# Individual variation in plasma testosterone levels and its relation to badge size in House Sparrows *Passer domesticus*: It's a night-and-day difference

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## ARTICLE INFO

## ABSTRACT

The steroid hormone testosterone (T) plays a central role in the regulation of reproduction in animals. Although seasonal variation in T levels is well-studied, differences between day and night have only been described in relatively few species, and daily within-individual variation has been largely neglected when evaluating the relationship between T and the expression of sexual ornaments or behavior. We measured plasma T levels during day and night in a captive population of House Sparrows, and analyzed their relationship with an important male ornament – badge size. T levels were on average twice as high at night than during daytime. This was true in all seasons, and in both males and females. Disturbance of the birds at night, but not during the day, led to significantly lower T levels, suggesting a rapid drop after an individual wakes up. The relationship between T levels and badge size depended on the time when T was measured. During the breeding season, badge size was strongly positively correlated with night-time, but not with daytime T levels. This suggests that badge size signals information related to an individual's maximum potential T level such as social dominance. Our study highlights that integrative research on the endocrine control of ornament expression needs to take diel variation in hormone levels into account.

## 1. Introduction

The steroid hormone testosterone (T) is well known to influence physiological, morphological and behavioral characteristics of animals [2], and individual variation in plasma T levels may reflect variation in these characteristics. Sources of individual variation in T include genetic, maternal, age, time-of-day, and social environment effects [31]. Variation in plasma T levels occurs seasonally [71], within seasons over different phases of reproduction [71], and over the 24 h period (e.g. [5,50]), which may partly be due to pulsatile T secretion [65]. In contrast to seasonal variation, patterns of diel variation in T levels have been studied in comparatively fewer species (all known studies are summarized in Table 1).

In human males T levels show considerable diel variation with a maximum occurring in the early morning [7,15,66]. Similarly, in both diurnal mammals and birds, male testosterone levels were generally higher at night than during daytime (Table 1). The only study on female birds showed similar patterns [23], suggesting a general increase in night testosterone levels. Earlier studies on human males also suggested a relationship between increased T levels and sleep [5,15,67], and more recent studies describe a correlation of diel variation in T levels with REM sleep [35–37]. The association of high T levels and sleep (rather than night-time per se) is strongly supported by patterns observed in nocturnal species, where T levels show the opposite pattern and peak during the day [14,22,27,49,68]. The functional significance of the diel variation in T levels, if any, remains unknown.

In behavioral or evolutionary ecology, among the most studied roles of T is its influence on ornament elaboration (reviewed in e.g. [10,31,53]), and on behaviors related to male–male competition or female choice, such as aggression, mate guarding, song output and courtship (e.g. [2,24,71]). In most studies, T levels are measured in a single blood plasma sample taken from individuals that were caught during the day. However, plasma T levels can show both pronounced daily rhythms and even shorter-term episodic pulses within the day [65]. Such short-term within-individual variation has not been taken into account when evaluating the relationship between individual T levels and ornament elaboration or behaviors. It also remains unclear whether the diel variation in T levels is more or less pronounced during the period when the influence of T on reproductive behavior and ornament expression is most important. We are unaware of any studies that have specifically addressed these issues.

To examine circadian variation of plasma T levels, we studied a population of captive birds (House Sparrows, *Passer domesticus*).

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**Table 1**
Review of studies that investigated diurnal variation in testosterone levels.

