1   **Migratory carryover effects and endocrinological correlates of reproductive decisions and**

2   **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 (e.g. 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 traits 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 characterised 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.

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 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

73 luteinizing hormone releasing hormone (LHRH) challenge, but they do not or cannot sustain

74 gonadal steroid production [5,14,15]. These studies suggest that the metabolic demands of

75 migration might down-regulate the reproductive axis in terms of gonadal steroid production, an

76 effect that might be mediated by, or simply correlated with, elevated corticosterone levels and/or

77 inhibition of LH. Few studies have considered individual variation in circulating gonadal

78 steroids at arrival in migratory birds in relation to subsequent reproduction. Furthermore, the

79 studies cited above only considered breeding decisions as breeding/not breeding (all-or-none

80 decisions), and did not extend the analysis of hormonal patterns at arrival to consider the

81 consequence of the decision to breed in terms of success or failure. Recently, Crossin et al. [8]

82 showed that migratory carryover effects can place constraints on the estrogen-dependent

83 production of yolk precursors by egg-producing females, with negative effects on egg sizes, and

84 that these effects are unrelated to variation in individual condition. However, the functional

85 significance of reduced or constrained sex-steroid production, during the migration-reproduction

86 transition, to reproductive success remains largely unknown. Nevertheless, what is emerging is

87 the recognition that condition (or resource state) at arrival need not be the only mechanism

88 affecting reproductive success. The endocrine processes controlling female reproduction may be

89 directly affected by migratory carryover effects, via constraints on, or incompatibilities between,

90 competing physiological regulatory systems [8,17,37].

91   Procellariiforme seabirds (the albatrosses and petrels) are pelagic species which also make

92 good models for exploring the effects of migratory activity on the hormonal processes underlying

93 reproduction. Like many seabirds, the Procellariiformes have a slow life-history characterized by

94 delayed age of reproduction, a single-egg clutch, prolonged parental care, and longevity, and they

95 make very long, pelagic migrations during a non-breeding period which lasts 6-16 months in

96 *Thalassarche spp*. [9,26]. Among breeding-age albatrosses and petrels, there is typically a high

97   proportion of non-breeding individuals, and among even the most experienced breeders, breeding

98   deferral for one or more years is a common tactic underlying lifetime fitness [27,36]. The annual

99   breeding pattern of black-browed albatrosses (*Thalassarche melanophris*) make this species a

100   particularly tractable model for the study of carryover effects. Each year, black-brows return to

101   breeding colonies from distant foraging areas occupied during the non-breeding period [26], and

102   in the weeks preceding arrival, birds are presumably balancing their own physiological

103   requirements against those needed for the initiation of reproduction. Upon arrival at breeding

104   colonies, they then experience one of three inevitable outcomes: they will defer reproduction

105   entirely until the next season, or they will lay and then either fail or succeed at fledging a chick

106   [26].

107   In this study, we investigate the physiological mechanisms that mediate reproductive

108   decisions (breeding or deferral) and reproductive outcomes (success or failure) in female black-

109   browed albatrosses during the transition between winter migration and spring breeding.

110   Specifically we consider condition or resource related traits (mass, hematology), as well as the

111   steroidogenic hormones and estrogen-dependent yolk precursor pathways underlying

112   reproductive readiness (*sensu* [8]), in mediating decisions and outcomes. We predicted that the

113   decision to lay or to defer would be condition-dependent, and that females deferring reproduction

114   would be in poorer condition (low body mass, low hematocrit [Hct] and hemoglobin [Hb]

115   concentrations) relative to breeding females. However, we also predicted that reproductive

116   success (e.g. fledging of chicks) might reflect differences in "reproductive readiness" upon

117   arrival at the colony, as indicated by the estrogen-dependent production of the yolk precursors

118   vitellogenin [VTG] and yolk-targeted very low density lipoprotein [VLDLy], as well as by

119   patterns of production of the reproductive steroid hormones progesterone [P4] and testosterone

120   [T].

