

## Physiological Condition and Reproductive Consequences in Adélie Penguins<sup>1</sup>

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**SYNOPSIS.** Animals must make “decisions” (*e.g.*, when or whether to breed, the effort to put into a breeding episode) by integrating physiological, environmental and social inputs. This integration can be studied only in a field context. In Adélie penguins (*Pygoscelis adeliae*) reproduction is constrained by foraging ecology, mode of transport, and the extreme latitude at which they live. The decision whether to breed in a given year is influenced by body conditions. Adélie penguins must fast for several weeks during the early reproductive stages and use stored fat for metabolic energy. Females that return to the colony, but do not breed, are 10–12% lighter than females that do breed. Birds that are relatively low in body mass tend to have lower reproductive success than heavier birds, and an individual’s reproductive success is positively correlated with the body fat stores it had on arrival. After eggs are laid, parents alternate in attending the nest. Nest failure occurs if one parent does not make a timely return and its fasting partner must eventually leave. During normal-length fasts plasma corticosterone and glucose levels do not change. Blood  $\beta$ -hydroxybutyrate levels gradually increase during the fast while uric acid levels remain low, but in birds with the longest fasts (>~50 days), ketone levels may fall and uric acid levels increase, indicative of a switch from using fat to using body proteins for metabolism. In incubating males, hematocrit and hemoglobin concentrations also increase, suggesting dehydration can accompany energy stress during the breeding fast.

### INTRODUCTION

Free-living animals have greater opportunity than captive animals to display the full range of behaviors indicative of underlying physical, physiological and social conditions. For example they can leave a site, which captive animals cannot do. In addition, there is probably a greater number of variables that can affect such decisions in the wild than under more highly controlled laboratory conditions. This presents both challenges and opportunities to field physiologists. Presumably free-living animals make the “right” decision based on integration of all available information, but what are these sources of information and the mechanisms that transduce such information into reaction? Our goal here is to identify critical input variables by examining physiological measurements that provide reliable predictors of animals’ reproductive decisions. Candidate physiological measurements that might predict breeding decisions include body mass and nutrient stores, and hematological measures that estimate hydration state, stress, and reliance of cellular metabolism on stored body fat or protein. Such measurements can be made non-destructively and repeatedly in free-living animals. These physiological and endocrine measurement should reveal the proximate mechanisms at the individual level that link environmental and population-level variation in animal reproductive success. Once this is accomplished, it becomes possible to move beyond such a correlational study to more critical experimental studies that can isolate and quantify the individual factors.

Penguins are flightless birds that are highly special-

ized for swimming and diving, and spend much of their life at sea (reviewed in Williams, 1995). Penguins must return to land to breed, often in large colonies, and this aspect of their natural history has facilitated several important long-term ecological studies (*e.g.*, Ainley *et al.*, 1983; Trivelpiece *et al.*, 1990; Fraser *et al.*, 1992). High latitude distribution and a short summer season have led to highly synchronized breeding schedules that simplify demographic tracking of population performance and numbers. Penguins fast while on land and many species show remarkable ability to subsist on body fat for long intervals, an adaptation that allows them to survive periods of fasting while molting, incubating, or (for chicks) over-wintering on land while parents are at sea (Groscolas and Chérel, 1992). One of the best-studied species is the Adélie penguin (*Pygoscelis adeliae*) (Ainley *et al.*, 1983). Its behavior (*e.g.*, Müller-Schwarze and Müller-Schwarze, 1980; Speirs and Davis, 1991), physiology and endocrinology (*e.g.*, Chappell *et al.*, 1993; Davis *et al.*, 1995; Vleck *et al.*, 1999) and ecology (*e.g.*, Ainley and Demaster, 1980; Davis and McCaffrey, 1986) have been studied at several sites within its Antarctic coastal distribution.

During the austral summers of 1995–96 and 1996–97, we banded approximately 1,000 Adélie penguins on Torgersen Island (64°46’S, 64°04’W) near Palmer Station on the Antarctic Peninsula. We determined sex of marked birds using copulation behavior and nest attendance pattern (Ainley *et al.*, 1983), condition of the cloaca and lipemic plasma in laying females, and differences between sexes in the CHD gene (Vleck, unpublished data; Griffiths *et al.*, 1996). We censused daily, weather permitting, to determine which birds were present and the stage of the nesting cycle, and weighed birds each time we handled them. From many of these birds we collected blood samples from the

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jugular vein. We used blood samples to follow reproductive hormones levels over the reproductive cycle (Vleck *et al.*, 1999, 2000a) and assessed stress levels through monitoring corticosterone and changes in the relative numbers of leukocyte types (Vleck *et al.*, 2000b). We also used our blood samples to measure important indicators of metabolic condition, hydration, and substrate utilization upon arrival in the colony and during the long fast associate with the early breeding season.

