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Seasonal patterns of prolactin and corticosterone secretion in an Antarctic seabird that moults during reproduction.

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Running head: Hormonal basis of breeding-moult overlap.

Abstract

In avian species that have evolved life-history strategies wherein molt and breeding overlap, there are potential conflicts between the regulatory roles of baseline prolactin and corticosterone in parental care (positive) and moult (negative). We describe seasonal patterns of hormonal secretion, moult, and parental behaviour in sibling species of giant petrels (*Macronectes spp.*) which begin moult during the incubation/early chick-rearing stage of reproduction. With the exception of male Southern giant petrels (*M. giganteus*), prolactin secretion and moult in Northern (*M. halli*) and female Southern giant petrels conformed to those observed in all other avian species, with the initiation of moult coincident with decreases from peak prolactin levels. However, male Southern giant petrels began moulting early in incubation when prolactin was increasing and had not yet peaked, which suggests a requirement of prolactin for incubation behaviour and a dissociation of prolactin from moult. Corticosterone showed little seasonal variation and no relationship with moult. When comparing prolactin, corticosterone, and moult in failed versus active breeders, we found that failed breeding enabled a more rapid down-regulation of prolactin, thus facilitating a more rapid moult. We present specific examples of the behavioural ecology of giant petrels which we conclude help mediate any potential hormonal conflicts between parental care and moult.

Keywords: Prolactin, corticosterone, physiological conflict, incubation, chick rearing, giant petrel, *Macronectes spp.*

1. Introduction

It has long been held that the avian annual cycle is structured so that reproduction and moult are temporally segregated, thereby minimizing the potential for energetic or physiological conflict [9,25,33,34,40], and the idea that these two activities are mutually exclusive persists [7]. However, many avian species have evolved moulting strategies which overlap to varying degrees with reproduction, including seabirds [1,4,17,32,38,44], raptors [28,43], passerines [19,20,26,41] and shorebirds [27]. Here we use the extensive moult-breeding overlap that occurs in giant petrels (*Macronectes spp.*; [30]) to further our understanding of the hormonal control of moult, and in particular to determine how birds resolve potential hormonal conflicts generated by 'pleiotropic' effects of prolactin and corticosterone in the regulation of parental care (positive regulatory effects) and moult (negative regulatory effects) [23,47].

Experimental and descriptive studies with a variety of bird species (e.g. European starlings [*Sturnus vulgaris*], Mute swans [*Cygnus olos*]) have shown that the endocrine control of moult is regulated in part by the hormone prolactin [35], and specifically it is the decrease from seasonal peaks rather than some absolute threshold which signals the initiation of moult [9,15]. Immunization against vasoactive intestinal peptide, the prolactin-releasing hormone in birds, inhibits photoperiodically induced prolactin secretion and moult [12]; thus, moult generally cannot be readily disengaged from prolactin secretion [9]. However, prolactin also affects parental behaviour, particularly incubation behaviour in the form of egg defense and thermoregulation, and elevated plasma prolactin is maintained in many species until relatively late in chick-rearing to facilitate parental care [55]. Therefore, if high plasma prolactin levels actually inhibit the initiation of moult, this could explain why for many (but not all) birds moult tends to begin late in chick-rearing when parental care is waning [2,10,13,15]. In species exhibiting an overlap between parental care and moult, there is an apparent conflict due to the pleiotropic effects of prolactin. How can some species initiate moult without a decrease in prolactin (as suggested by [15])? Conversely, how can birds provide effective parental care if prolactin decreases early in incubation/chick-rearing in order to initiate moult?

A similar conflict might emerge during a breeding-moult overlap via the pleiotropic effects of the glucocorticoid hormone corticosterone. In many respects, a physiological stress response should be 'adaptive' by increasing the likelihood that an individual will survive and cope with a transient environmental stressor, though over-stimulation due to chronic stress, or severe acute stressors, can lead to long-term negative effects [52]. Many studies of stress physiology have focused on the functional consequences of *maximum* or *stress-induced* corticosterone levels in response to acute stressors or unpredictable events [37,49,58,59,60]. The acute stress response mediates fairly rapid, short-term physiological and behavioural changes (e.g. the "emergency life history stage" [59]) to allow individuals to cope with environmental stressors, principally though a positive effect on locomotor and foraging behavior with negative effects on reproduction. More recent attempts to place this work in the context of life-histories has highlighted the small number of studies that have linked variation in glucocorticoids to variation in fitness, fecundity, reproductive success or survival, either for baseline corticosterone [6] or stress-induced corticosterone levels [8]. Variation in corticosterone at any single point in time can be positively, negatively, or non-significantly related to surrogates of fitness [6,8] and the relationships can (not surprisingly) vary within individuals at different times in their life history.

Along these lines, recent studies have shown that baseline (i.e. not stress-induced) corticosterone secretion can be adaptively up-regulated during the reproductive season to facilitate high rates of provisioning for growing chicks [2,36]. However, corticosterone is usually down-regulated prior to the onset of the post-breeding moult, presumably to avoid the catabolism of structural proteins needed for feather synthesis, as well as the corticosterone-dependent inhibition of new protein synthesis during feather growth [16]. Indeed, baseline corticosterone tends to be low in migratory passerines undergoing prebasic moult, and the adrenocortical response to stress is also down-regulated [48,51]. How do birds exhibiting a breeding-moult overlap reconcile the need to up-regulate baseline corticosterone during chick-rearing to support foraging against the need to down-regulate it to avoid negative effects on moult (e.g. delayed moult, poor feather quality; [14,51])?