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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 historically 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 then captured the newly arrived, previously unsighted 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 sec) 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 10,000 g, 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

145 as packed cell volume (%) following centrifugation of whole blood in microhematocrit tubes for

146 5 min at 10,000 g.   Hemoglobin (Hb, g dl^{-1} whole blood) was measured with the

147 cyanomethemoglobin method modified for use with a microplate spectrophotometer, using 5µl

148 whole blood diluted in 1.25 ml Drabkin's reagent (D5941 Sigma-Aldrich Canada, Oakville,

149 Ontario, Canada). Absorbance was measured at 540 nm.

150   Progesterone (P4) and testosterone (T) were assayed in duplicate from each of 33 samples

151 by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Both steroids were assayed

152 in a single injection, starting from a sample volume between 50 and 100 µl. All samples received

153 a deuterated internal standard representing a final concentration of 5 ng ml^{-1} P4-d9 and 1 ng ml^{-1}

154 T5-d2, and were diluted to 500µl with water. Sample preparation consisted of solid phase

155 extraction over C18, with elution in 1 ml of ethyl acetate. Samples were dried under nitrogen gas

156 and reconstituted in 50% MeOH. Liquid chromatography (Agilent 1200 SL system) used an

157 injection volume of 40 µl, a 100x300 mm Kinetex C18 Column (Phenomenex), and

158 water/methanol as mobile phases. Mass spectrometry (AB Sciex Q-trap 5500) used APCI +ve

159 mode, with the following MRM transitions: Progesterone 315/97; Progesterone-d9 324/100;

160 Testosterone 289/97; Testosterone-d2 291/99.

161   Quantification of E_2 was not possible at these volumes as sample preparation and optimal

162 LC-MS conditions at sample concentrations (based on pilot analysis of a pooled sample) differed

163 from the conditions for testosterone and progesterone. However, the principal role of E_2

164 secretion is the production of the yolk precursors vitellogenin (VTG) and yolk-targeted very low

165 density lipoprotein (VLDLy), which are a strong indicator of reproductive readiness [8 and

166 references therein].

167   Plasma samples were assayed in duplicate for vitellogenic zinc (Zn; zinc kit, Wako

168 Chemicals) and total triglycerides (Glycerol Reagents A and B, Sigma), as indices of the yolk

169 precursors VTG and VLDLy respectively (e.g. [8]). VTG and VLDLy are the two main yolk

170 precursors in birds and are transported from the liver to the ovary where they are deposited into

171 developing follicles. Of the two precursors, VTG is generally considered the more reliable

172 indicator of follicle development in birds [8]. All assays were measured using a Biotek 340i

173 microplate reader. Using a 19-week domestic laying hen (*Gallus domesticus*) plasma pool, intra-

174 assay coefficients of variation for VTG and for VLDLy ranged from 5.9-8.2% and 6.6-8.7%,

175 respectively. Inter-assay coefficients of variation were 7.8% for VTG and 6.9% for total VLDLy.

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177 *2.3. Statistical analyses*

178 Analyses were run with either the JMP 8.0 or SAS 9.0 software packages. All variables were

179 tested for normality, as were residuals from plots against predicted values, using Shapiro-Wilk

180 tests. Data transformations were applied when distributions were non-normal. Analysis of

181 covariance (ANCOVA) tests were used to examine breeding-group differences (e.g. successful,

182 failed, and deferring breeders) in VTG, VLDLy, Hct, Hb, T, P, and body mass. As many studies

183 show links between age and individual quality and breeding output, we included age and a

184 measure of individual quality as a covariates in all analyses. We defined individual quality as the

185 number of chicks fledged (i.e. breeding quality) during the previous 6 year period (i.e. breeding

186 experience). We also examined whether a female bred successfully or not, or deferred, in the

187 previous year alone (i.e. immediate breeding history). However, because age and the two quality

188 measures are co-linear, we used the covariates in separate models exploring each response

189 variable listed above. Tukey-Kramer *post-hoc* tests were run after univariate tests to identify

190 significant contrasts between breeding groups. Correlations among all variables with dates of

191 colony arrival and with individual quality measures were examined through a Pearson's

192 correlation matrix.

193

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 ± 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, VLDLy, T, P; all P>0.10, N~33 individuals per variable). Furthermore, no variables were related to age (mean age 22 years, range 12-30), or to individual experience 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 breeding experience in 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).

