

**The effect of group size, reproductive condition, and time period on sexual segregation patterns in three vespertilionid bat species**

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Running header: sexual segregation in roosting bats

**Abstract**

Sexual segregation is widespread across the animal kingdom, yet there is limited consensus on the factors that shape this behavioural phenomenon. Many of the existing theories are based on study species with high levels of sexual size dimorphism. Insights from studies on species with minimal sexual size dimorphism, such as vespertilionid bats, provide an opportunity to study the factors associated with segregation irrespective of body size effects. Using long-term data pooled from multiple bat box monitoring schemes across the U.K., we investigated segregation patterns in maternity roosts of three vespertilionid bat species, Bechstein's bats, *Myotis bechsteinii*, Natterer's bats, *M. nattereri*, and brown long-eared bats, *Plecotus auritus*. We used the Sexual Segregation and Aggregation Statistic (SSAS) to evaluate temporal trends of sexual segregation of roosts over the reproductive period (divided into pre-parturition, lactation, and post-lactation periods). Additionally, we used generalised linear mixed models and beta

regression models to investigate the effect of group size on segregation patterns. Our results showed that the size of the maternity group was an important covariate of inter- and intra-sexual segregation, with males and non-breeding females typically segregated from large maternity groups across all three periods. Additionally, we demonstrate that reproductive condition and period influence segregation patterns, with breeding females segregated from non-breeding females and males during the lactation period. Although sexual segregation may be caused by multiple mechanisms, our results show that group size, female reproductive condition, and time period are key factors associated with segregation within bat roosts. These findings make a valuable contribution to the understanding of inter- and intra-sexual segregation in vespertilionid bats and complement existing research on segregation in other mammalian taxa, providing further evidence that sexual size dimorphism is not a prerequisite for sexual segregation.

## **Keywords**

Breeding status, dimorphism, group composition, lactation, sociality, social organization

## **Introduction**

Sexual segregation, the spatial or temporal separation of males and females (Ruckstuhl, 2007), is widespread across vertebrate taxa (Ruckstuhl & Neuhaus, 2005; Wearmouth & Sims, 2008). The definition of this behaviour is separated into three potentially overlapping concepts: ‘social segregation’, whereby the sexes form separate social groups, ‘habitat segregation’, whereby the sexes differ in their habitat use, and ‘spatial segregation’, whereby the sexes differ in their space use within homogenous habitat (Ruckstuhl, 2007; Conradt, 1998; Bon & Campan, 1996).

Multiple hypotheses exist to explain the drivers of this phenomenon, and it is possible that several factors are involved, and that these can vary across taxa (Ruckstuhl, Clutton-Brock & Neuhaus, 2005). However, as research into sexual segregation has focussed on sexually dimorphic taxa, such as ungulates, the effects of body size have become embedded into many of these hypotheses (Ruckstuhl & Neuhaus, 2000; Bowyer, 2004). For example, as a result of size dimorphism, males and females may have different nutritional and energetic requirements, activity budgets, reproductive strategies, or social affinities, which may cause segregation (Ruckstuhl & Neuhaus, 2002; Ruckstuhl, 1998; Conradt, Ruckstuhl & Neuhaus, 2005; Bon & Campan, 1996). To understand drivers of sexual segregation independent of the effects of body size, it is useful to study non-dimorphic species (Ruckstuhl & Neuhaus, 2000). As bats often segregate, but frequently exhibit less extreme, absent, or reversed (female biased), sexual dimorphism (Myers, 1978; Williams & Findley, 1979; Stevens, Johnson & McCulloch, 2013), they are ideal candidate species to investigate factors other than size-dimorphism that might affect sexual segregation patterns (Ruckstuhl *et al.*, 2005).

