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ORIGINAL ARTICLE

Reproductive timing in bats: evidence of spring mating following hibernation

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Abstract

Temperate-zone bats typically mate in autumn, though anecdotal evidence suggests some species may also mate in spring. Using *Nyctalus noctula* as a model, we investigated post-hibernation reproductive status to assess the occurrence of spring mating. We examined male (buccal glands, testes, epididymides) and female (buccal glands, reproductive cytological status) traits and developed a minimally invasive vaginal lavage method suitable for field-based reproductive assessment in female bats. We found that male epididymides were frequently distended after hibernation, indicating retained sperm despite spermatogenesis being halted during autumn and hibernation. Vaginal cytology showed no evidence of immediate fertilisation after arousal, yet 15% of smears suggested recent mating. Buccal gland size was positively associated with season and epididymis size, consistent with both a sexual and broader social function. Collectively, these findings indicate that active mating in *N. noctula* can extend into spring, potentially providing opportunities for reproduction before migration. Additionally, we provide the first histological evidence of sperm phagocytosis by neutrophils in bats, supporting the hypothesis of leucocytic clearance of non-viable spermatozoa, as documented in other mammals.

Keywords *Nyctalus noctula* · Pregnancy test · Reproduction · Reproductive state · Non-invasive cytology · Buccal glands · Sperm phagocytosis · Sperm storage · Vaginal lavage

Introduction

Understanding reproduction is essential for conservation biology, as it is linked to population growth, effective population size (Wilson 1975), population recovery rate (McCracken 1989), and reintroduction success (Robertson et al. 2006). Due to their slow reproductive rates, bat populations are especially vulnerable to habitat loss, pollution, and disturbance. Notable declines have resulted from DDT use (Zukal et al. 2015) and white-nose syndrome (Langwig et al. 2017). Thus, a solid understanding of bat reproductive biology is essential for effective conservation and the recovery of threatened populations.

Bat reproductive timing and duration is closely linked to temperature and food availability (Racey 1969; Racey and Entwistle 2000; Mello et al. 2009), requiring adaptation to climatic fluctuations. As climate change intensifies, forecasting reproductive shifts becomes increasingly important. *Nyctalus noctula* was chosen as a model species for its demonstrated responsiveness to climatic variation, attributed to its high reproductive output, and strong dispersal capacity

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(Kravchenko et al. 2020a, b, 2025). In this species, mating typically occurs from late summer until autumn at migratory roosts (Petit and Mayer 1999; Lehnert et al. 2018) and occasionally at hibernation sites (Guthrie 1933; Gebhard 1995; Entwistle et al. 1997), with courtship peaking between August and October (Kleiman and Racey 1968).

Noctules form harem-based hierarchies at roosts, with younger males typically at the periphery of the roosting tree complex (Ocampo-Gonzalez et al. 2020) while older, territorial males occupy central positions and are more likely to reproduce (Racey and Tam 1974; Furmankiewicz and Szkudlarek 2001). *Nyctalus noctula* males may attract between one and eighteen females by mating calls marking roosts by rubbing buccal gland secretions on entrances (Gebhard 1993 in Gebhard and Bogdanowicz 2004), thus forming small harems within their territories (Sluiter and van Heerdt 1966).

Buccal glands, white-yellowish swellings at the mouth corners, have been shown to serve communicative functions in territorial and mating contexts (Lanza 1956; Kleiman and Racey 1968), though recent studies suggest that the volatiles they produce do not aid roost location (Ruczyński et al. 2007). However, during courtship, males rub their mouths on females' neck fur (Kleiman and Racey 1968), possibly to transfer glandular secretions from these buccal glands (Gebhard 1995 in Gebhard and Bogdanowicz 2004).

Like most temperate-zone bats, noctules exhibit delayed fertilisation (Oxberry 1979; Racey 1979): after autumn mating, both sexes store viable sperm through winter until spring (Racey 1972, 1979; Krishna and Bathnagar 2011). Females have been shown to store sperm for up to seven months (Wimsatt 1944; Racey and Tam 1974; Racey 1979), males for up to ten (Racey 1972). Ovulation and fertilisation are typically reported immediately post-hibernation (Oxberry 1979; Krishna and Abilasha 2000), at a time when male epididymal size is minimal and spermatogenesis starts to resume (Racey 1979; Entwistle et al. 1998).

Although most European bats mate in autumn, anecdotal reports suggest winter mating in some species (*Plecotus auritus*, *P. austriacus*, *Pipistrellus pipistrellus*, *N. noctula)* (Wimsatt 1945; Aubert 1963; Stebbings 1965; Gäth 2008; Krishna and Bathnagar 2011) and spring mating in others (*Pipistrellus pygmaeus*, *P. auritus*, *P. austriacus*, *Eptesicus fuscus*, *Myotis lucifugus*) (Moffat 1922; Guthrie 1933; Eisentraut 1935; Stebbings 1966; Horn 2006; Gäth 2008; Furmankiewicz et al. 2013). Yet, it is unclear if spring mating actually contributes to reproductive success. If spring mating contributes to reproduction, we would expect sperm to be retained in the epididymides post-hibernation, with gradual depletion through successive copulations. As testes recrudescence requires sustained euthermia (Wimsatt 1969; Kenagy and Trombulak 1986; Entwistle et al. 1997; Komar et al. 2022), testicular growth should be delayed until males can thermoregulate consistently. Buccal glands, if fulfilling mating-related functions, should also be enlarged in spring. To investigate these hypotheses, we assessed these reproductive traits minimal-invasively in male and female noctules during spring during annual population monitoring schemes.

