**Costs of reproduction and carryover effects in breeding albatrosses.**

Glenn T. Crossin1\*, Richard A. Phillips2, Christine R. Lattin3, L. Michael Romero3, Xavier Bordeleau1, Christopher M. Harris4, Oliver P. Love4, and Tony D. Williams5

1Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

2British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, United Kingdom

3Department of Biology, Tufts University, Medford, MA, 02155, United States

4Department of Biological Sciences and Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada

5Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

\* Corresponding author: gtc@dal.ca

Running title: Costs of reproduction and carryover effects in albatrosses.

**Abstract**

We investigated the physiology of two closely related albatross species relative to their breeding strategy: black-browed albatrosses (*Thalassarche melanophris*) breed annually, while grey-headed albatrosses (*T. chrysostoma*) breed biennially. Via observations of breeding fate and blood samples collected at the end of breeding in one season, and feather corticosterone levels sampled at the beginning of the next breeding season, we found that in both species, some post-breeding physiological parameters differed according to breeding outcome (successful, failed, deferred). Correlations between post-breeding physiology and fCort, and links to future breeding decisions, were examined. In black-browed albatrosses, post-breeding physiology and fCort were not significantly correlated, but fCort independently predicted breeding decision the next year, which we interpret as a possible migratory carryover effect. In grey-headed albatrosses, post-breeding triglyceride levels were negatively correlated with fCort, but only in females, which we interpret as a potential cost of reproduction. However, this potential cost didn’t carry-over to future breeding in the grey-headed albatrosses; none of the variables predicted future breeding decision. We suggest that biennial breeding in the grey-headed albatrosses may have evolved as a strategy to buffer against the apparent susceptibility of females to negative physiological costs of reproduction. Future studies are needed to confirm this.

**Keywords:** testosterone; hematocrit; feather corticosterone; glucocorticoids; triglycerides; migration

**Introduction**

There is considerable experimental evidence for costs of reproduction in birds, which include both short-term costs associated with specific stages of the breeding cycle (e.g. incubation or chick rearing; Monaghan *et al*. 1995, Monaghan *et al*. 1998), and longer-term costs that carry over to affect future reproductive investment (Daan *et al*. 1996). Carryover effects have been documented in several bird species, and influence traits such as the timing of breeding (Descamps *et al*. 2011, Harrison *et al*. 2011), breeding deferral (Ebbinge and Spaans 1995, Crossin *et al*. 2012, Crossin *et al*. 2013a) and breeding output (Ebbinge and Spaans 1995). The physiological mechanisms that underlie these costs however, and whether they involve limitations in food resource or energy, or some other non-resource based constraint arising from competing physiological systems, are uncertain (Williams 2012). Previous studies have, however, proposed a role for glucocorticoid hormones (Bortolotti *et al*. 2008, Crossin et al. 2013b), energy reserves (Harrison et al. 2011), and hematocrit or aerobic capacity (Williams 2012, Crossin et al. 2013a) as potential mediators of costs and carryover effects.

The role of the glucocorticoid hormones, specifically corticosterone and cortisol (called hereafter “Cort”), and whether they have a positive or negative relationship with particular life-history traits, depends on whether they are expressed at baseline or stress-induced levels (Bonier *et al*. 2009, Crossin *et al.* 2016). Given Cort’s fundamental role in energy acquisition and metabolism, Cort may be the key factor that explains individual variation in energy balance, body condition, and the condition-dependence of breeding activity (Descamps *et al*. 2011). In birds and mammals, an effective means of resolving such relationships involves the analysis of Cort in keratin-based structures, including feathers (Bortolotti *et al*. 2008, Lattin *et al*. 2011). Cort deposited into these structures from the general circulation is thought to provide an integrated measure of hypothalamo–pituitary–adrenal axis (HPA) activity over time-scales relevant to life-history, e.g. transitions between non-breeding and breeding stages of the annual cycle. In birds, measurement of feather Cort levels (fCort) has revealed links between previous breeding activity, migration, and future reproductive investment, thus revealing carryover effects onto key traits, including breeding decisions (whether to lay or defer), laying date, and egg sizes (Bortolotti *et al*. 2008, Crossin *et al*. 2013b, Kouwenberg *et al*. 2013).

 Other studies have suggested that variation in energy reserves (or condition) might also mediate carryover effects (see review by Harrison *et al*. 2011; in this context, condition is presumably linked to Cort as a metabolic regulator, although as noted above the dynamics between Cort and condition in mediating carryover effects are poorly defined). Links between pre-breeding condition and breeding investment have been demonstrated in birds (Harrison *et al*. 2011, O’Connor *et a*l. 2014), especially in capital breeders via modifications to laying date or breeding success, via trade-offs between current reproduction and survival (Prop *et al*. 2003), and via clutch size manipulations (Monaghan and Nager 1997). Harrison et al. (2011) note that low rates of pre-breeding energy intake can adversely affect reproductive traits in many birds and other species (Ebbinge and Spaans 1995, Prop *et al*. 2003). Plasma triglycerides provide one method for assessing individual condition, as these correlate with both energy intake and fattening rate in birds (Guglielmo and Williams 2003). As with body mass, variation in triglyceride levels in pre-breeding birds could signal a carryover effect with potential implications for subsequent investment decisions (Hennin *et al.* 2015).

