

Changes in reproductive hormones and body mass through the reproductive cycle in the Adélie Penguin (*Pygoscelis adeliae*), with associated data on courting-only individuals

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Antarctic penguins face constraints on reproduction imposed by their foraging ecology. We studied the reproductive endocrinology of Adélie Penguins (*Pygoscelis adeliae*) at Palmer Station on the Antarctic Peninsula by following 61 focal pairs through the 1995/96 breeding season, taking blood samples ca. every 10 days, and observing nesting behavior. During courtship testosterone levels in males increased up to 100-fold, but dropped to levels usually <1 ng/ml as soon as incubation began. Patterns of estradiol secretion in females were similar. Females also had elevated testosterone prior to egg laying, but testosterone levels were an order of magnitude lower than in males. Males exhibited elevated estradiol during courtship, but at lower levels than those found in females. Prolactin levels increased during courtship, peaked during mid-incubation, and were higher in females than in males. Prolactin levels declined slowly once chicks reached the crèche stage. Prolactin levels remained high in adults during long foraging bouts away from the colony (up to 2 weeks or more). Control of prolactin secretion in Adélie Penguins is influenced by extrinsic input such as egg contact less than in many birds. The body masses of males that did not successfully acquire a mate who laid eggs were not different from the body masses of males that mated with females who did lay eggs. In contrast, females that never laid eggs were 10 to 12% lighter than those females that did produce eggs. During the courtship phase, however, the reproductive hormone levels of these courting-only birds did not differ from those of the breeding birds, suggesting that body condition (fat stores) of the female may play an important role in determining whether or not a pair breeds.

INTRODUCTION

Free-living birds provide excellent systems for the study of endocrine control of behaviour because their behaviour can usually be observed readily. In addition diverse social systems in birds facilitate comparative study. The Adélie Penguin (*Pygoscelis adeliae*) is a particularly tractable species in which to study the hormonal control of reproductive behaviour. Due to their tameness and the openness of the habitat in which they live, penguins can be caught easily and repeatedly to take blood samples, and it is simple to observe their behaviour on land. Breeding site fidelity between years (Trivelpiece & Trivelpiece 1990) simplifies demographic studies and between-year comparisons.

Adélie Penguin reproductive biology is similar to that of many colonial, off-shore feeding seabirds. Biparental care is necessary for penguins to rear young successfully, and monogamy

within a breeding season is normal (Williams 1996). Both members of the pair incubate, defend the nest site, and feed and defend the young. Birds usually begin nesting at age three to five years (Ainley *et al.* 1983) and may nest yearly thereafter, although not all adults nest each year (Bucher unpubl. data). The annual breeding cycle is long, beginning in October, the austral spring, and continuing into March when the adults complete their post reproductive molt. Both sexes migrate to breeding sites and remain on land during establishment of territories and formation of pair-bonds, until the two eggs are laid. Thus the birds must undergo long periods of fasting during the reproductive season. During incubation and brooding stages, females and males alternate in attendance at the nest, fasting on land and foraging at sea. The length and number of shifts on the eggs varies among populations (Bucher & Vleck in press).

In the Antarctic climate, eggs would freeze in the exposed nests if not covered. In addition eggs and young chicks must be continuously guarded against avian predators, particularly skuas (*Catharacta* sp.), which prey on any eggs and small chicks that are not closely guarded. Consequently the coordination between parents of nest attentiveness is crucial to successful reproduction. During the period of care of eggs and young, a foraging bird must remain at sea long enough to replenish its own nutrient stores, yet return to the nest in time to relieve its fasting partner and feed the chicks. Parental shifts at the nest last many days, but if the foraging member of the pair does not return, the attending bird must eventually abandon the nest in order to forage for itself (Davis & Miller 1990). The physiological control of this attentiveness pattern must resolve a tradeoff between parental energetics (influenced by individual fat stores, foraging efficiency, prey abundance and distribution at sea) and parental behaviour necessary for successful reproduction. Our first objective toward understanding this tradeoff was to determine the hormonal and body mass changes that accompany the reproductive cycle of free-living Adélie Penguins.

Extensive work has been done on the endocrine physiology of fasting during the post-breeding molt season in penguins (e.g. Groscolas & Cherel 1992). The metabolic and hormonal correlates of fasting during the breeding season have been studied in the King Penguins (*Aptenodytes patagonica*) (Cherel *et al.* 1988; Cherel *et al.* 1994), as have the hormonal correlates of breeding (Garcia *et al.* 1996; Jouventin & Mauget 1996). Reproductive hormone cycles have also been studied in winter-breeding Emperor Penguins (*Aptenodytes forsteri*) (Groscolas *et al.* 1986; Groscolas *et al.* 1988), Adélie Penguins at Dumont d'Urville (Groscolas 1986) and at Cape Bird (Davis *et al.* 1995), and in Macaroni (*Eudyptes chrysolophus*) and Gentoo (*Pygoscelis papua*) Penguins at South Georgia (Williams 1992), and Gentoo Penguins in the Crozet archipelago (Mauget *et al.* 1995). The reproductive endocrinology of the Magellanic Penguin (*Spheniscus magellanicus*) at Punta Tombo, Argentina has also been studied (Fowler *et al.* 1994). The patterns of hormone secretion in these species are generally similar to those of other avian species. Males and females arrive on the breeding ground with elevated levels of luteinizing hormone and gonadal steroids or achieve these elevations soon after arrival, suggesting that environmental cues (probably photoperiod) stimulate recrudescence of the reproductive system. Peak levels of reproductive steroid hormones occur during the courtship and copulation phases of

the reproductive cycle, after which levels fall during the parental phase of the cycle and are low through the molt stage. Elevation in prolactin is associated with care of eggs and young, even when the chick rearing stage is very long (Cherel *et al.* 1994; Garcia *et al.* 1996).