Materials and methods

Study area

Sampling took place during regular population monitoring actions (bat box checking for counting, individual bat banding) in seven different forests equipped with bat boxes across the North East of Germany (Saxony-Anhalt, Mecklenburg Western-Pomerania, Brandenburg). In the studied regions, *Nyctalus noctula* is a tree-roosting species that either undergoes seasonal migration or remains to hibernate in tree cavities or installed bat boxes (Lehnert et al. 2018). As a temperate-zone bat with a so-called Type I reproductive pattern, it relies on a circannual clock to synchronize seasonal cycles (Erkert 1982; van der Vinne et al. 2014; Spitzenberger and Weiss 2020), depending on temperature. Commonly, arousal from hibernation takes place around mid-March, depending strongly on ambient temperature (Kravchenko et al. 2025). In the population under study, noctules either migrate to summer or nursery roosts or remain within hibernation sites that also serve as reproductive habitats, where males call for mates at migration stopover or at hibernation sites (Lehnert et al. 2018).

Sampling

Between February 27th and May 25th 2021, 627 noctules (235 males, 392 females) were captured from bat boxes across twelve sampling days. The sampling spanned late hibernation through arousal to early migration. Age classification was possible via mark-recapture data from the bat bandings. All bats were assessed within ~5 min of capture and immediately returned to their roosts. Fieldwork was conducted under permits issued by the relevant animal welfare regulations and approved by conservation (Permit No. 44.30-2021-217-Gru, VGA-16-030) and animal ethics authorities (Permit No. 203 m-42502-2-1668, 203.6.1-42502-2-1525LIZW_G). Temperature data were taken from the website of the CDC-Climate Data Centre (DWD-Deutscher Wetterdienst-Wetter und "Klima aus einer Hand", URL: dwd.de [09.08.2021]).

Male reproductive features

For males, we assessed the sizes of buccal glands, testes and epididymides (Table 1). Each reproductive feature was ordinally scaled based on Haarsma (2008) but using three instead of five categories for simplification: (0) not or minimally expressed/absent (-); (1) Intermediately expressed (+/-); (2) Maximally expressed/present (+). All reproductive features were scored by a single observer (X.M.) to ensure consistency across individuals.

Figure 1 shows the different stages of the size-increase of the buccal glands (a-c); different testes sizes (d-f), and different sizes filling states of the epididymides (g-i).

Female reproductive features

To assess female reproductive status, 126 vaginal lavage samples were collected (see Table 1) and buccal glands were categorized in analogy to those of the males.

As Table 2 shows, the female reproductive cycle was classified into four stages, based on the dominant vaginal cell types (Voigt and Schwarzenberger 2008; Racey 2009; Vela-Vargas et al. 2016).

Vaginal lavages have already been successfully applied to European bats (Kleiman and Racey 1968), oriental bats (Wang et al. 2007) and neotropical bats (Vela-Vargas et al. 2016; Stukenholz et al. 2018). To optimize field sampling, we tested sterile isotonic saline solution (0.9%) and sterile water at a pH of 5. A 2.5 µl drop was applied to the vaginal orifice using a sterile micropipette. Unlike in previous work, we did not insert the pipette into the vagina in order to avoid any mechanical harm, yet we were still able to collect sufficient amounts of epithelial cells in most samples (Table 1). The fluid was aspirated and re-suspended ~ 5 times until it became turbid, then smeared onto slides and air-dried. In the lab, samples were Gram-stained (Hucker's modification; Public Health England 2019) and examined microscopically at 400× magnification.

Slides were included in the analysis only when at least 25 cells could be evaluated, and reproductive status was assigned when more than 50% of cells corresponded to a specific stage. Applying these criteria resulted in 73 samples out of the 126 collected to be statistically analysed. No samples were dominated by PC or NC cells.

Social behaviour

To detect social calls or songs corresponding to mate attraction (see Pfalzer 2002), six batcorders (ecoObs GmbH, Batcorder 2.0) were deployed near bat boxes in a forest near Greifswald, Mecklenburg Western-Pomerania (natural roosts such as tree cavities were unknown); recording from

Table 1 Distribution of the 392 female samples (buccal gland expressions, number of vaginal smears in brackets) and 235 male samples (buccal gland, testes, epididymides expression) collected across the different field dates and sites

Date	27.02.-28.02.2021	19.03.2021	27.03.2021	12.04.-13.04.2021	25.04.2021	01.05.2021	07.05.2021	15.05.2021	25.05.2021
Site	Havelberg, Saxony Anhalt	Stahnsdorf, Brandenburg	Greifswald, Mecklenburg-Western Pomerania	Lubmin, Mecklenburg-Western Pomerania & Schwedt, Brandenburg	Havelberg, Saxony Anhalt	Havelberg, Saxony Anhalt	Lubmin, Mecklenburg-Western Pomerania	Havelberg, Saxony Anhalt	Rügen, Mecklenburg-Western Pomerania
♀	122 (10)	38 (10)	15 (14)	40 (38)	69 (12)	29 (10)	11 (10)	38 (11)	30 (11)
♂	85	21	6	14	35	42	13	12	7