The first penguins arrived in the colony on 13 October and 15 October in 1995 and 1996, respectively. In both years, most arriving birds we handled had food in their digestive tracts, so we assume that fasting began on arrival. We knew the length of the fast for some individuals that were banded upon arrival in the colony. For birds that were banded after arrival, we assumed that they had arrived and begun fasting on Oct 20 for males and Oct 25 for females. These are the mean arrival dates for each sex based on colony counts in 1995. Weather conditions made comparable counts impossible in 1996, but given the synchronous nature of penguin arrival, we estimate these dates are in error by no more than  $\pm 5$  days. We used these data to calculate the fast durations corresponding to our measurements of blood chemistry or body mass. We report here on data from 212 individuals during the early breeding fast, from which 386 body mass and blood sample measurements were obtained.

#### ARRIVAL CONDITION, BODY MASS, AND REPRODUCTIVE SUCCESS

The annual breeding cycle of Adélie penguins begins when birds arrive in October–November and continues into February when adults cease feeding their chicks. After arrival, females normally remain until their two-egg clutch is complete; then most females leave to forage at sea. Their mates remain, incubating the eggs and fasting until relieved by the returning female (Ainley *et al.*, 1983). At Torgersen Island, males return to the colony about 5 days before the females, take the first incubation shift averaging 8–12 days (Bucher and Vleck, 1998) and fast on average 37 days. If the female does not return, the male will usually continue incubating until his fast has extended to more than 50 days before abandoning the nest to return to the sea to feed. The long duration of these early breeding season fasts suggests that energy reserves at the time of arrival at the breeding colony are likely to be one important predictor of reproductive success. We have four lines of evidence that suggest energy reserves affect the decision to breed and the reproductive success of those birds that do breed.

In any given year, a substantial number of penguins can return to the colony in which they previously bred, yet fail to breed again, even when the previous mate is there (Bucher, unpublished data; Ainley *et al.*, 1983; Trivelpiece *et al.*, 1990). Trivelpiece *et al.* (1990) noted that in one of their survey years, 22% of banded Chinstrap Penguins (*Pygoscelis antarctica*) with pre-

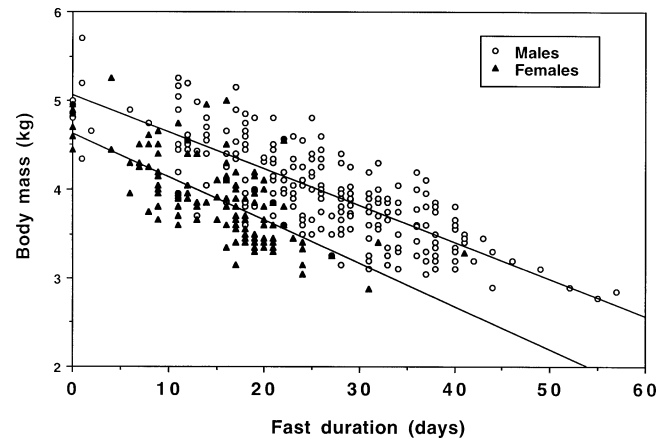


FIG. 1. Decrease in body mass of Adélie penguin during the early breeding season fast. Data ( $n = 367$ ) are from 212 individuals. Lines are best-fit linear regressions through the data for each sex, described by the equations: Male mass =  $5.06 - 0.042$  days fasting ( $r^2 = 0.64$ ); Female mass =  $4.63 - 0.049$  days fasting ( $r^2 = 0.50$ ).

vious breeding experience did not attempt to breed, and speculated those data indicated poor physiological condition of returning birds. Our data support the hypothesis that body condition at arrival affects a penguin's breeding decisions. During the 1995–1996 breeding season we followed 61 focal pairs of birds, obtaining blood samples and body masses from both males and females at approximately 10–14 day intervals (Vleck *et al.*, 1999). Of these 61 pairs, five courted and defended a nest site, but the female never laid eggs. Although the sex steroid hormones and prolactin levels did not differ between birds that only courted and those that laid eggs, the body masses of the courting-only females were significantly lower than those of females that laid eggs ( $F_{1,60} = 7.41$ ,  $P = 0.01$ ). These courting-only females were 10–12% lighter than laying females at the same time. Even though courting-only females were apparently reproductively competent as evidenced by their elevated reproductive hormones (Vleck *et al.*, 1999), they may not have had sufficient nutrient stores to lay eggs and complete the reproductive cycle. The courting-only males, in contrast, did not differ in body mass from successful males, and they presumably could have bred successfully given a mate that laid eggs. Poor-condition males may have never obtained a territory and mate. There is often a male-biased sex ratio in penguin colonies, including Adélie penguins (Williams, 1995), and thus some competent males each year may not have the opportunity to reproduce.