To address these questions, we present correlative data on the temporal patterns of prolactin and corticosterone secretion during the breeding-moult overlap in sibling species of giant petrels (*Macronectes spp.*) nesting sympatrically at a sub-Antarctic island. Giant petrels provide a good model for exploring the endocrine control of moult and the potential conflicts therein as both Northern (*M. halli*) and Southern (*M. giganteus*) giant petrels begin moulting their primary flight and body feathers towards the end of the incubation period or early-to-mid chick-rearing, though Southern males can begin moult during the initiation of incubation behaviours [30]. Hunter [30] speculated that the high availability of food resources and animal carrion might provide sufficient energy to facilitate both reproduction and moult, and due to sexual dimorphism in foraging behaviour this might favour males as they tend to forage more locally while females rely more on marine foraging [21]. Coupled with the fact that the two species differ both in the timing and rate of moult [30], giant petrels provide a powerful system to look at hormonal correlates of moult. By the time of hatching, giant petrels have begun moult, which continues throughout the rest of the breeding season, and both species fledge their chicks and out-migrate at the same time at the breeding season’s end, despite that Southern giant petrels begin breeding/moulting two months later than the Northern species [30]. Given the strong associations and empirically tested relationships between moult and both prolactin [9,15] and corticosterone [51], and between incubation behaviour and prolactin [2], we examined seasonal profiles of prolactin and corticosterone secretion and moult scores during the breeding-moult overlap. The widespread (though not universal [18]) role of prolactin in incubation behaviour led us to predicted that increasing prolactin would reveal a dissociation from moult in male Southern giant petrels, which, unlike the other species and sexes, begin moult early during incubation. In contrast, we predicted that female Southern and both sexes of Northern giant petrels, which begin moulting towards the end of incubation, decreasing prolactin would herald the start of moult, as observed in the majority of birds in which prolactin-moult dynamics have been investigated. With respect to corticosterone, we predicted that the relatively high availability of food resources during the breeding season would make an up-regulation of corticosterone to facilitate increased foraging activity unnecessary. Therefore, we did not predict that plasma corticosterone would show seasonal up-regulation. Rather, we predicted that plasma corticosterone would be maintained at a fairly low, baseline, level and without a direct association with moult.

At the end of the breeding season before giant petrels depart the breeding colony, we also compare hormonal patterns to test predictions regarding the successful maintenance of both breeding and moult, especially as prolactin and corticosterone can both be involved in breeding failure [2,3,6,22]. For example, failed breeders should have lower prolactin and more advanced moult relative to birds still actively engaged in chick rearing. Therefore, we also compared relationships between prolactin, corticosterone, and moult in failed and active breeders to understand how these dynamics change when birds are released from the constraints of parenthood.

2. Material and Methods

2.1 Field collection

Data were collected from Northern and Southern giant petrels breeding on Bird Island, South Georgia (54º00'S, 38º02'W) during the austral summer of 2008-2009. Northern giant petrels lay in mid September-mid October, chicks hatch in mid November-mid December, and fledge in mid March-early April. Southern giant petrels lay on average about 6 weeks later, in November, and chicks hatch in January and fledge in May [see 31], and British Antarctic Survey unpublished data). The duration of the initial guard phase before the chick is first left alone is considerably longer in northern than southern giant petrels (30 days vs. 16 days; see [31]). Sex of adult giant petrels can be determined reliably in the field based on bill dimensions [31], and all individuals in this study were part of the long-term population monitoring programme. A maximum of twenty males and 20 females of each species were sampled at four different times throughout the breeding season: after laying, before hatch, after hatch, and late in chick-rearing. This sequence spanned a 6 month period from October 2008 to April 2009. Only one bird was sampled from each nest, and no birds were subsequently re-sampled (e.g. we did not serially sample birds). Failed and successfully breeding petrels were also sampled during the last sampling period (late chick-rearing), just prior to dates of chick-fledging and adult out-migration. Dates of breeding failure for these late-season petrels were determined from weekly nest visits as part of the routine giant petrel monitoring program at Bird Island. Failure varied from 63-147 days prior to dates of sampling.

At most sampling periods, birds were approached at the nest. The exception was during late-chick rearing when active breeders (identified from the unique alphanumeric code on their plastic leg bands) are more likely found in small groups in open grassy areas in the general vicinity of their nests. Upon capture, blood samples (2.0 ml) were collected from the tarsal vein using a pre-heparinized syringes with 25 gauge needles. Blood was transferred to heparinized 2.5-mL Eppendorf vials, and a small sub-sample was removed with a micro-haematocrit tube and centrifuged for 5 min. at 10,000 g. The remaining blood was then centrifuged for 5 min. at 10,000 g and plasma transferred to labeled 0.6-mL vials for storage at -20˚ C until analysis. In nearly all cases (299 of 302), blood was collected in less than 3 minutes, which is necessary for interpreting baseline corticosterone levels [50]. Bill length and minimum depth, and tarsus length, were measured to the nearest 1.0 mm with calipers. Mass was measured to the nearest 10.0 g with Pesola spring scales. Mass-corrected primary feather moult was scored using the method outlined by [11]. To compare the progression of moult to a previous study of giant petrels on Bird Island, moult was also scored using the method outlined in [30]. Birds were then released with a dab of red paint on their breasts to ensure that they were not recaptured or disturbed on subsequent sampling dates. We did not make detailed notes on the effects of handling on subsequent chick egg/survival as the birds that we sampled were outside the designated area for giant petrel monitoring at Bird Island.