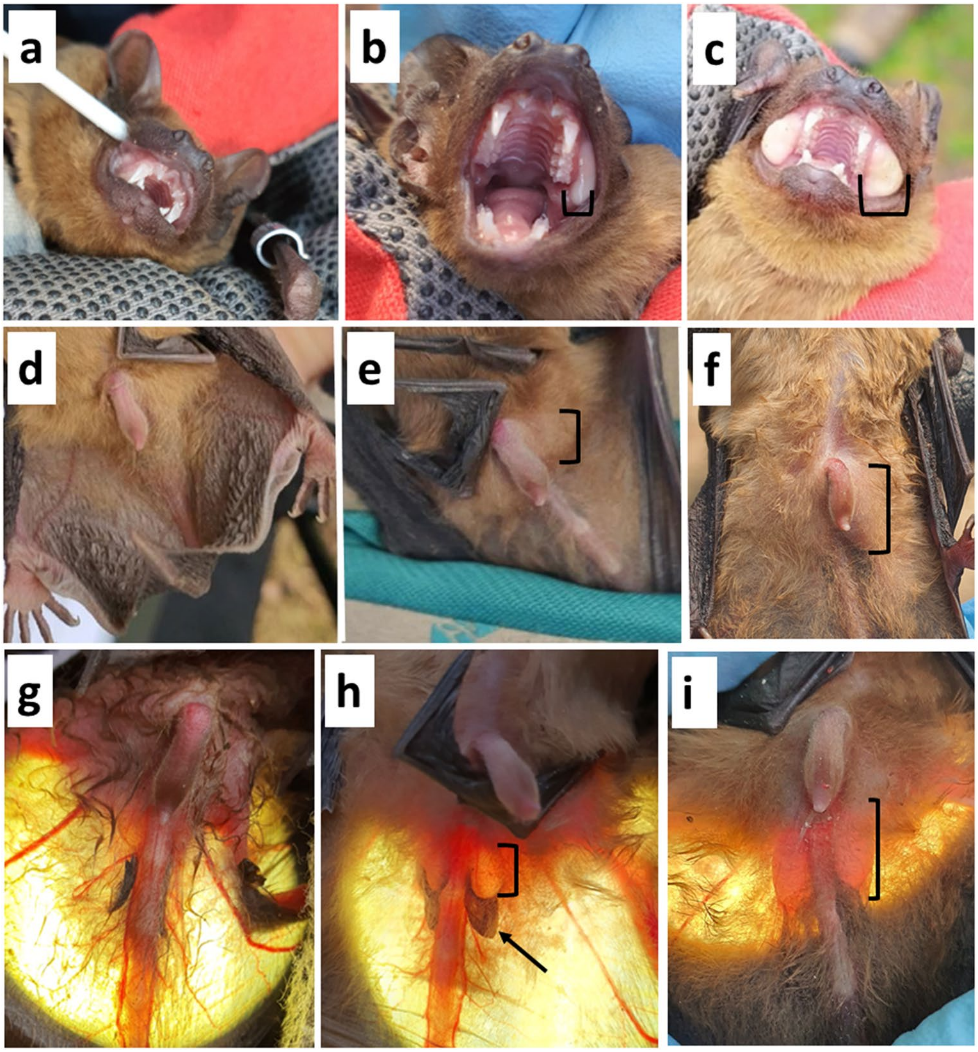


Fig. 1 (**a**-**c**) The three different buccal gland sizes for male and female noctule bats (*Nyctalus noctula*): (**a**) small size (BG0), (**b**) medium size (BG1), (**c**) large size (BG2). (**d**-**f**) The testes sizes of male noctule bats: (**d**) small size (Te0), (**e**) medium size (Te1), (**f**) large size (Te2). (**g**-**i**) Size (filing state) of the epididymides of male noctule bats: (**g**) not filled (E0, the absence of sperm filling results in a visibly contracted tunica vaginalis), (**h**) slightly filled (E1, in this state, the darkly pigmented tunica vaginalis becomes visible due to the partially emptied contents - see black arrow), (**i**) well-filled epididymides (E2, in this condition, the tunica vaginalis is distended due to the filling with sperm)

Table 2 The different reproductive stages of female bats and the most abundant vaginal cell type in the different stages

Reproductive status	Vaginal cell type
Prooestrous (0)	Nucleated cells (NC)
Oestrous (1)	Superficial anucleated cells (SAC)
Metoestrous (2)	Intermediate cells (IC)
Anoestrous (3)	Nucleated circular parabasal cells (PC)

March 27th to May 29th 2021 (7 pm – 6 am). Acoustic data were analysed using bcAdmin (version 2.35) to distinguish social from echolocation calls, and batIdent (version 1.5) for species identification. Additionally, bat box inspections across all sites included searches for harem groups as a typical mating structure in noctules.

Statistical analysis

Statistical analyses were conducted in R, version 4.5.1 (R Core Team 2025). Ordinal response variables (testes size, epididymides size, buccal gland size) were analysed using cumulative link models with a logit link implemented via the polr() function (package MASS). Female reproductive status (oestrous versus metoestrous) was analysed using binomial logistic regression. Due to quasi-separation in the female status data, Firth's penalized likelihood approach was applied using the logistf package.

Model structure and candidate models: For each response variable, we constructed a predefined set of biologically plausible candidate models. Sampling date was included as a primary predictor in all biologically relevant models, reflecting seasonal progression.

For males, candidate predictors included: sampling date, ambient temperature, body mass, reproductive organ covariates (e.g. testes or epididymides, depending on response variable).

For females, predictors included: sampling date, ambient temperature, body mass.

Female reproductive status was modelled as a binary response (oestrous = 1, metoestrous = 2 recoded to 0/1 for logistic regression).

Model selection was based on Akaike's Information Criterion (AIC). Candidate models were compared only when fitted to identical datasets, as AIC comparisons require equal sample sizes. When predictors contained missing values (notably body mass in females, ~28%, in males ~25%), reduced datasets including only complete cases were used for model comparison involving those predictors. Models within ΔAIC < 2 were considered statistically equivalent, and the most parsimonious model was selected.

Assumption checks: For ordinal logistic models, the proportional odds (parallel regression) assumption was assessed using the Brant test (brant package). Multicollinearity was evaluated using variance inflation factors (VIF; car package). VIF values < 3 were considered indicative of negligible collinearity.

Effect sizes are reported as odds ratios (OR) with 95% confidence intervals derived from standard errors on the log-odds scale. Odds ratios represent the multiplicative change in the odds of being in a higher response category per unit increase in the predictor.

To assess robustness against missing data structure, best-supported models were re-fitted on reduced datasets including only individuals with complete temperature and body mass data. Qualitative consistency of effect direction and magnitude between full and reduced datasets was interpreted as evidence of robustness.