Although it seems intuitive that carryover effects could be generated by energy or nutrient limitation (Harrison *et al*. 2011), conceivably they could reflect deficiencies in other physiological currencies. In many migratory birds, hematocrit (red blood cell percentage) is up-regulated during migration to increase oxygen-transport capacity (Piersma *et al*. 1996), which is a key trait for sustaining high aerobic performance for long-distance flight. However, hematocrit can be impacted negatively by reproductive processes, specifically by estradiol (E2) secretion during egg production, which can result in a debilitating reproductive anemia that can persist for up to a year (Kalmbach *et al.* 2004; Crossin *et al*. 2013a). Low hematocrit could therefore indicate a potential carryover effect in the form of reduced migratory performance and increased costs that ultimately constrain future reproductive investment. By extension, variation in testosterone levels could also be important as androgens are known to stimulate erythropoiesis, and affect breeding decisions in birds (Crossin *et al*. 2012). Previous work has shown that sex steroid expression (progesterone, testosterone) during the pre-breeding period can predict breeding decisions in albatrosses (Crossin *et al*. 2012, Crossin *et al*. 2013a). Sex steroids and Cort also affected breeding output in marine iguanas (*Amblyrhynchus cristatus*) and other reptiles (Vitousek *et al*. 2010).

Long-lived seabird species characterized by slow life-histories and single-egg clutches (Jouventin and Dobson 2002) provide ideal model species for exploring the physiological costs of reproduction and mechanisms involved in carryover effects. The congeneric black-browed albatross (*Thalassarche melanophris*) and grey-headed albatross (*T. chrysostoma*) breed sympatrically throughout much of their range in the sub-Antarctic, but display very different reproductive life-histories. As in the majority of bird species worldwide, black-browed albatrosses are annual breeders, although a small proportion breed in alternate years. In contrast, grey-headed albatrosses generally breed biennially if successful, as do all the great albatrosses (*Diomedea* spp.) and sooty albatrosses (*Phoebetria* spp.) (Tickell 2000). Sister species like the black-browed and grey-headed albatrosses, with markedly different breeding strategies, therefore provide a good contrast for exploring the potential physiological mechanisms underlying breeding frequency and links to costs of reproduction and carryover effects.

Biennial breeding is often associated with life-histories characterized by long periods of intensive bi-parental care. In wandering albatrosses and king penguins for example, birds usually take a year or more to fledge their chicks, making it impossible, or at least impractical, for them to initiate another breeding attempt whilst still rearing young from the previous season (but see Weimerskirch *et al*. 2015). However, biennial grey-headed albatrosses rear their chicks in a single summer, on a schedule that starts one week earlier and ends 2-4 weeks later than that of the sympatric, annually-breeding black-browed albatrosses. Despite this, most grey-headed albatrosses will not attempt to breed in the successive year as annual breeders generally do (although some may if they fail breeding early in the previous year; Ryan *et al.* 2007), even though parental care is not as prolonged as in some other biennial species. This begs the question: why don’t grey-headed albatrosses breed every year? It has been suggested that biennial breeding in grey-headed albatrosses is the result of a) the short time available to adults to recover body condition and replace flight feathers before the start of the following season (Ryan *et al.* 2007), which may either be related to, or resulting in, b) a physiological cost of reproduction that carries over to negatively affect future breeding effort (e.g. Crossin *et al*. 2013b).

In this study, we explore whether physiological costs link current to future reproduction, which would suggest a physiological carryover effect (Crossin *et al*. 2013b) We do so by comparing the annual and biennial breeding systems found within the genus *Thalassarche*. We determined the breeding fate (successful, failed, or deferred breeding) of black-browed albatrosses (annual) and grey-headed albatrosses (biennial) at the end of a breeding season, and sampled them for indicators of their energetic (triglycerides), aerobic (hematocrit), and hormonal (testosterone) condition. We then examined correlations between these indicators and Cort levels measured in tail feathers grown during the subsequent nonbreeding period. Assuming that variation in fCort levels indicate the “stress state” of non-breeding individuals after the breeding season, we then predict relationships between fCort and 1) previous breeding fate, and 2) post-breeding physiological condition of those individuals, as indicated by triglyceride, testosterone, and hematocrit levels. For example, if low triglyceride and low hematocrit levels correlated with high fCort in the breeding birds (successful and failed) but not in the non-breeding deferring birds, then this would suggest a cost of reproduction that carries over into winter. Finally, we predict that 3) fCort would itself carry over to affect future reproductive investment, e.g. the decision to breed, with high fCort indicative of deferred breeding in the following season. We discuss our results relative to hypotheses regarding the physiological costs of reproduction, and speculate on the evolution of annual versus biennial breeding strategies.

**Methods**

*Study site*

Fieldwork was conducted during austral summers 2008/09 and 2009/10 at Bird Island, South Georgia (54°01’S, 38°02’W), a sub-Antarctic island group that is one of the most important breeding sites, globally, for grey-headed and black-browed albatrosses. Individually banded albatrosses of known age and breeding history in long-term monitoring colonies were sampled (grey-headed albatrosses in Colonies B and E, and black-browed albatrosses in Colony J). All birds had bred at least once previously. Research was approved by the Ethics Committee of the British Antarctic Survey and carried out under permits issued by the Government of South Georgia and South Sandwich Islands. Sampling protocols conformed to guidelines established by the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 897B-8).