| Group | Species | Period of highest T | Study |
|---|---|---|---|
| Diurnal mammals | Human *Homo sapiens* | Early morning[a] | Evans et al. [15] |
| | | Early morning | Barberia et al. [7] |
| | | Early morning[a] | Aschoff and references therein [5] |
| | | Night | Schulz et al. [56] |
| | | Night | Luboshitzky et al. [36,37] |
| | | Early morning | Walton et al. [66] |
| | Bonnet Monkey *Macaca radiata* | Night | Kholkute et al. [32] |
| | Rhesus Monkey *Macaca mulatta* | Night[b] | Plant [50] |
| | | Night | Sehgal et al. [57] |
| | Ring-tailed Lemur *Lemur catta* | Night | Van Horn et al. [63] |
| | Stallion *Equus caballus* | Early morning | Sharma [58] |
| | Boar *Sus scrofa* | Afternoon and night | Claus and Giménez [11] |
| | Dog *Canis lupus* | Several peaks in T, but no clear diurnal rhythm | DePalatis et al. [13] |
| | NZ Rabbit *Oryctolagus cuniculus* | Peaks every 4–5 h | Moor and Younglai [44] |
| Diurnal birds | Domestic Fowl *Gallus gallus domesticus* | Night | Schanbacher et al. [55] |
| | Ring Dove *Streptopelia risoria* | Night | Balthazart et al. [6] |
| | Blue Tit *Cyanistes caeruleus* | Night[c] | Kempenaers et al. [31] |
| | Lapland Longspur *Calcarius lapponicus* | For some males[d]: night | Hau et al. [23] |
| | European Stonechat *Saxicola torquata rubicolor* | Higher T metabolite concentration in feces at night and in early morning | Goymann and Trappschuh [21] |
| Nocturnal mammals | Owl Monkey *Aotus trivirgatus* | Day | Dixson and Gardner [14] |
| | Thick-tailed Galago *Galago crassicaudatus* | Day | Wilson et al. [68] |
| | Lesser Mouse Lemur *Microcebus murinus* | Day | Perret [49] |
| | Djungarian Hamster *Phodopus sungorus* | Day | Hoffmann and Nieschlag [27] |
| | Sprague–Dawley Rat *Rattus norvegicus* | Day | Wilson et al. [68] |
| Nocturnal birds | Indian Spotted Owlet *Athene brama* | Day | Guchhait and Haldar [22] |
| Reptiles | Tuatara *Sphenodon punctatus* | No daily cycle | Cree et al. [12] |
| Insects | Sand Field Cricket *Gryllus firmus* | Cycles in Juvenile Hormone[e] in flight-capable[f] morph with peaks before dark | Zera et al. [72] |

[a] Suggested to be somewhat sleep dependent.
[b] Drop with lights.
[c] One study showed the opposite.
[d] Higher T levels at night in males kept in Alaska, but not in Seattle; no change in Alaska females, but a trend in May in Seattle females.
[e] JH is the insect analogue to T ([48,62] and references therein).
[f] JH might regulate aspects of flight.

This allowed multiple sampling of the same individuals during day and night in different seasons. Our study had three general objectives: (1) to describe individual differences in night and day T levels in males and females during four periods covering an entire year; (2) to examine the effect of disturbance during day and night by sampling immediately after disturbance or 30–60 min later; (3) to investigate the relationship between day and night T levels and a male sexual ornament (badge size). Note that our study specifically focuses on broad-scale diel changes in T (i.e. night versus day values). Since we cannot sample T levels continually throughout the course of a day our study does not provide enough resolution to infer shorter-term T pulses (e.g. [65]) although such episodic release of T may be an important additional source of individual variation.

The House Sparrow (*P. domesticus*) is one of the model organisms for studies on endocrine control of breeding behavior and ornamentation expression [4,24]. House Sparrows have an obvious ornament, the black bib or badge, which is present in males but not in females. Previous studies showed that badge size is related to social status, age, and variation in sexual behavior [34,40–42,46,47,64]. The link between individual daytime plasma T levels and badge size has also been studied, but the results differ among studies. Some studies found a positive correlation between plasma T levels measured around the time of the annual (pre-basic) molt and the size of the new badge [9,16,20]. However, Laucht et al. [33] found no correlation between badge size and daytime plasma T levels during any season. The difference between studies is difficult to explain, in particular because Evans et al. [16] also demonstrated a causal effect of testosterone via implants. However, information about diel variation in T levels and its influence on badge size is lacking. This is important because differences in ornaments (here badge size) could reflect differences in individual variation of T levels (e.g. [39]). This individual variation could be most prominent either in average T levels or in the increase of T levels above average (due to time, seasonal or social effects) and thus also leading to differences in maximal T levels.