If arrival condition is important to breeding success, variation between years that affects arrival condition should be reflected in differences in breeding success. We used measurements of body mass and its changes in males and females during the early breeding season fast over the two years of our study to look for such effects (Fig. 1). Arrival masses differed between years ( $F_{1,363} = 4.191$ ,  $P = 0.04$ ), and between males and females ( $F_{1,363} = 171$ ,  $P < 0.0001$ ), but the rate at

TABLE 1. *Breeding success and body condition of fasting Adélie penguins during the early breeding season fast.\**

	Successful Breeders	Unsuccessful Breeders	Significance Test
Females	-0.19 (SEM = 0.05, N = 35)	-0.31 (SEM = 0.05, N = 34)	$t_{67} = 2.77$ $P = 0.101$
Males	0.14 (SEM = 0.04, N = 53)	0.04 (SEM = 0.04, N = 72)	$t_{123} = 2.67$ $P = 0.105$

\* Condition is the difference between measured body mass (kg) and the average body mass predicted for the population on the date of measurement.

which mass declined during fasting (about 42 g/day) did not differ between sexes (ANCOVA:  $F_{1,362} = 1.97$ ,  $P = 0.16$ ) or between years (ANCOVA:  $F_{1,362} = 0.0001$ ,  $P = 0.99$ ). This suggests that energy demands while in the breeding colony did not differ between years. Overall, the average arrival mass for female penguins at the Torgersen colony was 4.75 kg, whereas arrival mass of males averaged 5.02 kg, but the average mass of birds at any point during their initial fast was 122 g greater in 1995 than in 1996.

The winter preceding the 1995–1996 breeding season was characterized by greater amounts of sea ice than the 1996 winter, a condition that is associated with a more plentiful supply of krill, the principle food source for Adélie penguins (Fraser *et al.*, 1997; Ainley *et al.*, 1998). Penguins in 1995–1996, with 122 g more body reserves, would have been able to withstand about 3 extra days of fasting compared to birds in 1996–1997 before their energy stores were depleted to the same extent. Reproductive success was significantly higher among our banded pairs in 1995–1996 (83% of 153 pairs raised at least one chick to independence) than in 1996–1997, when only 39% of 253 pairs raised at least one chick. The lower success in 1996–1997 appeared to be due mostly to low feeding rates of hatched chicks, not losses during the initial breeding fast, but poorer body condition of birds in 1996–1997 may have contributed directly to decreasing chick feeding rates. Alternatively, body condition may have had no direct effect on chick feeding rates, but instead represented an early signal of an environmental variable (food distribution and abundance?) that limited reproductive success later in the breeding season. Field correlations such as these often point out the need for experimental work. Although food supplementation would be difficult in free-living penguins, natural variation in food availability, both within and between seasons could be used to explore these questions. In fact Antarctic penguins have been critical foci in studies on change in energy flow in the Antarctic ecosystem (Le Maho *et al.*, 1993; Mangel and Switzer, 1998; Croxall *et al.*, 1999) and studies on individual foraging behavior and nesting success are in progress (see Ballard *et al.*, 2001).

In the section above we compared population means of body masses during fasting, estimated from regression, with population reproductive success. We can examine the same relationship on an individual basis. For this population of penguins, body mass declined with time, as described by the regression equation:

$$M = 5.04 - 0.038 \cdot \text{time}$$

$$(r^2 = 0.62, F_{2,365} = 394, P < 0.0001)$$

where body mass (M) is in kg and time is days after 15 October. The residuals from this equation are the differences between the measured body mass for an individual and the average body mass for all fasting individuals on that date. Residual masses greater than zero identify individuals with body condition better than average, and residuals less than zero identify individuals with body condition worse than average. Table 1 compares the residual body masses (kg) of successful breeders with residual masses for breeders who nested, but failed to rear young to the crèche stage. For birds that were weighed more than once during the fasting period, we used the average residual mass. Successful females averaged 120 g heavier than unsuccessful females, and successful males averaged 100 g heavier than unsuccessful males. Neither of those differences reached significance at the 0.05 confidence level, although they are consistent with the hypothesis that higher body mass improves reproductive success. Using a size adjusted mass (see Hood *et al.*, 1998) did not improve the relationship. It would be interesting to know if controlling for either condition of the bird's mate or mass-independent sources of mortality would improve the predictive value of individual body condition for breeding success.