*Study species*

The breeding and foraging ecology of grey-headed and black-browed albatrosses at Bird Island is well studied (Prince 1985, Phillips *et al*. 2004, Crossin *et al*. 2012, Crossin *et al*. 2013a). Both species are monogamous and lay a single egg. After a long incubation period, the single chick is reared by both parents over the next 4-5 months. Grey-headed albatrosses (GHA) arrive at the breeding colony in spring, usually around mid September, approximately a fortnight earlier, have a slightly longer incubation period (72 vs. 68 days), and a longer chick-rearing period (141 vs. 116 days), which makes their breeding season ~45 days longer than black-browed albatrosses (BBA). There is a degree of niche divergence partially mediated by differences in flight performance (Phillips *et al*. 2004), and although the diets overlap, chicks of grey-headed albatrosses are fed more squid, and those of black-browed albatrosses are fed more krill and fish. The lower energy density of squid contributes to the slower growth rate and longer fledging period of grey-headed albatross chicks. At South Georgia, approximately 80% of the breeding population of black-browed albatrosses returns to breed the following year, the absence of the remainder due principally to either natural mortality or loss of partner (Croxall *et al.* 1998). In contrast, only ~1% of successfully breeding grey-headed albatrosses will breed again the following year, 25-80% two years later, and all but a small minority within three or four years, whereas those that fail in incubation or early chick-rearing in one year will generally breed the next year (Ryan *et al.* 2007). All of our analyses however examined the post-breeding physiology and fCort relative to breeding activity in the very next year, not two or more years later.

*Sampling design*

A timeline of our sampling protocol is presented in Fig. 1. From 24 January to 6 February 2009, we sampled non-breeding (i.e. deferring) black-browed and grey-headed albatrosses at nests in their respective colonies. Daily monitoring records of the albatross colonies at Bird Island, maintained by the British Antarctic Survey, allowed us to know the breeding status of all individuals. Deferring albatrosses return to and spend time in the colony each year, despite not breeding, presumably to re-establish pair bonds (Tickell 2000). Deferring black-browed albatrosses typically depart South Georgia for winter migration in early February (Phillips *et al*. 2005), so our sampling plan aimed to capture these birds and deferring grey-headed albatrosses before their departures (see Table 1 for numbers of birds sampled). From 30 March to 2 April, we sampled successful and failed breeders before their out-migration in mid-April. Blood samples (2 ml) were collected from the brachial vein using syringes with 25 gauge needles, and returned to the laboratory where the plasma was separated by centrifugation and stored at -20 °C. In all cases, blood was collected in less than 3 min from first approach to the bird. Ultimately, we sampled 125 albatrosses at the end of the 2008/09 season (BBA=62, GHA=63), 68 of which were resampled when they subsequently returned to breed in the following season in 2009/10. Therefore, the analyses conducted in this study were restricted to the 68 birds sampled in both 2008/09 and 2009/10 (BBA=50, GHA=18). Upon arrival at breeding colonies in 2009/10, a single rectrix was collected from each bird. Specifically, we collected only the outer-most rectrix from the right side of the bird, by cutting the feather with scissors at the base of the feather shaft (calamus). This was done to ensure that we sampled newly moulted feathers, as the sequence of rectrix moult is from outer to inner (Prince *et al.* 1993). These were stored in labeled bags and kept in the dark at 4 °C until analysis of feather Cort.

*Physiological assays*

Plasma samples and known standards were assayed in duplicate for total triglyceride levels (Trig) using a commercial triglyceride kit (Glycerol Reagents A and B, Sigma), and measured using a Biotek 340i microplate reader. Additionally, multiple duplicates of a domestic hen (*Gallus domesticus*) plasma pool were also assayed to provide a mean intra-assay coefficient of variation was 7.1%. The inter-assay coefficient of variation was 6.7%.

Hematocrit (Hct) was measured in fresh whole blood by centrifugation in microhematocrit tubes (2 per individual) for 5 min at 10,000 *g*, and is reported as packed cell volume (%).

Testosterone (T) was measured by first extracting plasma samples in dichloromethane and then quantifying hormone levels using a commercially available enzyme-linked immunosorbent assay (Cayman Chemicals Kit 582701). For each species, an extracted plasma pool was found to be parallel to the standard curve, and samples were assayed in triplicate at a 1:10 dilution. Samples were assayed across 6 plates yielding inter and intra-assay coefficients of variation of 4.6% and 6.3%, respectively. Extraction efficiency was assessed by spiking 4 randomly-chosen samples for each species with a known amount of testosterone standard immediately before extraction and comparing measured hormone levels to their corresponding normally-assayed (unspiked) levels. Average recovery was found to be 76.4% for black-browed albatrosses and 79.7% for grey-headed albatrosses. Statistical analyses were run on values corrected for these extraction efficiencies.

Corticosterone measured in the feathers of the same birds upon their return to Bird Island reflect plasma Cort levels in the weeks-months immediately after their departure from the breeding colony when tail feathers are moulted and regrown (Prince *et al*. 1993, Catry *et al.* 2013, Bugoni *et al.* 2015). To assay fCort, we used a standard radioimmunoassay as per Bortolotti *et al*. (2008), modified by Lattin *et al*. (2011), and following the protocol detailed in Crossin *et al.* (2013b). We determined the intra-assay coefficient of variation by measuring differences between duplicates, and the inter-assay variation by measuring differences between standard samples (using two standards: a known amount of Cort, and pulverized, homogenized feathers) (Romero and Fairhurst 2016). Intra-assay variation was 4.0%; inter-assay variation was 7.4%.

*Statistical analyses*

 All blood variables were examined for correlations with the time required to collect the blood sample and the date of collection. Due to known differences in life-history, behaviour, and physiology, we ran models for each species separately. To test our first prediction that fCort levels are related to previous breeding fate, we examined fCort, for each species separately (ANOVAs), by sex and breeding fate (successful, failed, deferred) as categorical factors, along with their interaction (sex\*fate). We then ran similar models to describe the species’ post-breeding physiological state (Trig, T, Hct) at the end of the 2008/09 breeding season. To test our second prediction of a relationship between end-of-breeding season physiological indicators and fCort, Pearson’s correlations by species and sex were examined. To test our third prediction that variation in fCort carries over to affect future breeding decision by each species, we used backwards stepwise generalized linear models with binomial response distributions to explore the variables most related to future breeding decision (breed or defer), in the subsequent breeding season only (2009/10 season). Explanatory predictors included fCort, as well as bird sex, previous breeding status (breeder or non-breeder), T, Trig, and Hct. We restricted this comparison to the 2009/10 year only so that the physiological links from one season to breeding in the next could be compared between species simultaneously and on identical time scales.