## 2. Material and methods

### 2.1. Study population

A population of 150 male and 9 female House Sparrows was held at the Max Planck Institute for Ornithology, Seewiesen, Germany, in $1.2 \times 2.0 \times 4.0$ m aviaries. At all times, the birds had *ad libitum* access to food, drinking and bathing water, and sand for dust-bathing. The light–dark cycle and temperature regime in the aviaries were close to natural conditions, as the aviaries were semi-outdoor with one side enclosed only by chicken wire. All sparrows were after-hatch year at the time of the study, and were either caught in rural areas in Bavaria, Germany (under license: Permit No. 55.1-8642.3-3-2006 of the "Regierung Oberbayern", with several extensions) and held in captivity for at least eight months (136 males, 4 females) or raised in captivity (14 males, 5 females). Males raised in captivity did not have different T levels compared to birds that were caught in the wild and held in

captivity (linear mixed effect model: $z = 0.45$, $n = 710$ observations, $p = 0.65$, random effects: bird ID ($n = 150$), season ($n = 4$), time-of-day ($n = 66$)). Males were kept in groups of five or six per aviary, and females were housed together in one aviary around the time of sampling. Aviary ID only explained 4.6% of variation in T levels and including it in models did not qualitatively change our results. For simplicity, we do not further include it. Further details about the study population can be found in Laucht et al. [33].

## 2.2. Individual sampling and measuring

We caught all individuals during four periods over the course of an entire year: 26 September – 3 November 2006 ("fall"), 15 January – 2 February 2007 ("winter"), 8–16 March 2007 ("spring"), and 31 May – 22 June 2007 ("summer"). During each season, all birds were blood sampled twice, once during the day and once during the night, with a range of 4–21 days recovery time between bleeding events. For the daytime sampling, we captured all males either in the morning or in the afternoon at approximately the same times and took biometric measurements, standardized photographs of the badge, and a blood sample. For the night-time samples, we caught a subsample of the males we sampled during the day (36 individuals in fall, 36 individuals in winter, 17 individuals in spring, and 53 individuals in summer). In fall, winter and spring all individuals were caught between midnight and 1:00. In summer, we caught six groups of individuals between 22:00–04:30. At night in fall, winter and spring each time we caught a different set of birds. In summer, about half of the birds had been caught at night in the previous season. Excluding these birds did not qualitatively change the results of the day–night comparison. All night-time sampling was performed at 45 min (for first summer samples) or more than five hours (other seasons) after sunset and all birds were roosting during this time.

In winter, half of the birds ($n = 18$) were sampled first at night and second during the day, while the other half were sampled first during the day, and second at night. We did this to check for an influence of previous bleeding on subsequent T-values, and found there was no such effect (linear model: $t_{33} = -1.30$, $p = 0.204$). In the other three seasons, all night-time samples were taken after daytime bleeding.

During night-time sampling in fall, winter and summer, we sampled half of the birds immediately after waking them up and the other half approximately 30–60 min after they woke up. For the daytime samples in spring and summer, we first sampled birds from one group of aviaries and 30–60 min later sampled individuals from a second group of aviaries.

In the summer, we additionally tested whether diel changes in testosterone occurs in a small sample of females ($n = 9$). These individuals were sampled once in the morning (at 07:45) and once at night (at 01:45, 8 days later).

During each sampling event, we took 150–200 μl of blood from the wing vein within 15 min after first starting to catch the birds. We collected the blood in 75 mm Na-heparinised micro-hematocrit capillaries, centrifuged it at 13,000 rpm for three minutes, separated the plasma, and stored it at $-80\ °C$ until analysis. The time passed since first starting to catch birds did not have an influence on T levels (linear mixed effect models with season, daytime, and bird ID as random effects: day: $z = 1.13$, $p = 0.26$, $n = 551$ [33]; night: $z = 0.41$, $p = 0.68$, $n = 159$).

## 2.3. Determination of plasma T levels

Frozen plasma samples were sent to the endocrine laboratory of the Leibniz Institute for Zoo and Wildlife Research in Berlin, Germany, where T levels were determined blindly by enzyme immunoassays (for further details on the methods see [54]; see also

[33]). The inter-assay coefficient of variation (CV) for the enzyme immunoassay was 12.3% and the intra-assay CV was 9.0%. To calculate the true repeatability (i.e. the intra-class correlation coefficient) of measuring serum T-levels, we split 122 plasma samples of several males into duplicates right after centrifugation. Based on samples from the whole year, the repeatability of these plasma T estimates was $R = 0.967 \pm 0.006$ (SE) ($F_{121,\ 122} = 59.24$, $p < 0.001$). We assumed that all values of zero were below the detection limit (9 out of 282 cases) and assigned them the lowest value measured (15 pg/ml). T levels are reported in pg per ml, but were ln-transformed for statistical analyses. Daytime T levels are the same as reported in Laucht et al. [33].