Results

Male buccal gland size

Male buccal gland size increased over the study period (Fig. 2a). The best-supported model included date and epididymides size (ΔAIC = 0, AIC weight = 0.70, Table 3). A model including testes size also received support (ΔAIC = 1.71, weight = 0.30), whereas all remaining models had ΔAIC > 14 and negligible support (Table S1). The summed AIC weight of models including date and epididymides was approximately 1.00, indicating strong evidence that both predictors contributed to variation in buccal gland size. In contrast, testes size received little overall support. Buccal gland size increased strongly with date (OR = 1.05 per day, 95% CI: 1.03–1.06). In addition, buccal gland size was positively associated with epididymides development (linear contrast: OR = 3.25, 95% CI: 1.86–5.68), whereas the quadratic contrast was not supported (OR = 0.92, 95% CI: 0.58–1.46). The proportional odds assumption was not substantially violated (Brant omnibus: $p = 0.07$), and

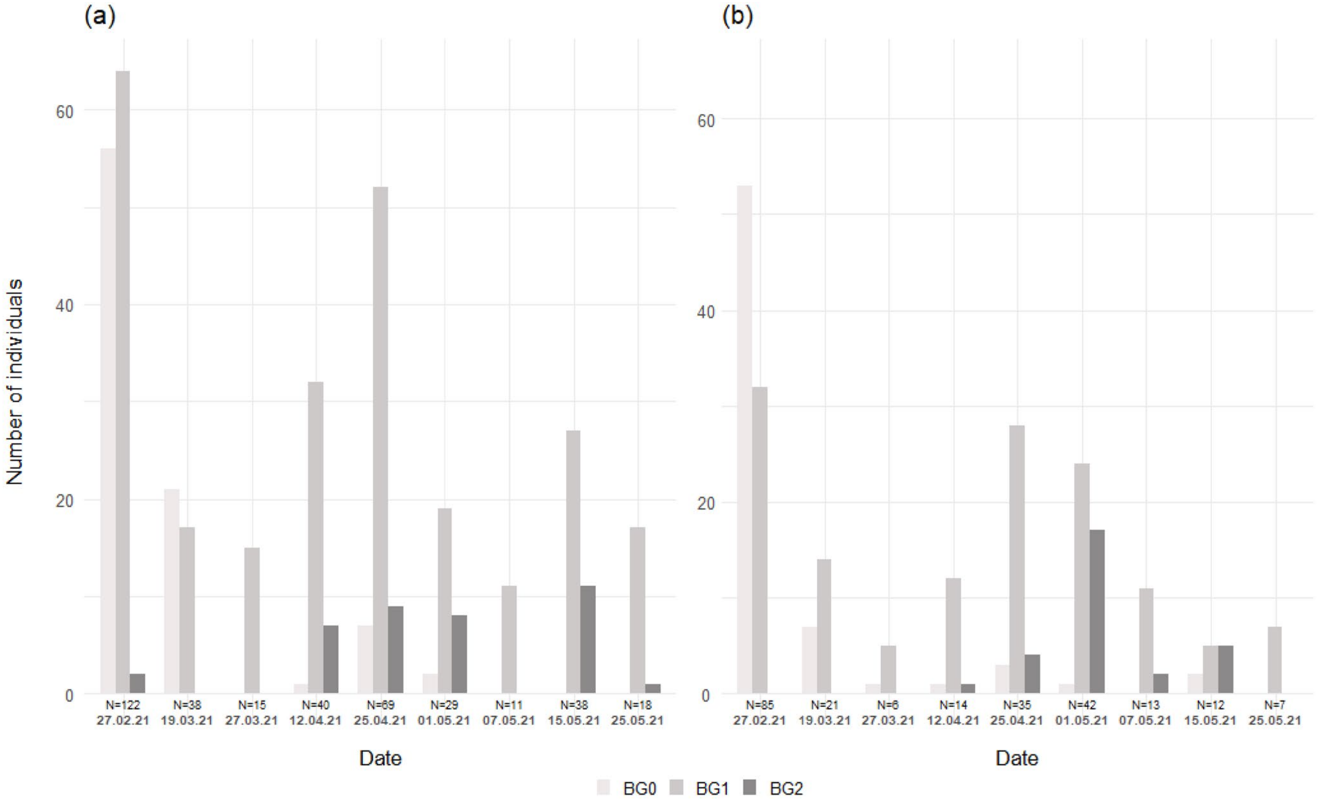


Fig. 2 (**a**) Female and (**b**) male buccal gland size categories from February to May 2021. Bars indicate the number of individuals assigned to small size (BG0, light grey), medium size (BG1, medium grey), and large size (BG2, dark grey) on each sampling date. Sample sizes (N) for each date are listed below the x-axis

Table 3 Results of the best-supported regression models for male and female reproductive traits identified using Akaike's Information Criterion (AIC) from predefined sets of biologically plausible multifactorial candidate models. Effect sizes are presented as odds ratios (OR) with 95% confidence intervals. Odds ratios represent the multiplicative change in the odds of being in a higher reproductive category per unit increase in the predictor. Ordinal traits (epididymides, testes, buccal glands) were analysed using cumulative logit models. Female reproductive status (oestrous vs. metoestrous) was analysed using Firth-penalized logistic regression due to quasi-separation. Only predictors retained in the best-supported models are shown

Sex	Response variable	Predictor	OR	95% CI
Male	Epididymides	Date	1.01	0.99–1.02
		Buccal gland size	2.82	1.72–4.63
	Testes	Date	1.01	0.99–1.04
	Buccal glands	Date	1.05	1.03–1.06
		Epididymides (linear)	3.25	1.86–5.68
		Epididymides (quadratic)	0.92	0.58–1.46
Female	Buccal glands	Date	1.04	1.03–1.06
	Reproductive status	Date	1.05	1.00–1.18

multicollinearity was negligible (GVIF^(1/(2*Df))$\leq$1.01). These results indicate a pronounced seasonal increase in buccal gland size and a strong linkage between buccal gland development and epididymal state.

Testes

Following arousal, all males exhibited small testes, with a gradual increase in medium-sized testes by late May. However, no individuals reached the maximal testis size over the course of the study (Fig. 3a).

AIC-based model selection identified the model including date only as best supported (ΔAIC = 0, AIC weight = 0.52, Table 3). However, several alternative models had ΔAIC < 4, indicating considerable model uncertainty (Table S1). No predictor consistently improved model fit, and effect sizes were small. Testes size showed a weak positive association with date (OR = 1.01 per day, 95% CI: 0.99–1.04), but the confidence interval overlapped 1, indicating no clear seasonal trend in testis enlargement during the study period. The proportional odds assumption was met (Brant test: $p = 0.98$).