**Results**

As albatrosses were sampled in two different seasons (end of 2008/09 season for blood, and beginning of 2009/10 season for feathers), we explored whether any of the physiological variables correlated with sampling time or date. In 2008/09, there were no correlations between sampling time (number of seconds to collect the blood sample) or date with any of the blood parameter levels (Pearson’s correlations with Trig, T, and Hct, all P>0.09).

The end-of-season physiological results were determined for each species by ANOVA models examining differences by sex and breeding fate (Fig. 2). In the black-browed albatrosses (N=50), Trig and T levels of females, but not males, were significantly higher in deferring breeders than in successful or failed breeders (Trig sex\*fate interaction ß=0.142, P=0.004. T: sex\*fate interaction ß=53.42, P=0.008). Hct levels did not differ by sex, but were generally lower in deferring than in successful or failed black-browed albatrosses (sex ß=-0.886, P=0.126; fate ß=-1.030, P=0.010; sex\*fate ß=-0.418, P=0.163).

In the grey-headed albatrosses (N=18), Trig did not differ between sexes or among breeding fates (sex ß=0.044, P=0.567; fate ß=-0.097, P=0.095; sex\*fate ß=0.013, P=0.437). However, T was significantly higher in deferring males than in successful and failed males, while females did not differ among fates (sex ß=-24.78, P=0.030; fate ß=5.30, P=0.187; sex\*fate ß=-9.140, P=0.043). Hct did not differ by sex, but was generally higher in successful and failed breeders than in deferring breeders (sex ß=-0.980, P=0.171; fate ß=-1.540, P=0.001; sex\*fate ß=-0.345, P=0.625). Finally, fCort did not differ among grey-headed sexes or fates (sex ß=-0.476, P=0.151; fate ß=0.109, P=748; sex\*fate ß=-0.071, P=0.897).

Contrary to our first prediction, fCort did not differ between the sexes or among breeding fates in either species (BBA N=50: sex ß=-0.584, P=0.070; fate ß=0.034, P=0.778; sex\*fate ß=-0.056, P=0.925. GHA N=18: sex ß=-0.476, P=0.151; fate ß=0.109, P=748; sex\*fate ß=-0.071, P=0.897). However, we found partial support for our second prediction in that fCort showed a significant negative correlation with Trig levels in female grey-headed albatrosses (r=-0.703, N=12 P=0.011), but not in male grey-headed albatrosses (r=-0.202, N=15, P=0.471) or either black-browed albatross sex (females, r=-0.022, N=23 P=0.921; males, r=-0.314, N=21, P=0.116) (Fig. 3). The only other significant correlation in this set of analyses was a negative relationship between T and Hct in female black-browed albatrosses (r=-0.577, N=30, P<0.001; data not shown).

Comparisons of generalized linear models examining future breeding decisions by black-browed albatrosses are presented in Table 2. The most parsimonious model identified via AICc selection identifies a significant effect of fCort on breeding decision, such that when fCort is high there is greater tendency to defer breeding (P=0.028; Table 3), which supports our third prediction that variation in fCort indicates a carryover effect to future reproduction. For grey-headed albatrosses, no variables were significant predictors of breeding decision, with the null model receiving the best support (Tables 2 and 3).

**Discussion**

As the tail feathers of albatrosses are moulted and re-grown after their departure from breeding colonies (Prince *et al*. 1993, Catry *et al.* 2013), we predicted that variation in fCort levels would reflect the state or condition of individuals after breeding and suggest a cost of reproduction related to levels of breeding investment (e.g. successful, failed, deferred breeding). Working from the assumption that feather Cort levels are a key link between current and future reproduction, we found limited evidence with a link between post-breeding physiological state and fCort, and between fCort and future breeding decision. However, these results are very species specific, which we suggest may reflect the contrasting life-histories of black-browed and grey-headed albatrosses, e.g. annual versus biennial breeding. For example, in the black-browed albatrosses fCort did not correlate with any of the physiological parameters measured at the end of the breeding season, but fCort nevertheless predicted future breeding decision. Conversely, in the grey-headed albatrosses there was limited evidence for a link between post-breeding physiology and fCort, via a negative correlated with circulating triglyceride levels. But despite this correlation, fCort did not predict future breeding decision in the grey-headed albatrosses.

So what do our results suggest? For the black-browed albatrosses, the link between fCort and future breeding investment, in the absence of any clear link to previous breeding investment, might indicate that the more immediate experience of pelagic migration immediately after departure from breeding colonies influences Cort deposition more so than previous breeding activity does, and that it is winter experience itself that generates carryover effects onto future breeding decisions. Certainly, this and many other recent studies support a role of fCort in carryover effects and future breeding investment (Crossin *et al.* 2013, Kowenberg *et al.* 2013, Fairhurst *et al.* 2015, Harms *et al.* 2015). For the grey-headed albatrosses, we observed the opposite – post-breeding triglyceride levels showed a negative correlation with fCort (significant in females and trending in males), but neither variable had any discernable affect on future breeding decision. Why this might be for the grey-headed albatrosses we do not know, but it may that genetic programming for biennial breeding provides an buffer against physiological carryover effects. However, our sample of grey-headed albatrosses was low, which may limit our power to detect physiological links between breeding states, and so interpretation of our results should be made cautiously. Furthermore, concerning links to fCort, successful grey-headed albatrosses do not necessarily complete tail moult in a year’s time (Prince et al. 1993), which may have been the case for some of the seven successful breeders that we resampled the following year. So our power to detect a carryover effect might be low. However, we do not believe that this changes our general conclusions about future breeding decisions by the grey-headed albatrosses.