216   Supporting our first prediction, body mass at colony arrival differed among females with

217   differing breeding outcomes (whole model, $F_{2,30}$=4.7, P=0.011), independently of age effects

218   (P=0.26). Successful females weighed 3.83 ± 0.07 kg, which was significantly heavier (*post hoc*

219   contrast, P=0.006) than deferring females which weighed 3.53 ± 0.10 kg. Failed breeders were

220   intermediate in mass, and weighed 3.62 ± 0.10 kg (Fig. 1A) which was not significantly different

221   from either successful (P=0.150) or deferring (P=0.289) females. Therefore, as predicted,

222   deferring birds were significantly lighter than successfully breeding birds. When we pooled

223   failed and successful birds and compared them to deferring birds, the breeding birds were still

224   significantly heavier than deferring birds (3.79 ± 0.06 kg vs. 3.52 ± 0.09 kg; $F_{2,30}$=4.4, P=0.016).

225   Hct, but not Hb, also supported our first prediction (Fig. 1B-C), and Hct levels followed the same

226   trend as body mass. Hct was significantly higher in successful (41.7 ± 0.8%; P=0.004) and failed

227   (41.3 ± 1.2%; P=0.033) breeders than in deferring breeders (37.8 ± 1.0%) ($F_{2,30}$=5.18, P=0.012).

228   Hb did not differ significantly between breeding categories ($F_{2,30}$=0.739, P=0.486).

229   Also supporting our second prediction, differences in reproductive readiness, as measured

230   by differences in sex steroid hormone and yolk precursor levels (Fig. 1D-G), varied in relation to

231   reproductive status independently of age and breeding quality effects. Differences in P4 (Fig.

232   1D) showed that successful (P=0.009) and failed (P=0.030) females had higher (up-regulated)

233   concentrations than deferring females (successful = 1.71 ± 0.38 ng ml^{-1}, failed = 1.41 ± 0.57 ng

234   ml^{-1}, deferring = 0.34 ± 0.53 ng ml^{-1}; $F_{2,26}$=4.10, P=0.028). T was significantly higher in failed

235   females (2.79 ± 0.50 ng ml^{-1}) relative to both successful (P=0.003) and deferring (P=0.016)

236   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;

237   Fig. 1E). VTG varied significantly ($F_{2,21}$=7.33, P=0.004; Fig. 1G), with higher concentrations in

238   breeding females (2.91 ± 0.34 μg Zn ml^{-1}) than in both deferring (0.74 ± 0.42 μg Zn ml^{-1}, P=0.0

239   12) and failed (1.21 ± 0.46 µg Zn ml^{-1}, P=0.032) females. VLDLy concentrations however did

240   not vary significantly among deferred, failed and successful females ($F_{2,22}$=2.05, P=0.15; Fig.

241   1F).

242   Among the breeding birds, T and P4 were related to VTG concentrations but in opposite

243   directions: P4 was positively related with VTG in a natural log manner (r=0.64, P=0.001, N=14,

244   Fig. 2A), while T was negatively related to VTG (r=-0.56, P=0.007, N=16, Fig. 2B). T and P4

245   were not significantly correlated in the breeding birds (r=-0.22, P=0.32, N=24), or in all breeding

246   and deferring birds collectively (r=-0.15, P=0.42, N=33).

247   Using regression analysis, date of breeding failure (known for 7 of the 8 failed females)

248   was significantly related to pre-breeding T, VTG, and VLDLy concentrations (N=7 females, all

249   P<0.017, Fig. 3A-C). T tended to be higher in females which failed shortly after laying relative

250   those failing later. Similarly, VTG and VLDLy were generally lower in females failing early,

251   and higher values were associated with later failure. In contrast, pre-breeding body mass was not

252   significantly related to date of breeding failure (P=0.128, Fig. 3D). Multiple logistic regression

253   analysis revealed a significant role of VTG in predicting breeding success or failure (χ^2=4.98,

254   P=0.026), but the influence of body mass was not significant (χ^2=1.13, P=0.287). In other words,

255   resource-independent VTG effects were more strongly predictive of breeding success than

256   resource-dependent mass effects.

