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ABSENCE OF DAILY RHYTHMS OF PROLACTIN AND CORTICOSTERONE IN ADÉLIE PENGUINS UNDER CONTINUOUS DAYLIGHT

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Abstract. Plasma prolactin and corticosterone levels were measured in free-living Adélie Penguins (*Pygoscelis adeliae*) at Torgersen Island, Antarctica (64°S latitude), at 4-hr intervals throughout the day during early January 1997 and examined for evidence of a 24-hr rhythm. At this season and latitude, natural daylight is continuous. No significant change in the plasma level of either corticosterone or prolactin was found across the day in this population. In contrast, hormone levels in birds at lower latitudes typically fluctuate between night and day. Our data would not have revealed circadian rhythms within individuals even if they exist, because each bird was only sampled once. The lack of hormone rhythms in the population, however, suggests that changes in light intensity at this latitude in the Antarctic summer are not sufficient to entrain, or perhaps even to maintain, circadian rhythms of individuals.

Key words: *Adélie Penguin, continuous daylight, corticosterone, diel rhythm, light intensity, prolactin, Pygoscelis adeliae.*

Ausencia de Ciclos Diarios de Prolactina y Corticosterona en *Pygoscelis adeliae* bajo Luz Solar Continua

Resumen. A principios de enero de 1997 en la Isla Torgersen, Antártica (latitud 64°S), se midieron cada 4 horas los niveles de prolactina y corticosterona en el plasma de *Pygoscelis adeliae* en busca de evidencia de un ciclo hormonal de 24 horas. Durante esta estación del año y a esta latitud, la luz solar es continua. No se encontraron cambios significativos en los niveles

de prolactina ni de corticosterona en el plasma a través del día en esta población. En contraste, los niveles hormonales en aves en menores latitudes fluctúan típicamente entre el día y la noche. Aún si existiesen, nuestros datos no habrían revelado la existencia de ritmos circadianos para cada individuo, dado que cada animal fue muestreado una sola vez. Sin embargo, la ausencia de ciclos hormonales a nivel poblacional, sin embargo, indica que los cambios de luz a esta latitud en el verano antártico no son suficientes para sincronizar, o quizás ni siquiera para mantener, ritmos circadianos en los individuos.

In most vertebrates that have been studied, hormone concentrations in plasma exhibit a diel pattern. The timing of hormone secretion is thought to be important for the initiation, maintenance, and termination of a variety of physiological processes, often associated with daily and seasonal events (Gwinner 1975). The light-dark cycle, or photoperiod, is the primary environmental cue that entrains daily and annual rhythms in hormone secretion. However, photoperiod may be an unreliable cue for seasonal changes at very low latitude, where it changes very little, or in polar regions during the summer or winter. At high latitudes daylight and darkness are continuous during the summer and winter respectively, although daily changes in light intensity do occur during the summer. That raises the question of whether diel cycles in light intensity at high latitudes are adequate to entrain circadian rhythms in hormone levels, or whether these rhythms disappear or become free-running, as often happens in the laboratory under constant light (Yamada et al. 1988).

Studies of how continuous natural daylight in the Antarctic affects a free-living animal's ability to maintain daily rhythms are few. These have generally fo-

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cused on daily rhythms in body temperature and activity (reviewed in Cockrem 1990) and plasma melatonin concentrations (Cockrem 1991, Miché et al. 1991). Rhythms in melatonin levels in Antarctic animals are suppressed or absent under continuous daylight, which accords with the suppressive effect of light on melatonin secretion (Yamada et al. 1988), although this phenomenon varies among species (Kumar et al. 2000).

In temperate-zone birds, corticosterone and prolactin levels also exhibit daily rhythms (Joseph and Meier 1973, Proudman 1991, Breuner et al. 1999). These two hormones are of particular interest because Meier (1972) suggested that the phase relationship between the diel rhythms of corticosterone and prolactin controls and coordinates many physiological conditions in vertebrates including development, migration, and reproduction. In this study, we examined plasma prolactin and corticosterone levels in Adélie Penguins (*Pygoscelis adeliae*) exposed to 24 hr of continuous natural daylight during the Antarctic summer.

