based on pilot analysis of a pooled sample) differed from the conditions for testosterone and progesterone. However,

the principal role of E_2 secretion is the production of the yolk precursors vitellogenin (VTG) and yolk-targeted very low density lipoprotein (VLDL_y), which are a strong indicator of reproductive readiness [8 and references therein].

Plasma samples were assayed in duplicate for vitellogenic zinc (Zn; zinc kit, Wako Chemicals) and total triglycerides (Glycerol Reagents A and B, Sigma), as indices of the yolk precursors VTG and VLDL_y, respectively (e.g. [8]). VTG and VLDL_y are the two main yolk precursors in birds and are transported from the liver to the ovary where they are deposited into developing follicles. Of the two precursors, VTG is generally considered the more reliable indicator of follicle development in birds [8]. All assays were measured using a Biotek 340i microplate reader. Using a 19-week domestic laying hen (*Gallus domesticus*) plasma pool, intra-assay coefficients of variation for VTG and for VLDL_y ranged from 5.9% to 8.2% and 6.6% to 8.7%, respectively. Inter-assay coefficients of variation were 7.8% for VTG and 6.9% for total VLDL_y.

2.3. Statistical analyses

Analyses were run with either the JMP 8.0 or SAS 9.0 software packages. All variables were tested for normality, as were residuals from plots against predicted values, using Shapiro-Wilk tests. Data transformations were applied when distributions were non-normal. Analysis of covariance (ANCOVA) tests were used to examine breeding-group differences (e.g. successful, failed, and deferring breeders) in VTG, VLDL_y, Hct, Hb, T, P, and body mass. As many studies show links between age and individual quality and breeding output, we included age and a measure of individual quality as a covariates in all analyses. We defined individual quality as the number of chicks fledged (i.e. breeding output) during the previous 6 year period (i.e. recent breeding experience). We also examined whether a female bred successfully or not, or deferred, in the previous year alone (i.e. immediate breeding history). However, because age and the two quality measures are co-linear, we used the covariates in separate models exploring each response variable listed above. Tukey–Kramer *post-hoc* tests were run after univariate tests to identify significant contrasts between breeding groups. Correlations among all variables with dates of colony arrival and with individual quality measures were examined through a Pearson's correlation matrix.

3. Results

Female black-browed albatrosses in this study returned to the breeding colony on 10–17 October 2008, which is consistent with data from previous years (e.g. [26]). Of the 33 females that we sampled, 8 deferred reproduction entirely, 8 laid eggs which either failed to hatch ($N=5$), or hatched but failed shortly thereafter ($N=3$), and 17 fledged chicks successfully. Mean dates of egg-laying occurred approximately 15 days after arrival (range 8–18 days), with a median lay-date of 26 October. Based on observed arrival and laying dates, all 24 females that laid were sampled during the 1–2 day period when newly arrived birds reunite with their mates at nest sites before departing for an approximately 10–12 day pre-laying exodus. So on average, all breeding females were sampled approximately 15 days into the rapid yolk deposition phase of egg production when VTG levels are held at maximal levels (in black-browed albatrosses, $RYD = 20.4 \pm 1.1$ days; [3]). All 8 deferring females were sampled over the same 7-day period as all the breeding females.

No measured variables were significantly correlated with arrival date (body mass, tarsus length, bill length, Hct, Hb, VTG, VLDL_y, T, P; all $P > 0.10$, $N \sim 33$ individuals per variable). Furthermore, no variables were related to age (mean age 22 years, range 12–30), or to individual quality when defined as the number of chicks fledged

during the previous 6 year period, or whether a female bred in the immediately preceding year (in all models, both $P > 0.10$). In other words, variation in age and reproductive quality (i.e. reproductive output over the last 6 years) did not significantly affect our subsequent analyses and conclusions. Body mass did not correlate with either tarsus or bill length (both $P > 0.20$), and so the analysis of body mass did not necessitate correction for structural differences in body size (e.g. analysis of covariance or principal components analysis).