## 2.4. Determination of badge size

During each season, we took four pictures of the black bib of each male. For each picture, we held the birds ventrally such that the throat and bib were stretched and presented to the camera. We rearranged the bird's position between each photograph. SL measured the size of the badge from the photographs by encircling and measuring the area of the bib in pixels using the program ImageJ 1.36b [1] and later converting it into $cm^2$ using an area standard present in each photograph. For analyses, we used the average of all four pictures for each bird. These scores were highly repeatable within individuals ($R = 0.943$, estimated according to Falconer and Mackay [17] from repeatability of single pictures). See Laucht et al. [33] for additional details.

## 2.5. Statistical analyses

We performed all statistical analyses using R 2.8.0 [51] (packages: lme4, nlme, RODBC) at the significance level $\alpha = 0.05$. We compared day and night T levels in each period both at the population level (including those individuals for which only day T-values were available; $t$-test) and at the individual level (paired $t$-test). To analyze the relationship between badge size or disturbance and plasma T levels, we used linear models and linear mixed effect models as indicated in the results. There was no need for model simplification. For analyses on badge size, we used the summer scores (means of the four photos), because badge size changed due to abrasion of the white feather edges in fall and early spring [43]. The results did not qualitatively change when using means of spring and summer badge scores.

Note that sample sizes differ due to missing data points for single individuals and due to the exclusion of males in breeding aviaries for the summer samples.

## 3. Results

### 3.1. Day/night variation in T levels

In males, plasma T levels were on average 1.3–4.8 times higher during the night than during the day and this was significant during all four seasons (Fig. 1a and b and Table 2). However, the within-individual correlation between day and night levels was rather weak (Spearman rank correlation: fall: rho = 0.29, $n = 36$, $p = 0.091$; winter: rho = 0.38, $n = 35$, $p = 0.023$; spring: rho = $-0.12$, $n = 17$, $p = 0.640$; summer: rho = 0.27, $n = 53$, $p = 0.054$) indicating that males with the highest day levels did not necessarily have the highest night levels (Fig. 1b). Female plasma T levels were also significantly higher at night than during the day, at least in summer (Fig. 1a and Table 2).

During daytime there was no effect of previous disturbance on T levels (Fig. 2; linear models: in spring, in summer, or in both periods combined; all $p > 0.59$). However, at night T levels were signif-
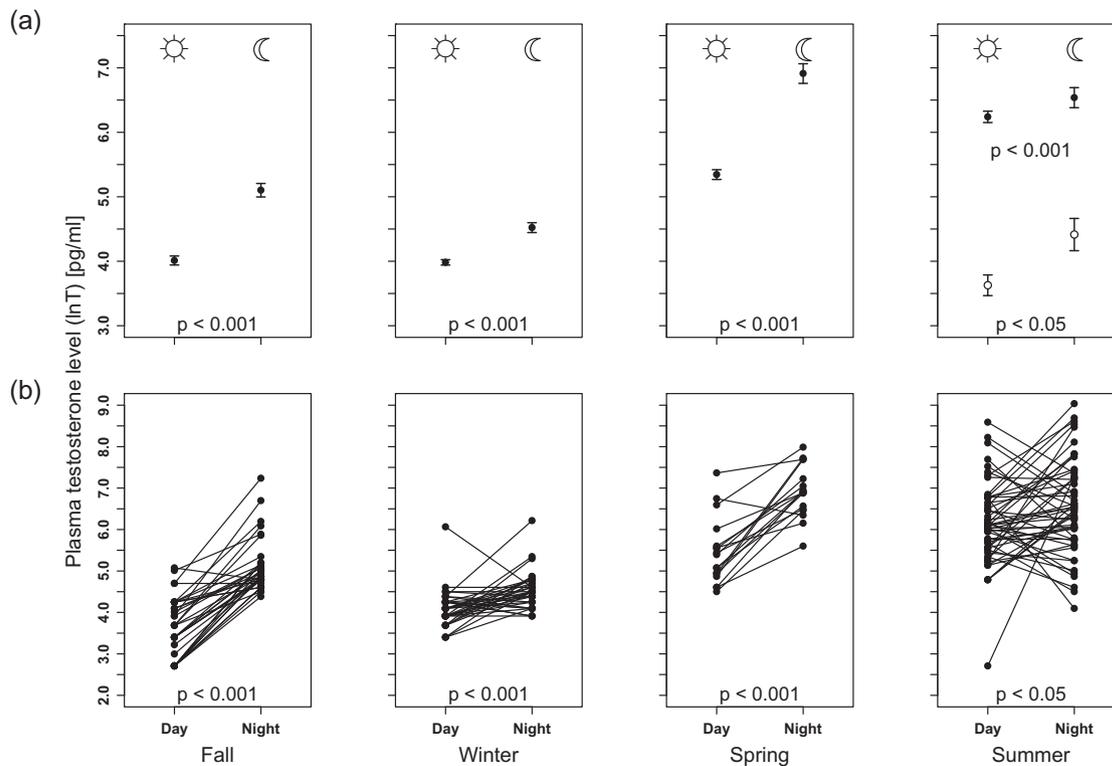
**Fig. 1.** Day and night plasma testosterone levels of male (closed circles) and female (open circles) House Sparrows at four different seasons. (a) At the population level: mean ± standard error. (b) At the individual level. For statistics and sample sizes see Table 2.