Fat stores fuel most of the metabolic demands of penguins in their early breeding season fast (Chappell *et al.*, 1993), and we have a small data set that allows us to estimate fat content at the time of arrival. We measured body mass and estimated total body water (TBW) of 22 arriving Adélie penguins at Torgersen Island using deuterium-enriched water dilution (Nagy, 1975). Similar measurements have been made on Adélie penguins on Torgersen Island by Chappell *et al.*, 1993. We identified birds as new arrivals if they were seen emerging from the water, were still wet or suddenly appeared in well-censused colonies. Mean estimated water content of these arriving individuals was 50% (SEM = 1%) (Bucher and Vleck, unpublished data). It tended to decrease with increasing body mass (Fig. 2), although the relationship was not as tight as we would have predicted (correlation coefficient,  $r = -0.52$ ). Free water content of fat and lean body tissue have been measured in Adélie penguins at Torgersen Island (Chappell *et al.*, 1993). If we assume the penguins we studied had similar percent water content, we can use our estimates of TBW and total mass to esti-

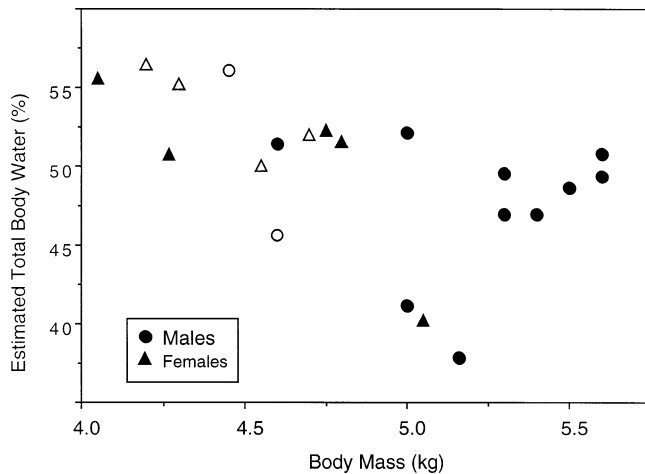


FIG. 2. Estimated total body water (TBW) based on deuterium-enriched water dilution in Adélie penguins upon arrival in the breeding colony in 1995. Open symbols are from birds that were not known to breed. Closed symbols are from successful breeders. Deuterium data from Bucher and Vleck (unpublished data).

mate fat stores. The 22 penguins in our sample had body water contents from 38% to 56% of total mass, corresponding to fat stores of 0.94 to 2.74 kg (mean = 1.65 kg). This is similar to the depot fat in arriving males reported by Chappell *et al.*, 1993. The use of isotope dilution to estimate body composition has been validated in other sea birds (reviewed in Groscolas *et al.*, 1991). Chappell *et al.* (1993) based their estimate of water content in different tissue types on measurements from four freshly dead penguins at Torgersen Island. Uncertainties in these measurements could produce errors of about 5–7% in estimates of depot fat (Chappell *et al.*, 1993).

Our data suggest that there is considerable individual variation in fat stores, and hence energy stores, at the time of arrival in the breeding colony. Of these 22 penguins, 16 mated with other birds, and hatched chicks that survived at least to the crèche stage. The remaining 6 birds eventually left our breeding colony without forming successful pairs, though we cannot be certain that they did not nest elsewhere. The birds that departed without forming pairs had significantly less depot fat than those that bred ( $t_{20} = -2.263$ ,  $P = 0.03$ ). In this sample we could not identify an effect of sex ( $P = 0.15$ ). Non-breeders averaged 52% (SEM = 2) water with 1.3 kg of depot fat on arrival, while birds that bred successfully averaged 48% (SEM = 1) water with 1.8 kg of depot fat at arrival.

These data support the hypothesis that energy reserves in Adélie penguins affect the decision to breed and the reproductive success of those birds that do breed. Returning females with low body mass are less likely to lay eggs and breed than females of higher body mass. In a year in which body masses of returning and fasting birds was significantly lower than birds at the same stage in the previous year, there was also substantially lower reproductive success. Those birds with lower body fat depots on arrival are significantly

less likely to breed than individuals with higher fat depots. In Magellanic penguins (*Spheniscus magellanicus*), body mass has also been identified as a predictor of reproductive success (Fowler *et al.*, 1994). Adélie penguins can live up to 20 yr (Williams, 1995), and annual survival is lower for birds that breed than those that do not breed (Spurr, 1975; Ainley and Demaster, 1980). These life history traits suggest that the most adaptive strategy for a bird in sub-optimal condition may be to forego reproduction for that year, rather than attempt to breed, because breeding is likely to be unsuccessful and may compromise future reproductive value.