With this caution in mind, we suggest that fundamental differences in breeding life-history can explain the patterns that we observed. Both species undertake long-distance migrations during the non-breeding period, lasting 6-16 months (Croxall *et al*. 2004, Phillips *et al*. 2005). In both species, there is a degree of condition dependence to breeding investment and decision making, which might reflect altered hypothalamus-pituitary-gonadal axis signaling in response to conditions experienced during migration (Crossin *et al*. 2012, Crossin *et al*. 2013a). In a species capable of breeding every year, like the black-browed albatross, the experiences and conditions encountered during the winter are likely to influence Cort dynamics, and thus carry over to affect pre-breeding condition in the spring. By then, previous breeding activity and its relative costs, if any, might be overshadowed. In contrast to the black-browed albatrosses, a significant negative correlation between end-of-season Trig levels and fCort was observed in the biennial grey-headed albatrosses, which might reflect a physiological or energetic cost of reproduction, although we acknowledge again that our samples size was small. However, it is not uncommon to observe negative correlations between measures of body condition (e.g. body mass, Trig levels) and Cort levels in birds (Love *et al.* 2004). The correlation between circulating Trig and fCort in our study suggests a cost of reproduction, wherein some individuals were in poorer energetic condition relative to others. But ultimately, neither Trig, fCort or any other post-breeding measure (T, Hct) predicted the decisions of either male or female grey-headed albatrosses to breed in the very next year. This raises intriguing questions about the physiological determinants of biennial breeding strategies, which we discuss below.

A previous attempt to explain the biennial breeding pattern in albatrosses took a comparative approach and examined how the duration of the breeding season and distance to foraging grounds predicted the breeding frequency in 12 species (Jouventin and Dobson 2002). The authors hypothesized that the length of the rearing period for some albatrosses is simply too long and energetically demanding for adults to recover sufficient body condition to breed again in a consecutive year. In support of this, the analysis showed that across all 12 species, those species with the longest rearing periods were generally biennial breeders. However, we would argue that it is perhaps less obvious how the comparatively small difference in breeding season duration would lead to energetic shortfalls that cannot be restored during the ~4 months of marine foraging before the next breeding attempt, unless there is a significant effect on post-breeding moult dynamics (Rohwer *et al.* 2011) – a topic that should be explored in future studies.

The second hypothesis addressed by Jouventin and Dobson (2002) to explain the breeding patterns of albatrosses, predicts that travel to distant foraging areas during the breeding season should reduce reproductive rates, presumably due to increased effort, but also because longer chick feeding intervals could lengthen the breeding season. There was some support for this when comparing across 12 species. However, it is unclear how this might ultimately determine the breeding strategies of black-browed and grey-headed albatrosses, as although grey-headed albatrosses have longer foraging ranges in incubation, there is little difference during chick-rearing, which accounts for the last 5 months of the season (Phillips *et al*. 2004). Moreover, there is some sexual segregation during incubation, which was attributed to differences in wing loading and flight performance, as opposed to competitive exclusion (Phillips *et al*. 2004). Consequently, the slightly longer distances travelled during this stage by grey-headed albatrosses should not therefore be interpreted as marginalization to poorer foraging areas, with negative consequences for overall condition. Indeed, although we detected physiological differences in albatrosses at the end of the season that related to their breeding fate (successful, failed, deferred; Fig. 2), there were no significant differences between species or sexes (Fig. 2). Worthy of note was the significantly high T levels in deferring male grey-headed and deferring female black-browed albatrosses. Hector *et al.* (1986) observed significant increases in T levels in both species and sexes at the end of breeding, although the breeding status of those individuals was not known. The functional significance of these increases is not readily apparent. Although T had no bearing on future reproductive activity, high T in deferring individual could reflect intraspecific interactions (e.g. aggression) prior to out-migration, which might also have relevance for the onset and pattern of winter migrations as seen in some passerines (Silverin *et al.* 1989).

We did, however, observe a significant negative correlation between plasma Trig at the end of the breeding season and fCort levels in (female) grey-headed albatrosses, but not black-browed albatrosses. This could have functional consequences as grey-headed albatrosses usually migrate considerably longer distance during winter migrations than black-browed albatrosses (Croxall *et al.* 2005, Phillips *et al.* 2005). Together, low Trig and high fCort may signal relative need to recover lost body reserves after breeding, and thus indicate a cost (Love *et al*. 2004, Hennin *et al.* 2015). However, other studies are needed to more fully explore this possibility. Electronic tracking of individual winter migrations and foraging activity would lend insights to this possibility. However, as previously indicated, variation in fCort did not ultimately predict breeding decision in the next year, as it did in the black-browed albatrosses. We therefore suggest that biennial breeding may have evolved as a bet-hedging strategy in grey-headed albatrosses as a means for buffering the apparent susceptibility of females to negative physiological costs of reproduction (e.g. the negative Trig~fCort correlation, Fig. 3). There are a very few individual grey-headed albatrosses that attempt to breed in successive years (Ryan *et al.* 2007), and presumably only those in the best relative condition are able to do so (Crossin *et al*. 2013a).

