No Evidence of Pre-Hibernation or Pre-Migratory Body Mass Gain in Miniopterus natalensis in North-Eastern South Africa

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No evidence of pre-hibernation or pre-migratory body mass gain in *Miniopterus natalensis* in north-eastern South Africa

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Abstract. Migration and hibernation are survival strategies that require physiological preparation using fattening. Bats employ both strategies in times of resource shortages. However, because males and females vary seasonally in their reproductive physiological needs, they may employ different fattening patterns. Whilst fattening, migration and hibernation are common in temperate bats, little is known about subtropical migratory insectivores. This study investigated seasonal variation in body mass of the regionally migrating Natal long-fingered bat *Miniopterus natalensis* to determine if males and females show fattening in preparation for migration/hibernation. Seasonal change best explained the variation observed in overall body mass, whilst sex and forearm length explained the variation to a lesser extent. Body mass between males and females differed significantly by reproductive category among the four seasons. Forearm length was a significant predictor of the body mass of males. Scrotal males had a higher body mass in summer compared to autumn. This pattern of mass gain was not observed in non-scrotal males. The summer body mass of nonpregnant and post-lactating females was not significantly higher than the autumn body mass of nonpregnant females, which did not support the hypothesis that females would exhibit fattening during summer before migration. Results suggest that males and females employ different mass-gain strategies related to reproductive investment rather than fattening preparation for migration or hibernation.

Key words: bats, body mass, fattening, hibernation, migration
Introduction

Seasonal changes in resource availability induce various adaptations in animals. Some notable adaptations, particularly for vertebrates, include hibernation (prolonged periods of inactivity to save energy) and migration (periodically relocating to geographically distinct areas; Zubaid et al. 2006, Kronfeld-Schor & Dayan 2013). The conditions and selective pressures at these geographically different sites affect individual body condition and subsequent adult survival during migration, reproduction and hibernation (Webster & Marra 2005). Bats are unique among mammals, because they employ both migration and hibernation as adaptive strategies to changes in resource availability (Fleming & Eby 2003). The majority of bat migration studies have focused on temperate species (Fleming 2019). These studies show that migrating temperate bats exhibit different patterns of migratory behaviour: (i) regional migration movements of 100-500 km between summer and winter roosts and (ii) long-distance migration (>1,000 km) between seasonal roosts (Fleming & Eby 2003, Hutterer 2005). To prepare for either type of migration, bats may undergo hyperphagia and fat deposition (McGuire et al. 2009, Fleming 2019). Studies have highlighted fat as the primary fuel for bat migration (Hedenström 2009, McGuire & Guglielmo 2009, Krauel 2014) and fat deposition can increase bat body mass by 12-26% (Krulin & Sealander 1972, Kunz et al. 1998, Speakman & Rowland 1999). Unlike birds that fuel only migration through fattening (Rubolini et al. 2002), bats must use fat stores for both migration and the subsequent hibernation, but the two fattening strategies are not mutually exclusive or easily separated (McGuire & Guglielmo 2009, McGuire et al. 2013, 2014).

Migration is less common in tropical and subtropical bat species and, in the majority of cases, migration is not associated with hibernation (Fleming & Eby 2003), although the use of torpor is widespread (Geiser & Stawski 2011). Whereas temperate species migrate to avoid unfavourable winter weather conditions, tropical and subtropical bats migrate among seasonally ephemeral resource patches (Popa-Lisseanu & Voigt 2009, Fleming 2019). Due to the low availability of flying insects during the winter season (Janzen & Schoener 1968, Boultet et al. 2011), the breeding season of insectivorous bats is significantly reduced compared to frugivorous and nectarivorous species (McNab 1969, Mares & Wilson 1971, Bernard 2002). Currently, it is unclear whether subtropical migrating insectivorous bats also prepare for the journey through fattening like Northern-latitude bats.