257

258   **4. Discussion**

259   In this study we show that after long-distance migrations to a breeding colony, reproductive

260   decisions (breeding or deferring) and reproductive outcome (successful or failed) in female

261   black-browed albatrosses are associated with marked differences in patterns of circulating

262 gonadal steroids (P4, T) and steroid-dependent traits (yolk precursors). Previous studies of

263 black-browed albatrosses have shown that all breeding-age females generally return to natal

264 colonies at the start of the breeding season, whether they breed or not, and that plasma LH levels

265 are elevated upon arrival [18]. It is therefore parsimonious to assume that day-length drives the

266 general timing of their return to colonies, indicating a general seasonality of migratory behaviour,

267 and that this is sufficient to "switch on" the reproductive axis at the level of the hypothalamus

268 and pituitary as in most temperate-breeding birds [10]. However, our data clearly show that

269 female black-browed albatrosses are not all equally prepared for reproduction upon arrival in

270 terms of "downstream" components of the reproductive axis. As we predicted, deferring females

271 had low plasma progesterone (P4), and consequently low testosterone (T) and low estrogen-

272 dependent vitellogenin (VTG) levels, which suggests that the decision to defer reproduction by

273 these annually breeding birds was made prior to arrival perhaps due to "stressful" metabolic

274 demands incurred at sea during the over-winter or migratory phase, i.e. carryover effects. Also as

275 predicted, successful females had high plasma P4 and high VTG, but low T, which indicates a

276 responsiveness of the ovary to stimulation by pituitary LH. The low plasma T in these

277 successfully breeding birds is presumably due to rapid conversion (aromatisation) to E_2 to

278 support vitellogenesis, as indicated by the high VTG levels that we measured. The failed

279 breeders however are especially interesting as they had high P4 and high T, which like the

280 successful breeders indicates gonadal sensitivity to LH secretion, but these females had low VTG

281 which suggests a possible disruption of aromatisation (as per [12]) in the E_2-mediated

282 vitellogenic pathway. Ultimately, and rather amazingly, VTG in pre-laying females predicted not

283 only their breeding success or failure, but it also the length of time from their arrival at the colony

284 to breeding failure (Fig. 3B). This suggests very strongly that hormonal differences among

285 individuals at colony arrival, during the transition from migratory to reproductive states, affect

286   not only breeding decisions but also the degree of reproductive readiness with consequences for

287   reproductive success. Furthermore, these effects influence processes at the *downstream* end of

288   the reproductive axis, at the level of ovary steroidogenesis and E_2-dependent regulation of hepatic

289   vitellogenesis.

290   Collectively, our data suggest that reproductive decisions (breeding or deferral) and

291   reproductive success are influenced by two very different mechanistic pathways in female black-

292   browed albatrosses. In addition to hormonal differences, the decision to defer reproduction was

293   associated with more traditional measures of individual condition, characterized by lower body

294   mass and lower hematocrit levels at arrival relative to breeding individuals. This is consistent

295   with previous studies of breeding deferral in seabirds (blue petrels [7]; gulls [19]; shearwaters

296   [21]), and with the idea that the initiation of breeding attempts is resource- or condition-

297   dependent. Furthermore, the low P4 and T levels in deferring birds is a hormonal profile that has

298   been linked to poor condition in seabirds [13,14,15]. These finding support our first prediction:

299   breeding deferral is characterized by low body mass, low blood hematocrit, and a generally

300   compromised condition upon arrival at the breeding colony after overwinter migration. In

301   contrast, and supporting our second prediction, reproductive success in albatrosses that initiated a

302   breeding attempt was not related to body mass or to hematocrit (i.e. to resource state or

303   condition) but showed a significant relationship with the potentially "resource-independent"

304   endocrine processes underlying follicle development [8,17,37].

305   It is important to note that, contrary to many studies, female age and breeding experience

306   were never significant effects in our models. However, this is perhaps not surprising as all but

307   three of the birds we sampled (30 of 33) were experienced breeders between 17-25 years of age

308   and within the range of peak breeding performance of black-browed albatrosses [1]. The

309   significance of this is that we have found hormonal differences in female albatrosses at the peak

310 of breeding performance which were predictive of reproductive success, failure, and deferral, and

311 which were unrelated to senescence or inexperience.