METHODS

Our study site was located on Torgersen Island, an Adélie Penguin rookery located ~ 1 km offshore from Palmer Station (64° 46'S, 64°04' W), a US National Science Foundation base on the Antarctic Peninsula. This island is inhabited by several colonies of Adélie Penguins in the breeding season, containing up to 8,000 pairs in some years. During the 1995/1996 field season we banded 61 focal pairs of Adélie Penguins as, or soon after they arrived in the colony and set up territories. These birds were followed through the course of the breeding season. Birds were captured by hand or with a small net and weighed. A blood sample (1 to 2 ml) was taken by jugular venipuncture every 10 to 15 days. All blood samples were obtained between about 0800 and 1200 h (Greenwich -4 h). Blood was kept cold, but protected from freezing until we returned to the lab, at which time the plasma was separated and frozen at -70° C for storage. We surveyed the colonies daily, weather permitting, to determine which member(s) of the pair were present and whether they were incubating eggs or caring for chicks.

We assayed plasma hormone levels of testosterone, estradiol, and prolactin in duplicate or triplicate at Iowa State University by radioimmunoassay (RIA). For the steroids we used kits obtained from Diagnostics Systems Laboratories, Inc. (Webster, Texas). These RIA kits measure steroid concentrations in plasma directly using an I¹²⁵ labeled steroid. The kits were modified in the following ways. We ran all volumes at 25% of the suggested kit volume and standards were made by adding known amounts of steroid to charcoal-stripped chicken plasma. Serial dilutions of penguin plasma produced curves which were parallel to the standard curves. For the prolactin RIA we used purified chicken prolactin as a standard and a rabbit antiserum raised against prolactin (both obtained from Dr. A. F. Parlow, Director of the Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, California). We radiolabeled the prolactin with I¹²⁵ using chloramine-T. Dilutions of Adélie Penguin plasma bind to the antibody in a manner parallel to the standard curve.

Statistical analyses were carried out using analysis of variance. We compared masses and hormone levels in males and females penguins which bred during the season with penguins that established nests and actively courted, but never laid eggs. There was no egg-lay date for the courting-only pairs, so to compare early season data in breeding vs. courting-only birds we parsed the data as a function of calendar date into three courtship intervals: early courtship = 15 October to 31 October, mid-courtship = 1 November to 15 November, and late courtship = 16 November to 2 December. For the breeding pairs we analyzed the effect of sex on mass and hormone level over the entire season. We divided the season into ~12-day intervals, relative to the first laid egg, from 38 days prior to egg laying through 87 days after egg laying. For females only, we included an interval for samples obtained on the day of laying. Sample sizes and other

statistics are shown in Table 1. A P level of < 0.05 was used as our criterion for statistically significant differences.

RESULTS

Nesting chronology

Of the 61 focal pairs, 56 laid eggs, nine lost both eggs during incubation, three lost their chicks after hatching, and 44 raised at least one chick to the crèche stage. The mean date on which the first egg was laid for these pairs was 14 November (S. D. = 3.8), and the mean date of the first hatching was 20 December (S. D. = 4.0). The mean interval from laying of the first egg to its hatching was 36 days (S. D. = 1.6), from laying of the second egg to its hatching was 34 days (S. D. = 1.4), and between laying of the first and second eggs was 3 days (S. D. = 0.6). The average pair of penguins thus spent 37 days incubating. Upon hatching Adélie chicks were brooded or guarded nearly continuously until they were about three weeks old (Davis 1982). After reaching thermal independence, the chicks joined crèches while both parents foraged simultaneously, returning every day or so to feed the chicks. By mid-February when we left the field station, some chicks were beginning to leave the colony to forage independently, and a few adults had begun their post-season molt.

Courting-only vs. breeding pairs

Five of 61 focal pairs of penguins courted and defended a nest site, but never laid eggs. These courting-only birds were seen in the colony for many days, but most left the colony by early December and most were seen sporadically in the colony thereafter. The courting-only birds were still courting and defending their nest-site together during the second half of November (our late courtship stage). The breeders had, for the most part, laid eggs by late-November and were on average either 8 (males) or 12 (females) days into incubation when sampled during that “late courtship” calendar interval.

