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Do night-active birds lack daily melatonin rhythms? A case study comparing a diurnal and a nocturnal-foraging gull species

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Abstract Plasma melatonin concentrations in most animals investigated so far increase at night regardless of whether individuals are day or night active. Nevertheless, daily melatonin amplitudes are often seasonally adjusted to ecological conditions, with birds that breed at high latitudes and migrate during the night showing lower daily amplitudes. Here we investigate whether nocturnal seabirds, gulls that feed at night, also show a low melatonin amplitude because they have to be active predominantly during the night but also intermittently during the day. We sampled free-living nocturnal-foraging swallow-tailed gulls (*Creagrus furcatus*) on two Galapagos islands every ~4 h and compared their plasma melatonin concentrations with those of related black-headed gulls (*Larus ridibundus*) sampled in the Netherlands. Like most seabirds, the black-headed gulls showed generally low melatonin concentrations, but clear diel cycles. The swallow-tailed gulls, on the other

hand, had similarly low absolute melatonin concentrations, but no detectable diel changes. Despite problems inherent in comparisons between two species and field/lab setups, our data lend support to the hypothesis that the lack of a diel melatonin rhythm allows animals to be active at any time.

Keywords Swallow-tailed gull · *Creagrus furcatus* · Black-headed gull · *Larus ridibundus* · Melatonin · Daily rhythm · Nocturnal · Diurnal · Galápagos · Seabird · Hormones · Activity

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Introduction

Most animals are active either during the day or during the night [1, 2]. However, many animal species also face the problem that at times during their life they have to be active at odd hours. For example, diurnal songbirds migrate at night [3] and birds breeding at high latitudes (high Arctic, Antarctic) may have to be active continuously to defend their territories [4]. Here we ask how the circadian system adjusts to situations in which birds potentially have to be active at any time during the day. In birds, the pineal gland is one of the most important components of the circadian system (for reviews see, e.g., [5–9]). The pineal gland synthesizes the hormone melatonin (5-methoxy-*N*-acetyltryptamine) and releases it into the blood stream. Mammals use changes in plasma concentrations of the hormone melatonin as time signals, such that diurnal mammals become sleepy with high melatonin concentrations whereas nocturnal mammals are active with high melatonin concentrations [1]. In birds, melatonin production also occurs in a diel manner, with high melatonin secretion at night and low secretion during the day, corresponding to the light-dark cycle [6, 10]. This diel pattern has been found in most species investigated to date, irrespective of whether the species is active during the day or at night. The only exceptions are owl species, such as the nocturnal barn owl [*Tyto alba*; 11], which may have low nocturnal

melatonin levels as a prerequisite for activity at night. We hypothesize, following Gwinner et al. [8], that, in general, melatonin lacks a diel rhythm in bird species that have the challenge of being active during the night and also intermittently during the day [12–15]. To address this hypothesis we compare melatonin concentrations in a nocturnal-foraging and a diurnal gull species.

Swallow-tailed gulls (*Creagrus furcatus*) and black-headed gulls (*Larus ridibundus*) are good model species for investigating the physiological processes that accompany and enable a switch in activity time. Swallow-tailed gulls are exceptional among gulls because of their night-foraging activity [16–18]. Night activity is very rare in gulls and swallow-tailed gulls appear to possess several adaptations for nocturnal foraging including extraordinarily large eyes and possibly ultrasound communication [19]. Though swallow-tailed gulls forage at night they are also active at times throughout the day, exhibiting many behaviors including flying, mating displays, and preening [17]. Black-headed gulls, on the other hand, are largely diurnal, and generally only move at night when disturbed [20].

Here we investigate whether a difference in activity pattern in these two related gull species is reflected in a difference in their plasma melatonin rhythms. We are aware of the problems inherent in two-species comparisons [21] but believe that the melatonin secretion patterns are sufficiently phylogenetically conserved such that deviations from these patterns will be heuristically informative. We predicted that to allow for activity at night (and intermittently during the day), swallow-tailed gulls should either have a damped, or no amplitude in their melatonin rhythm compared to black-headed gulls [11]. For the black-headed gulls, we predicted a typical melatonin rhythm with increased levels at night and low levels during the day. We also expected overall low plasma concentrations of melatonin in both species, because seabirds appear to have lower levels of melatonin than most other species [22].