METHODS

FIELD PROCEDURES

We studied Adélie Penguins at a rookery on Torgersen Island, near the U.S. base (Palmer Station) on Anvers Island, Antarctic Peninsula (64°46'S latitude, 64°05'W longitude). Blood samples were collected primarily from 5–6 January 1997 ($n = 30$) with a few additional samples taken between 7–13 January 1997 ($n = 11$). At this time light is continuous, although it decreases in intensity between about 21:00 and 04:00 (Fig. 1). Even at the lowest light intensity, it was bright enough for us to collect blood samples and record data without the use of artificial light. We captured the birds by hand at their nests and individually marked them with a metal flipper band for identification. All birds sampled were tending one or two chicks. Blood samples of 1–2 mL ($n = 41$ for prolactin; $n = 36$ for corticosterone) were collected from the jugular vein using a heparinized syringe and 20-gauge needle and then transferred immediately to heparinized containers. The mean \pm SE elapsed time between first approaching the bird and obtaining the blood sample was 1.9 ± 0.2 min (range 1 to 6 min). Blood samples were obtained at approximately 4-hr intervals from different individuals so that each bird was bled only once. The sex of most birds could not be determined at the time of sampling, but was determined later using the CHD gene (Griffiths et al. 1996). The blood was kept cold, but protected from freezing until the plasma could be separated and frozen at -70°C for storage (1–6 hr after collection). All animal procedures were approved by the Animal Care and Use Committee at Iowa State University under guidelines established by the National Institutes of Health. All collecting was done under permits as required by the Antarctic Conservation Act.

HORMONE MEASUREMENTS

Plasma hormone levels were assayed in triplicate by radioimmunoassay (RIA). For the prolactin RIA, we used purified chicken prolactin (reference preparation AFP-10328B) as a standard and a rabbit antiserum (AFP-151040789) raised against chicken prolactin

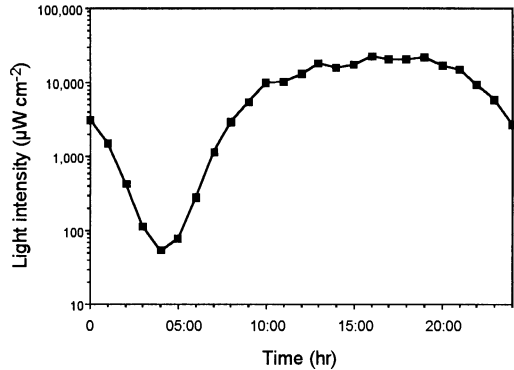


FIGURE 1. Mean light intensity 5–7 January 1995–1997, determined by integrating hourly spectral irradiance input over the visible wavelengths of 400 to 600 nm at Palmer Station, Antarctica. Light intensity is plotted on a logarithmic scale. Data are from NSF Polar Programs UV Radiation Monitoring Network 1995 to 1997 v 6.0.a Data Set (Biospherical Instruments, Inc., San Diego, CA).

(Vleck, Ross, et al. 2000). Plasma corticosterone concentration was measured by radioimmunoassay following the technique of Wingfield and Farner (1975), but without chromatography (Vleck, Vertalino, et al. 2000). The least detectable concentration for each hormone was about 2 ng mL^{-1} . All samples were run in a single assay for each hormone.

STATISTICAL ANALYSES

A basic sinusoid model was constructed (Bloomfield 1976) to determine if the plasma corticosterone and prolactin levels exhibited any circadian rhythm over a 24-hr period of measurement. The model used was $y(t) = \beta_0 + \beta_1 \cos(\omega t) + \beta_2 \sin(\omega t) + \varepsilon_i$, where $y(t)$ is hormone level at time t (min); β_0 is the intercept; and ε is the error term. The frequency term ω is a fixed constant where $\omega = (2\pi \text{ period}^{-1})$ and the period equals the total number of minutes (1440) in 24 hr. The data were fitted by least squares to derive a set of rhythm parameter estimates, and ANOVA was used to determine if the parameters were significantly different from zero. Differences were considered significant if $P < 0.05$, and the values are reported as means \pm SE.

RESULTS

Prolactin levels were significantly higher in females (mean = $28.3 \pm 1.1 \text{ ng mL}^{-1}$) than in males (mean = $25.1 \pm 1.1 \text{ ng mL}^{-1}$; ANOVA, $F_{1,39} = 4.2$, $P = 0.05$), as we earlier reported in Adélie Penguins tending chicks (Vleck, Ross, et al. 2000). As a consequence, male and female prolactin data were analyzed separately in the sinusoid model. There was no effect of sex on the corticosterone levels ($F_{1,33} = 1.7$, $P = 0.20$), so male and female data were combined. There were no significant daily rhythm components to prolactin levels in either males or females (males: $F_{2,19} = 3.0$, $P = 0.07$; females: $F_{2,16} = 0.8$, $P = 0.45$; Fig. 2a). Basal corticosterone levels were low (mean = $4.0 \pm 0.2 \text{ ng mL}^{-1}$) in all individuals, relative to the levels