Supporting our first prediction, body mass at colony arrival differed among females with differing breeding outcomes (whole model, $F_{2,30} = 4.7$, $P = 0.011$), independently of age effects ($P = 0.26$). Successful females weighed 3.83 ± 0.07 kg, which was significantly heavier (*post hoc* contrast, $P = 0.006$) than deferring females which weighed 3.53 ± 0.10 kg. Failed breeders were intermediate in mass, and weighed 3.62 ± 0.10 kg (Fig. 1A) which was not significantly different from either successful ($P = 0.150$) or deferring ($P = 0.289$) females. Therefore, as predicted, deferring birds were significantly lighter than successfully breeding birds. When we pooled failed and successful birds and compared them to deferring birds, the breeding birds were still significantly heavier than deferring birds (3.79 ± 0.06 kg vs. 3.52 ± 0.09 kg; $F_{2,30} = 4.4$, $P = 0.016$). Hct, but not Hb, also supported our first prediction (Fig. 1B and C), and Hct levels followed the same trend as body mass. Hct was significantly higher in successful ($41.7 \pm 0.8\%$; $P = 0.004$) and failed ($41.3 \pm 1.2\%$; $P = 0.033$) breeders than in deferring breeders ($37.8 \pm 1.0\%$) ($F_{2,30} = 5.18$, $P = 0.012$). Hb did not differ significantly between breeding categories ($F_{2,30} = 0.739$, $P = 0.486$).

Also supporting our second prediction, differences in reproductive readiness, as measured by differences in sex steroid hormone and yolk precursor levels (Fig. 1D–G), varied in relation to reproductive status independently of age and breeding quality effects. Differences in P4 (Fig. 1D) showed that successful ($P = 0.009$) and failed ($P = 0.030$) females had higher (up-regulated) concentrations than deferring females (successful = 1.71 ± 0.38 ng ml⁻¹, failed = 1.41 ± 0.57 ng ml⁻¹, deferring = 0.34 ± 0.53 ng ml⁻¹; $F_{2,26} = 4.10$, $P = 0.028$). T was significantly higher in failed females (2.79 ± 0.50 ng ml⁻¹) relative to both successful ($P = 0.003$) and deferring ($P = 0.016$) females (successful = 1.20 ± 0.28 ng ml⁻¹, deferring = 0.68 ± 0.39 ng ml⁻¹) ($F_{2,27} = 5.62$, $P = 0.009$; Fig. 1E). VTG varied significantly ($F_{2,21} = 7.33$, $P = 0.004$; Fig. 1G), with higher concentrations in breeding females (2.91 ± 0.34 μg Zn ml⁻¹) than in both deferring (0.74 ± 0.42 μg Zn ml⁻¹, $P = 0.012$) and failed (1.21 ± 0.46 μg Zn ml⁻¹, $P = 0.032$) females. VLDL_y concentrations however did not vary significantly among deferred, failed and successful females ($F_{2,22} = 2.05$, $P = 0.15$; Fig. 1F).

Among the breeding birds, T and P4 were related to VTG concentrations but in opposite directions: P4 was positively related with VTG in a natural log manner ($r = 0.64$, $P = 0.001$, $N = 14$, Fig. 2A), while T was negatively related to VTG ($r = -0.56$, $P = 0.007$, $N = 16$, Fig. 2B). T and P4 were not significantly correlated in the breeding birds ($r = -0.22$, $P = 0.32$, $N = 24$), or in all breeding and deferring birds collectively ($r = -0.15$, $P = 0.42$, $N = 33$).