312

313 *4.1. Resource-dependent breeding decisions*

314 Female black-browed albatrosses showed considerable inter-individual variation in body mass

315 upon arrival at the breeding colony, the extremes of which differed by as much as 1 kg (range

316 3.25-4.25 kg, N=33). The initial breeding decision (breed/defer) was very strongly associated

317 with body mass, independent of age or quality, and the simplest explanation for this pattern is

318 that returning females can assess their post-migratory physiological state, and if some relative

319 threshold of condition has not been attained they will forgo reproduction thereby minimizing

320 fitness costs (as per life-history theory, [29]). This might be mediated in part by the effect of

321 body condition on hormonal processes which affect HPG functioning [13,14,15], and in our study

322 P4 and T were not up-regulated in deferring females to a degree that would support gamete

323 production. Previous studies of carryover effects in migratory animals, including seabirds,

324 provide some evidence for this by arguing that the environmental conditions experienced during

325 the non-breeding period can influence resource state at the onset of reproduction ([16] and

326 references therein). The low body masses and hematocrit levels of deferring females suggest that

327 these albatrosses had very different or difficult experiences during the nonbreeding period (e.g.

328 different foraging opportunities, feeding successes, distributions, migration routes or

329 oceanographic conditions) relative to successful females, and that deferring females were less

330 able to cope with the physical demands of migration, perhaps via reduced hematocrit-dependent

331 oxygen transport capacities. However, direct observation of migratory behaviour via telemetry is

332 needed to confirm these links. Nevertheless, as in other avian species [31,32], the deferring

333 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 provision an egg 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 17β-estradiol which is then secreted to general circulation to stimulate the production of yolk precursors VTG and VLDLy by the liver (reviewed by [35]). VTG and

357 VLDLy are the principal sources of protein and lipid for developing embryos, so it might be

358 expected that a constraint on either precursor would have deleterious effects on embryonic

359 development and egg/chick survival. In fact, we show that failed females had significantly lower

360 VTG concentrations than successful females, and that VTG more strongly predicted

361 success/failure than body mass (Fig. 1G). Furthermore, when we examined VTG and VLDLy

362 levels in failed females (Fig. 3), we found that low levels were related to failure shortly after

363 laying, whereas higher levels delayed egg/chick mortality by some weeks/months. Overall, failed

364 females had a mean VTG value which was nearly identical to that of the deferring, non-breeding

365 females which did not produce eggs, and closer to those of females sampled 6 months after the

366 egg-producing stage of the annual cycle just prior to winter out-migrations (mean 0.17 μg Zn ml^{-1}

367 ^{1}, G.T. Crossin, unpublished data). In contrast, pre-breeding body mass was not significantly

368 related to the date of failure (Fig. 3). Some caution may be warranted when interpreting the

369 effect of VTG on breeding failure as the number of failed albatrosses in this study was somewhat

370 low (N=8). Nevertheless, even with a low sample size, we were still able to resolve significantly

371 lower VTG concentrations in failed versus successful breeders; generally, low samples sizes

372 increase the risk of a Type II statistical error, but this is not the case here.

373   The consequences of low circulating maternal VTG concentrations for egg and offspring

374 quality have been demonstrated in experimental studies with female birds. Experimental

375 reduction in circulating vitellogenin decreased yolk size and quality [33,34], with deleterious

376 effects on offspring quality and survival [8,30]. In the absence of any age or breeding experience

377 effects, we therefore propose that the provisioning of constituents to the yolks of failed albatross

378 eggs was probably insufficient to sustain normal embryonic development, which as indicated in

379 Fig. 3 could result in either relatively rapid or delayed mortality. The mechanism(s) responsible

380 for this constraint in post-migratory females is not known, but might involve a glucocorticoid-

381 mediated carryover effect or inhibition of reproductive physiology [4,8].