Previous work with other biennial species lends support to a fundamental role of Cort and body condition in mediating biennial versus annual breeding strategies (Vitousek *et al*. 2010). For example, in the biennially-breeding iguana (*Amblyrhynchus cristatus*) females in poor physiological condition at the onset of breeding are likely to produce smaller hatchlings with poor survival prospects, and risk their own survival. Iguanas that defer reproduction tend to have higher glucocorticoid levels and sensitivity, which might be a response to some exogenous factor like local resource availability, thus enhancing survival probability and subsequent fitness.

Although our study provides evidence for both costs of reproduction and carryover effects, and suggests plausible mechanisms, it is observational by nature. An alternative approach to advance our understanding of physiological control of breeding frequency and glean insights into the evolution of biennial breeding strategies, would be controlled experimental manipulations of glucocorticoid levels at the onset of the non-breeding season, in tandem with electronic tracking (Crossin *et al.* 2014), especially of female albatrosses.

**Acknowledgements**

We thank Derren Fox and Andy Wood at the British Antarctic Survey for field and data support. We also thank the suggestions of two anonymous reviewers. Financial support was provided by the Antarctic Science International Bursary, awarded to GTC. Additional support was provided by the British Antarctic Survey through a Natural Environment Research Council (NERC) Collaborative Gearing Scheme awarded to RAP and others, a Natural Sciences and Engineering Research Council of Canada (NSERC) Post-doctoral Fellowship to GTC, NSERC Discovery Grants to TDW, OPL and GTC, and a National Science Foundation (USA) grant IOS-1048529 to LMR. This study represents a contribution to the Ecosystems component of the British Antarctic Survey Polar Science for Planet Earth Programme, funded by NERC.

**Author contributions**

 GTC performed the field work, collected the data, performed the triglyceride and hematocrit assays, analyzed the data, and wrote the manuscript. RAP assisted with the coordination of the field plan, provided funding and logistical support through BAS, and contributed to the writing. XB assisted with statistical analysis. CRL and LMR ran the corticosterone assays. CH and OPL ran the testosterone assays. TDW provided logistical and intellectual guidance with respect to the field plan, the analyses, and the framing of the manuscript, as well as providing financial and other support. All authors reviewed and edited the final submitted manuscript.

**References**

BONIER, F., MARTIN, P.R., MOORE, I.T. & WINGFIELD, J.C. 2009. Do baseline glucocorticoids predict fitness? Trends in Ecology and Evolution, 24, 634-642.

BORTOLOTTI, G.R., MARCHANT, T.A., BLAS, J. & GERMAN, T. 2008. Corticosterone in feathers is a long-term integrated measure of avian stress physiology. Functional Ecology, 22, 494-500.

BREUNER, C.W., PATTERSON, S.H. & HAHN T.P. 2008. In search of relationships between the acute adrenocortical response and fitness. General and Comparative Endocrinology, 157, 288-295.

BUGONI, L., NAVES, L.C. &FURNESS, R.W. 2015. Moult of three Tristan da Cunha seabird species sampled at sea. Antarctic Science, 27, 240-251.

CATRY, P., POISBLEAU, M., LECOQ, M. & PHILLIPS, R.A. 2013. Differences in the timing and extent of annual moult of black-browed albatrosses *Thalassarche melanophris* living in contrasting environments. Polar Biology, 36, 837-842.

CROSSIN, G.T., PHILLIPS, R.A., TRATHAN, P.N., FOX, D.S., DAWSON, A., WYNNE-EDWARDS, K.E. & WILLIAMS, T.D. 2012. Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses. General and Comparative Endocrinology, 176, 151-157.

CROSSIN, G.T., PHILLIPS, R.A., WYNNE-EDWARDS, K.A. & WILLIAMS, T.D. 2013a. Post-migratory body condition and ovarian steroid production predict breeding decisions by female gray-headed albatrosses. Physiological and Biochemical Zoology, 86, 761-768.

CROSSIN, G.T., PHILLIPS, R.A., LATTIN, C.R., ROMERO, L.M. & WILLIAMS, T.D. 2013b. Corticosterone mediated costs of reproduction link current to future breeding. General and Comparative Endocrinology, 193, 112-120.

CROSSIN, G.T., COOKE, S.J., GOLDBOGEN, J.A. & PHILLIPS, R.A. 2014. Tracking fitness in marine vertebrates: current knowledge and opportunities for future research. Marine Ecology Progress Series, 496, 1-17.

CROSSIN, G.T., LOVE, O.P., COOKE, S.J. & WILLIAMS, T.D. 2016. Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context, and reproductive state. Functional Ecology, 30, 116-125.

CROXALL, J.P., PRINCE, P.A., ROTHERY, P. & WOOD, A.G. 1998. Population changes in albatrosses at South Georgia. Pages 69-83 in G. Robertson and R. Gales, editors. Albatross biology and conservation. Surrey Beatty and Sons, Chipping Norton.

DAAN, S., DEERENBERG, C. & DIJKSTRA, C. 1996. Increased daily work precipitates natural death in the kestrel. Journal of Animal Ecology, 65, 539-544.

DESCAMPS, S., BÊTY, J., LOVE, O.P. & GILCHRIST, G.H. 2011. Individual optimization of reproduction in a long-lived migratory bird: a test of the condition-dependent model of laying date and clutch size. Functional Ecology, 25, 671-681.

EBBINGE, B.S. & SPAANS, B. 1995. The importance of body reserves accumulated in spring staging areas in the temperate zone for breeding in dark-bellied brent geese *Branta b. ber- nicla* in the high Arctic. Journal of Avian Biology, 26, 105-113.