**Table 2**

Seasonal and daily variation in plasma T levels of male and female House Sparrows. Shown are the geometric mean (± standard errors) values (in pg/ml) during the day and the night, population level t-test, and individual level (paired) t-tests comparing day and night values. Because all analyses were performed on log-transformed values, geometric means (the back-transformed mean of log-transformed values) are reported here. Standard errors were calculated as the back-transformed differences of means plus standard errors of log-transformed T-values. Overall mean was calculated as the means of seasonal means. Means population level statistics were calculated from all birds sampled but for the individual level statistics only those individuals that were sampled during day and night were included.

| | | Means ± SE of T | | | | Population level | | Individual level | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day | $n$ | Night | $n$ | Statistic | $p$-Value | Statistic | $p$-Value |
| Males | Fall | 55.22 ± 3.96 | 142 | 164.38 ± 18.18 | 36 | $t_{68.9} = 8.67$ | <0.0001 | $t_{35} = 12.45$ | <0.0001 |
| | Winter | 53.66 ± 2.33 | 140 | 91.99 ± 7.35 | 35 | $t_{56.7} = 6.14$ | <0.0001 | $t_{34} = 4.55$ | <0.0001 |
| | Spring | 209.46 ± 16.62 | 139 | 1003.77 ± 164.54 | 17 | $t_{24.9} = 9.22$ | <0.0001 | $t_{16} = 6.23$ | <0.0001 |
| | Summer | 522.21 ± 39.84 | 103 | 690.43 ± 116.02 | 53 | $t_{74.4} = 13.2$ | <0.0001 | $t_{52} = 2.17$ | 0.035 |
| Females | Summer | 37.64 ± 6.55 | 9 | 82.61 ± 23.45 | 9 | $t_{13.6} = 2.65$ | 0.019 | $t_8 = 2.67$ | 0.028 |

icantly lower when birds had been awake for 30–60 min before being sampled (Fig. 2 and Table 3; linear mixed effects model: all periods combined: $z = -3.12$, $n = 124$ observations, $p = 0.002$, crossed random effects: bird ID ($n = 100$ individuals) and season ($n = 3$)). The effect was observed in every season (Fig. 2), but it was only significant in winter (t-tests; fall: $t_{34} = -1.80$, $p = 0.082$; winter: $t_{33} = -3.22$, $p = 0.003$; summer: $t_{51} = -1.79$, $p = 0.081$).

### 3.2. Night T levels and badge size

Badge size was related to night plasma T levels in summer (linear model: $t_{43} = 2.54$, $R = 0.36$, $p = 0.015$; Fig. 3), but not significantly in any of the other sampled seasons (all $p > 0.48$). Because badge size was not related to day plasma T levels in June (Fig. 3, data from Laucht et al. [33]), we further tested whether the relationship between T levels and badge size differed depending on the time when plasma samples were collected. This was indeed the case (interaction with time period: linear mixed effect model:

$z = 2.54$, $p = 0.015$, $n = 180$ observations, random effect: bird ID ($n = 135$ individuals); Fig. 3).