Fat accumulation depends on sex and age, because different ages and sexes vary in their physiological needs and prioritise behaviours and resource acquisition differently (Ruckstuhl & Neuhaus 2002). Reproductive effort and the associated energetic investment are asynchronous between bat sexes, because of the ability to store sperm or delay implantation (Willis 2017). One strategy of delayed reproduction is that males spend the majority of their reproductive effort during the late summer and autumn periods, whilst mated females store sperm during the winter and depend on autumn fat reserves to commence gestation in early spring (Crichton 2000). Variation in behaviour, habitat selection, foraging and physiology between breeding and non-breeding females reflects the vast energetic cost of reproduction and lactation (Bernard & Davison 1996, Safi et al. 2007, Pretorius et al. 2019). Therefore, pre-migratory body condition is crucial not only for survival during hibernation but also successful reproduction in the subsequent spring/summer period, whereas males, at least in the northern hemisphere, are not under the same selective pressure (Jonasson & Willis 2011). Subsequently, the differences in reproductive strategies between males and females to maximize fitness may result in differences in foraging behaviour, food intake and, ultimately, seasonal variation in body mass (Rughetti & Toffoli 2014). In temperate zone bats, fattening in spring may also be a strategy to recover from hibernation rather than preparation for migration (Dechmann et al. 2017). Focusing research on individual-level effects, such as body mass, during these periods provides insight into the ecologically relevant variation in reproductive strategies and may ultimately also provide insight into the evolution of sex-biased migration (Gros et al. 2009, Bowlin et al. 2010).

The Natal long-fingered bat Miniopterus natalensis Smith, 1833 (Chiroptera, Miniopteridae) is a small, migratory insectivorous bat that occurs widely throughout southern Africa (van der Merwe 1975, Simmons 2005). The species is not size-dimorphic (Monadjem et al. 2010) but exhibits female-biased migration and, in north-eastern South Africa, migrates in large numbers regionally (150 km) between maternity caves in the Limpopo province.
and hibernacula caves in the Gauteng Province/ North-west Province (van der Merwe 1975, Miller-Butterworth et al. 2005, Monadjem et al. 2010). Females are monoestrus and copulation occurs at hibernacula sites during the late summer and autumn, followed by winter hibernation in May through to July/August (Bernard et al. 1996, Mason et al. 2010). Females delay the implantation of the blastocyst until mid-late winter (July/August; van der Merwe 1986, Cumming & Bernard 1997), to give birth to a single pup at the end of spring/early summer (late November/early December; van der Merwe 1979). Subsequently, the duration of the gestation period is eight months (van der Merwe 1980, 1987). Females then consistently depart the maternity site en masse during early February (Pretorius et al. 2020). In males, spermatogenesis occurs in summer and sperm may then be stored in the testes for up to five months (van der Merwe 1987).

This study investigates the seasonal variation in body mass of male and female M. natalensis. Migration and hibernation strategy (e.g. fattening) should shape seasonal differences in male and female body mass. Different sex-specific reproduction strategies should also translate into differences in male and female body mass. If females prepare, through fattening, for local migration prior to departure at the maternity site, body mass was expected to be higher during the late summer, before migration occurs in February. Females were expected to show the lowest body mass in late spring/early summer due to the energetic requirements of lactation. Males were expected to show a higher body mass during summer and early autumn in preparation for mating and hibernation.