2.2 Hormonal assays

Prolactin was assayed in duplicate 20 μl plasma samples in a recombinant-derived starling prolactin assay [5]. The sensitivity of the assay was 1.0 ng ml^{-1}, and 50% displacement was obtained with 12.1 ng ml^{-1}. All samples were measured in a single assay and the intra-assay coefficient of variation was 6.5%. For some samples, an extra 10 μl was measured to ensure parallelism (Fig. 1). Corticosterone was determined by double antibody radioimmunoassay (^{125}I-RIA, MP Biomedicals, 07-120103) with modifications validated for several avian species [42,54,57]. The assay detection limit was 3.13 pg corticosterone per tube (i.e. the lowest corticosterone standard, 12.5 ng ml^{-1}, using a 50 μl assay volume). The low corticosterone control and a consistent native plasma sample were analyzed in each assay to determine an inter-assay coefficient of variation (5.10%). Intra-assay coefficient of variation was 8.79% for the

Northern giant petrels and 10.71% for the Southern giant petrels. Serially diluted native plasma samples were parallel to the corticosterone standard curve (coefficients of variation for final concentrations were 9.94% for Northern giant petrels (N=3) and 7.48% for Southern giant petrels (N=3) following methods outlined in [45].

2.3 Statistics

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 variance (ANOVA) tests were used to examine differences among species and breeding stages and between sexes with regard to plasma prolactin, corticosterone, and moult score. We also used regression analysis to compare prolactin, corticosterone, and moult score differences between successful and failed breeders sampled at the end of the breeding season just prior to dates of chick fledging and adult out-migration. Tukey-Kramer *post-hoc* tests were used to identify significant contrasts in all tests. Linear regression was used to explore relationships between moult and hormonal levels in birds that were captured at the end of the season, and were thus all in moult. Values presented in figures are untransformed, least-squares means ± SEM.

3. Results

We sampled a total of 302 giant petrels (Northern = 70 females, 70 males; Southern = 81 females, 81 males) throughout the 6-month reproductive season. There was little variation in body mass, and without any consistent seasonal pattern between species or sexes (Table 1). There was significant variation in plasma prolactin levels in relation to breeding stage in each species and sex (ANOVA, all $P<0.01$; Fig. 2). In each case there was a unimodal pattern with low values around egg-laying, significant increases occurring around hatching, with prolactin then decreasing to lowest levels at the end of the breeding season. Moult generally did not begin until eggs hatched, except for *M. giganteus* males which initiated moult in the days following egg laying when prolactin had not yet peaked (Fig. 2).

In contrast to prolactin, there were no significant variations in plasma corticosterone relative to breeding stage in male and female Northern or in female Southern giant petrels (ANOVA, all P>0.55; Fig. 2). In male Southern giant petrels however, plasma corticosterone was significantly lower at the first sampling stage, just after egg-laying (3.42 0.83 ng ml^{-1}; $F_{3,70}$=3.698, P=0.024), but at no other stages were differences significant (Fig. 2), and in no species or sex did mean corticosterone levels exceed 10 ng ml^{-1}. Among the different sampling stages, mean baseline corticosterone ranged from 6.7-9.1 ng ml^{-1} in females and 3.9-8.4 ng ml^{-1} in males (Fig. 2). With regard to sampling time effects on corticosterone, after removing the 3 outlier samples (of 302) which were collected in >3 min, there were no significant relationship between sample collection time and baseline corticosterone concentrations (r=0.128, N=299, P=0.079) or prolactin (r=-0.028, N=299, P=0.633).

By examining moult scores and hormone concentrations in failed and successful breeders at the end of the season when all birds were in moult, a significant negative relationship was observed between plasma prolactin and moult in male and female Southern as well as male Northern giant petrels (Southern males, R^2=0.34, N=49, P<0.001; Southern females, R^2=0.32, N=19, P=0.026; Northern males, R^2=0.26, N=19, P=0.030) (Fig. 3), but for female Northern giant petrels, the relationship was only marginally non-significant (R^2=0.25, N=10, P=0.058). However, the slopes of the relationships are similar among all species and sexes, so we attribute the non-significance in Northern females to the small sample size relative to the others. In neither species nor sex was corticosterone significantly correlated with moult score (all P>0.440) (Fig. 3). There were no significant correlations among prolactin, corticosterone, and body mass in any species, sex or reproductive stage (Pearson's correlations, all P>0.298).

When comparing active (Northern females N=9, males N=18; Southern females N=14, males N=15) and failed breeders (Northern females N=8, males N=5; Southern females N=7, males N=4) at the late chick-rearing stage, significant differences in moult score were observed, with moult at a more advanced stage in failed breeders in both species and sexes (ANOVA, all P<0.001; Fig. 4). Prolactin showed an inverse pattern, with low levels when moult scores were high, but only in Southern giant petrels (ANOVA, both P<0.001; Fig. 4); there was no difference in prolactin between Northern giant petrels that had failed and those still raising chicks (both sexes, P>0.187). We ran an ANOVA model to compare prolactin in successful birds sampled next to the nest vs. successful birds sampled further way to explore the effect of sampling distance on concentrations. The models were not significant (sex-by-species; all P>0.233), meaning that we did not detect a difference based on sampling distance from the nest. Corticosterone levels did not differ between failed and active breeders in either species or sex (all P>0.37; Fig. 4).