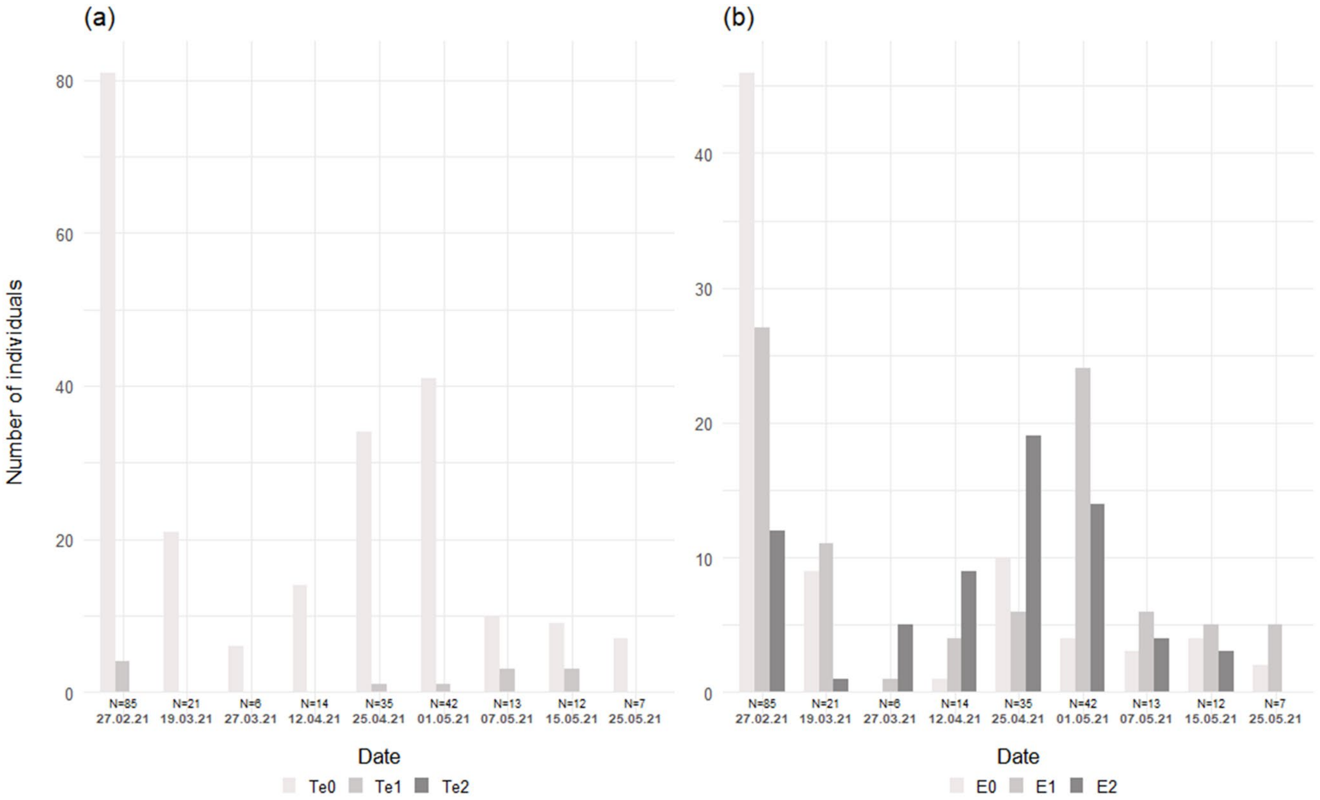


Fig. 3 (**a**) Testes and (**b**) epididymides size categories of from February to May 2021. Bars indicate the number of individuals assigned to respective size categories on each sampling date: (**a**) small size testes (Te0, light grey), medium size (Te1, medium grey), and large size (Te2, dark grey). (**b**) small size epididymides (E0, light grey), medium size (E1, medium grey), and large size (E2, dark grey). Sample sizes (N) are listed below the x-axis

Epididymides

Epididymides were enlarged in 52% of males in early spring, peaking from late March to April, and declined in May (Fig. 3b). Model selection based on AIC supported the model including date and buccal gland size (ΔAIC = 0, AIC weight = 0.63, Table 3). A slightly more complex model additionally including testes size showed similar support (ΔAIC = 1.04, weight = 0.37). All remaining candidate models had ΔAIC > 15 and negligible support (Table S1). Ordinal logistic regression indicated that the probability of larger epididymides increased markedly with buccal gland size (OR = 2.82, 95% CI: 1.72–4.63). The effect of date was comparatively weak (OR = 1.01 per day, 95% CI: 1.00–1.02), with the confidence interval slightly overlapping 1. The proportional odds assumption was not violated (Brant test: omnibus $p = 0.23$), and no problematic multicollinearity was detected (all VIF ≤ 1.36). Thus, males with more developed buccal glands were substantially more likely to exhibit enlarged epididymides, whereas seasonal progression alone had only a minor influence.

Robustness analysis of male reproductive features

Because body mass and temperature contained a substantial proportion of missing values (> 25%), these variables were not included in the primary AIC-based model selection. To assess robustness, models were re-fitted on a reduced dataset including body mass and temperature ($n = 176$). The overall pattern of results remained qualitatively unchanged. For epididymides, buccal gland size remained positively associated (linear contrast: OR = 2.81, 95% CI: 1.18–6.72; quadratic contrast: OR = 0.43, 95% CI: 0.26–0.73). Body mass showed an additional positive effect (OR = 1.26, 95% CI: 1.08–1.47), whereas temperature did not exhibit a consistent association (OR = 1.21, 95% CI: 0.80–1.83). For buccal glands, date remained strongly positive (OR = 1.06, 95% CI: 1.04–1.08), and the association with epididymides persisted (linear contrast: OR = 2.92, 95% CI: 1.51–5.66). Neither temperature nor body mass altered the overall pattern. For testes, no predictor showed a consistent or biologically meaningful effect in the reduced dataset. Overall, the principal results were robust to the inclusion of additional covariates.