## 4. Discussion

We found that in captive male and female House Sparrows plasma T levels were significantly higher during the night than during the day. T levels on average doubled, demonstrating dramatic daily within-individual fluctuations in plasma T levels. The higher night T levels were observed in males throughout the year. These results are in accordance with findings from diurnal mammals, and some diurnal bird species (Table 1). The only other study that has examined diel variation in T levels in female birds also found a trend towards higher night levels [23]. However, the sample size in this as well as in our study was small and birds were only sampled at one time point during the night during one season. Overall, however, our results indicate strongly that nocturnal increases in T levels occur in diurnal species, independent of season and sex. Hence, there
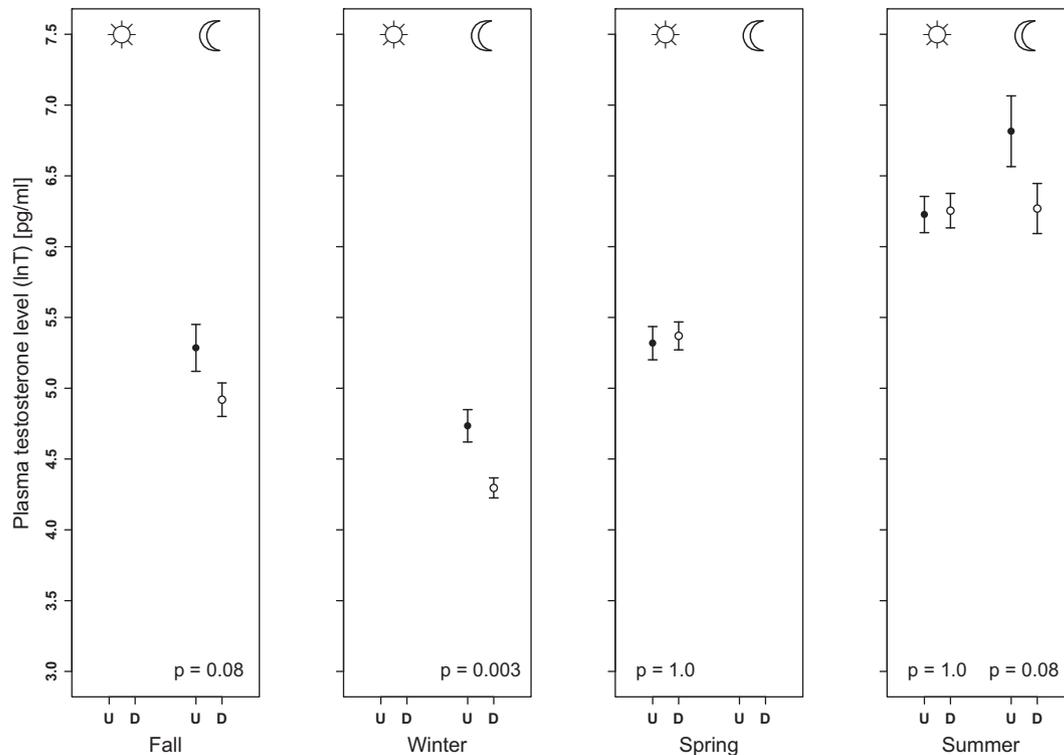
**Fig. 2.** Plasma testosterone levels of male House Sparrows at three different seasons in undisturbed (U, closed circles) and disturbed (D, open circles) groups. Presented are means ± standard errors. For statistics and sample sizes see Table 3.

**Table 3**
Variation in night plasma T levels of male House Sparrows in disturbed and undisturbed groups. Shown are the geometric mean (± standard errors) values (in pg/ml), results of $t$-tests comparing levels of disturbed and undisturbed groups, and results of $t$-test comparing disturbed groups and daytime levels. Because all analyses were performed on log-transformed values, geometric means (the back-transformed mean of log-transformed values) are reported here. Standard errors were calculated as the back-transformed differences of means plus standard errors of log-transformed T-values.