4. Discussion

Our primary aim was to determine how giant petrels mediate the potential conflict between the roles of prolactin and corticosterone in the regulation of parental care (positive effects) and moult (negative effects) when reproduction and moult operate simultaneously (i.e. the breeding-moult overlap). All the giant petrels in this study showed a clear overlap between moult and reproduction, and in female Southern giant petrels and both sexes of Northern giant petrels, patterns of prolactin and corticosterone secretion were as predicted for the facilitation of moult

[16], i.e. both hormones were decreasing or at low levels throughout moult, even though these birds maintained parental care. These giant petrels therefore appear to have evolved behavioural or ecological means for avoiding any deleterious effects that low prolactin levels might exact on parental care, which we will discuss in greater detail below. (However, we do not know the levels at which prolactin were maintained in the non-breeding birds at this stage of the season; it is possible that the breeding birds still had relatively higher levels than non-breeding birds, which would indicate a continued role of prolactin in parental care. In contrast, male Southern giant petrels initiated moult at a time when prolactin and corticosterone were both increasing, which stands as a rare exception among the majority of birds for which a decrease from seasonal prolactin peaks seems required for the initiation of moult [9]. This suggests that in male Southern giant petrels moult must be regulated by some alternate physiological pathway (e.g. the thyroid hormones [46], but see [9]) so as to avoid the negative effects of low prolactin on incubation behaviour when moult starts very early in reproduction. Indeed, the pattern of increasing prolactin in male Southern giant petrels conforms to many descriptive and experimental studies which show a positive link between prolactin and incubation behaviour [2].

As noted, with the exception of male Southern giant petrels, patterns of prolactin secretion and moult conformed to those observed in other species [9]: moult was initiated only once prolactin began decreasing from seasonal peaks, and for the Northern giant petrels and female Southern giant petrels, this decrease occurred when their eggs began hatching. What is interesting is that this decrease preceded a major shift in parental behaviour, after which young chicks are left unguarded by both parents (see black arrows in Fig. 2; [30]), thus heralding the functional endpoint of prolactin-mediated parental care (e.g. physical protection and thermoregulation of chicks) and conforming to a general decreasing pattern observed in other Procellariiforme species [9,15,24]. It is probable that this aspect of their behavioural ecology is what allows giant petrels to avoid any negative pleiotropic effects of decreasing prolactin on parental behaviour, and may provide a means through which to mediate a potential hormonal conflict between parental care and the initiation of moult. Parent birds still provide care in the form of frequent food deliveries to growing chicks, but the brooding behaviours most often associated with prolactin secretion, including the defence and thermo-protection of chicks, are not exhibited by parents at this stage of chick development. Parents are thus free, in theory, to down-regulate prolactin and initiate moult without any apparent cost to the chick, which is the case with many bird species [2]. However most birds do not moult during the breeding season, so studies examining endocrinological differences between breeding-moult overlap species and non-overlap species are still needed. But, as noted, the Southern males do not conform to this model, and moult is initiated early in the incubation stage when prolactin is *increasing* (see Fig. 2). Unlike their Northern relatives and Southern female conspecifics, prolactin peaks in Southern males near the time of egg hatching and decreases only when chicks are left unguarded. This suggests a requirement for high prolactin in Southern male petrels in order to foster the behaviours that are vitally necessary for egg survival (e.g. defence and thermoregulation), and further suggests a disconnect between prolactin and moult. Southern Giant petrels begin breeding and moulting two months later than the Northern species, but both fledge their chicks and out-migrate at the same time before winter [30]. Rates of chick development are therefore faster in the Southern species, but rate of moult is slower, especially in males. This may present a requirement for male Southern giant petrels to begin moult so early during incubation, and selection for a de-coupling of prolactin from the dynamics of moult. This is supported by the fact that moult takes longer in the Southern than in the Northern species, and longer in males than females [30]. However, whether this disconnect comes without any real cost to the dynamics of moult, or whether direct, negative pleiotropic effects are indeed manifest in the quality or composition of newly grown feathers [14] is not presently known. Comparative analysis of feather structure and physiology between *Macronectes* species and sexes would be needed to discern this, though presumably selection pressure would be high. Furthermore, the location and density of prolactin receptors may vary among species, sexes and stages. Examination of receptor expression might lend insights to the mechanics of parental care, even when prolactin levels are comparatively low, and to how male Southern giant petrels are able to initiate moult so early during incubation.