The courting-only pairs differed from those breeding pairs that produced an active nest in some ways. Courting-only females that never laid eggs were significantly lower in body masses during early and middle courtship intervals than females that laid eggs (Fig. 1) ($F_{1,45} = 5.10$, $P = 0.03$ and $F_{1,60} = 7.41$, $P = 0.01$). Body masses of the courting-only males did not differ from the breeding males during any of these three courtship intervals (Fig. 2) ($P > 0.31$). Testosterone levels did not differ in either females or males between courting-only birds and breeders ($P > 0.26$), except that the courting-only males had higher levels of testosterone than the breeding males in the last interval ($F_{1,57} = 51.41$, $P < 0.001$), because testosterone in the breeders dropped remarkably with the onset of incubation (see below). Similarly, in the females, estradiol did not differ between courting-only birds and breeders ($P > 0.42$), except during the last interval when estradiol in those birds which were incubating was significantly less than it was in the courting-only females ($F_{1,51} = 14.94$, $P < 0.001$). Prolactin levels increased over the courtship phase in both sexes (Figs. 1 and 2) and did not differ significantly between courting-only and breeding birds ($P > 0.09$), except in the females in the third interval when prolactin in the breeders was higher than in the courting-only females ($F_{1,54} = 5.15$, $P = .02$).

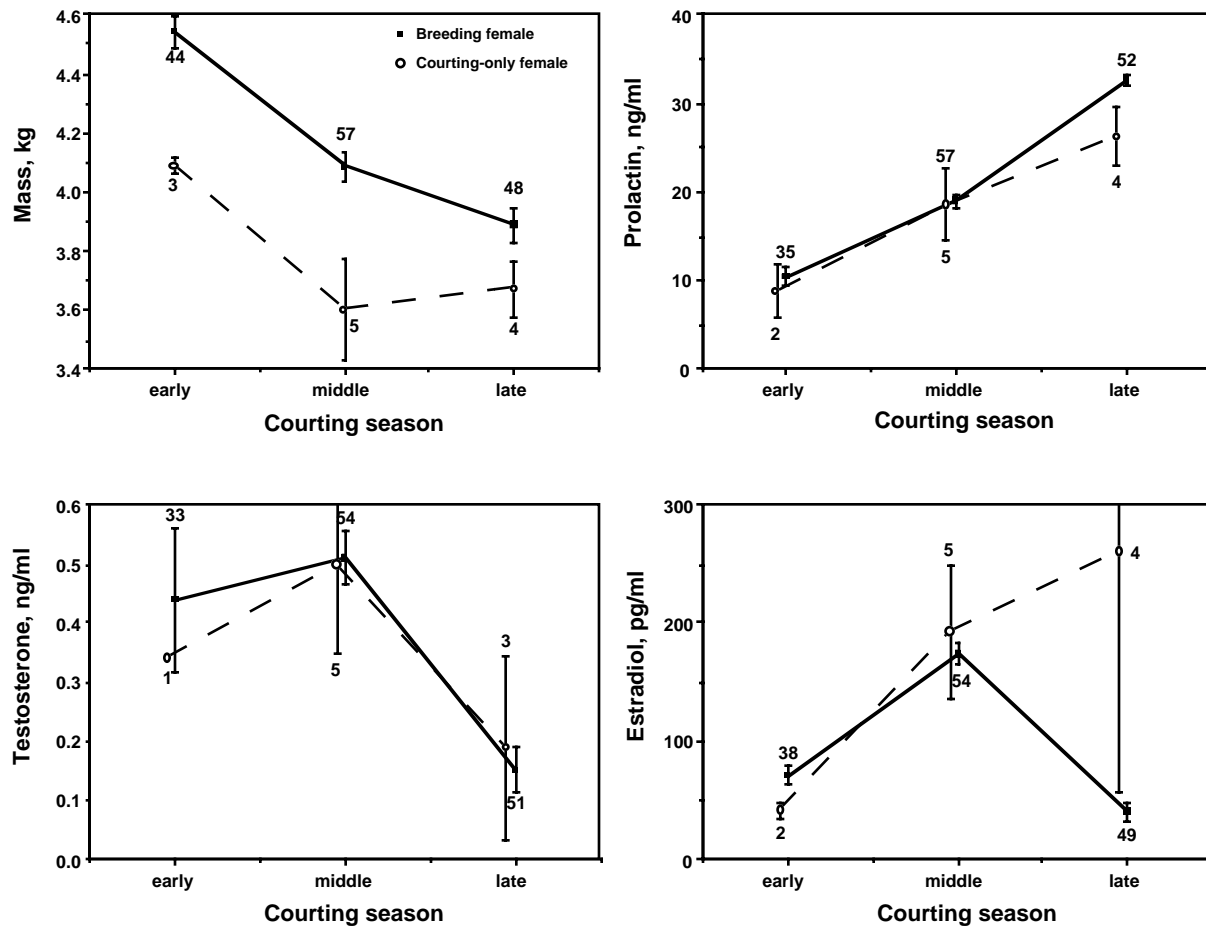


Fig. 1 The change in body mass, prolactin, testosterone, and estradiol across the courting season in female Adélie Penguins that actively courted and defended a nest-site, but never laid eggs (unfilled circle and dotted line); and females that laid eggs (filled squares and solid line). For courting season, early = last half of October, middle = first half of November, late = last half of November to 2 December. During the last interval most of the breeding females had eggs. Error bars indicate \pm S. E. Sample size is shown for each point.