FAIRHURST, G.D., BOND, A.L., HOBSON, K.A., & RONCONI, R.A. 2015. Feather-based measures of stable isotopes and corticosterone reveal a relationship between trophic position and physiology in a pelagic seabird over a 153-year period. Ibis, 157, 273-283.

GUGLIELMO, C.G. & WILLIAMS, T.D. 2003. Phenotypic flexibility of body composition in relation to migratory state, age, and sex in the western sandpiper (*Calidris mauri*). Physiological and Biochemical Zoology, 76, 84-98.

HARMS, N.J., LEGAGNEUX, P., GILCHRIST, H.G., BÊTY, J., LOVE, O.P., FORBES, M.R., BORTOLOTTI, G.R., & SOOS, C. 2015. Feather corticosterone reveals effect of moulting condition in the autumn on subsequent reproductive output and survival in an Arctic migratory bird. Proceedings of the Royal Society B, 282, 000-000.

HARRISON, X.A., BLOUNT, J.D., INGER, R., NORRIS, D.R. & BEARHOP, S. 2011. Carryover effects as drivers of fitness differences in animals, Journal of Animal Ecology, 80, 4-18.

HECTOR, J.A.L., FOLLETT, B.K. & PRINCE, P.A. 1986, Reproductive endocrinology of the Black-browed albatross *Diomedea melanophris* and the grey-headed albatross *D. chrysostoma*. Journal of Zoology, London, 208, 237-253.

HENNIN, H.L., LEGAGNEUX, P., BêTY, J., WILLIAMS, T.D., GILCHRIST, H.G., BAKER, T.M. & Love, O.P. 2015. Pre-breeding energetic management in a mixed-strategy breeder. Oecologia, in press.

JOUVENTIN, P. & DOBSON F.S. 2002. Why breed every other year? The case of albatrosses. Proceedings of the Royal Society B, 269, 1955-1961.

KALMBACH, E., GRIFFITHS, R., CRANE, J.E. & FURNESS, R.W. 2004. Effects of experimentally increased egg production on female body condition and laying dates in the great skua *Stercorarius skua*. Journal of Avian Biology, 35, 501-514.

KOUWENBERG, A.L., HIPFNER, J.M., MCKAY, D.W. & STOREY, A.E. 2013. Corticosterone and stable isotopes in feathers predict egg size in Atlantic puffins. Ibis, 155, 413-418.

LATTIN, C.R., REED, J.M., DESROCHERS, D.W. & ROMERO, L.M. 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. Journal of Avian Biology, 42, 247-252.

LOVE, O.P., BREUNER, C.W., VÉZINA, F. & WILLIAMS, T.D. 2004. Mediation of a corticosterone-induced reproductive conflict. Hormones and Behaviour, 46, 59-65.

MONAGHAN, P., BOLTON, M. & HOUSTON, D.C. 1995. Egg production constraints and the evolution of avian clutch size. Proceedings of the Royal Society of London B, 259, 189-191.

MONAGHAN, P. & NAGER, R.G. 1997. Why don’t birds lay more eggs? Trends in Ecology and Evolution, 12, 270-272.

MONAGHAN, P., NAGER, R.G. & HOUSTON, D.C. 1998. The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. Proceedings of the Royal Society of London B, 265, 1731-1735.

O’CONNOR, C.M., NORRIS, N.R., CROSSIN, G.T. & COOKE, S.J. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. Ecosphere, 5, 1-11.

PRINCE, P.A. 1985. Population and energetic aspects of the relationships between black-browed and grey-headed albatrosses and the Southern Ocean marine environment. In Antarctic nutrient cycling and food webs: Proceedings of the SCAR symposium on Antarctic biology, 473-477. Siegfried, W.R., Condy, P.R. & Laws, R.M. (Eds). Berlin: Springer Verlag.

PRINCE, P.A., RODWELL, S., JONES, M. & ROTHERY, P. 1993. Moult in black- browed and grey-headed albatrosses *Diomedea melanophris* and *D. chrysostoma*. Ibis, 135, 121-131.

PHILLIPS, R.A., SILK, J.R.D., PHELAN, B., CATRY, P. & CROXALL, J.P. 2004. Seasonal sexual segregation in two *Thalassarche* albatross species: competitive exclusion, reproductive role specialization or foraging niche divergence? Proceedings of the Royal Society of London B, 271, 1283-1291.

PHILLIPS, R.A., SILK, J.R.D., CROXALL, J.P., AFANASYEV, V. & BENNETT, V.J. 2005. Summer distribution and migration of nonbreeding albatrosses: individual consistencies and implications for conservation. Ecology, 81, 2386-2396.

PIERSMA T., EVERAARTS, J.M. & JUKEMA, J. 1996. Build-up of red blood cells in refueling bar-tailed godwits in relation to individual migratory quality. Condor, 98, 363-370.

PROP, J., BLACK, J.M. & SHIMMINGS, P. 2003. Travel schedules to the high arctic: barnacle geese trade-off the timing of migration with accumulation of fat deposits. Oikos, 103, 403- 414.

ROHWER, S., Viggiano, A. & Marzluff, J.M. 2011. Reciprocal tradeoffs between molt and breeding in albatrosses. The Condor, 113, 61-70

ROMERO, L.M. & FAIRHURST, G.D. 2016. Measuring corticosterone in feathers: strengths, limitations, and suggestions for the future. Comparative Biochemistry and Physiology A, 000, 000-000.

RYAN, P.G., PHILLIPS, R.A., NEL, D.C. & WOOD, A.G. 2007. Breeding frequency in grey-headed Albatrosses. Ibis, 149, 45-52.