The male Southern giant petrels also showed a significant increase in corticosterone at the onset of moult, unlike the female Southern and both Northern giant petrels, but this increase was small in scale. Shultz and Kitaysky [53] have interpreted baseline corticosterone as an indicator of nutritional stress, which shows negative correlations with food availability in kittiwakes (*Rissa tridactyla*). This idea is consistent with our study; we have observed very low levels of corticosterone throughout the breeding season in a system with high forage availability. However, other studies have also suggested that up-regulation of baseline (not stress-induced) corticosterone during reproduction can be a tactic in some species to facilitate high rates of chick provisioning [2,36], but one that presents a potential conflict with regard to moult as up-regulation can diminish the structural and thermoregulatory properties of newly grown feathers [51]. Despite the initial increase in the male Southern petrels, there was little overall variation in plasma corticosterone throughout the breeding season for both sexes and species, and corticosterone levels were similar in all the petrels (on average, all below 10 ng ml^{-1}). Furthermore, corticosterone concentrations did not correlate with either prolactin or the rate of moult. This raises the possibility that a relative up-regulation of corticosterone to support foraging may not be necessary for giant petrels given their unique behavioural ecology. High abundances of seal (*Acrtocephalus gazella*) and penguin (gentoo *Pygoscelis papua*, macaroni

Eudyptes chrysolophus) carrion from November to the end of January [see 29], as well as high levels of local marine production [39], may reduce foraging demands and offset any requirement for up-regulation of baseline corticosterone, or low baseline levels may reflect less nutritional stress [53]. Coupled with their tendency to leave their young chicks unattended, both parents are free to forage [29]. Either way, low corticosterone levels during moult, and the lack of any discernable pattern of increasing corticosterone, may reflect a reduced urgency to forage relative to terrestrial bird species in which one parent often remains on or in the vicinity of the nest to defend chicks. These low and generally constant baseline levels might then be related to the high seasonal food abundance, and probably facilitates a breeding-moult overlap by minimizing any potentially deleterious effects of protein catabolism in newly forming feathers. This provides a rare example of a potential hormonal conflict being resolved, in part, by aspects of behavioural ecology. Annual difference in environmental conditions and food availability may influence corticosterone secretions, and this could then have affects of the quality of newly moulted feathers. Experimental manipulations reflecting inter-annual variation of corticosterone levels and subsequent analysis of feather quality would lend support to this idea.

Our second aim was to investigate the inter-relationships between prolactin, corticosterone, and moult when breeding failure released birds from hormonal conflict between moult and parental care. In long-lived species, such as Procellariiform seabirds, life-history theory predicts that individuals should partition resources towards self-maintenance, survival and future fecundity, rather than for current breeding attempts when ecological conditions are poor [56], and studies have linked corticosterone in mediating this trade-off via suppression of prolactin [3]. Our data clearly show that that the loss of chicks allowed parent birds to shift from investment in parental care towards self-maintenance by directing time and resources to moult. In both male and female Southern giant petrels, a rapid down-regulation of prolactin was correlated with an accelerated rate of moult, and this is evident in Figs. 3 and 4 which show a significant inverse relationship between prolactin and moult among failed and successful individuals in both sexes. However, that there was no discernable difference in mean prolactin between failed and successful Northern giant petrels (Fig. 4) is probably due to reproduction beginning six weeks earlier in Northern than in Southern giant petrels, which may have provided sufficiently more time for both failed and successful breeders to down-regulate prolactin relative to the Southern species. Ultimately, there were no differences in body mass or corticosterone levels in failed and successful breeders, but these variables were measured weeks to months after the loss of eggs and chicks, and were presumably no longer representative of physiological state at the time of failure.

5. Conclusion

In summary, we suggest that giant petrels avoid any potential conflicts between the regulatory roles of prolactin and corticosterone in parental care (positive) and moult (negative) during their extensive moult-breeding overlap due to unique aspects of their behavior and foraging ecology. Despite moult being initiated early in parental care, moult was nevertheless associated with a decrease from seasonal prolactin peaks, supporting the hypothesis for a role in the regulation of moult [10,15]. The exceptions were male Southern giant petrels, which began moulting early during incubation and appear to have dissociated prolactin from moult in order to maintain high prolactin levels and preserve incubation behaviour. By early chick-rearing however, prolactin levels in all giant petrels were decreasing, but any potential ‘costs’ to parental care were presumably negligible as nest defence and thermo-protection of chicks are not typical parental behaviours in giant petrels at this stage of the reproductive season. Although Dawson [9] and Dawson and Sharp [13] highlight a concordance between *decreasing* prolactin and initiation of moult, our results nevertheless revealed significant negative relationships between moult score and prolactin levels, which suggests that absolute plasma prolactin levels might play a role in regulating the progression of feather replacement once moult begins. With respect to plasma corticosterone, levels were low throughout the period of parental care, which was consistent with the hypothesis that low levels are required to avoid the negative effects on new feather quality [16]. This may be especially true for male Southern giant petrels, which had significantly low corticosterone levels at the onset of incubation, which is when they begin moulting. There was however no evidence for an up-regulation of corticosterone to support increased foraging demands of chick-rearing, perhaps because of the high abundance carrion and/or high levels of local marine production may reduce foraging demands. Our study thus highlights the need to consider various behavioural, ecological, and evolutionary contexts when attempting to explain the putative hormonal mechanisms controlling the physiology of life-histories.

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Table 1: Body mass of giant petrels (*Macronectes spp.*) measured throughout a breeding season at Bird Island, South Georgia. Values are least square means ± SEM. Raised letters indicate statistically significant contrasts within species and sex.