#### EFFECT OF FASTING ON BODY CONDITION

Long-term fasting is a feature of the biology of many secondarily marine vertebrates that, like penguins, must go ashore to reproduce (reviewed in Castellini and Rea, 1992). Penguins, particularly the large *Aptenodytes* species, have served as model organisms for the study of long-term fasting and protein sparing. Male emperor penguins (*Aptenodytes forsteri*) fast for up to 120 days during winter breeding and incubation (Groscolas, 1986). Male king penguins (*Aptenodytes patagonicus*) have early breeding fasts about as long as those of Adélie penguin, but king penguin chicks may fast for up to five months during the winter when parents leave the colony (Cherel *et al.*, 1987). The physiological changes that accompany these fasts in *Aptenodytes* penguins have been worked out in classic studies by Groscolas, Cherel, and colleagues. Three stages of fasting are categorized by differences in rates of mass loss and in the substrates used to support cellular metabolism (Cherel and Le Maho, 1985; Cherel *et al.*, 1988; Le Ninan *et al.*, 1988; Castellini and Rea, 1992). Stage I, the beginning of a fast, is characterized by rapid mass loss as the last meal is digested and processed and the birds switch from oxidizing dietary fuels to oxidizing primarily stored fat. Uric acid, the principal nitrogenous waste of birds, is often elevated in the blood during early Stage I in penguins because of their high protein diet. In Stage II, which may last for weeks, metabolic rates and activity decrease and fat stores are the major fuel source (Davis *et al.*, 1989; Robin *et al.*, 1998). Markers of lipid metabolism (free fatty acids and ketones like  $\beta$ -hydroxybutyrate) in the blood increase, while protein oxidation and nitrogenous waste products are minimized (Groscolas, 1986; Cherel *et al.*, 1987, 1988; Le Ninan *et al.*, 1988). In stage III, fat stores are largely depleted and body protein catabolism increases, so blood uric acid levels rise, blood ketone levels drop, and locomotor activity and the rate of mass loss often increase (Robin *et al.*, 1988, 1998; Cherel *et al.*, 1994a). Plasma corticosterone, the avian stress hormone, rises in some species (Le Ninan *et al.*, 1988; Cherel *et al.*, 1994a). Increased activity in stage III may signal a “drive to refeed” that will ultimately lead to a decision to abandon the nesting attempt (Groscolas, 1986; Cherel *et al.*, 1994a; Robin *et al.*, 1998). Less work has been done with the

smaller penguin species, although various metabolic indicators of fasting have been reported for macaroni (*Eudyptes chrysolophus*) (Williams *et al.*, 1992), gentoo (*Aptenodytes papua*) and chinstrap (*A. antarctica*) penguins (Cherel *et al.*, 1993; Ferrer *et al.*, 1994).

Adélie penguins weigh about 5 kg when they come ashore, but may fast as long as king penguins that weigh three times as much and nest at lower latitudes. To determine the physiological consequences of this fast, we collected heparinized blood samples from free-living birds for which we knew fast duration. Avian blood sediments rapidly and field conditions precluded sample preparation in the field, so blood samples were taken to the field station, then vortexed to resuspend the cells. Pair-wise comparison of fresh field samples and resuspended lab samples demonstrated that this protocol produces cell suspensions matching fresh blood (Vleck *et al.*, 2000b). After resuspension, subsamples were taken immediately to measure hemoglobin and hematocrit, and for preparation of blood smears. Hemoglobin samples were preserved in Drabkin's solution and measured within one week using Sigma Diagnostics kits. The remaining blood was centrifuged to separate cells and plasma. Osmotic concentrations of some plasma samples were determined in duplicate with a Wescor vapor pressure osmometer. Plasma was stored at  $-70^{\circ}\text{C}$ , and ultimately transported to Iowa State University where plasma uric acid, glucose, and  $\beta$ -hydroxybutyrate were measured using spectrophotometric assays from Sigma Diagnostics, and corticosterone levels were measured by radioimmunoassay (Vleck *et al.*, 2000b). In the following analysis we include only one sample (the latest sample) per bird, because we did not have repeated measures on enough individuals to test for individual effects in most analyses (Vleck, 2001).