382   Although we did not directly measure plasma E_2, variation in P4 and T provide some

383 indication why VTG levels were so low in post-migratory female albatrosses which subsequently

384 failed in their breeding attempt. P4 is the common precursor for T and E_2, and after a series of

385 enzymatic reactions P4 is converted to T, which can then be aromatized to E_2. Elevated levels of

386 both P4 and E_2 are required for normal reproductive function in females, mediating oviduct

387 development, yolk precursor production (e.g. P4 is synergistic with E_2 binding on vitellogenin

388 gene II, [6]), follicle development, and reproductive behaviours. This is supported by the strong,

389 positive relationship we observed between P4 and E_2-dependent plasma VTG levels (Fig. 2A),

390 which we argue is indicative of greater "reproductive readiness" *sensu* [8]. In contrast, females

391 which failed had significantly higher T levels and lower VTG levels than successful birds (Fig.

392 2B). This suggests a potential stress-related inhibition of P_{450} aromatase which prevented

393 conversion of ovarian T to E_2 in these birds (as occurs in the brain nuclei of various bird species

394 [4,12]), and which effectively constrained E_2-dependent vitellogenesis. This supports the idea of

395 reduced reproductive readiness in females with high T. It is important to reiterate that the

396 variables we examined were measured during the pre-breeding period, weeks to months before

397 ultimate reproductive outcome. Although we found no significant associations between pre-

398 breeding body mass and reproductive success, this does not discount the possibility of a resource

399 link to reproductive success later during the incubation or chick rearing stage. In fact, one may

400 very well assume that resource-dependent and resource-independent factors work synergistically

401 to affect ultimate reproductive fate (but as we have indicated, the focus of this present study was

402 on the pre-breeding physiology and condition of female albatrosses). Ultimately, we have shown

403 that at this early stage of the breeding season, immediately following overwinter migration, there

404 was a very clear and significant effect of reproductive physiology on breeding success,

405 independent of resource effects, which we attribute to a migratory carryover effects on

406 reproductive readiness operating downstream in the reproductive axis.

407   We have thus far used the words "resource-dependence" and "condition-dependence"

408 synonymously, but there exists the possibility that reproductive success might be resource-

409 dependent but not condition-dependent. For example, we have shown that there is a relative

410 resource or condition threshold below which breeding does not occur, and here both terms are

411 generally synonymous with "body mass". However, once this body mass threshold was met and

412 egg production proceeded in the breeding albatrosses, then a possible distinction between

413 "resource" and "condition" emerged. Our data show that both failed and successful breeders

414 were similar in mass, and thus in similar condition. However, the resources available to these

415 birds to allocate towards reproduction (e.g., steroidogenesis, vitellogenesis, etc.) might still be

416 subject to limitation. Protein limitation is thus one possible explanation for the presumed

417 inhibition of P450 aromatase activity and the low VTG levels that distinguished failed from

418 successful breeders. As such, it is possible that breeding failure is resource- but not condition-

419 dependent.

420

421 **5. Conclusion**

422 We present data which suggest that migratory carryover effects can impact two very different

423 aspects of reproductive effort. In the short term, carryover effects constraining the acquisition of

424 resources and body condition during the pre-breeding period mediated the trade-off between

425 current and future reproductive investment (e.g. breeding decision), via hormonal mediators.

426 However, for females deciding to initiate reproduction, the steroidogenic processes underlying

427 yolk precursor production become more directly involved in ultimate breeding success, which in

18

428   this case involved a constraint on the E_2-mediated yolk-precursor production pathway

429   independent from any condition-related effect. As few studies reporting carryover effects on

430   reproductive success show strong or consistent relationships with measures of pre-breeding

431   resource allocation or condition in females [8,16,22,28], it is important to consider a much

432   broader set of potential mechanisms [17,35], especially as virtually none of the studies reviewed

433   by Harrison et al. [16] show definitive links between resource state and improved breeding

434   success in females. Having controlled for age and experience related effects, the pleiotropic

435   interactions and incompatibilities among endocrine and other physiological regulatory systems

436   like those described in this study are the most likely candidates underlying reproductive success.