Materials and methods

We studied 23 swallow-tailed gulls on Genovesa Island (November 1998) and 48 swallow-tailed gulls at Punta Cevallos, Isla Española (89°37'W, 1°23'S; March 2000) in the Galápagos archipelago. In addition, we took blood samples from 20 black-headed gulls residing in aviaries at the Biological Centre of the University of Groningen, the Netherlands, in May 2002. Some of these birds were sampled repeatedly because not enough birds were available for single samples. However, individuals were not sampled twice on the same day. Birds were captured with hand nets in both the field and aviaries, and each individual in the field was blood-sampled only once. Blood samples were obtained by puncturing the superficial brachial wing vein with a 26-gauge needle. Blood (200–400 µl) was collected from the wing vein with heparinized microcapillaries. All blood

samples were taken within five minutes after disturbing the birds. Plasma was separated from blood cells by centrifugation (at 588×10^2 m/s for 4 min) within 15 min after collection (swallow-tailed gulls) and 30 min in black-headed gulls, and subsequently treated with 10 µl of a 0.2% β-propiolactone solution to destroy viruses, according to U.S. import regulations for avian blood. Samples were then stored at –4°C in the field and below –20°C for transport and until analysis. Research conducted on swallow-tailed gulls during this project was permitted under the regulations of the University of Illinois, Urbana-Champaign Office of Laboratory Animal Research, and research conducted on black-headed gulls was permitted under the regulations of the Institutional Animal Care and Use Committee of the University of Groningen, under license number DEC2159. All work adheres to NIH standards for the use of animals in research.

Hormone analysis

Plasma concentrations of melatonin were determined by indirect radioimmunoassay [RIA; see 23, 11, 24]. All samples were analyzed in one single melatonin assay. In short, aliquots of about 100 µl of plasma were equilibrated with 3,000 dpm of tritiated label overnight at 4°C for estimation of recovery. Samples were first extracted by adding 120 µl NaOH and 4 ml chloroform and again by adding 2 ml chloroform. Samples were dried in a 40°C water bath under nitrogen gas and redissolved in 300 µl PBSG buffer. Samples equilibrated with buffer overnight at 4°C. Fractions of 100 µl were taken for duplicates and fractions of 50 µl were used for recovery (melatonin antibody, Stockgrand, Surrey, UK). Average recovery levels were 73%. Blanks were below detection limit, and intra-assay variation (for a total of eight standards) was 9%, lower detection limit was at 0.01 ng/ml. Data were analyzed with SPSS 10.0 for Windows (SPSS, Chicago). Values below the lower detection limit were set at that limit as a conservative estimate for statistical analysis. For the analysis of diel rhythms in hormones we used analysis of variance (ANOVA) followed by post hoc comparisons with LSD post hoc tests. We did not detect any effect of body mass (included as a covariate) on hormone concentrations thus we did not use body mass in the analysis. Significance of tests was accepted at the $\alpha = 0.05$ level.

Results

Black-headed gulls showed clear diel changes in plasma melatonin concentrations, with samples during the night (0 and 3 h) being higher than those during the day (Fig. 1a, ANOVA, $F_{5,36} = 2.6$, $P = 0.04$; posthoc LSD tests: sample at 0 h significantly different from samples at all other times except 3 h, $P < 0.05$; time 3 h significantly different from 16 h, $P < 0.05$). Swallow-tailed