Most female Adélie penguins fasted for 20 days or less and over this interval there was little change in either uric acid or  $\beta$ -hydroxybutyrate (Fig. 3). Males that fasted longer did experience noticeable changes in blood chemistry related to fasting. The plasma levels of  $\beta$ -hydroxybutyrate were low in birds during the early fast and rose steadily reaching a peak averaging about 1.7 mM after 35–40 days of fasting, a level similar to that in king penguins at the end of Stage II fasting (Cherel *et al.*, 1988). Individuals with high  $\beta$ -hydroxybutyrate levels had low uric acid levels ( $r = -0.37$ ). Uric acid levels in some individuals were high when they arrived in the colony, presumably because they were still metabolizing dietary protein, but over the first 30 days of fasting, plasma uric acid was generally low, averaging about 0.2 mM (Fig. 3). As fasting continued beyond 30 days, some individuals had higher uric acid levels. Uric acid was conspicuously elevated and  $\beta$ -hydroxybutyrate was low in two males that fasted for longer than 55 days, indicating they had probably entered Stage III fasting. Their uric acid levels (mean = 0.8 mM) were, however, lower than those reported for king penguins (about 1.4 mM) after 50 days of fasting (Cherel *et al.*, 1988). Most Adélie pen-

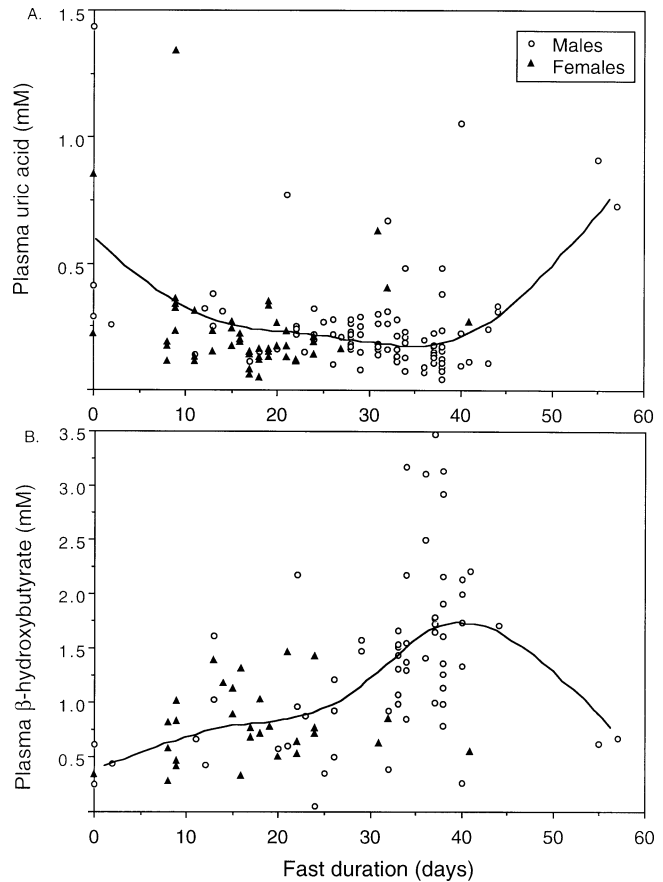


FIG. 3. A. Plasma uric acid concentration and B.  $\beta$ -hydroxybutyrate in Adélie penguins during the early breeding season fast. The lines shown are spline fits to the male data only.

guins appear to be able to leave the colony and forage before they approach the end of Stage II fasting, but there is considerable variation between individuals in both uric acid and  $\beta$ -hydroxybutyrate.

Levels of plasma corticosterone also did not change with fast duration in Adélie penguins ( $P = 0.59$ ; Fig. 4). In females, corticosterone did increase as residual mass (as calculated above) decreased, indicating the lightest females in the population tended to have the highest level of stress hormone ( $F_{1,70} = 4.03$ ;  $P = 0.05$ ), but this was not found in males ( $P = 0.45$ ). Corticosterone levels averaged 5.2 ng/ml (SEM = 0.3,  $n = 195$ ). Even in males that had fasted for more than 40 days there was no increase in corticosterone levels, except for a single bird sampled at 55 days of fasting. In contrast, Cherel *et al.* (1988) reported corticosterone levels in king penguins increased 2–3 fold after 40–50 days of fasting. Our corticosterone measurements are all from samples collected within 5 min of first approaching the bird, before the level of corticosterone increases due to handling stress (Vleck *et al.*, 2000b).