**Material and Methods**

Miniopterus natalensis bats were trapped from December 2011 until August 2018 over 49 trap nights at the Madimatle Cave (also known as the Gatkop Cave; 24°37′ S 27°39′ E) and Meletse Bat Research and Conservation Training Centre (600 m from the cave entrance), Limpopo Province, South Africa. The area is characterised by savanna grasslands, Sweet Lowveld Bushveld and Mixed Lowveld Bushveld vegetation types and typically experiences summer rainfall, with a hot and humid climate throughout the spring and summer months (October-February) and cool, dry winters (June-August) (Mucina & Rutherford 2006, Zhu & Ringler 2010). Sample times were structured to capture annual and seasonal variation in the bat population, with sessions conducted during spring (1 September-30 November), summer (1 December-28 February), autumn (1 March-31 May) and winter (1 June-31 August) each year according to the South African seasons (Schulze 1997). Sampling sessions were limited to three consecutive nights per sample month. Processing of bats was undertaken with University of Pretoria Animal Ethics Committee Project numbers SOP008-13, ECO14-13, ECO29-17, ECO30-117, EC054-14 and conducted under permits from the Limpopo Provincial Government, Department of Economic Development, Environment and Tourism (permit numbers CPM 011805, ZA/LP/83642 and ZA/LP/73972).

Bats were captured using one Austbat 2-bank 4.2 m harp trap and one 2-bank cave strainer trap (Chege et al. 2015). Traps were open an hour before sunset at the cave entrance and the training centre, and were dismantled after two hours (mean ± SD: 175.28 ± 95.29 minutes). The majority of bats were captured within the first 10-30 minutes after emergence. However, to control for bats captured later that may have fed, individuals (other than pregnant females) with a body mass > 14 grams were excluded from analyses (n = 9) in accordance with the known average fed body mass of M. natalensis from the same cave (Pretorius et al. 2019). During adverse weather conditions (heavy lightning and storms), trapping was only conducted at the training centre.

In total, 670 adult bats (365 females and 305 males) were sexed, aged and weighed shortly after capture using electronic and spring Pesola balances (± 0.1 g). Forearm length was measured with a calliper (dialMax® Clock Callipers; ± 0.1 mm). Bats were aged using the degree of ossification of the phalangeal epiphyses by trans-illuminating the wing bones (Brunet-Rossini 2009). Only adults were used in this study. Females were classified based on characteristics associated with gestation and lactation (Racey 2009), indicating reproductive status determined by presence of white mammary tissue (lactating – L or post-lactating – PL), and pregnancy status by abdominal palpation (pregnant – P or not obviously pregnant (hereafter referred to as nonpregnant) – NOP). Males were classified based on scrotum characteristics (testes enlarged and descended: scrotal – S or non-scrotal – NS). While male mass increases could be associated
with testes mass in some species, testes mass of Schreiber's long-fingered bat M. schreibersii and the least long-fingered bat M. minor comprises only approximately 1% of body mass (McWilliam 1988, Wilkinson & McCracken 2003), suggesting that the contribution of testes mass to overall body mass is negligible for male M. natalensis. Lastly, bats were tattooed with a unique identifying number and wing-biopsies were taken, then individuals were released.

To assess body mass differences in M. natalensis, methodology followed McGuire et al. (2018), who advocated using body mass as a measure of body fat content since body condition indices (e.g. dividing body mass by forearm length) confound body condition results. Forearm length was therefore kept as a separate explanatory variable in analyses. First, linear mixed effects models (lmer) were used to test whether sex, forearm length, season or a combination of these fixed effects would best explain variation in mean body mass of male and female M. natalensis using the “lme4” package (Bates et al. 2014). Year of capture was set as a random effect to account for possible seasonal variability in body mass within years. No random dependence upon the year of capture (Likelihood ratio test $\chi^2 = 1.53, P = 0.22$) or site of capture ($\chi^2 = 0.02, P = 0.86$) was observed and data from different years and sites were pooled for further analysis.

To investigate changes in body mass, a global general linear model (GLM) was constructed: mass $\sim$ forearm + sex + season. Next, the analyses were focused by treating male and female mass data separately and introducing reproductive category by season as a combined fixed effect (repro-season) in addition to forearm length. To select the best candidate GLMs, we used the “dredge” function of the “MuMln” package (Bartoń 2019). The Akaike Information Criterion (AIC), the difference between the top-ranked model and the $i$th model ($\Delta_i$) with AICc weight ($W_i$) was used to rank candidate models. Models where $\Delta_i < 3$ were deemed the most informative (Burnham et al. 2011). The relative importance of each predictor variable ($x_i$) was then calculated as the sum of the AIC weights of each informative model that the predictor was included in (Burnham & Anderson 2002). All statistical analyses were conducted using R (R Core Team 2017) in RStudio Desktop Software (version 1.1.456), with $\alpha = 0.05$. 