Bats are characterised by extreme sociability, with diverse social systems encompassing a range of segregation patterns (Altringham *et al.*, 2005), however research focussing on segregation in bats is scant compared to other mammalian taxa. Segregation in bats may occur at the roost level, within the foraging habitat, and at wider spatial scales. Segregation within roosts is common among many species throughout the nursery season (Altringham *et al.*, 2005), however there is a lack of evidence to support a generalisable explanation. One leading theory attributes segregation within roosts to different thermoregulatory and energetic requirements between the sexes, which are particularly apparent during the reproductive season (May-September for northern temperate species) (Senior, Butlin & Altringham, 2005; Ruckstuhl *et al.*, 2005; Kerth & van Schaik, 2012). In spring and early summer, reproductive females have

higher energetic demands, and require warmer roosts for juvenile development during pregnancy and lactation, whereas males have lower energy requirements and may further reduce energy expenditure by entering torpor in cooler roosts (Willis, 2006; Grinevitch, Holroyd & Barclay, 1995).

Another proposed explanation relates to competition for foraging areas close to the roost (Kunz, 1974). Lactating females must balance the demands of foraging and returning to the roost to nurse their young during the night, and are known to forage nearby to the roost (Henry *et al.*, 2002). If males present in the nursery roost share the females' foraging area, as is the case for some species, e.g. Daubenton's bats (Senior *et al.*, 2005), this increased competition could compromise the foraging efficiency of females and the survival of their young (Tuttle, 1979; Altringham *et al.*, 2005). It is unclear if females exclude males from their home range, however there is evidence demonstrating a higher relative abundance of females compared to males in higher quality foraging habitat with high prey availability (Lintott *et al.*, 2014; Senior *et al.*, 2005; Russo, 2002; Angell, Butlin & Altringham, 2013; Encarnação *et al.*, 2005). This could be a result of male exclusion from high quality habitats (Senior *et al.*, 2005; Encarnação *et al.*, 2005), or differential resource use due to the lower energetic needs of males (Levin *et al.*, 2013).

Whilst both hypotheses are plausible, there are limited empirical data to support them, leaving scope for alternative interpretations. Furthermore, due to the single species focus of many existing studies, the factors associated with varying levels of segregation between ecologically and physically similar species are not apparent. Although the results from these single species studies have been compared to highlight overall variation (Altringham *et al.*, 2005), there are a lack of comparative empirical data to explore these differences in detail. Research into inter-

and intra-specific variation in segregation may yield important common factors associated with this behaviour, and inform a more generalisable theory of segregation for bats.

In this study, we investigated factors associated with inter- and intra-sexual segregation in maternity roosts of three ecologically similar vespertilionid bat species: Bechstein's bats, *Myotis bechsteinii*, Natterer's bats, *Myotis nattereri*, and brown long-eared bats, *Plecotus auritus*. These medium-sized vespertilionids display similar low levels of female-biased sexual dimorphism (<6 % weight difference, this study) and different patterns of sexual segregation, providing an opportunity to clarify segregation mechanisms independent of the effects of body size dimorphism. These species are widespread across Europe, with life cycles typical of temperate zone vespertilionid bats, and colonies displaying fission-fusion sociality (Fleischmann & Kerth, 2014; Zeus, Reusch & Kerth, 2018). *M. bechsteinii* females roost in large sexually segregated maternity groups (Kerth & Morf, 2004; Kerth & Petit, 2005; Kerth, Safi & König, 2002), while in *M. nattereri*, both mixed groups and segregated groups are common (Altringham, 2014; Funakoshi, 1991; Park, Masters & Altringham, 1998; August *et al.*, 2014). In contrast, *P. auritus* differs from most other temperate zone vespertilionids, as there is no evidence of sexual segregation in maternity groups during the breeding period (Entwistle, Racey & Speakman, 1997, 2000).

Roosting within maternity groups involves a trade-off between costs and benefits: benefits include social thermoregulation, reduced risk of predation, and social benefits of co-operation and information transfer (Altringham *et al.*, 2005), whilst costs include parasite and disease transmission (Lourenço & Palmeirim, 2007; Brown & Brown, 1986), and resource competition (Krause & Ruxton, 2002). These trade-offs vary with species, sex, breeding status, and between reproductive periods. For instance, breeding females benefit from social thermoregulation and

are known to roost together (Kerth & Konig, 1999; Kerth & van Schaik, 2012). However, due to the different physiological requirements of males and non-reproductive females, compared to reproductive females, and the additional costs of high parasite load in maternity roosts, non-reproductive bats would be expected to maximize their own fitness by segregating from maternity roosts and entering torpor (Pretzlaff, Kerth & Dausmann, 2010; Lourenço & Palmeirim, 2007). Furthermore, as ectoparasite load is known to peak during the lactation period, and is particularly prevalent among vulnerable juveniles and breeding females with reduced immune and behavioural defences (Christe, Arlettaz & Vogel, 2000; McLean & Speakman, 1997; Speakman, 2008), higher levels of segregation may be expected during this period (Lourenço & Palmeirim, 2007).