Plasma glucose also did not vary with fasting time ( $P = 0.28$ ; Fig. 4). Plasma glucose levels averaged 16.2 mM (SEM = 0.4,  $n = 136$ ) and were quite variable between individuals, particularly in comparison

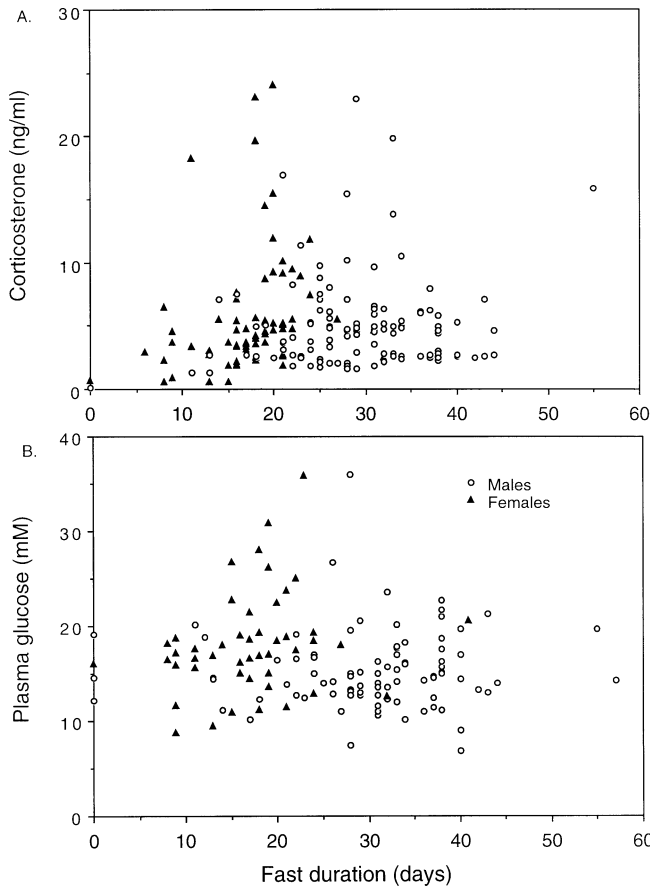


FIG. 4. A. Plasma corticosterone concentration and B. glucose concentration in Adélie penguins during the early breeding season fast.

to those in mammals. The highest levels of blood glucose occurred in females in the 10 days prior to egg laying, with many values over 20 mM. There was no relationship between levels of blood glucose and plasma corticosterone in those females ( $P = 0.55$ ) or in the data set as a whole ( $P = 0.15$ ). Plasma glucose levels also do not vary greatly with fasting in other penguins (Groscolas and Rodriguez, 1981; Groscolas, 1986; Cherel *et al.*, 1988). Blood glucose levels do fall in late stage III fasting in some penguins (Groscolas, 1986; Cherel *et al.*, 1988).

During incubation, penguins may experience dehydration as well as inanition. Incubating Adélie penguins do not leave the nest and usually have no access to free water unless snow surrounds the nest. As a result, fasting males may be subject to dehydration after incubation begins. Females are unlikely to experience dehydration during the initial breeding fast. Incubating birds eat snow if they can reach it, and when relieved by their mate often visit a snowbank and consume snow before leaving to forage (personal observation). Surprisingly, there is little information about water budgets of penguins during the fast. Researchers studying fasting penguins in confinement usually provide water or snow *ad lib.* (Groscolas, 1986; Cherel *et al.*, 1987, 1988; Robin *et al.*, 1998). In our free-

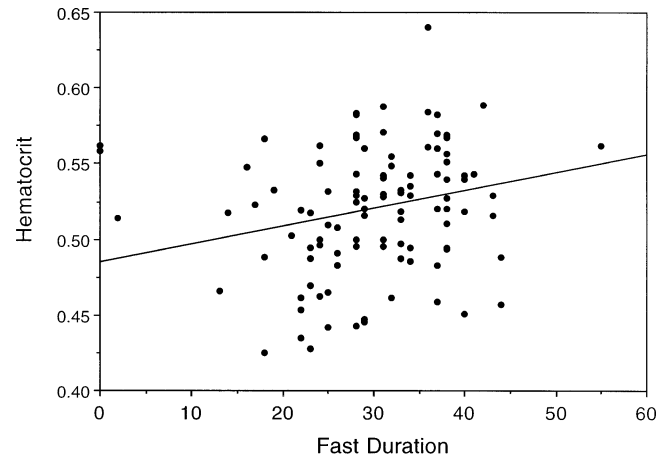


FIG. 5. A. Hematocrit and concentration in male Adélie penguins during the early breeding season fast. The line is the best-fit linear regressions through the data, described by the equation: Hematocrit =  $0.485 + 0.012$  days fasting ( $r^2 = 0.07$ ).