### Table 1. Body mass (g) ± SD (n in parentheses) of female and male Miniopterus natalensis across different seasons from December 2011 until August 2018 at the Madimatle Cave, Limpopo Province, South Africa. No females were captured during winter sampling. The last column shows the ratio of females (F) compared to males (M) seasonally.

| Season | Female | Male | Sex ratio F:M |
|--------|--------|------|---------------|
| Spring | 11.01 ± 1.22 (202) | 10.82 ± 0.75 (134) | 2:1 |
| Summer | 11.20 ± 1.06 (132) | 11.44 ± 0.94 (54) | 2:1 |
| Autumn | 10.77 ± 0.63 (31) | 11.07 ± 0.70 (61) | 1:2 |
| Winter | - | 10.47 ± 0.73 (56) | 0:56 |

### Table 2. Ranked Akaike Information Criterion (AIC), difference between the top-ranked model and the $i$th model ($\Delta_i$) with AICc weight ($W_i$) from generalized linear models investigating if forearm length (FA), sex and/or season explain the variation in the mean body mass of Miniopterus natalensis. The top-ranked model ($\Delta_i = 0$) is shown in bold.

| Model | df | AIC | $\Delta_i$ | $W_i$ |
|-------|----|-----|------------|-------|
| Season | 5 | 1,885 | 0 | 0.505 |
| FA + season | 6 | 1,886.6 | 0 | 0.226 |
| Season + sex | 6 | 1,887 | 1.6 | 0.186 |
| FA + season + sex | 7 | 1,888.6 | 2 | 0.083 |
| Sex | 3 | 1,908.7 | 3.6 | 0 |
| FA + sex | 4 | 1,909.4 | 23.73 | 0 |
| FA | 3 | 1,910.5 | 25.27 | 0 |

### Table 3. Model-averaged parameter estimates (average model coefficients), adjusted standard error (SE) and associated z-values for variables for five top-ranked generalized linear models testing the effects of season, forearm length and sex on the variation of Miniopterus natalensis body mass. Note that male and female capture only overlapped for autumn, summer and spring.

| | Estimate ± SE | z-value | Pr (>|t|) |
|---|----------------|----------|----------|
| Intercept | 10.96 ± 0.50 | 21.63 | < 0.001 |
| Spring | -0.03 ± 0.11 | 0.28 | 0.779 |
| Summer | 0.30 ± 0.21 | 2.37 | < 0.01 |
| Winter | -0.50 ± 0.17 | 2.97 | < 0.001 |
| FA | 0.003 ± 0.01 | 0.27 | 0.78 |
| Sex (M) | 0.00 ± 0.02 | 0.02 | 0.98 |
Results

*Miniopterus natalensis* male body mass (±SD) averaged 10.65 ± 0.83 g (n = 305) and females (excluding pregnant females) averaged 10.77 ± 1.13 g (n = 345). Male forearm length averaged 44.50 ± 0.90 mm (n = 305) and was 44.90 ± 2.53 mm (n = 365) for females. Different proportions of males and females were captured across the four seasons, with no females captured during winter sampling (Table 1).

Season best explained the overall variation in *M. natalensis* body mass (relative importance $x_1 = 100\%$), as well as forearm length ($x_2 = 31\%$) and sex ($x_3 = 27\%$) to a lesser extent (Table 2). Overall, mean body mass in summer was significantly higher than body mass in autumn (Table 3). In winter, body mass was significantly lower than in autumn but in spring, body mass was not significantly different to body mass in autumn (Table 3).