Female buccal gland size

Ordinal logistic regression identified sampling date as the best-supported predictor of female buccal gland size (Table 3). The model including date alone had the lowest AIC (ΔAIC = 0, AIC weight = 0.49), whereas adding temperature or body mass did not improve model fit (ΔAIC < 2), indicating moderate model uncertainty (Table S1). Buccal gland size increased with date (OR = 1.04, 95% CI: 1.03–1.06), indicating a progressive enlargement over the course of spring. Neither ambient temperature nor body mass provided additional explanatory value in model comparisons.

Robustness: Because body mass contained missing values (28%), we re-fitted the best-supported model on a reduced dataset including only individuals with complete temperature and body mass data. The positive association between date and female buccal gland size remained virtually unchanged (OR = 1.04, 95% CI: 1.03–1.05), confirming that the seasonal effect was robust and not driven by missing data.

Female reproductive status

Both saline and water proved equally effective for vaginal lavage. Over the course of the experiment, two cell types were identified (SAC and IC). In early samples (mid-March), SACs were frequently observed, indicating oestrous (unfertilised) status. By late March, increasing IC prevalence suggested ovulation and transition to metoestrous, with only two exceptions in late April and early May (Fig. 4). Thus, vaginal cytology yielded 73 evaluable samples, of which eight females were classified as oestrous (stage 1, see Table 1) and 65 as metoestrous (stage 2). The two other stages (0 = prooestrous, 3 = anoestrous) were not recorded. Because the low number of oestrous females resulted in quasi-complete separation in standard logistic regression, reproductive stage (oestrous, metoestrous) was analysed using bias-reduced logistic regression (Firth method).

Reproductive stage showed a positive seasonal trend. The probability of oestrous increased with date ($\beta = 0.048$, SE = 0.031), corresponding to an odds ratio of 1.05 per day (95% CI: 1.00–1.18, Table 3). The likelihood ratio test approached statistical significance ($\chi^2 = 3.81$, $n = 21$, $p = 0.051$). Ambient temperature alone did not significantly predict reproductive stage (likelihood ratio $p = 0.108$). Moreover, adding temperature to the date model did not improve model fit (likelihood ratio comparison $p = 0.736$), indicating that seasonal progression was primarily explained by date rather than temperature. Buccal gland size was not included as a predictor in the regression model because its inclusion resulted in quasi-complete separation and unstable parameter estimates due to the strong seasonal structuring of both variables. Descriptively, buccal gland enlargement co-occurred predominantly with metoestrous females, suggesting parallel seasonal progression of gland development and reproductive stage.

Sperm was detected on 11 vaginal cytology slides (15% of samples) on five different dates: 27.02, 28.02, 13.04, 01.05, and 15.05.2021. On 01.05.2021, a young female

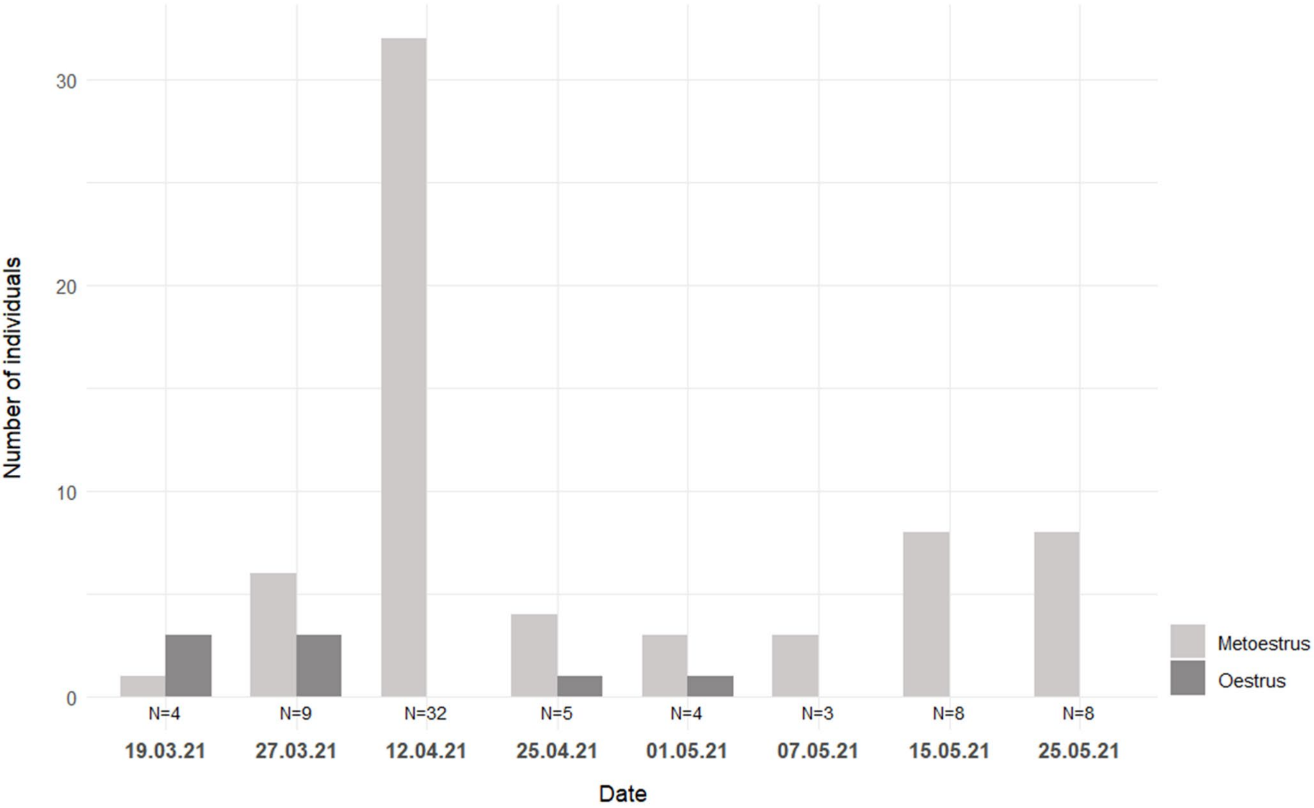
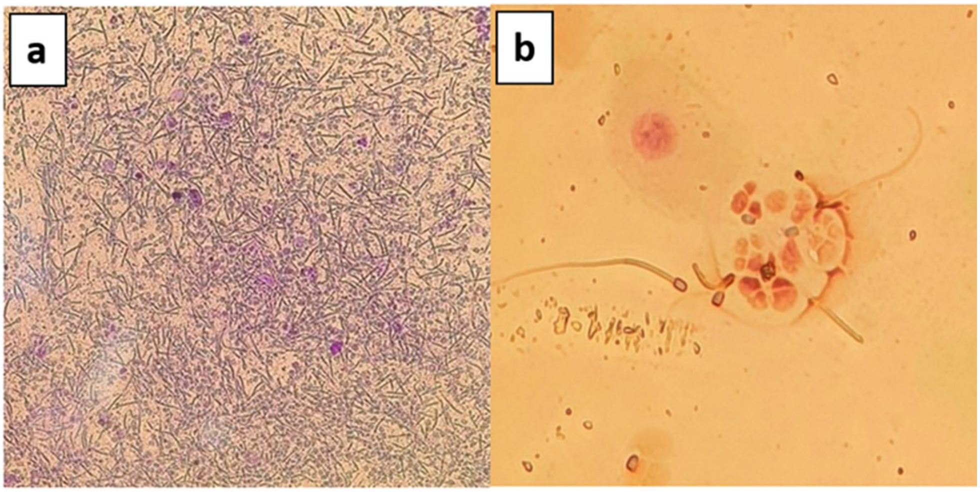