Changes in mass and hormone levels in breeding birds

We compared the effects of sex and breeding stage on mass and hormone levels in the breeding penguins over the course of the season (Table 1). For those pairs that lost their eggs or chicks, we included only those values prior to nest-loss. Adélie Penguins arrived in the breeding colonies with large fat reserves, and body masses decreased as the birds fasted through the courtship phase and, in the case of males, the first incubation shift (Fig. 3A). Males were significantly heavier than females both upon arrival in the colony and at all subsequent stages except mid-incubation. Males lost mass at a rate of ca. 43 g per day and females lost mass at a rate of ca. 38 g per day (Vleck & Bucher in press). The majority of females had arrived by about 25 October and the mean date on which they left after laying the second egg was 17 November, at which point the average female arriving at a mass of 4.7 kg would have lost about 912 g or ~19% of her arrival mass. Males generally arrive in the colony before females and take the first shift of incubation,

which in our colonies averaged 10.7 day (range 3-33 days) (Bucher & Vleck in press). Over a typical 35 day courtship/incubation fast, a male arriving with a mass of 5.3 kg would have lost 1500 g or 28% of his arrival mass. When the birds were alternating between foraging at sea and tending chicks or eggs, their body masses were fairly stable (males) or declined at a slow rate (females) (Fig. 3A).

Fig. 2. The change in body mass, testosterone, and prolactin across the courting season in male Adélie Penguins that actively courted and defended a nest-site, but whose mate never laid eggs (unfilled circle and dotted line); and males that eventually had a nest with eggs (filled squares and solid line). For courting season, early = last half of October, middle = first half of November, late = last half of November to 2 December. During the last interval most of the breeding males were incubating eggs. Error bars indicate \pm S. E. Sample size is shown for each point.

Testosterone rose during the courtship stage prior to egg laying in both males and females (Fig. 3B), although the levels were significantly higher in males than females (Table 1). As soon as the eggs were laid plasma testosterone in males decreased to less than 2% of the value measured prior to egg laying. The egg must be covered by either the female or male as soon as it is laid to protect it from extreme temperatures and egg predators, so incubation behaviour begins with the first egg, even if the egg is not brought to incubation temperature for a few days. Estradiol levels in females show the same pattern with an 84% drop after egg laying (Fig. 3C). Plasma estradiol was slightly elevated in males during the courtship phase, but was significantly lower than in females (Table 1).

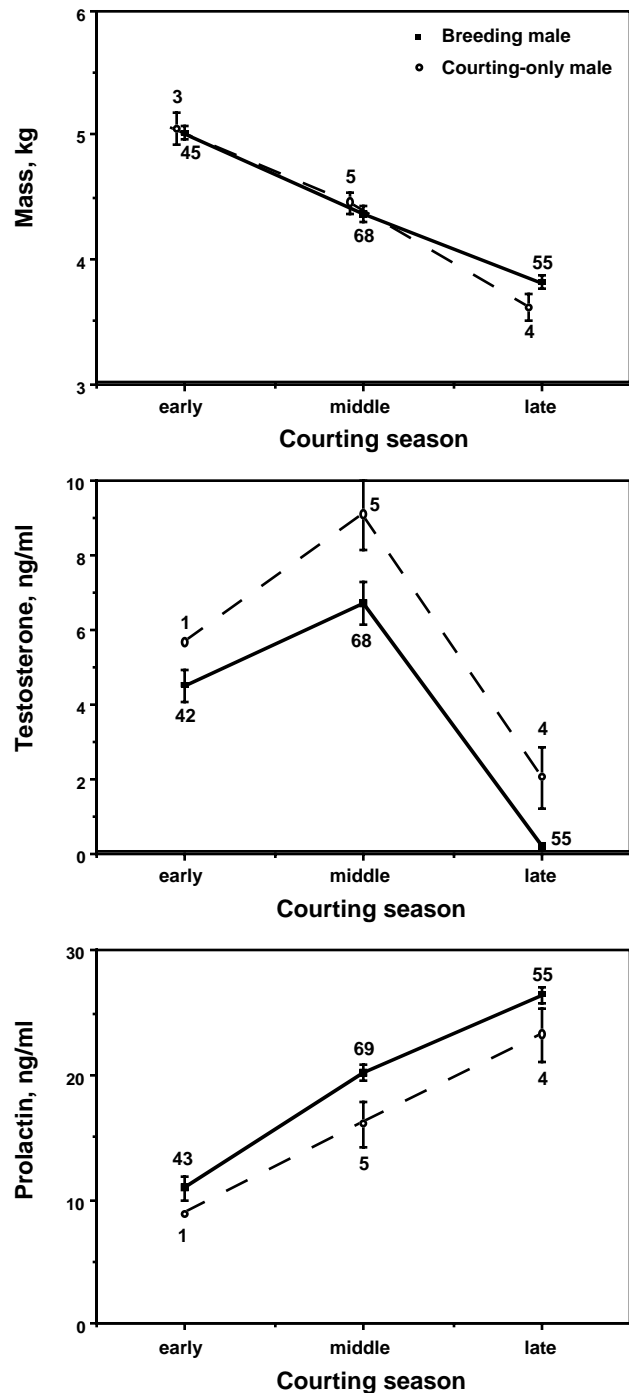


Table 1. Sample sizes, *F* and *P* values for comparisons of breeder female and male Adélie penguins at different stages of the reproductive cycle. Dependent variables include body mass and plasma testosterone (*T*), estradiol (*E2*) and prolactin (*Prl*) levels.