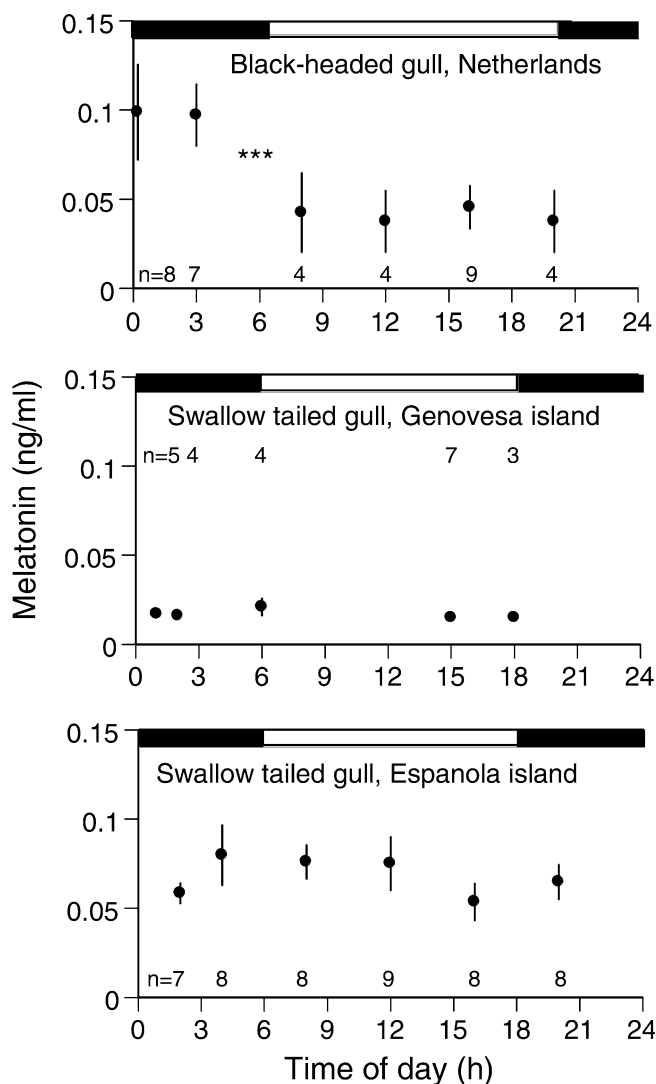


Fig. 1 Daily plasma melatonin concentrations for black-headed gulls from the Netherlands and two populations of swallow-tailed gulls from the Galapagos archipelago, Ecuador. Bars above each graph show night (black) and day (open), sample sizes are indicated in the figures, asterisks indicate statistical significance ($P < 0.05$)

gulls on Genovesa Island did not show a significant diel difference in plasma melatonin concentrations (Fig. 1b, ANOVA, $F_{4,23} = 1.1$, $P = 0.3$; all posthoc tests $P > 0.05$). Similarly, we did not detect significant diel differences in melatonin in swallow-tailed gulls on Española Island (Fig. 1c, ANOVA, $F_{5,48} = 0.7$, $P = 0.6$; all posthoc tests $P > 0.05$).

Discussion

Our data confirm the prediction that night-active swallow-tailed gulls have no measurable daily melatonin rhythm whereas black-headed gulls show a clear melatonin rhythm, with higher plasma melatonin concentrations at night as expected for day-active birds. Overall, melatonin levels in both species were low

compared to other avian species, as previously described for seabirds [22, 25], perhaps because these species always show some activity during the night. As we were only able to compare two wild populations of gulls in the Galapagos with one captive population of a different species in the Netherlands, we suggest that our data should only be used to derive testable hypotheses.

We suggest that the differences we found in the circadian system of these related species could be adaptive in the following way: Swallow-tailed gulls forage at night and in addition potentially have to be active at any time of day, such as for social interactions or breeding [17]. Black-headed gulls, on the other hand, generally only have to be active during the daytime [20]. Thus for black-headed gulls it makes sense to physiologically signal the night as a time when little or no activity will take place—a task generally ascribed to the increase in plasma melatonin in day-active birds [26]. On the other hand, we suggest that the main physiological reason for tonically low melatonin levels in swallow-tailed gulls is to keep their circadian pacemaker less self-sustained [7, 8, 13, 14]. A poorly self-sustained pacemaker would allow the animals to be active intermittently and potentially also permit an easier or faster synchronization with tidal or moonlight patterns, which could be important determinants of (nocturnal) foraging times in seabirds [27]. We further hypothesize that birds that have continuously low melatonin levels could also fall asleep more easily or quickly at any time of day (in sunlight) because they are not physiologically primed to be awake at specific times.

Differences in overall activity levels could also explain the different concentrations of plasma melatonin we found in the two Galapagos populations, i.e., nocturnal levels of melatonin secretion could be suppressed dynamically by the degree of activity. Alternatively, activity might only be a proximate indicator of differences in energy levels, which in turn are often linked to corticosterone secretion [28]. As melatonin and corticosterone secretion are often coupled (sometimes inversely), activity (or energy level) could influence melatonin levels via hormonal interaction mechanisms. These hypotheses are entirely testable in both the field and the laboratory. It is thus far unclear whether moonlight and other environmental factors influence absolute levels of plasma melatonin in swallow-tailed gulls.