living birds, plasma osmotic concentrations averaged 299 mOsm (SEM = 2,  $n = 67$ ) and did not change with fast length in either sex ( $P > 0.68$ ), so fasting birds osmoregulate effectively. They may still be dehydrated, however, because salt excretion via the salt glands could maintain osmotic balance and cell volume regulation while extracellular fluid volume dropped. In males, but not females, both hematocrit (Fig. 5;  $P < 0.008$ ) and hemoglobin ( $P < 0.0001$ ) increased with fast duration. Over a 50 day fast, average hematocrit in fasting males increased from 0.49 to 0.54. Incubating birds in negative water balance may maintain constant erythrocyte volume even as total blood volume falls, to ensure adequate blood oxygen capacity when they return to sea. If total blood cell volume remained constant, the hematocrit change we observed would imply a 9% decrease in blood volume due to plasma loss over a 50 day fast. Plasma losses would be lower if blood cell volume declined. In contrast, king penguins with access to free water show no significant change in hematocrit through stage II fasting (Cherel *et al.*, 1987, 1988). For Adélie penguins, water deprivation during incubation may add another physiological challenge to that of fasting, an insight that emerged only from studying fasting animals under natural conditions.

We followed and sampled 212 free-living penguins during natural fasts, locating each animal nearly daily, so we are reasonably certain that they did not feed between samples. Many other studies of fasting penguins rely on serial samples from confined individuals (Groscolas, 1986; Cherl *et al.*, 1988; Robin *et al.*, 1988) and suggest a tight profile for the changes in blood metabolites. An important feature of the data from free-living birds is the magnitude of the variation between individuals. For example, at 40 days of fasting, ketone levels in seven individual penguins varied from 2.1 mM (probably late stage II fasting) to 0.3 mM (possibly late stage III fasting). Robin *et al.*

(1998) reported that fasting times before emperor penguins reach a critical body mass and enter Stage III fasting vary from 60 to 110 days. This variation could result from differences in initial fuel stores, though plotting our data against body mass rather than fast duration does not reduce the scatter. Variation in ketone levels could also result from individual differences in metabolic rates or abilities to oxidize ketones, which merits further study. The identification of such variation under natural conditions suggest that we still have much to learn about those factors that limit fast duration in individuals.

Fasting birds ultimately abandon their eggs if their mate does not return, and that decision may be precipitated by the progression from stage II to stage III fasting (Groscolas, 1986; Cherel *et al.*, 1994b; Robin *et al.*, 1998). Given their long life span, the optimal strategy for an incubating bird entering stage III fasting may be to abandon reproduction for that year, rather than risk its own survival. Given the time and distance that can separate a foraging adult from its nest, it is surprising how rarely abandonment occurs in Adélie penguins. Female Adélie penguins nearly always return to relieve the mate, probably under the influence of continuously elevated prolactin levels (Vleck *et al.*, 2000a). In our samples, many males were near the end of stage II fasting before being relieved, and elevated uric acid and low blood ketones suggest some had entered stage III, so safety margins may be small for many breeders, even on the relatively benign Antarctic peninsula. We do not know how long Adélie penguins can survive after entering stage III fasting. Larger king penguins, held for 15 days after entering stage III, still appeared to be in good health (Cherel *et al.*, 1988). Our data show that some Adélie penguins will continue incubation until they have fasted for as much as 57 days. Because of the tractable nature of working with penguins, it is possible to alter the behavior of one member of the pair (hold it in a pen) in order to study the effect on the mate (Miller and Davis, 1993). Thus it should be possible experimentally to study the physiological and behavioral responses of birds undergoing prolonged incubation fasts under entirely natural conditions.

#### CONCLUSIONS

Adélie penguin reproductive biology is similar to that of many colonial, off-shore feeding seabirds, but like other Antarctic penguins, Adélie penguins face special constraints on reproduction that are difficult to study except under field conditions. Body condition (particularly fat stores to sustain long fasts) influences reproductive success. Birds in poor condition (particularly females) do not attempt to breed. Condition at arrival is important to penguins because they must fast from the time they arrive at the breeding colony through courtship and mating, and cannot leave to feed at sea until the eggs are laid (females), or their mate returns (males). Incubating birds may also experience dehydration. Adélie penguins undergoing normal-

length fasts are not usually near the limits of their ability to tolerate fasting. Birds that must undergo unusually long fasts show signs of fat store depletion and body protein catabolism that may dictate a decision to abandon the nest. Penguins provide a model system for studying how physiological parameters affect breeding decisions, because of the relative ease of obtaining data from a large number of individuals all engaged in the same set of well-defined behaviors. Variation among individuals and between years or sites provides opportunity to test hypotheses about physiological and environmental input into those decisions, as would experimentation to increase the length of fasting under field conditions.

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