|  | Mean ± SE of T | | | | Disturbed–undisturbed | | Disturbed-day | |
|---|---|---|---|---|---|---|---|---|
|  | Undisturbed | $n$ | Disturbed | $n$ | Statistic | $p$-Value | Statistic | $p$-Value |
| Overall | 319.62 ± 57.91 | 62 | 207.75 ± 30.88 | 62 | $t_{118.12} = 1.99$ | 0.049 | $t_{79.64} = 3.02$ | 0.003 |
| Fall | 197.41 ± 35.52 | 18 | 136.87 ± 17.20 | 18 | $t_{30.79} = 1.80$ | 0.082 | $t_{30.23} = 6.62$ | <0.0001 |
| Winter | 113.83 ± 13.77 | 18 | 73.42 ± 5.37 | 17 | $t_{28.12} = 3.27$ | 0.003 | $t_{29.28} = 3.80$ | 0.0007 |
| Summer | 911.84 ± 258.79 | 26 | 528.19 ± 102.22 | 27 | $t_{45.39} = 1.78$ | 0.081 | $t_{35.55} = 0.06$ | 0.953 |

seems to be a general mechanism that causes T levels to increase at night.

### 4.1. Elevated nocturnal testosterone and sleep

Higher T levels may be associated with sleeping, as opposed to night-time per se. Studies on human males showed a correlation between the first REM sleep and the increase in T levels [15,35] and a significant delay in this T rise when sleep was fragmented [37] or shifted to daytime [8,15]. Consistent with the hypothesis that increased T levels are linked to sleep and not to night-time per se, is the observation that nocturnal animals show highest T levels during the day (Table 1). Our results are also consistent with this hypothesis: we found that T levels at night were lower when the birds were sampled 30–60 min after being disturbed (Fig. 2), suggesting a quick drop in T levels after waking up. An alternative explanation is that T levels dropped due to increased stress associated with disturbance. However, we would then have expected a decrease in T levels after disturbance during the day, which we did not observe (Fig. 2). Nevertheless, it remains possible that the House Sparrows experienced disturbance during the night as a much stronger stressor, and that this explains the difference in T level changes.

An alternative explanation for higher night T levels in diurnal animals, is that T levels are affected by social interactions, assuming that such interactions generally lead to a decrease in plasma T levels. Lower plasma T levels are expected in individuals that lose out in competitive interactions (e.g. subordinate individuals) (reviewed in [2]). Under this scenario, we would expect individuals with low day T levels (e.g. subordinate individuals) to show the strongest increase during the night, whereas those with the highest T levels should not show a further increase. One would then expect a lower variance in T levels at night than during the day, for which we found no evidence ($F$-test for equality of variances: each season analyzed separately, $p = 0.31$–$0.93$). However, we note that particularly during the breeding season not all individuals had higher T levels at night (Fig. 1b). More detailed studies on the effects of sleep, (nocturnal) stress, and social interactions on within-individual variation in T levels are needed.

### 4.2. Function of elevated nocturnal testosterone

The patterns observed here and in other species (Table 1) raise an intriguing and important question. What is the functional significance – if any – of the nocturnal increase in plasma T? T may simply accumulate during the night (i.e. accumulation occurring
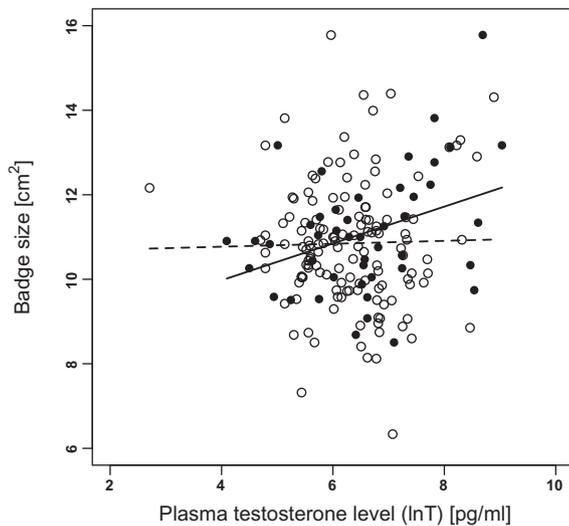
**Fig. 3.** Badge size in relation to June plasma night (closed circles, straight line; $R = 0.35$, $t_{43} = 2.44$, $p = 0.019$) and day (open circles, dashed line; $R = 0.03$, $t_{133} = 0.31$, $p = 0.76$) testosterone levels for male House Sparrows. We defined badge size as the June measurements. The relationship between T levels and badge size differed between day and night: interaction with time period: linear mixed effect model: $t_{43} = 2.54$, $p = 0.015$, $n = 180$, random effect: bird ID.