437

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534

535 **Figure Legends**

536 Fig. 1: Body mass (A), hematological (B-C), hormonal (D-E), and yolk precursor (F-G) profiles

537 in pre-breeding female black-browed albatrosses (*Thalassarche melanophris*) upon arrival at a

538 breeding colony at Bird Island, South Georgia. Birds are grouped according to breeding

539 outcome, and bars represent least square means ± SEM. Numbers at the base of bars indicate

540 sample sizes. Differing letters indicate statistical significance when $\alpha=0.05$. Note that when

541 failed and successful birds are pooled in Panel A, the deferring birds are significantly lighter

542 (see Results section for details). Abbreviations: Hct- hematocrit; Hb- hemoglobin; P4-

543 progesterone; T- testosterone; VLDLy- yolk-targeted very low density lipoprotein; VTG-

544 vitellogenin.

545

546 Fig. 2: Relationship between plasma vitellogenin and progesterone (A) and testosterone (B) in

547 pre-breeding female black-browed albatrosses (*Thalassarche melanophris*) which eventually

548 laid eggs. Open circles indicate failed breeders and closed circles are successful breeders.

549 Lines are natural log fits. See Figure 1 for key to abbreviations.

550

551 Fig. 3: Relationships of testosterone (A), vitellogenin (B), very low density lipoprotein (C), and

552 body mass (D) with date of breeding failure in female black-browed albatrosses. Lines are