| | | Days before first egg | | | | Days after first egg laid | | | | | | |
|------------|----------|-----------------------|--------|--------|---|---------------------------|--------|--------|--------|--------|-------|-------|
| | | 38-25 | 24-13 | 12-1 | 0 | 1-12 | 13-24 | 25-36 | 37-48 | 49-60 | 61-72 | 73-87 |
| Mass | n_f^1 | 7 | 48 | 45 | 3 | 13 | 65 | 45 | 48 | 48 | 9 | 10 |
| | n_m^1 | 7 | 56 | 38 | | 51 | 43 | 44 | 48 | 46 | 16 | 6 |
| | F^2 | 19.13* | 19.34* | 18.45* | | 4.24* | 2.34 | 25.14* | 44.77* | 7.62* | 6.98* | 5.33* |
| | <i>P</i> | <0.001 | <0.001 | <0.001 | | 0.04 | 0.13 | <0.001 | <0.001 | 0.01 | 0.01 | 0.04 |
| <i>T</i> | n_f | 5 | 39 | 43 | 3 | 15 | 66 | 45 | 48 | 47 | 9 | 10 |
| | n_m | 7 | 53 | 38 | | 51 | 42 | 43 | 49 | 44 | 16 | 7 |
| | <i>F</i> | 4.89* | 107.9* | 130.2* | | 0.002 | 4.43* | 4.60* | 1.68 | 0.09 | 1.04 | 0.15 |
| | <i>P</i> | 0.05 | <0.001 | <0.001 | | 0.96 | 0.04 | 0.03 | 0.20 | 0.76 | 0.32 | 0.70 |
| <i>E2</i> | n_f | 5 | 41 | 45 | 4 | 12 | 63 | 44 | 43 | 41 | 9 | 10 |
| | n_m | 5 | 26 | 7 | | 8 | 7 | 8 | 8 | 8 | 3 | 2 |
| | <i>F</i> | 6.67* | 12.55* | 13.80* | | 7.27* | 7.56* | 4.04* | 8.91* | 6.55* | 2.19 | 2.91 |
| | <i>P</i> | 0.04 | <0.001 | <0.001 | | 0.02 | 0.01 | 0.05 | 0.01 | 0.01 | 0.17 | 0.12 |
| <i>Prl</i> | n_f | 4 | 43 | 44 | 4 | 15 | 67 | 45 | 48 | 47 | 9 | 10 |
| | n_m | 7 | 54 | 38 | | 52 | 43 | 44 | 48 | 44 | 16 | 6 |
| | <i>F</i> | 0.009 | 2.49 | 0.31 | | 26.19* | 20.32* | 23.25* | 17.91* | 25.70* | 6.63* | 0.16 |
| | <i>P</i> | 0.93 | 0.12 | 0.58 | | <0.001 | <0.001 | <0.001 | 0.001 | <0.001 | 0.02 | 0.70 |

¹ n_f and n_m are sample sizes for females and males respectively.

²Degrees of freedom for each comparison are (1, $n_f + n_m - 2$). An asterisk after the *F*-value indicates significant difference between sexes.

Prolactin levels in males and females were not significantly different from each other during the courtship phase (Fig. 3D). Prolactin increased gradually through courtship and reached significantly higher level in females than in males by early incubation, remaining higher in females than in males through the early crèche stage (Table 1). Maximum levels in prolactin were not reached in either sex until mid-way through incubation. Prolactin remained relatively high through the early chick stage, decreasing slowly once adults were no longer brooding or guarding the chicks, but never reaching the low levels typical of the early courtship stage.

DISCUSSION

For many species of birds, we have a good understanding of the changes in plasma levels of hormones through a breeding cycle and of how predictive environmental information (often annual photocycles) and other supplementary information (e.g. social interactions with conspecifics or supply of food) interact with endogenous rhythms to control the timing of reproduction (reviewed in Wingfield & Moore 1987). Much of the basis for this understanding, however, has been derived from studies on mid- to high-latitude terrestrial species that are generally monogamous, defend breeding territories, and often migrate to wintering grounds. Penguins differ from most terrestrial birds that have been studied. They are highly colonial and unable to forage close to the nest. Penguins differ from most other seabirds in the length of time it takes them to reach foraging grounds from the breeding colonies because they swim rather than fly to foraging grounds, often swimming distances of 15-80 km or farther (Williams 1995). Consequently the length of time that one member of the pair must remain fasting in the colony, waiting for the other member to return is greater in penguins than in most birds.