	Females		Males	
Species & reproductive stage	N	Body mass (kg)	N	Body mass (kg)
M. halli				
Early egg incubation	20	3.38 ± 0.07	20	4.31 ± 0.10 ^{a}
Late egg incubation	10	3.79 ± 0.10	10	4.73 ± 0.14 ^{b}
Early chick rearing	20	3.49 ± 0.08	20	4.91 ± 0.10 ^{b}
Late chick rearing	20	3.59 ± 0.11	20	4.67 ± 0.15 ^{b}
M. giganteus				
Early egg incubation	20	3.84 ± 0.09 ^{a,b}	20	4.70 ± 0.11
Late egg incubation	20	3.89 ± 0.09 ^{a}	20	4.92 ± 0.11
Early chick rearing	20	3.59 ± 0.09 ^{b}	20	4.80 ± 0.11
Late chick rearing	21	3.56 ± 0.10 ^{b}	21	4.59 ± 0.14

Figure Captions

Fig. 1: Six plasma samples from Northern giant petrels were assayed for prolactin concentration at 10 L and 20 L volumes to examine assay parallelism. The lines connect points which show counts per minute for the pairs of samples plotted against the standard curve (bold dashed line).

Fig. 2: Plasma prolactin (closed circles), corticosterone (open circles), and moult scores (closed triangles) in breeding giant petrels (*Macronectes spp.*) at Bird Island, South Georgia. The progression of points follows seasonal stages of incubation and chick-rearing: first points represent early incubation, then late incubation, then early rearing, ending at late rearing. Points are least square means ± SEM. Solid arrows indicate approximate dates when chicks are first left unguarded by both parents. Open triangles in the *M. halli* panels are moult scores collected from separate groups of breeding birds to complement moult scores collected in *M. giganteus* on the same day.

Fig. 3: Relationships of moult with prolactin and corticosterone in giant petrels (*Macronectes spp.*) measured at the end of the breeding season when all birds were moulting. Closed circles signify *M. halli* Open and circles *M. giganteus*. Lines are best linear fits. Dashed line represents a non-significant relationship.

Fig. 4: Comparison of moult scores and plasma hormones in failed and successfully breeding giant petrels (*Macronectes spp.*) sampled at the end of the breeding season just prior to out-migration. Bars indicate least square means ± SEM.

Fig. 1

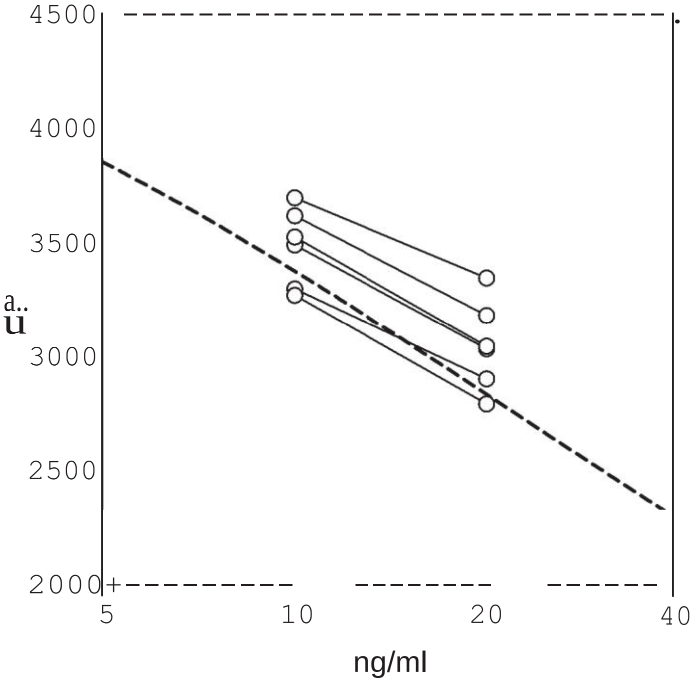
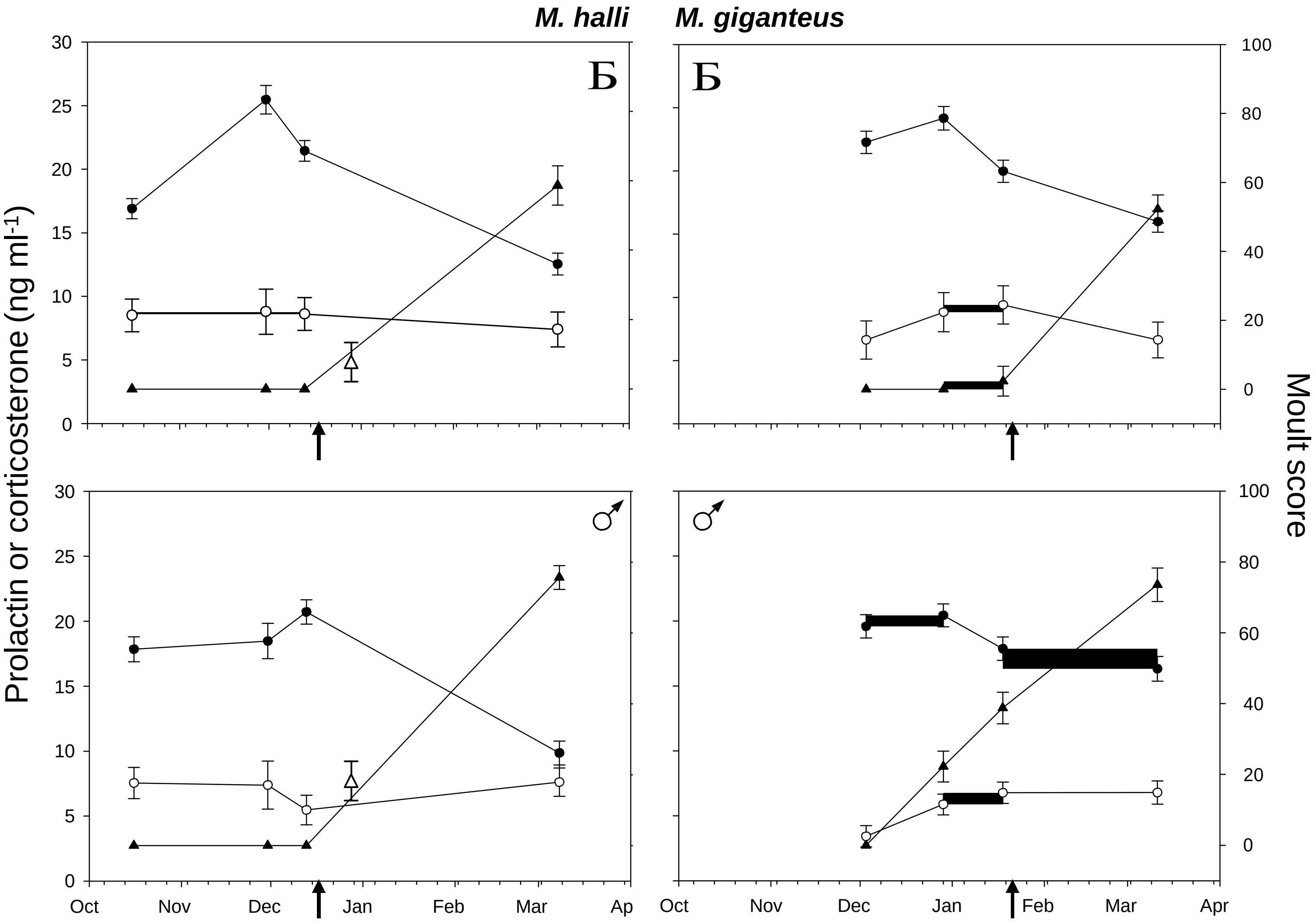