SILVERIN, B., VIEBKE, P.A. & WESTIN, J. 1989. Hormonal correlates of migration and territorial behavior in juvenile willow tits during autumn. General and Comparative Endocrinology, 75, 148-156.

TICKELL, W.L.N. 2000. *Albatrosses*. Sussex, UK: Pica Press.

VITOUSEK, M.N., MITCHELL, M.A., ROMERO, L.M., AWERMAN, J., WIKELSKI, M. 2010. To breed or not to breed: physiological correlates of reproductive status in a facultatively biennial iguanid. Hormones and Behavior, 57, 140-146.

WEIMERSKIRCH, H., DELORD, K., GUITTEAUD, A, PHILLIPS, R.A. & PINET, P. 2015. Extreme variation in migration strategies between and within wandering albatross populations during their sabbatical year, and their fitness consequences. Scientific Reports, 5, 8853. DOI: 10.1038/srep08853

WILLIAMS, T.D. 2012. Physiological adaptations for breeding in birds. Princeton University Press, Princeton, New Jersey, USA.

Table 1 | The numbers and fates of black-browed (*Thalassarche melanophris*, BBA) and grey-headed (*T. chrysostoma*, GHA) albatrosses sampled in two consecutive breeding seasons at Bird Island, South Georgia (austral summer 2008/09 to 2009/10). To link current 2008/09 breeding to future 2009/10 breeding, the dataset was restricted to albatrosses sampled for tail feathers in 2009/10 (future breeding).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 2008/09 |  |  |  | 2009/10 |  |
| Species | Sex | Successful | Failed | Deferred |  | Bred | Deferred |
| BBA (total N=50) | Female | 15 | 9 | 4 |  | 22 | 6 |
|  | Male | 11 | 5 | 6 |  | 19 | 3 |
|  |  |  |  |  |  |  |  |
| GHA (total N=18) | Female | 5 | 3 | 1 |  | 3 | 6 |
|  | Male | 2 | 4 | 3 |  | 4 | 5 |

Table 2: Comparison of models exploring the effects of breeding status (successful breeder, failed breeder, or deferred breeder) and post-breeding season physiological condition of black-browed and grey-headed albatrosses on reproductive decision a year later. Output were generated from a backward stepwise generalized linear model with a binomial response and logit link function. Species were run separately due to known differences in breeding strategy and physiology.

|  |  |  |
| --- | --- | --- |
| **Species** | **Model** | **AICc** |
| BBA (N=50) | sex + breeding status + hematocrit + triglyceride + testosterone + fCort | 38.04 |
|  | breeding status + hematocrit + triglyceride + testosterone + fCort | 35.21 |
|  | breeding status + triglyceride + testosterone + fCort | 32.99 |
|  | breeding status + triglyceride + fCort | 31.02 |
|  | **breeding status + fCort** | **28.80** |
|  | null (~1) | 37.99 |
|  |  |  |
| GHA (N=18) | sex + breeding status + hematocrit + triglyceride + testosterone + fCort | 40.49 |
|  | sex + breeding status + hematocrit + testosterone + fCort | 33.14 |
|  | sex + breeding status + hematocrit + fCort | 27.49 |
|  | **null (~1)** | **25.30** |

Table 3 | Results of a generalized linear model comparing the binomial breeding decision (breed or defer) of black-browed (*Thalassarche melanophris*, BBA) and grey-headed (*T. chrysostoma*, GHA) albatrosses. Models are the most parsimonious as determined by AICc model comparisons (Table 2). Given differences in breeding strategy (BBA are annual breeders, and GHA are biennial breeders), separate models were run for the two species. Significant model effects are indicated by bold text.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species (N) | Effects | estimate | z | P |
| BBA (50) | Previous breeding fate | -19.567 | -0.005 | 0.996 |
|  | **fCort** | **-0.955** | **-2.196** | **0.028** |
|  |  |  |  |  |
| GHA (18) | Null (intercept) | 0.357 | 0.724 | 0.469 |

**Figure legends**

Fig. 1 | Schematic representing annual cycle of black-browed (BBA, *Thalassarche melanophris*) and grey-headed (GHA, *T. chrysostoma*) albatrosses at Bird Island, South Georgia during the austral summer of 2008/09 (breeding season 1) and 2009/10 (breeding season 2). Brackets indicate times when physiological samples were collected. The yellow bars indicate the period when tail feathers are moulted and regrown; the dashed segment indicates that the duration of the period of tail feather growth is presently unknown. See Table 1 for samples sizes.

Fig. 2 | Physiological variables in black-browed (BBA, *Thalassarche melanophris*) and grey-headed (GHA, *T. chrysostoma*) albatrosses measured at the end of the 2008/09 breeding season. Feather Cort levels reflect circulating Cort in the weeks/months after breeding, when tail feathers are moulted and then replaced. Data are classified according to species and breeding fate (S=successful breeders, F=failed breeders, D=deferred breeders). Females are indicated with triangles and males with circles, while GHA values are in grey and BBA values are in black. Values represent least squares means ±SEM.

Fig. 3 | Correlations between plasma triglyceride levels and residual feather corticosterone levels in black-browed (BBA, *Thalassarche melanophris*) and grey-headed (GHA, *T. chrysostoma*) albatrosses. Triglyceride levels were measured at the end of breeding in the 2008/09 season, while fCort levels reflect circulating Cort at the time of feather regrowth in the early to mid nonbreeding season. Females are indicated with triangles and males with circles, while GHA values are in grey and BBA values are in black. All feathers (whether from breeding or deferring albatrosses) were sampled at the beginning of the 2009/10 season.