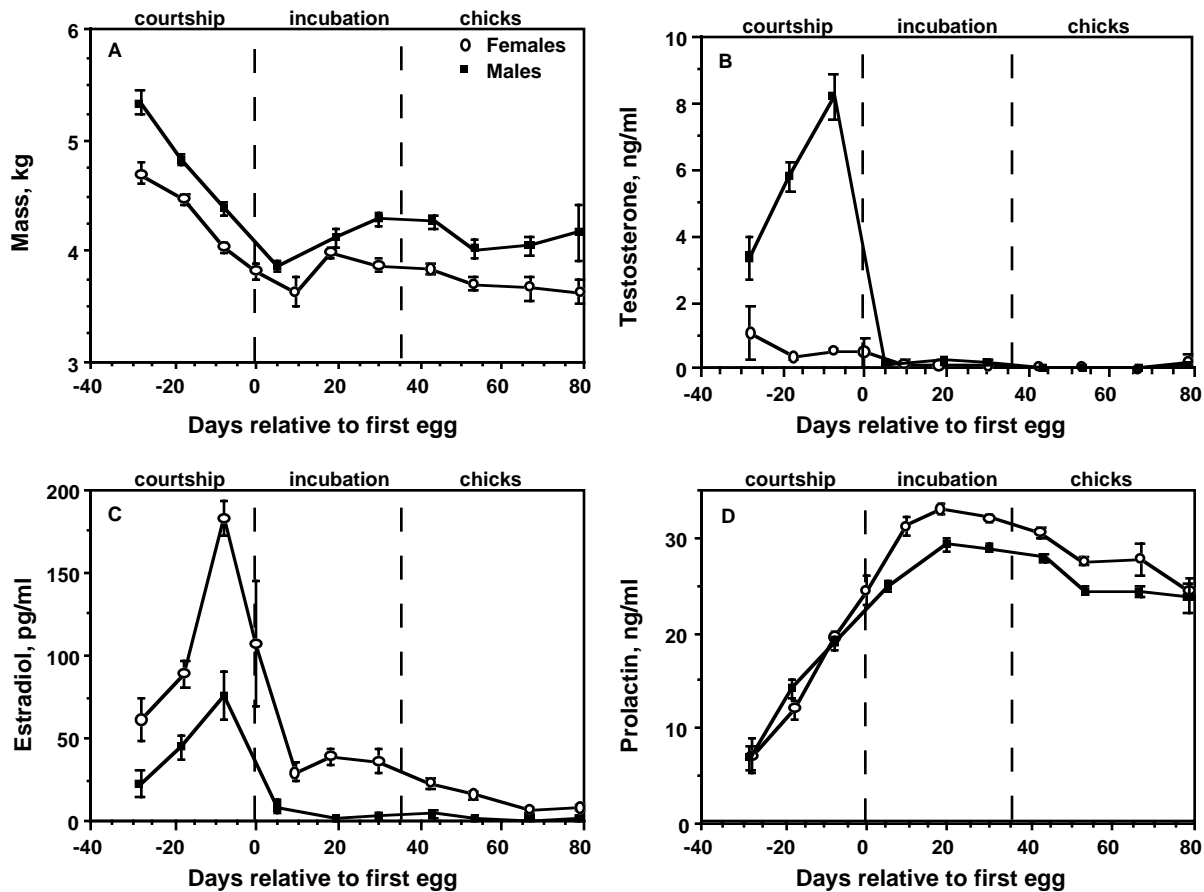


Fig. 3. The change in breeding Adélie Penguins across the reproductive season for (A) body mass, (B) testosterone, (C) estradiol, and (D) prolactin. Data are plotted relative to the first laid egg; dashed lines demarcate the incubation stage from the courtship and chick stages. Female data are represented by an unfilled circle. Male data are represented by a filled square. Error bars indicate \pm S. E. Sample sizes are shown in Table 1, as are significant differences between the sexes.

Establishment of individual recognition and a strong pair-bond is crucial to successful reproduction in penguins. These birds engage in pair-bond displays (bowing, mutual greeting) (Ainley *et al.* 1983) throughout the season, whenever both are at the nest-site (which happens frequently during incubation and brooding and very infrequently during the crèche stage). In the courtship phase these displays may be stimulated by the elevated levels of the sex steroids. The continued display of these behaviours is clearly not dependent on constantly elevated sex steroids, because the levels of testosterone and estradiol fall precipitously after incubation begins. Either the display of these pair-bond and individual recognition displays is independent of sex steroids after egg laying, or their display depends only on the slight levels that remain. It would be interesting to know whether or not birds in the non-breeding season and/or away from the colony recognize and greet their former mates. If they do not, this would suggest that these behaviours may depend on some aspect of the nest-site as well as on a particular hormonal milieu.