Fig. 1


M. halli
M. giganteus
Б
Б
♂
♂
Prolactin or corticosterone (ng ml-1)
Moult score
0
5
10
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25
30
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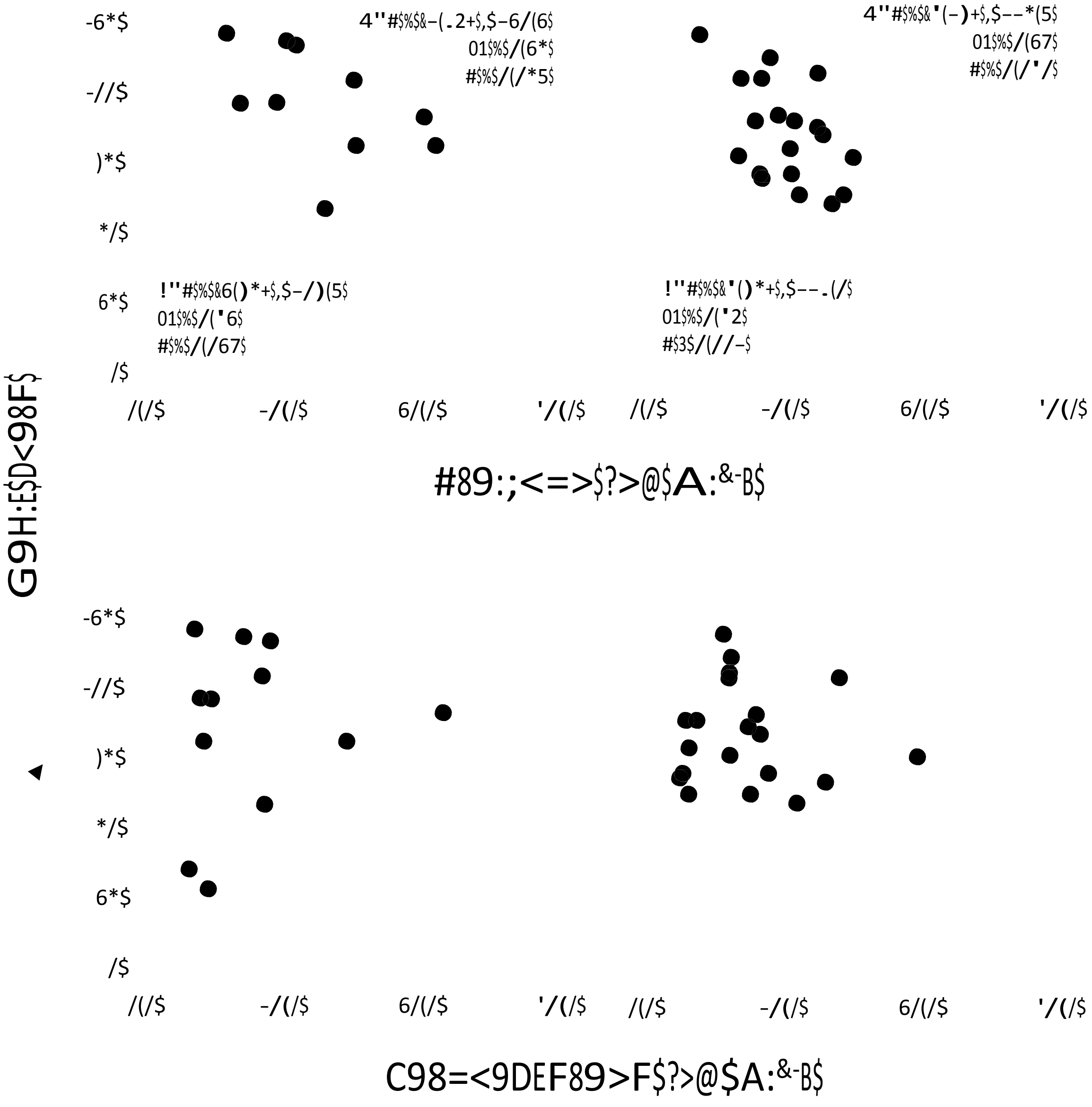
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G9H:E$D<98F$
C98=<9DEF89>F$?>@$A:&-B$