Using regression analysis, date of breeding failure (known for 7 of the 8 failed females) was significantly related to pre-breeding T, VTG, and VLDL_y concentrations ($N = 7$ females, all $P < 0.017$, Fig. 3A–C). T tended to be higher in females which failed shortly after laying relative those failing later. Similarly, VTG and VLDL_y were generally lower in females failing early, and higher values were associated with later failure. In contrast, pre-breeding body mass was not significantly related to date of breeding failure ($P = 0.128$, Fig. 3D). Multiple logistic regression analysis revealed a significant role of VTG in predicting breeding success or failure ($\chi^2 = 4.98$, $P = 0.026$), but the influence of body mass was not significant ($\chi^2 = 1.13$, $P = 0.287$). In other words, resource-independent VTG

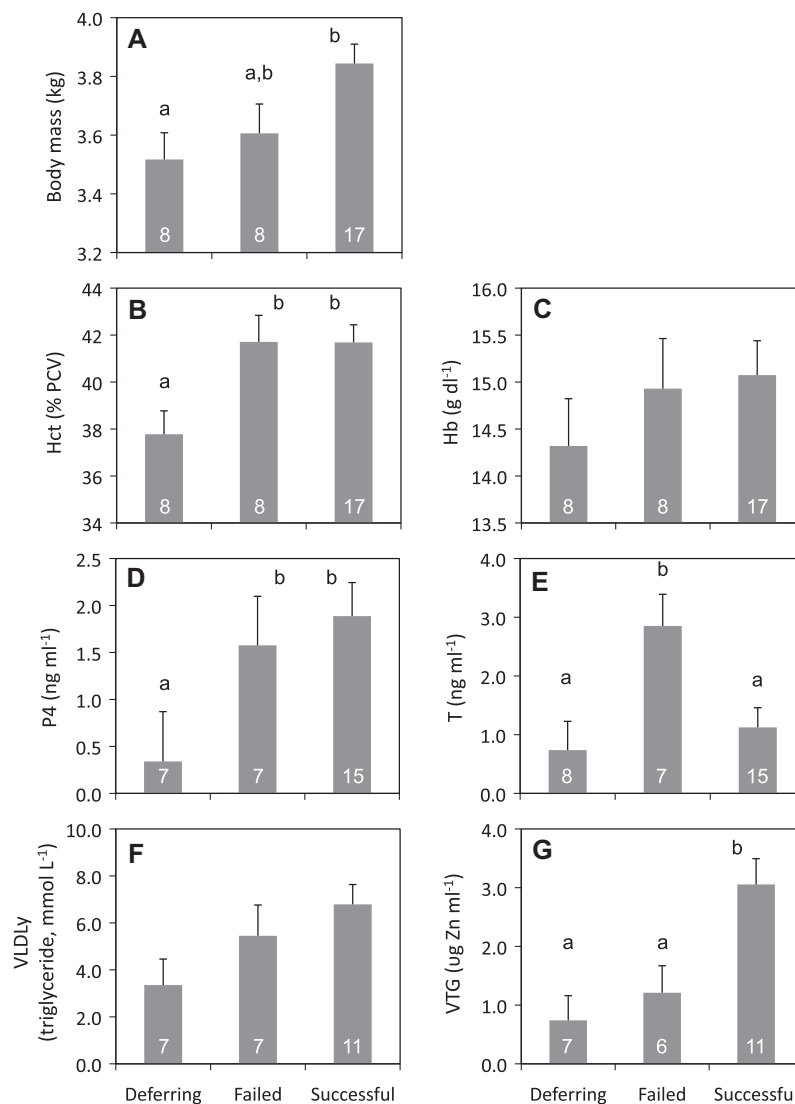


Fig. 1. Body mass (A), hematological (B and C), hormonal (D and E), and yolk precursor (F and G) profiles in pre-breeding female black-browed albatrosses (*Thalassarche melanophris*) upon arrival at a breeding colony at Bird Island, South Georgia. Birds are grouped according to breeding outcome, and bars represent least square means \pm SEM. Numbers at the base of bars indicate sample sizes. Differing letters indicate statistical significance when $\alpha = 0.05$. Note that when failed and successful birds are pooled in Panel A, the deferring birds are significantly lighter (see Section 3 for details). Abbreviations: Hct, hematocrit; Hb, hemoglobin; P4, progesterone; T, testosterone; VLDL, yolk-targeted very low density lipoprotein; VTG, vitellogenin.