The high levels of testosterone and possibly estradiol early in the season may be associated with competition for nest-sites in the colony. Moderately elevated testosterone levels have been observed in the females of other penguin species as well (Williams 1992; Cherel *et al.* 1994; Fowler *et al.* 1994). Elevated testosterone in females may potentiate nest-site acquisition and defense by females, even against males, behaviours that are often observed in penguins (Williams 1995). The moderate elevation of estradiol that we observed in male Adélie Penguins during courtship has been observed in the males of other penguin species as well (Williams 1992; Cherel *et al.* 1994; Fowler *et al.* 1994). The behavioural function of male estradiol is not known, but Fowler *et al.* (1994) suggested it may be implicated in control of copulatory behaviour.

Testosterone levels decline after egg laying in many monogamous birds in which the males participate in parental care (reviewed in Wingfield *et al.* 1990), but the rapidity and the extent of this decline in male Adélie Penguins is striking. Within just a few days testosterone levels in males drop from being highly significantly different, to being indistinguishable from those in females (Table 1). There is considerable variation in testosterone in males prior to egg laying, possibly associated with individual variation in the extent of male-male agonistic interactions. In no bird, however, did we find testosterone levels elevated to courtship-stage levels while they were caring for eggs or young. Testosterone can become elevated in breeders that have lost their eggs or chicks (Vleck unpubl. data; Fowler *et al.* 1994). The cue for the decline in testosterone in males at egg laying is not known. It could be the appearance of the egg itself (which serves as a strong stimulus for incubation behaviour in both sexes), or it could be precipitated by some subtle change in the female's behaviour or chemistry as she approaches egg laying. Supplying courting pairs of penguins with an extraneous egg before they had their own egg and tracking testosterone could yield an answer to this question. In any case, it seems likely that this rapid fall in testosterone in both males and females is instrumental in changing the primary focus of their behaviour from nest defense and courtship to care of the eggs, a change that is crucial to protect the eggs from the persistent and intense aerial predation by skuas.

In general, high levels of testosterone are thought to be incompatible with the display of male parental behavior (e.g. Vleck & Dobrott 1993). Anti-androgens can bring about a premature onset of incubation (Oring & Fivizzani 1991). Furthermore, androgen supplementation is known to disrupt parental behavior in several free-living species (Silverin, 1980; Hegner and Wingfield, 1987; Oring *et al.*, 1989; Ketterson *et al.*, 1992). Testosterone does not necessarily block the display of parental behavior, but rather seems to increase the likelihood that the birds will engage in others non-parental behaviors (e.g. courtship or territorial defense) at the expense of parental behavior. Such behavior would likely be fatal to penguin eggs.

Prolactin levels are associated with parental behaviour in essentially all birds that have been studied (reviewed in Buntin 1996). In most, but not all birds, prolactin levels reach their peak in early incubation and then often decline precipitously after hatching, especially in species with precocial chicks that are not brooded extensively. The gradual rise in prolactin through incubation seen in Adélie Penguins is similar to the pattern seen in Columbiformes (Cheng & Burke 1983)

and in Pied Flycatchers (*Ficedula hypoleuca*) (Silverin & Goldsmith 1983). Brood patch formation is dependent on elevated prolactin in some species (Jones 1971). It is found in both males and female penguins, but does not reach its full development until after incubation has begun in some species (Farner 1958; St. Clair 1992). We found that most Adélie Penguins had well vascularized brood patches within a few days of the initiation of incubation (unpubl. data). Whether the gradual increase in prolactin early in incubation in the Adélie Penguins is associated with an increase in the intensity of incubation, as measured by a rise in egg temperature is not known. In *Eudyptes* penguins, with dimorphic eggs, peak egg temperatures may not be found until about 10-15 days into incubation, but in Gentoo and Jackass (*Spheniscus demersus*) Penguins, egg temperatures over 30° C are reached in the first week of incubation (Burger & Williams 1979). A rise in the persistence of nest attentiveness does not seem to be dependent upon the rise in prolactin since Adélie Penguins attend the egg with great tenacity as soon as the females lay.

Prolactin levels were higher in females than in males during the parental care phases of reproduction, despite the observation that both males and females develop a brood patch, incubate and feed the young, and in fact the male generally spends more days incubating the eggs than the female. Prolactin levels at some stages of the reproductive cycle are also higher in females than in males in King Penguins (Cherel *et al.* 1994; Garcia *et al.* 1996) and Macaroni and Gentoo Penguins (Williams & Sharp 1993), although no sex difference in prolactin levels was reported in Gentoo Penguins by Mauget *et al.* (1995). In some species of birds in which the parents share equally in parental duties, prolactin levels do not differ between the sexes, e.g. *Diomedea* albatrosses (Hector & Goldsmith 1985). In other species in which one member of the pair incubates more than the other member, prolactin levels are usually higher in the sex that carries out most of the parental duties (e.g. Harris' Hawks, *Parabuteo unicinctu*) (Vleck *et al.* 1991).

Tactile stimulation of the brood patch is required in a number of poultry species to maintain prolactin secretion and continuous broody behaviour (El Halawani *et al.* 1980; Hall 1987; Sharp *et al.* 1988). This is clearly not the case in Adélie Penguins. Females left the colony for an average of 11 days after egg laying, and some females were absent for over two weeks before returning. When they returned the females' prolactin levels were higher than they were right after egg laying. Similar patterns in prolactin secretion during long absences from the nest were found in *Diomedea* albatrosses (Hector & Goldsmith 1985). In addition we found that even when penguins lost their eggs, their prolactin levels dropped only very slowly and never reached pre-breeding levels during that breeding season (Ross 1997), a finding similar to that from King Penguins (Garcia *et al.* 1996; Jouventin & Mauget 1996).