Male and female *M. natalensis* in various reproductive categories were captured differently across the four seasons, with lactating females captured during late spring and summer (Fig. 1A) and scrotal males captured during summer and autumn (Fig. 1B). Non-scrotal males were captured year-round. Between and within the four seasons, reproductive category was an important predictor of both female and male body mass, with forearm length explaining the variation in female body mass to a lesser extent (Table 4). The pregnant and lactating reproductive categories significantly and positively affected body mass of female *M. natalensis* in spring and summer (Table 5). Pregnant females had a significantly higher body mass in spring (2.17 g). Lactating females had a significantly higher body mass in summer, being 1.34 g heavier than nonpregnant females (Fig. 2A). In summer, post-lactating female body mass was significantly lower than that of lactating females (1.29 g). Nonpregnant females increased body mass by 0.51 g from spring to summer, although this difference was not statistically significant.

Fig. 1. Boxplot showing medians and interquartile ranges (Tukey-style whiskers extend to 1.5 × IQR) of the body mass (g) of A) female and B) male *Miniopterus natalensis* in different reproductive categories (lactating − L, nonpregnant − NOP, pregnant − P, post-lactating − PL, non-scrotal − NS and scrotal − S) across spring, summer, autumn and winter measured from December 2011 until August 2018 at the Madimatle Cave, Limpopo Province, South Africa.
In addition to reproductive category, forearm length was an important and significant positive predictor of the body mass of males in different reproductive categories (Table 5). Male body mass was significantly and positively correlated with forearm length, with greater male body mass at longer forearm lengths, but this pattern did not appear related to reproductive category (Fig. 3).
body mass of scrotal males was highest in summer, with mean scrotal male body mass increasing by 0.88 g from spring (Fig. 2B). Scrotal male autumn body mass was not significantly different to non-scrotal autumn body mass. Non-scrotal male body mass was lowest in winter, decreasing by 0.62 g from autumn, but did not differ significantly between spring and summer. Mean body mass of different reproductive categories in the different seasons can be found in Table S1.

Discussion

For *M. natalensis*, body mass changed seasonally and with a reproductive category-dependent pattern of variation between males and females. These mass differences were probably related to both the effects of variable seasonal food availability and also the evolutionary pressures on sex-specific reproductive strategies (Rughetti & Toffoli 2014). Female body mass was lower, but not statistically significant, in spring than summer, somewhat supporting our hypothesis that females would show the lowest body mass in late spring/early summer. Lactating females had the highest body mass in summer compared to nonpregnant and post-lactating females. Lactating female body mass was significantly higher than autumn body mass of nonpregnant females. However, the summer body mass of nonpregnant and post-lactating categories was not significantly higher than the autumn body mass of nonpregnant females, which did not

| Model | df | AIC  | Δi | Wᵢ |
|-------|----|------|----|----|
| Female | Repro-season | 9   | 1,031.8 | 0  | 0.714 |
|        | FA + repro-season | 10  | 1,033.7 | 1.83 | 0.286 |
|        | FA | 3   | 1,131 | 99.12 | 0 |
| Male   | FA + repro-season | 8   | 676.8 | 0  | 1  |
|        | repro-season | 7   | 710  | 33.25 | 0  |
|        | FA | 3   | 726.4 | 49.63 | 0  |

Table 4. Ranked Akaike Information Criterion (AIC), difference between the top-ranked model and the ith model (Δi) with AICc weight (Wᵢ) from generalized linear models investigating if reproductive category (repro), forearm length (FA) and season explain the variation in the mean body mass of male and female of *Miniopterus natalensis*. The top-ranked models (Δi = 0) are shown in bold.