Fig. 3

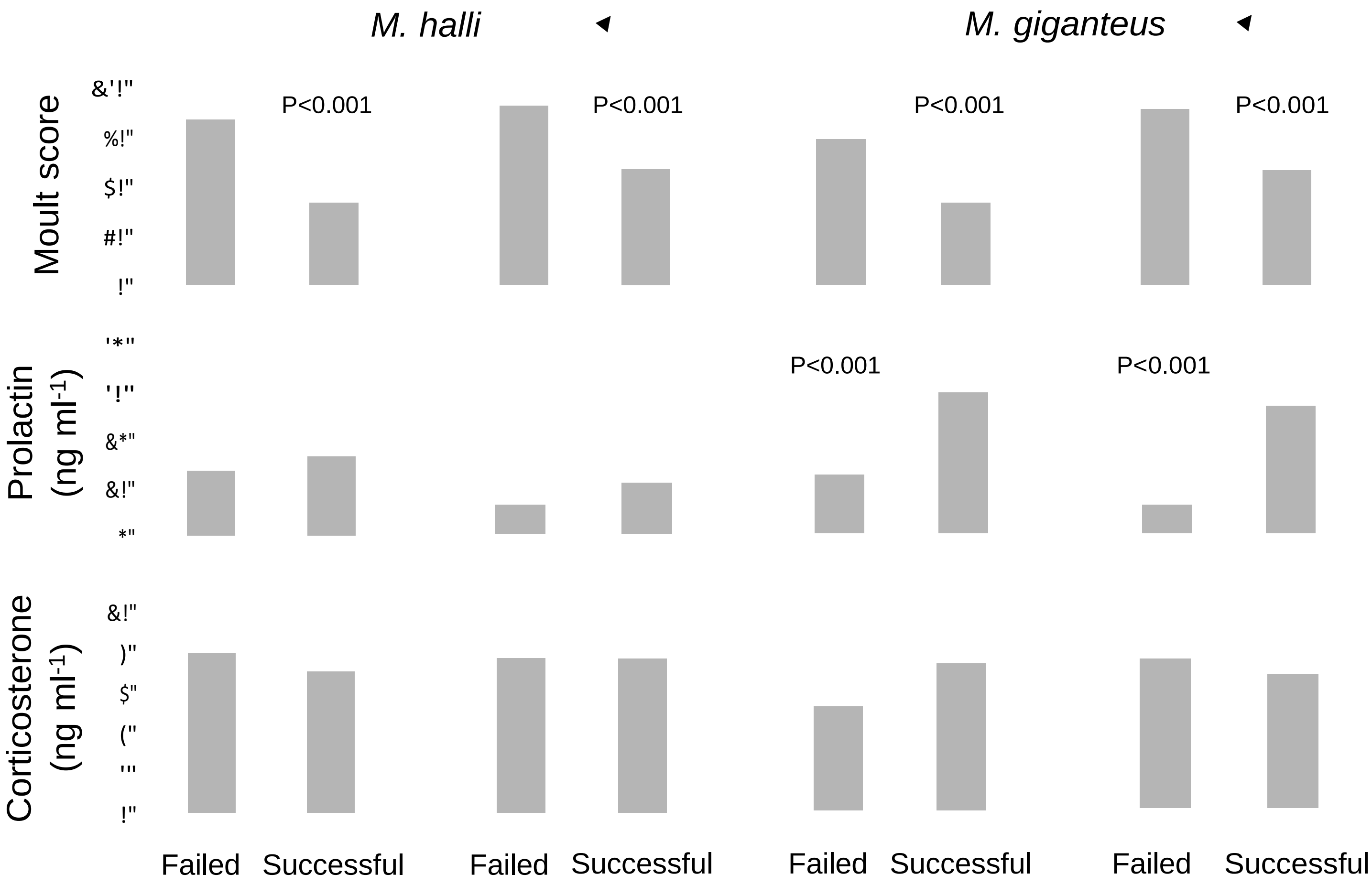
M. halli
M. giganteus
Moult score
&'!"
%!"
$!"
#!"
!"
P<0.001
P<0.001
P<0.001
P<0.001
Prolactin
(ng ml-1)
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Fig. 4