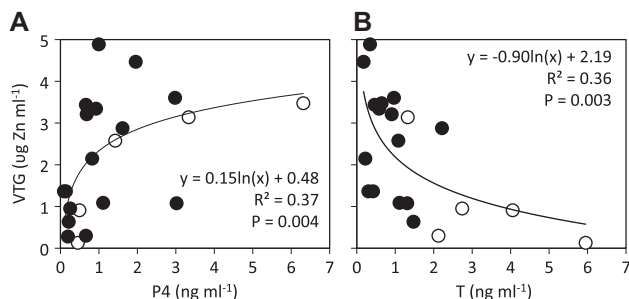


Fig. 2. Relationship between plasma vitellogenin and progesterone (A) and testosterone (B) in pre-breeding female black-browed albatrosses (*Thalassarche melanophris*) which eventually laid eggs. Open circles indicate failed breeders and closed circles are successful breeders. Lines are natural log fits. See Fig. 1 for key to abbreviations.

4. Discussion

In this study we show that after long-distance migrations to a breeding colony, reproductive decisions (breeding or deferring) and reproductive outcome (successful or failed) in female black-browed albatrosses are associated with marked differences in patterns of circulating gonadal steroids (P4, T) and steroid-dependent traits (yolk precursors). Previous studies of black-browed albatrosses have shown that all breeding-age females generally return to colonies at the start of the breeding season, whether they breed or not, and that plasma LH levels are elevated upon arrival [18]. It is therefore parsimonious to assume that day-length dictates the timing of their return to colonies, and that this apparently programmed seasonality in migratory behaviour is sufficient to “switch on” the reproductive axis at the level of the hypothalamus and pituitary as in most temperate-breeding birds [10]. However, our data clearly show that female black-browed albatrosses are not all equally prepared for reproduction upon arrival in terms of “downstream” components of the reproductive axis. As we predicted, deferring females had low plasma progesterone (P4), and

effects were more strongly predictive of breeding success than resource-dependent mass effects.

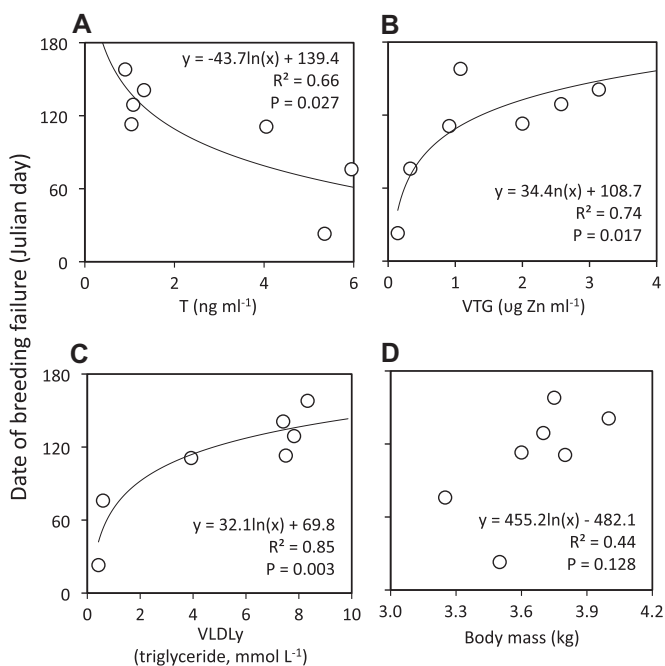


Fig. 3. Relationships of testosterone (A), vitellogenin (B), very low density lipoprotein (C), and body mass (D) with date of breeding failure in female black-browed albatrosses. Lines are natural log fits. See Fig. 1 for key to abbreviations.