due to diel changes in the half life of testosterone or of its binding proteins, in testosterone secretion or in the secretion of other hormones such as LH or prolactin), either because it is not used up (non-functional) or because individuals physiologically prepare for a (functionally) high T level in the early morning. The highest T levels should then be found when morning activity starts, which is indeed true for human males (highest levels found just before sunrise: [7,36,56,66]). However, other studies (Table 1) suggest that in some species T levels may fluctuate throughout the night, rather than gradually increase until early morning.

Alternatively, increased nocturnal T may itself be adaptive. A change in the secretion of T (rather than metabolic clearance rate) [66] could be functional, if higher T levels are required for short-term organizational functions such as neuronal development or memory consolidation, functions also suggested for sleep [59]. Additionally, the observation that nocturnal increase in T levels occurred independently of season also suggests that T might play a role in regulating diurnal changes in physiology, i.e. function as a "sleep hormone".

An alternative organizational function of increased nocturnal T is that it is associated with increased spermatogenesis. Early studies on male House Sparrows found that spermatogenesis takes place at night with greatest activity between 02:00 and 04:00 [3,19,52]. It was further suggested that it is related to the drop of body temperature that occurs during sleep, which itself might be triggered by increased T [18]. In Bonnet Monkeys (*Macaca radiata*), long term suppression of night-time T peaks had a negative influence on testis activity in general and on spermatogenesis in particular [60]. However, we also found elevated night T levels in males during the fall when the testes are regressed, and in females. Although this does not refute the idea that one function of elevated night T is increased spermatogenesis; it is clear that other functional explanations cannot be excluded.

### 4.3. Night-time testosterone and ornamentation

We found a significant correlation between badge size of male House Sparrows and night-time plasma T levels in the peak breeding season (June). This contrasts with our previous finding (on the same population) that badge size was not correlated with daytime

T levels in any season [33], but is in agreement with other studies that found correlations between badge size and post-breeding or breeding daytime T levels [9,20], and an effect of artificially increased T levels on badge size [16].

Previous studies suggest that – in House Sparrows and other bird species – agonistic interactions or challenges cause a short-term increase in T levels above breeding baseline levels (the "Challenge Hypothesis") [24,25,39,69,71]. We suggest that night-time T levels reflect maximum T levels achieved during challenges and that these levels reflect competitive ability better than day levels do. Under the hypothesis that night-time T levels indeed reflect maximum T levels (see also below), the relationship between night-time T levels and badge size suggests that badge size could be an "honest" signal of status. Maximum T levels during challenges could maintain signal honesty via social costs (i.e. keep the signal evolutionarily stable by preventing cheating via high costs; the badge of status hypothesis [28,38,61]). Badge size could therefore indicate an individual's dominance, level of aggression, and ability to defend itself in agonistic interactions during the breeding season.

Although the relationship between night-time T levels and maximal T levels during social challenges needs to be tested directly, there is indirect evidence that they may be similar. An experimental paradigm that is often used to estimate an individual's maximum T level is an injection with gonadotropin-releasing hormone (GnRH) [29,70]. Previous studies showed that plasma T levels measured after GnRH challenge correlate positively with elevated T levels after social challenges [39]. Studies in several bird species and in several seasons showed that plasma T levels on average increased 2.1-fold (range: 1.0–5.7-fold) after GnRH challenge [26,29,30,45]. Our results indicate that plasma T levels increased on average 2.6-fold (range 1.3–4.8) from day to night (all seasons, males and females), which is similar to the effects shown by the GnRH challenge.

### 4.4. Conclusions

In summary, we found that plasma T levels of male and female House Sparrows were much higher at night than during the day, and we provide evidence that higher nocturnal T could be associated with sleep because disturbance at night, but not during the day, reduces T levels. Additionally, we found that male badge size, an ornament generally associated with dominance, was related to night-time T levels, but not to daytime levels. Overall, our results imply that diel cycles need to be considered in studies using measurements of plasma T levels.

### Acknowledgments

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