Evidence is accumulating that birds facing specific demands on their activity times physiologically adjust to these challenges by changing their plasma melatonin concentrations. Mechanistically, such changes in melatonin concentrations could either be achieved via suppression of melatonin by light [29, 30], but not in night-active birds, or by an activity feedback on melatonin secretion itself [31–34]. An activity feedback could explain the fact that Lapland longspurs (*Calcarius lapponicus*) breeding under continuous daylight in the high Arctic have strongly suppressed overall melatonin levels, but nevertheless show slight but significant daily melatonin rhythms [24]. Low-amplitude melatonin patterns reflect

activity levels of longspurs in the high Arctic, where birds can be active throughout the polar day, but individuals nevertheless show distinct troughs and peaks in activity even under continuous sunlight [4] (Romero and Wingfield, personal communication). Similar (depressed but rhythmic) melatonin patterns are found in several penguin species in the Antarctic [35–37] and in high Arctic Svalbard ptarmigans (*Lagopus mutus*; [38]). Furthermore, songbirds that migrate at night show higher plasma melatonin concentrations when fat reserves are insufficient for migration and thus the birds are inactive during the night [39], supporting a hypothesized activity feedback mechanism on melatonin secretion [40].

Very few routinely night-active birds have been investigated with regard to their daily melatonin concentrations. Indian spotted owlets (*Athene brama*) have slightly lower melatonin concentrations during the daytime compared to the night, but also show a slight melatonin increase in the early afternoon [41]. This increase has been interpreted as coinciding with hiding behavior. Nevertheless, overall melatonin levels in the owlets are very low, and melatonin amplitudes are minor compared to day-active birds. Other owl species such as the Ural owl (*Strix uralensis*) or the barn owl (*Tyto alba*) also showed no discernable diel melatonin pattern, and again overall plasma melatonin concentrations were very low (10–60 pg/ml; [11, 42]). We hypothesize that all of these night-active species face similar ecological constraints on their daily activity pattern. Nocturnal-foraging species need to be highly alert and active during part or most of the night, i.e., during a time when melatonin levels peak in all other bird species investigated so far. Because high melatonin levels generally make birds sleepy, nocturnal species might support their nocturnal activities by maintaining continuously low melatonin concentrations. Future experiments including hormonal manipulations on nocturnal-foraging birds in the wild will shed light on these hypotheses and on the evolution of melatonin profiles in birds in general.

Zusammenfassung

Haben nachtaktive Vögel keine Melatonin-Rhythmik?
Eine vergleichende Studie an tag- und nachtaktiven
Möwen

Die meisten bisher untersuchten Tieren haben nachts niedrige Plasma-Melatoninkonzentrationen, ungeachtet, ob sie tag- oder nachtaktive sind. Unabhängig davon ist die tägliche Melatoninamplitude oft an ökologische Gegebenheiten angepasst. So zeigen viele arktische Brutvögel ebenso wie viele Zugvögel geringe tägliche Melatonschwankungen. In der vorliegenden Studie untersuchten wir, ob Möwen, die nachts Futter suchen, ebenfalls geringe tägliche Melatonschwankungen aufweisen. Gleichmäßig niedrige Melatoninwerte könnten es Individuen erlauben, sowohl nachts Futter zu suchen

als auch tagsüber (z.B. sozial) aktiv zu sein. Wir nahmen von nachtaktiven Gabelschwanzmöwen (*Creagrurus furcatus*) auf zwei Galapagosinseln ca. alle vier Stunden Blutproben und verglichen deren Melatoninwerte mit denen von Lachmöwen (*Larus ridibundus*), die in den Niederlanden in Freiflugvolieren gehalten wurden. Wie bei Seevögeln üblich hatten die Lachmöwen generell niedrige Melatoninwerte mit aber einem klaren Tagesgang mit erhöhten Nachtwerten. Dagegen zeigten die Gabelschwanzmöwen bei ebenfalls geringen Melatoninwerten keine Tagesschwankungen. Obwohl ein direkter Vergleich zwischen nur zwei Arten in unterschiedlichen Haltungsbedingungen problematisch ist, vermuten wir, dass Tiere, die potentiell zu jeder Tages- und Nachtzeit aktiv sein müssen, geringe oder fehlende tägliche Melatonschwankungen haben.

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