consequently low testosterone (*T*) and low estrogen-dependent vitellogenin (VTG) levels, which suggests that the decision to defer reproduction by these annually breeding birds was made prior to arrival perhaps due to “stressful” metabolic demands incurred at sea during the over-winter or migratory phase, i.e. carryover effects. Also as predicted, successful females had high plasma P4 and high VTG, but low *T*, which indicates a responsiveness of the ovary to stimulation by pituitary LH. The low plasma *T* in these successfully breeding birds is presumably due to rapid conversion (aromatization) to E_2 to support vitellogenesis, as indicated by the high VTG levels that we measured. The failed breeders however are especially interesting as they had high P4 and high *T*, which like the successful breeders indicates gonadal sensitivity to LH secretion, but these females had low VTG which suggests a possible disruption of aromatization (as per [12]) in the E_2 -mediated vitellogenic pathway. Ultimately, and rather surprisingly, VTG in pre-laying females predicted not only their breeding success or failure, but also the length of time from their arrival at the colony to breeding failure (Fig. 3B). This suggests very strongly that hormonal differences among individuals at colony arrival, during the transition from migratory to reproductive states, affect not only breeding decisions but also the degree of reproductive readiness with consequences for reproductive success. Furthermore, these effects influence processes at the *downstream* end of the reproductive axis, at the level of ovarian steroidogenesis and E_2 -dependent regulation of hepatic vitellogenesis.

Collectively, our data suggest that reproductive decisions (breeding or deferral) and reproductive success are influenced by two very different mechanistic pathways in female black-browed albatrosses. In addition to hormonal differences, the decision to defer reproduction was associated with more traditional measures of individual condition, characterized by lower body mass and lower hematocrit levels at arrival relative to breeding individuals. This is consistent with previous studies of breeding deferral in seabirds (blue petrels [7]; gulls [19]; shearwaters [21]), and with the idea that the initiation of breeding attempts is resource- or condition-dependent. Furthermore, the low P4 and *T* levels in deferring birds

is a hormonal profile that has been linked to poor condition in seabirds [13–15]. These findings support our first prediction: breeding deferral is characterized by low body mass, low blood hematocrit, and a generally compromised condition upon arrival at the breeding colony after overwinter migration. In contrast, and supporting our second prediction, reproductive success in albatrosses that initiated a breeding attempt was not related to body mass or to hematocrit (i.e. to resource state or condition) but showed a significant relationship with the potentially “resource-independent” endocrine processes underlying follicle development [8,17,37].

It is important to note that, contrary to many studies, female age and breeding experience were never significant effects in our models. However, this is perhaps not surprising as all but three of the birds we sampled (30 of 33) were experienced breeders between 17 and 25 years of age and within the range of peak breeding performance of black-browed albatrosses [1]. The significance of this is that we have found hormonal differences in female albatrosses at the peak of breeding performance which were predictive of reproductive success, failure, and deferral, and which were unrelated to senescence or inexperience.