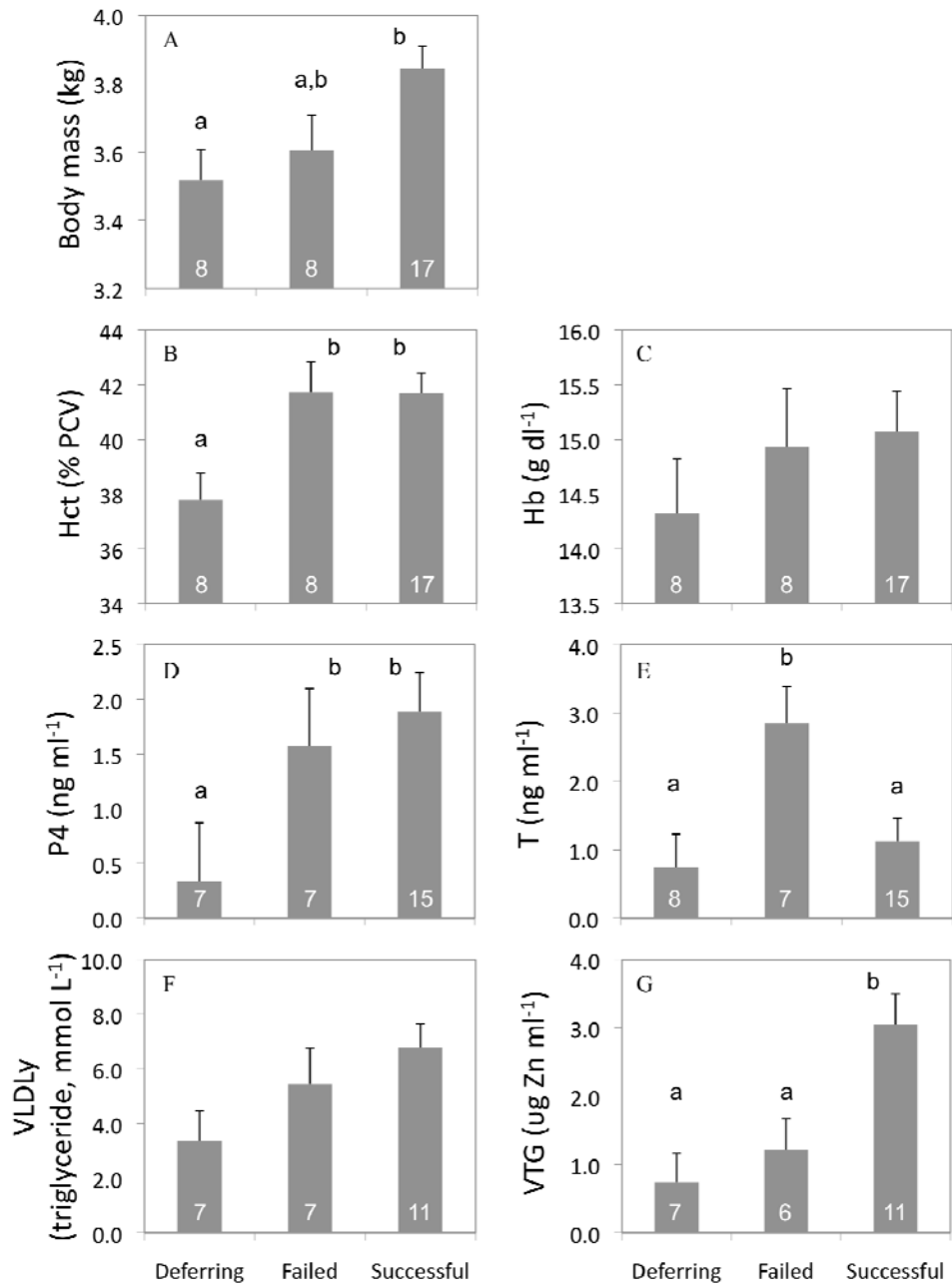553 natural log fits. See Figure 1 for key to abbreviations.
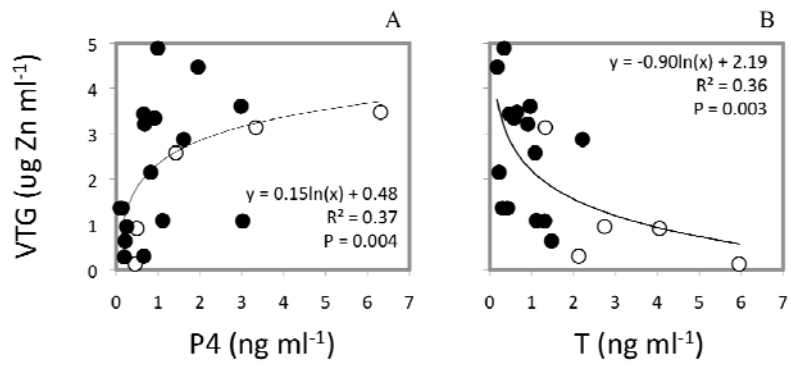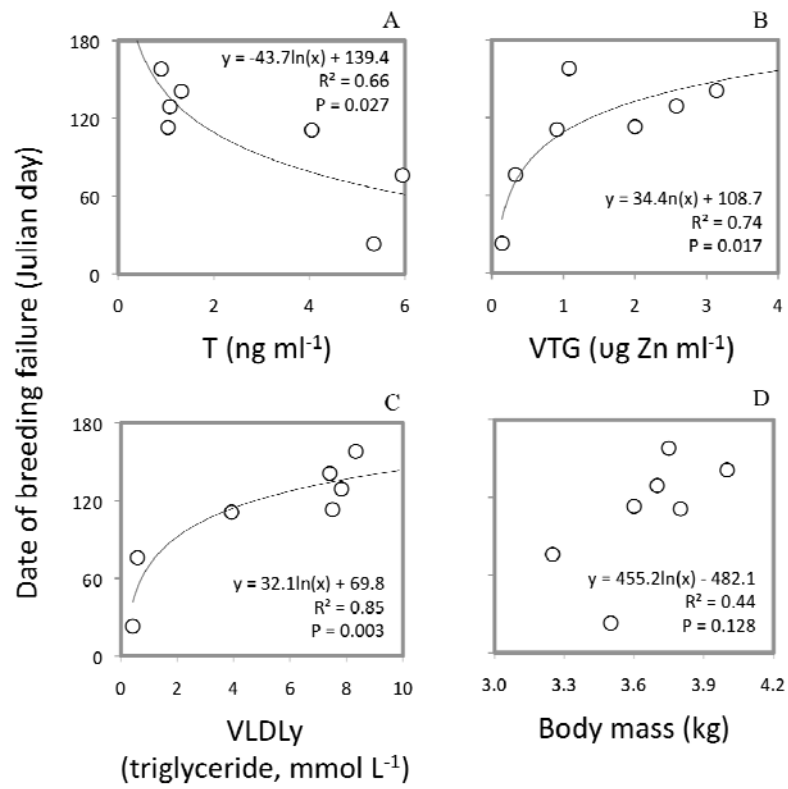
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Fig. 1

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Fig. 2

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Fig. 3

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