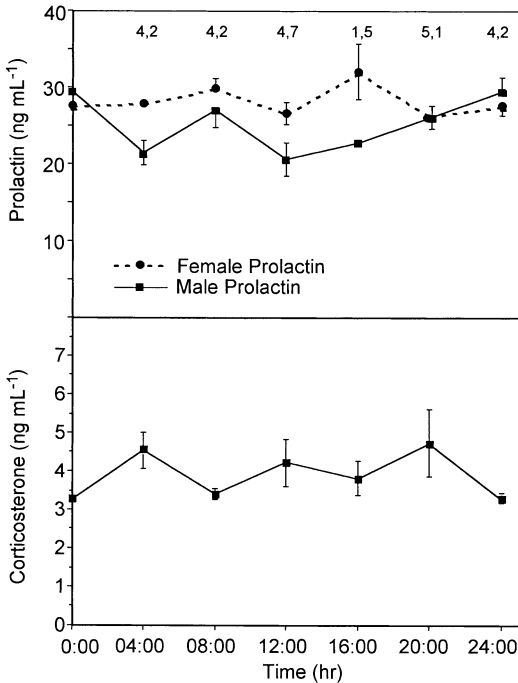


FIGURE 2. Variations in plasma prolactin and corticosterone (ng mL^{-1}) in Adélie penguins plotted against time of day in early January 1997. (a) Prolactin measurements for male and female are shown with the sample size (male, female) indicated above each data point. (b) Corticosterone levels are combined male and female data and each point represents $n = 6$. Vertical bars are standard errors about the mean hormone concentration for that time.

achieved when the birds are held for up to 30 min (Vleck, Verticalino, et al. 2000). There were no significant changes in corticosterone level across the 24-hr period ($F_{2,33} = 0.1$, $P = 0.90$; Fig 2b).

DISCUSSION

Plasma prolactin and corticosterone concentrations measured in different Adélie Penguins did not show any daily rhythm components during early January when daylight was continuous. Both corticosterone and prolactin levels do differ significantly among individual Adélie Penguins (Vleck, Verticalino, et al. 2000, Vleck, unpubl. data), and it is possible that individuals have circadian rhythms in hormone levels that are out of synchrony with other animals in the population, due to the weakness of environmental zeitgebers. In temperate-zone birds maximal corticosterone levels usually occur during the nocturnal or inactive period (Joseph and Meier 1973, Breuner et al. 1999, Romero and Remage-Healey 2000). In nocturnal Western Screech-Owls (*Otus kennicottii*) corticosterone levels are significantly higher during daylight hours than at night (Dufty and Belthoff 1997). There are few studies on plasma prolactin over 24 hr in birds, although in diurnal mammals, prolactin levels are well

known to increase during the night (Wehr 1998). In incubating domestic turkey hens (*Meleagris gallopavo*) plasma prolactin rises through the night to peak at the start of the photophase and corticosterone shows two peaks, in the middle of the light and dark periods (Proudman 1991).

Other workers have also reported reduced hormone fluctuations under the continuous daylight found at high latitudes in the summer. Weddell seals (*Leptonychotes weddelli*) studied at about 77°S latitude in Antarctica under continuous light dived most actively during the early morning hours, and one study reported a peak in melatonin secretion at about 17:00 with low levels the rest of the day (Griffiths et al. 1986). In another study, however, no diel pattern in either melatonin or cortisol was found (Barrell and Montgomery 1989). These authors suggested "that the increase recorded by Griffiths et al. (1986) was an aberrant result" (p. 448), and that pineal activity was shut off in these seals under the continuous natural light of the Antarctic summer. At the same latitude, melatonin levels in Adélie Penguins sampled during the summer period of continuous light showed no relationship with time of day, although some (but not all) serially sampled individuals had elevated melatonin during the period of reduced light intensity (Cockrem 1991). Emperor Penguins (*Aptenodytes forsteri*) studied throughout the year at about 66°S latitude had a well-defined diel pattern in melatonin during the spring and fall, which disappeared during both the summer and winter solstice (Miché et al. 1991). Under constant darkness all birds had very low circulating melatonin levels over the 24-hr period, but under constant light, two of five individuals serially sampled did show persistent small amplitude peaks in melatonin level.