Table 5. Parameter estimates (average model coefficients), standard error and associated t-values for variables for the top-ranked generalized linear models testing the effects of forearm length and reproductive category within season on the variation of male and female body mass of *Miniopterus natalensis*.
support the hypothesis that females would exhibit fattening during summer before their journey to the hibernacula. Scrotal males had a higher body mass in summer compared to autumn, supporting the hypothesis that male *M. natalensis* prepare for mating, since spermatogenesis is known to coincide with the austral summer months (van de Merwe 1987, Bernard et al. 1996). However, this pattern was not observed in non-scrotal males, suggesting that male *M. natalensis* do not prepare for hibernation or migration through fattening.

Females may not exhibit summer fattening to facilitate a lower wing load for migration to the hibernacula (Lehnert et al. 2018). Wing loading (body mass vs. wing area) is an important aspect of flight performance (Norberg & Rayner 1987), but since wing dimensions are fixed, variation in seasonal or daily fattening may determine flight success (Senar et al. 2002). This strategy has been observed in some bird species (van der Veen & Lindström 2000, Senar et al. 2002), but remains untested in bats. The fattening period and the initial spring body condition of female common noctules *Nyctalus noctula* does not affect migratory decisions but is rather associated with ideal environmental conditions for migration (such as fast tailwinds in the direction of travel; Dechmann et al. 2017). Migratory decisions in female *M. natalensis* may, therefore, be driven not by body condition, but rather by reproductive needs.

In summer, reproductive female *M. natalensis* are still under the enormous energetic burden of lactation. Lactation is one of the most energetically costly periods for female mammals (Millar 1978). Lactating female bats, including *M. natalensis*, compensate for energetic demands by increasing resource intake relative to non-reproductive females (McLean & Speakman 1999, Pretorius et al. 2019), supported by the higher summer body mass observed in lactating females in this study. Contrastingly, post-lactating females had a significantly lower body mass during summer. In post-lactating females, a decrease in body mass reflects the demands imposed by lactation for some vesper bats (Burnett & Kunz 1982), and the constraints of warm maternity roosts on the use of torpor (Koehler & Barclay 2000). In the brown long-eared bat *Plecotus auritus*, body mass steadily declined over 40 days after parturition, whereas the body mass of non-reproductive females continued to increase (McLean & Speakman 2000). In this study, nonpregnant females had a slightly higher, albeit non-significant, body mass in summer. Therefore, despite a higher insect resource availability during summer which would facilitate hyperphagia and fattening (Janzen & Schoener 1968, Parrish 2000, Boulter et al. 2011), reproductive females are probably constrained in their ability to fatten for migration by the energetic cost of lactation.

Female *M. natalensis* may also not exhibit fattening in preparation for migration simply because migration for this species may not be overly costly. Current known migratory distances for *M. natalensis* in north-eastern South Africa range over 150 km from a hibernaculum in Gauteng to a maternity cave in Limpopo (van der Merwe 1975). Whilst female *N. noctula* migrate further than males, there is no difference in body condition between males and females at the start of the journey (Dechmann et al. 2014). Migratory bats can spend long hours foraging, six hours as observed in the hoary bat *Lasiurus cinereus* (Barclay 1989). They can also cover vast distances during nocturnal commutes (Best & Geluso 2003). *Miniopterus schreibersii* in southern France fly more than 30 km on nocturnal foraging commutes, with flight speeds reaching 40-50 km/h, foraging continuously throughout the night (Vincent et al. 2010). Therefore, unlike the long-term fattening strategies employed by various bird species or temperate migratory bats, bats like *M. natalensis* may use shorter-term strategies to fuel their comparatively short migrations (Dechmann et al. 2017). Migrating *N. noctula* stop over frequently forage (Dechmann et al. 2014). Thus, bats may use recently ingested nutrients to fuel migration rather than reserves stored up for hibernation (Voigt et al. 2010). This reduces flight costs incurred through increased body mass and allows for rapid energy gain from directly combusted fuel (Voigt et al. 2010). Bats may also use torpor during daytime resting periods, decreasing the overall cost of migration and shortening stopover times (McGuire et al. 2012). Additionally, due to the short migratory distance, it is unlikely that *M. natalensis* employs strategies like reducing digestive organs as observed in long-distance migrators such as *L. cinereus* (McGuire et al. 2013). Hence, *M. natalensis* preparation for migration may not be completed at the maternity site before migration as predicted, but sometime throughout, or even after, the migratory journey.