Fig. 4 Reproductive stages of female noctule bats sampled between March and May 2021, assessed through vaginal cytology. Bars represent the absolute number of individuals per sampling date assigned to oestrous (dark grey, predominance of superficial anucleated cells (SAC)) and metoestrous (light grey, predominance of intermediate cells (IC)). Sample sizes (N) are listed below the x-axis

Fig. 5 (**a**) Smear of expelled vaginal fluid from a noctule bat collected on 01 May 2021, showing a high concentration of sperm cells. (**b**) Evidence of sperm phagocytosis by neutrophils in another vaginal lavage obtained on the same date



(ringed as a juvenile the previous year) with dried sperm visible on the vulva expelled sperm-rich fluid after vaginal NaCl lavage (smear result see Fig. 5a). The bat was still in oestrous, while all other females were already in metoestrous. Neutrophils were detected in vaginal smears from mid-April onward, exclusively in assumedly fertilised females in metoestrous. In three samples (13.04 and twice on 01.05.2021), neutrophils were observed phagocytosing sperm, with heads appearing retained and tails detached (Fig. 5b).

Social behaviour

Bat box inspections revealed only mixed colonies in spring (multiple males and females, data not included), with no clear harem structures. Bioacoustic recordings detected orientation and sporadic social calls, but no distinct mating calls such as ‘type A calls’ or songs (‘type D calls’) according to Pfalzer (2002).

Discussion

In our study on common noctule bats (*Nyctalus noctula*), we found evidence consistent with spring copulation following hibernation. This pattern was reflected in several male reproductive traits, including enlarged buccal glands and epididymides, as well as in female characteristics such as buccal gland expression and signs of oestrous, all of which indicate reproductive readiness. In addition, fresh sperm detected in vaginal lavages provides direct evidence that copulation occurred during this period. However, the extent to which these spring mating events contribute to subsequent fertilization and reproductive success remains unresolved.

Male reproductive features

Male noctule testes stayed small until late May, reflecting suppressed spermatogenesis during hibernation due to the concomitant reduction in metabolic rate (Wimsatt 1969; Kenagy and Trombulak 1986; Entwistle et al. 1997; Lee 2020), as optimal temperature for spermatogenesis in mammals ranges from 34 to 35 °C (Dantas 2022; Jorban et al. 2024).

Contrary to previous reports of minimal epididymal size at arousal (Racey 1979; Entwistle et al. 1998), we found that epididymides were often fully distended in spring, peaking at the end of hibernation and in May, which implies overwinter and spring sperm storage. This surprising increase in epididymal size over the course of spring may be explained by cold-induced cremasteric retraction that may make early-spring genital organs appear smaller (Bahat et al. 2012). Spermatogenesis may be affected by body condition (Racey and Entwistle 2000; Entwistle et al. 1998; Komar et al. 2020), so that males with higher body mass can produce more sperm in the summer months due to higher energy reserves. In accordance, statistically, epididymal size should correlate with body mass, which we could not prove in our study. On the other hand, bigger males should have attracted more females and mated more often, so that they should be left with fewer sperm stored in spring as compared to virgin males.

High autumn food availability aligns male peak condition with female receptivity, enabling females to assess mate quality (Sandell 1990), and the synchrony of sperm release into the cauda epididymides with female readiness underscores strong autumn mating demand and confers an early mating advantage (Pfeiffer and Mayer 2012). However, considering the energetic investment required for sperm production and presumably also for storage and fertility maintenance, we assume sperm storage until spring to be advantageous for hibernating bats. Nevertheless, earlier mates have been shown to be able to secure better sperm-storage sites within the female tract (Crichton et al. 1990; Pfeiffer and Mayer 2012) and may allow an extended opportunity for cryptic female choice (Eberhard 1996). Winter and spring mating may thus be driven by low-ranking “sneaker” males when dominant males’ reserves are spent. First-year males also exhibited enlarged epididymides, confirming other studies about sexual maturity already complete before the age of 1 (Kozhurina and Morozov 1994). Similar spring gonadal properties and spring mating occurs in *Plecotus auritus* and *P. austriacus* (Moffat 1922; Gäth 2008; Furmankiewicz et al. 2013), point to an ancestral pattern of continuous reproduction interrupted only by hibernation (Pfeiffer and Mayer 2012).