The resulting size of the group is also a consequence of this trade-off between costs and benefits (Silk, 2007). As group size increases, costs such as higher parasite load, or risk of disease transmission, increasingly outweigh benefits of group living (Côté & Poulinb, 1995; Krause & Ruxton, 2002). Furthermore, with increased levels of social warming in larger groups, the temperatures would be too high for non-reproductive bats to enter torpor. Therefore, we expect increased inter- and intra-sexual segregation in larger groups, as males and non-breeding females avoid the increased costs. Moreover, breeding females found in smaller roosts may not reap the full rewards of group living, e.g., social thermoregulation, and consequently may be driven to roost in larger groups under these conditions (Thirgood, 1996). Therefore, we expect group size to be an important covariate of inter- and intra-sexual segregation for bats.

Although assortative mixing among vespertilionid bats has previously been described (Zeus *et al.*, 2018; Patriquin *et al.*, 2010), the factors associated with overall roost segregation are poorly understood. Comparative empirical data drawn from multiple species are key to an improved

understanding of segregation in bats, providing support to a growing body of work studying this phenomenon (Altringham *et al.*, 2005). Furthermore, whilst group size is known to influence segregation in other mammalian species (Thirgood, 1996), this factor has not previously been explored in bats, despite their propensity to form large groups. In this study, we used a long-term individual-based dataset drawn from multiple bat box monitoring schemes in southwest England to investigate the influence of group size, reproductive condition, and time period on inter- and intra-sexual segregation patterns in maternity roosts of three ecologically and physically similar species. This study aimed to test the following predictions:

- i) breeding females will segregate from non-breeding females and males,
- ii) inter-sexual (between males and females) and intra-sexual (between breeding and non-breeding females) segregation will be highest during the lactation period, and
- iii) larger maternity groups will have higher levels of inter- and intra-sexual segregation.

## **Materials and methods**

Data used in this study were pooled from separate bat box monitoring schemes across eight sites in southwest England, collected between 1998 and 2018. The sites were predominantly heterogenous deciduous woodland with varied species composition and mixed management histories. Surveys were conducted by licensed bat workers and trainees under their direct supervision, all under separate English Nature/Natural England project licenses. Each scheme monitored at least one of our three target species (*M. bechsteinii*, *M. nattereri*, and *P. auritus*) through surveys of artificial bat roosts (Table 1). Due to the long-term nature of the monitoring schemes, we were satisfied that bats observed roosting in boxes were representative of the population at each site. Surveys occurred during the spring, summer, and autumn months. No

surveys occurred during the winter months as the bats were not detectable during this time. Data collection complied with ASM guidelines for research on live animals (Sikes & Gannon, 2011).

Bats were ringed (banded) on first capture using 2.9 mm aluminium rings (Bat Conservation Trust, UK, under licence from the SNCO) and age, sex, and reproductive status were determined in the hand using protocols described in (Linton & Macdonald, 2018). Bats were categorised as juveniles or adults, and roost groups containing bats that could not be confirmed as juvenile or adult were removed from our analyses. Females were categorised as breeding females (BF) if they were recorded as lactating until the post-lactation period that year. Pregnant bats whose outcome was unknown (only seen during the pre-parturition period that year) were also classified as BF. Females were categorised as failed or non-breeders (FoNB) if they were observed during the lactation period in a post-lactating condition, or without enlarged nipples. Roost groups containing adult females of unknown reproductive status that breeding season were excluded from the analyses. Only roosts where the full group composition (sex, age class, and reproductive status of females) of all bats present was known were used in analyses of segregation and aggregation (Table 2).