4.1. Resource-dependent breeding decisions

Female black-browed albatrosses showed considerable inter-individual variation in body mass upon arrival at the breeding colony, the extremes of which differed by as much as 1 kg (range 3.25–4.25 kg, $N = 33$). The initial breeding decision (breed/defer) was very strongly associated with body mass, independent of age or quality, and the simplest explanation for this pattern is that returning females can assess their post-migratory physiological state, and if some relative threshold of condition has not been attained they will forgo reproduction thereby minimizing fitness costs (as per life-history theory, [29]). This might be mediated in part by the effect of body condition on hormonal processes which affect HPG functioning [13–15], and in our study P4 and *T* were not up-regulated in deferring females to a degree that would support gamete production. Previous studies of carryover effects in migratory animals, including seabirds, provide some evidence for this by arguing that the environmental conditions experienced during the non-breeding period can influence resource state at the onset of reproduction ([16] and references therein). The low body masses and hematocrit levels of deferring females suggest that these albatrosses had very different or difficult experiences during the non-breeding period (e.g. different foraging opportunities, feeding successes, distributions, migration routes or oceanographic conditions) relative to successful females, and that deferring females were less able to cope with the physical demands of migration, perhaps via reduced hematocrit-dependent oxygen transport capacities. However, direct observation of migratory behaviour via telemetry is needed to confirm these links. Nevertheless, as in other avian species [31,32], the deferring albatrosses were in a poorer post-migratory/pre-breeding condition independent of arrival date, age, or recent breeding history, and the low steroid hormone levels (P4 and *T*) and near-basal yolk precursor levels suggests that they had ‘decided’ before their arrival at the breeding colony to forgo steroidogenesis and thus egg formation. We therefore propose that a migratory carryover effect(s) constraining resource acquisition prior to arrival is the most parsimonious explanation for breeding deferral.

4.2. Reproductive readiness and breeding success

Once a female albatross decides to devote resources to egg production, and that egg is laid, she will then either fail or succeed at producing a chick. Among the female albatrosses that initiated a breeding attempt, reproductive success was not related to

measures of resource state or physiological condition – neither body mass nor hematocrit differed significantly between failed and successful females upon their arrival at the colony (and reproductive success was independent of age and experience). In contrast, success was strongly related to the steroidogenic processes underlying follicle development and yolk precursor production. In fact, multiple logistic regression indicated a significant role of VTG, but not body mass, in reproductive success. This strongly suggests that the reproductive failure of female black-browed albatrosses in this study was most likely the result of a constraint on the endocrine pathways regulating normal yolk precursor production.

So how might variation in pre-breeding VTG concentrations affect breeding success or failure weeks to months after laying? The answer may lie in the dynamics of egg production. During the course of normal follicle development, testosterone synthesized in ovarian follicle cells is converted to 17-estradiol which is then secreted to general circulation to stimulate the production of yolk precursors VTG and VLDL by the liver (reviewed by [35]). VTG and VLDL are the principal sources of protein and lipid for developing embryos, so it might be expected that a constraint on either precursor would have deleterious effects on embryonic development and egg/chick survival. In fact, we show that failed females had significantly lower VTG concentrations than successful females, and that VTG more strongly predicted success/failure than body mass (Fig. 1G). Furthermore, when we examined VTG and VLDL levels in failed females (Fig. 3), we found that low levels were related to failure shortly after laying, whereas higher levels delayed egg/chick mortality by some weeks/months. Overall, failed females had a mean VTG value which was nearly identical to that of the deferring, non-breeding females which did not produce eggs, and closer to those of females sampled 6 months after the egg-producing stage of the annual cycle just prior to winter out-migrations (mean $0.17 \mu\text{g Zn ml}^{-1}$, Crossin, unpublished data). In contrast, pre-breeding body mass was not significantly related to the date of failure (Fig. 3). Some caution may be warranted when interpreting the effect of VTG on breeding failure as the number of failed albatrosses in this study was somewhat low ($N = 8$). Nevertheless, even with a low sample size, we were still able to resolve significantly lower VTG concentrations in failed versus successful breeders; generally, low samples sizes increase the risk of a Type II statistical error, but this is not the case here.

The consequences of low circulating maternal VTG concentrations for egg and offspring quality have been demonstrated in experimental studies with female birds. Experimental reduction in circulating vitellogenin decreased yolk size and quality [33,34], with deleterious effects on offspring quality and survival [8,30]. In the absence of any age or breeding experience effects, we therefore propose that the provisioning of constituents to the yolks of failed albatross eggs was probably insufficient to sustain normal embryonic development, which as indicated in Fig. 3 could result in either relatively rapid or delayed mortality. The mechanism(s) responsible for this constraint in post-migratory females is not known, but might involve a glucocorticoid-mediated carryover effect or inhibition of reproductive physiology [4,8].