Female reproductive features

A strong point for the notion that active mating happens in spring is the finding of fresh sperm outside the vaginal opening of a female. Most temperate bats (as of noctules) store sperm in their uterine horns after copulation (Racey 1979; Krishna and Bhatnagar 2011), with the cervix normally preventing passive sperm-flow back to the vagina (Rasweiler and Badwaik 2000). Therefore, we attribute this sperm which could be detected macroscopically as a secretion) to a recent mating event. Alternatively, the sperm found outside of the vagina could also represent the release of excessive sperm from the uterus around the time of or shortly after fertilization.

Cytological assessment indicated that 75% of females were in oestrous at arousal in February, suggesting that ovulation likely occurred later in the season, presumably in late March. This pattern contrasts with reports describing ovulation immediately after hibernation (Oxberry 1979; Crichton 2000; Krishna and Bhatnagar 2011). However, cytological patterns reflect underlying endocrine processes only indirectly and therefore do not allow precise determination of ovulation timing. Accordingly, conclusions regarding reproductive timing should be interpreted as best-supported estimates rather than exact physiological thresholds. Nevertheless, vaginal cytology represents an established and minimally invasive method to assess reproductive stage based on the predominance of specific epithelial cell types. Additionally, we found neutrophil phagocytosis of sperm, supporting evidence for leukocytic clearance of non-viable spermatozoa (Crichton 2000). All cytological evidence is derived from our new minimally invasive lavage method which proved to be field-suitable for long-term monitoring studies. However, we suspected that the methods we used may under-detect mating due to rapid sperm clearance from the vagina, so that further methods such as hormonal analyses, histology, or high-resolution ultrasonography will be needed to decisively determine the exact time-point of ovulation and fertilization.

Failures of insemination, fertilization, or implantation — as seen in two late-season oestrous females — could drive additional copulations (Entwistle et al. 1998) or, as known only from tropical bats, a second ovulation (Rasweiler and Badwaik 2000). Reproductive status correlated strongly with date (progression of season), just as in noctules from warmer climates, indicating shorter hibernation periods under warm conditions (Godlevska 2015; Kravchenko et al. 2025). The extended time interval between oestrous and actual fertilisation may have been due to low temperatures in the respective spring this study was conducted. With climate change leading to shorter and warmer winters, noctule reproduction may on the one hand benefit by allowing more participants to join the pool of successfully reproducing individuals (Ibáñez 1997; Zahn 1999; Frick et al. 2010; Sherwin et al. 2012), but on the other hand it might decrease their individual survival as trade-off cost (Mundinger et al. 2021).

Buccal glands

The function of buccal glands in noctules is still a matter of study: Lanza (1956) and Racey (1969) reported that autumn buccal glands are smaller and contain less fatty substance in females, whereas Sokolov et al. (1994) found no sex differences. Buccal glands enlarge seasonally in males—linked to testosterone and sexual maturity (Lanza 1956; Racey 1969). We found spring phenological differences between sexes, implying different sex-specific functions in spring. In males, epididymal size was positively associated with buccal gland size, supporting the interpretation of a sexually related function of buccal glands. We propose buccal glands serve pheromone-based communication— for example as a scent-mark for roost entrances to repel rivals (males) or nursery marking and pup tagging (females). Targeted studies of gland phenology and chemistry are needed to confirm these functions.

Social behaviour

Classical harems were not observed in the bat boxes, instead, we found mixed-sex groups of varying sizes. However, due to limited knowledge of noctule social structures (Torch and Parsons 2012), distinguishing harems from non-harems in spring remains challenging. Post-hibernation colonies included multiple males and exceeded the typical harem size of one male with up to eighteen females (Sluiter and Van Heerdt 1966), suggesting spring mating may differ from autumn patterns. In tropical bats, peripheral “sneaker” males occasionally mate with harem females (Knörnschild et al. 2009; Fasel et al. 2016). A similar strategy may occur in noctules, offering non-mated males a last chance to reproduce and females an opportunity to replenish lost or absent sperm reserves. Since our data shows that sperm is still available, the cost for males is low. Species with sperm competition often mate despite low reproductive success (Pfeiffer and Mayer 2012; Starratt and Shackelford 2023). Such alternative reproductive tactics, including sneaker males and the “raffle model,” are well documented across other animals (Parker 1990; Mesterton-Gibbons 1999; Kustra and Alonzo 2020).

Conclusions

Our study provides evidence suggesting that mating in *Nyctalus noctula* is not restricted to autumn but may extend into spring, potentially offering a “last-chance” reproductive opportunity. Retained sperm in epididymides, delayed ovulation in a substantial proportion of females until late March, and vaginal cytology findings—including sperm detection in 15% of samples—are consistent with obvious active spring copulations. Buccal gland enlargement in both sexes, partly independent of gonadal metrics, further supports a reproductive or communicative function extending beyond immediate copulation.

We introduce a minimally invasive vaginal lavage method adapted for European bats and document histological observations consistent with neutrophil-mediated sperm clearance in Chiroptera. While these findings align with Crichton’s (2000) hypothesis of leukocytic sperm clearance, further studies are needed to clarify the physiological mechanisms involved.

Our results suggest a degree of plasticity in bat reproductive timing, which may facilitate adaptive responses to changing climatic conditions in some species, including the common noctule (Kravchenko et al. 2020a, b). However, the capacity for rapid adjustment is likely to vary among species (e.g. Radchuk et al. 2019).

A deeper understanding of reproductive biology is essential for effective conservation of temperate bat species. Long-term and multi-population studies will be crucial to determine how reproductive strategies respond to ongoing environmental change.

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Data availability The raw data underlying the conclusions in this manuscript are available from the corresponding author upon reasonable request. Contact Marcus Fritze at marcus.fritze@uni-greifswald.de to access the data.

Declarations

Ethics approval Field work including study on live bats was conducted under permits issued by the relevant animal welfare regulations and approved by conservation (Permit No. 44.30-2021-217-Gru, VGA-16-030) and animal ethics authorities (Permit No. 203 m-42502-2-1668, 203.6.1-42502-2-1525LIZW_G).

Competing interests The authors declare no conflicts of interest.

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