The findings of this study suggest that male *M. natalensis* do not prepare for hibernation or
Migration and hibernation are survival strategies that require physiological preparation using fattenning. It is possible that non-scrotal male *M. natalensis* do not show the expected autumnal increase in body mass because they do not migrate and remain at the maternity cave, as suggested by the male-only sex ratio observed during winter. These males also may not fatten for hibernation because the population remaining at the Madimatle maternity cave continue to forage throughout winter (Pretorius et al. 2020). Male *M. natalensis* do, however, increase body mass during summer in preparation for mating, with scrotal males increasing their body mass by approximately 8%. This is indicative of spermatogenesis and readiness to mate (Krutzsch 2000, Wilkinson & McCracken 2003) and to buffer the loss of body mass during mating activities (Welbergen 2011). Most male aerial hawkers generally build up their winter fuel reserves before mating, with body condition related to reproductive status and mating competition (Becker et al. 2013, Gallant & Broders 2015). This may be why forearm length is a predictor of body mass in males, as larger individuals are heavier and larger male body size is related to more successful competition for mating opportunities (Crichton & Krutzsch 2000, Clutton-Brock 2007).

In *M. schreibersii* (now *M. natalensis*) in Zimbabwe, males initiate spermatogenesis in late December, with maximum spermatogenic activity observed by March/April (Bernard et al. 1996). Therefore, male *M. natalensis* time their peak reproductive readiness to coincide with female ovulation (Bernard et al. 1996, Mason et al. 2010). Even though the mating systems and levels of intrasexual competition between *M. natalensis* are still unknown, a higher body mass likely gives some males a competitive reproductive advantage over rivals during this period (Clutton-Brock 2007, Strauss et al. 2007), or a better likelihood of surviving through winter (Turbill et al. 2011). However, body condition may not always be related to reproductive success (Gallant & Broders 2015) and more detailed studies are required to investigate the implications of the results presented here.

**Conclusion**

Migration and hibernation are survival strategies that require physiological preparation using fattenning (Bauer et al. 2011, McGuire 2012). Bat physiology is unique compared to other migrants, like birds, since various species employ both strategies to survive resource shortages, and fuel deposition before hibernation is also used as the main energy source for migration (McGuire & Guglielmo 2009). Additionally, sex-differences are also expected to influence fattenning (Dechmann et al. 2014). Fattenning is a common strategy in both long-distance and regionally migrating temperate insectivorous bat species (Fleming 2019), but less common in tropical and subtropical species, particularly insectivores (Fleming & Eby 2003). This study investigated seasonal differences in the body mass of the regionally migrating *M. natalensis* to determine if these bats prepared for migration/hibernation through fattenning. Males and females in different reproductive categories showed different patterns in their seasonal mass, suggesting different fattenning strategies related to reproductive investment by the different sexes rather than preparation for migration or hibernation.

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**Supplementary online material**

**Table S1.** Body mass (g) ± SD (n in parentheses) of female and male *Miniopterus natalensis* in different reproductive categories (lactating – L, nonpregnant – NOP, pregnant – P, post-lactating – PL, non-scrotal – NS and scrotal – S) across different seasons from December 2011 until August 2018 at the Madimatle Cave, Limpopo Province, South Africa. Blank cells indicate that individuals from that reproductive category were not captured in that season (https://www.ivb.cz/wp-content/uploads/JVB-vol.-70-1-2021-PretoriusM.-et-al.-Table-S1.docx).