Daily rhythms in both body temperature and activity have also been recorded throughout the summer in penguins at various locations (reviewed in Cockrem 1990). Four out of ten Adélie Penguins had a significant rhythm in body temperature with an amplitude of about 0.9°C under natural continuous daylight, but individuals with the highest level of locomotor activity showed no body-temperature rhythm. Adélie Penguin activity in the summer is often highest at about midnight and lowest at about noon, but there is considerable variation among birds and between sites that appears broadly related to the amplitude of the light intensity cycle and shading effect at different locations (Cockrem 1990). We did not quantify activity at our site, although we found birds asleep at all hours, and likewise birds walking between the sea and the colony could always be found.

The alternation between day and night is the most reliable rhythmic cue in the environment under most natural conditions (Refinetti 2000). Around both solstices, however, the strength of this cue decreases in polar regions. Light intensity during the Antarctic summer does exhibit a regular daily cycle that could be used as a reliable zeitgeber if the change were great enough. During early January there is generally about a 350-fold difference (2 orders of magnitude) in light intensity across the day at our field site. In temperate areas, however, the change in light intensity between full sun and full moon spans 5 to 6 orders of magni-

tude, and the change between full sun and starlight is 8 to 9 orders of magnitude (Gates 1980). Lack of, or attenuation of, the expected rhythms in hormone levels, body temperature, and activity in Antarctic animals suggests that variation in light intensity during midsummer is not a strong enough signal to maintain clear diel rhythms or to synchronize free-running rhythms among individuals. Others have suggested that the small daily amplitude of light intensity in the high-Arctic summer may not entrain diel rhythms (Krüll 1976) especially in nocturnal species (Swade and Pittendrigh 1967), although Pohl (1999) showed that changes in the spectral composition of light could entrain activity rhythms in migratory passerines in the Arctic. Pohl further suggested that species originating from temperate-zone latitudes may be more sensitive to subtle zeitgebers than resident animals. Kumar et al. (2000) recently reviewed effects of light intensity on circadian patterns in activity and melatonin in birds and mammals and suggested that light-dark cycles using dim light (e.g., 0.2 lux) led to higher interindividual variability and lower amplitude rhythms than using bright light (e.g., 500 lux). Clearly animals can respond to different light intensities (see also Bentley et al. 1998, Rani and Kumar 2000).

Some aspects of the Adélie Penguin lifestyle suggest the need for an accurate sense of time. The breeding season at these latitudes is short relative to the breeding cycle and Adélie Penguins are highly synchronized in their arrival at the colony, egg laying, and the post-breeding molt (Ainley et al. 1983, Vleck, Ross, et al. 2000). These events in the annual cycle, however, take place when daylight is not continuous. An accurate biological clock is also needed for sun-compass orientation. Such a feature is probably involved in Adélie Penguin migration between the breeding and foraging grounds, which can be more than 100 km apart (Davis et al. 1988). The main food of penguins is krill, which make daily vertical movements in the water column, and a biological clock would be useful for coordinating foraging behavior with their prey (Cockrem 1990). Chappell et al. (1993) reported that Adélie Penguins in the same colony we studied do display a diel rhythm in hunting effort with decreased activity between 22:00 and 04:00 (data collected in December through February). Surprisingly however, they found no circadian pattern in the foraging depth data, and suggested that the magnitude of daily vertical migration of krill at this site and time may be minimal. Chinstrap Penguins (*Pygoscelis antarctica*) in the King George Island archipelago in Antarctica decrease foraging intensity under low light, but continue to dive down to 22 m at the lowest light intensities (Wilson and Peters 1999).

Glucocorticoids like corticosterone influence carbohydrate metabolism (Remage-Healey and Romero 2000), and the phase relationship of corticosterone and prolactin has been shown to influence the cyclical changes in body fat stores that occur in animals (Meier and Martin 1971, Cincotta et al. 1993). Penguins show fluctuations in body fat that can exceed 40% of body mass over the annual cycle (Vleck and Vleck 2002). The cyclical metabolic adjustments that control hyperphagia in penguins have not been studied, but the hy-

perphagia associated with the postbreeding molt begins just as daylight becomes discontinuous and perhaps when hormone rhythms are re-established.

The ecological advantages of a sense of time may be reduced at high latitudes during midsummer, because environmental conditions are relatively constant. Available data are limited, but suggest that in Antarctica, hormone rhythms are reduced in amplitude or desynchronized among individuals during times of constant light exposure.

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