Although we did not directly measure plasma E_2 , variation in P4 and T provide some indication why VTG levels were so low in post-migratory female albatrosses which subsequently failed in their breeding attempt. P4 is the common precursor for T and E_2 , and after a series of enzymatic reactions P4 is converted to T , which can then be aromatized to E_2 . Elevated levels of both P4 and E_2 are required for normal reproductive function in females, mediating oviduct development, yolk precursor production (e.g. P4 is synergistic with E_2 binding on vitellogenin gene II, [6]), follicle development, and reproductive behaviours. This is supported by the strong, positive relationship we observed between P4 and E_2 -

dependent plasma VTG levels (Fig. 2A), which we argue is indicative of greater “reproductive readiness” *sensu* [8]. In contrast, females which failed had significantly higher T levels and lower VTG levels than successful birds (Fig. 2B). This suggests a potential stress-related inhibition of P_{450} aromatase which prevented conversion of ovarian T to E_2 in these birds (as occurs in the brain nuclei of various bird species [4,12]), and which effectively constrained E_2 -dependent vitellogenesis. This supports the idea of reduced reproductive readiness in females with high T . It is important to reiterate that the variables we examined were measured during the pre-breeding period, weeks to months before ultimate reproductive outcome. Although we found no significant associations between pre-breeding body mass and reproductive success, this does not discount the possibility of a resource link to reproductive success later during the incubation or chick rearing stage. In fact, one may very well assume that resource-dependent and resource-independent factors work synergistically to affect ultimate reproductive fate (but as we have indicated, the focus of this present study was on the pre-breeding physiology and condition of female albatrosses). Ultimately, we have shown that at this early stage of the breeding season, immediately following overwinter migration, there was a very clear and significant effect of reproductive physiology on breeding success, independent of resource effects, which we attribute to a migratory carryover effects on reproductive readiness operating downstream in the reproductive axis.

We have thus far used the words “resource-dependence” and “condition-dependence” synonymously, but there exists the possibility that reproductive success might be resource-dependent but not condition-dependent. For example, we have shown that there is a relative resource or condition threshold below which breeding does not occur, and here both terms are generally synonymous with “body mass”. However, once this body mass threshold was met and egg production proceeded in the breeding albatrosses, then a possible distinction between “resource” and “condition” emerged. Our data show that both failed and successful breeders were similar in mass, and thus in similar condition. However, the resources available to these birds to allocate towards reproduction (e.g. steroidogenesis, vitellogenesis, etc.) might still be subject to limitation. Protein limitation is thus one possible explanation for the presumed inhibition of P450 aromatase activity and the low VTG levels that distinguished failed from successful breeders. As such, it is possible that breeding failure is resource- but not condition-dependent.

5. Conclusion

We present data which suggest that migratory carryover effects can impact two very different aspects of reproductive effort. In the short term, carryover effects constraining the acquisition of resources and body condition during the pre-breeding period mediated the trade-off between current and future reproductive investment (e.g. breeding decision), via hormonal mediators. However, for females deciding to initiate reproduction, the steroidogenic processes underlying yolk precursor production become more directly involved in ultimate breeding success, which in this case involved a constraint on the E_2 -mediated yolk-precursor production pathway independent from any condition-related effect. As few studies reporting carryover effects on reproductive success show strong or consistent relationships with measures of pre-breeding resource allocation or condition in females [8,16,22,28], it is important to consider a much broader set of potential mechanisms [17,35], especially as virtually none of the studies reviewed by Harrison et al. [16] show definitive links between resource state and improved breeding success in females. Having controlled for

age and experience related effects, the pleiotropic interactions and incompatibilities among endocrine and other physiological regulatory systems like those described in this study are the most likely candidates underlying reproductive success.

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