At Torgersen Island birds return to the nesting colonies year after year, but may not breed each year. Between 30-40% of birds in the colonies during courtship may not reach the egg-laying stage in some years (Bucher unpubl. data). Similar data were presented for Adélie Penguins at Cape Bird (Spurr 1975). Trivelpiece *et al.* (1990) noted that in one of their survey years 22% of banded Chinstrap Penguins (*Pygoscelis antarctica*) with previous breeding experience did not

attempt to breed. They interpreted those data as indicating poor physiological condition of returning birds.

In our study five of 61 focal pairs courted, built a nest, and defended it; but the females never laid eggs. The unsuccessful males did not differ from the successful males in body mass and we suggest that they would have been successful breeders, given a mate capable of laying eggs. There is often a male-biased sex ratio in penguin colonies, including Adélie Penguins (Williams 1995), and thus some competent males each year will probably not have the chance to reproduce. Female-choice of mates in Adélie females seems to be determined primarily by the male's location in the colony and prior history with that female rather than by differences in body size (Davis & Speirs 1990). This may explain why we found no difference in body mass between breeding and courting-only males. In contrast to males, the non-laying females were on average 10-12% lower in body mass than the laying females at the same time. These non-laying females may be unsuccessful because they do not have sufficient fat stores to undergo reproduction and/or they may be young, pre-breeding birds that have returned to the colony and keep company with another bird, but do not lay eggs (Ainley *et al.* 1983). The significant elevation in sex steroids and prolactin that these courting-only birds exhibited (Fig. 1) suggests that they were reproductively competent and that egg-laying may have failed because of poor body condition as indicated by low body mass.

The mechanism by which poor body condition inhibits ovulation and egg production is not well known. It may involve the effects of nutritional status on follicle stimulating hormone and subsequent effects on follicular development and atresia (Scanes & Grimminger 1990). Nutritional regulation of reproduction may be fairly common in high latitude penguins. This is supported by the large variance between years in the number of breeding pairs in colonies of several penguin species (Williams 1995), although weather conditions that affect access to the nesting colonies early in the season can also have a large effect on the number of breeding pairs in any given year (Spurr 1975; Ainley *et al.* 1983). The rise in reproductive hormones (exhibited by both successful and unsuccessful birds) probably follows a precise annual cycle as does return to the colony by most adults. This annual cycle is likely regulated by photoperiod, but the culmination of this preparation for reproduction, i.e. egg laying, may depend heavily on body conditions because of the generally high energetic costs of reproduction in penguins. The extended fasts as well as multiple trips between the foraging grounds and breeding grounds means that reproduction in Adélie Penguins is energetically costly (Chappell *et al.* 1993). In Magellanic Penguins, the body mass at the beginning of incubation is a good predictor of eventual reproductive success (Fowler *et al.* 1994). Adélie Penguins can live up to 20 years (Williams 1995), and annual survival is reported to be higher for nonbreeders than for breeders (Spurr 1975; Ainley & Demaster 1980). These life history traits suggest that the most adaptive strategy for a bird in sub-optimal condition is to forego reproduction for that year, rather than attempt to breed, since breeding is likely to be unsuccessful and may compromise future reproductive value.

In many other birds, the nesting date can be adjusted to compensate for poor body condition (Carey 1996). This strategy is not available to high-latitude penguins because the reproductive

cycle is long relative to the time when conditions in the colony (absence of unbroken sea ice and of deep snow pack) permit nesting. Gentoo Penguins nesting at South Georgia (latitude ca. 54° S) have a highly synchronized egg laying (Williams 1990), whereas Gentoo Penguins at more northern latitudes (ca. 45° S) have an extended laying period and may even undergo a second breeding attempt after a reproductive failure (Mauget *et al.* 1995). At Torgersen Island (latitude ca. 65° S), nearly two-thirds of the Adélie females lay eggs within one week of each other. Similar synchrony in lay date was reported by Ainley *et al.* (1983) for Adélie females at Cape Crozier (latitude ca. 77° S). Delay of nesting can also adversely affect the timing of the post-reproductive molt in adults, the age of the chick at the crèche stage, and chick survival (Viñuela *et al.* 1996; Moreno *et al.* 1997). In addition, because the birds do not forage once they come to the breeding colony until after clutch completion, delay of laying once birds come ashore, even by a few days, will only contribute to the decreased fat stores with which the birds begin incubation.

In summary, Adélie Penguins undergo a four-month breeding cycle that is highly synchronized within dense colonies. Body condition of the female appears to play an important role in determining whether a courting pair will achieve an active nest and follow through with reproduction in any given year. Hormonal cycles probably play an important role in maintaining a strong pair-bond and coordinating behaviour between members of a pair, particularly in the switch from early courtship and nest-defense behaviour to parental behaviour.

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