To demonstrate the levels of sexual size dimorphism across the species, we used body weight measurements, recorded using a spring balance to the nearest 0.1 g. We used measurements of adult bats with known breeding status and age. Individuals had between 1-29 repeated measurements (mean  $3.33 \pm 3.24$  SD) over the study period. Our dataset comprised 83 *M. bechsteinii* (72 female, 11 male), 1868 *M. nattereri* (1275 female, 593 male), and 659 *P. auritus* (307 female, 352 male) body weight measurements.



199 *Statistical analyses*

200 Statistical analysis was performed in R (R Core Team, 2019). To assess levels of sexual size  
201 dimorphism in each species, we used a linear mixed effects model using the R package “lme4”  
202 (Bates *et al.*, 2015). Fixed effects included species, sex, reproductive status, and age, with  
203 individual ID and year as random intercept effects to account for repeated measures of  
204 individuals and variation between years. Age was calculated as years since capture for  
205 individuals initially captured as juveniles, with bats of unknown age removed from the analysis.  
206 We compared models to the null hypothesis model using ANOVA, and identified the top model  
207 candidate using Akaike Information Criterion scores (AIC). Visual inspection of diagnostic  
208 plots confirmed homogeneity of variances and normally distributed residuals. Significance of  
209 random effects was tested using likelihood ratio tests.

210  
211 We used the Sexual Segregation and Aggregation Statistic (SSAS) (Bonenfant *et al.*, 2007) to  
212 assess sexual segregation and aggregation patterns of roosts. This simple index, derived from  
213 the chi-square statistic, is widely used to study segregation patterns in a variety of vertebrate  
214 species (Wang *et al.*, 2018; Hawkins *et al.*, 2019; Singh *et al.*, 2010; Ficetola, Pennati &  
215 Manenti, 2013). SSAS provides an index value between 0 (complete aggregation) and 1  
216 (complete segregation), according to the following equation:

$$SSAS = 1 - \frac{N}{XY} \sum_{i=1}^k \frac{X_i Y_i}{N_i}$$

217  
218  
219  
220 Where, for  $k$  groups,  $X$  is the total number of animals sampled in class X, and  $Y$  is the total  
221 number of animals sampled in class Y, and  $N$  is the sum of animals sampled in both classes, X  
222 and Y. This statistic can be used to assess segregation between sexes, or between different

classes, based upon breeding status and age, even when a large proportion of animals are observed alone (Wang *et al.*, 2018; Ficetola *et al.*, 2013; Bonenfant *et al.*, 2007). A randomization procedure (10,000 permutations) was used to build a distribution of SSAS under the null hypothesis of random association between classes, which was compared to the observed statistic using a significance level with a Bonferroni correction ( $\alpha = 0.00833$ ) to account for multiple comparisons (Bonenfant *et al.*, 2007). To assess segregation by reproductive period we divided our roost dataset into three reproductive periods: pre-parturition (PP), lactation (L), and post-lactation (PL), and calculated the statistic separately for each period. These periods were assigned separately for each roost according to the reproductive status of breeding females, and presence of juveniles.

To test for differences in maternity group size between species and reproductive periods, we used a generalised linear model (GLM) with a Poisson error distribution, as is commonly done for count data (Warton *et al.*, 2016). Maternity roosts were classified as any roost containing multiple females with at least one reproductive female. Fixed effects included reproductive period (PP, L, and PL), and species. We tested the significance of random intercept effects of box type and year using likelihood ratio tests and found these to be insignificant ( $p = 1$ ), so we did not include random effects in the model. We compared models to the null hypothesis model using ANOVA and identified the top model candidate using Akaike Information Criterion scores (AIC).

To examine the influence of maternity group size on segregation patterns, we extracted the maternity roosts that had one or more adult males present ( $n = 216$  groups) and fitted a generalised linear model with proportion of adult males in the group as the response variable and maternity group size as a fixed effect. As the response was a proportion, we used a beta

regression structure for the model using the R package “betareg” (Ferrari & Cribari-Neto, 2004). As “betareg” does not allow for random effects, we also examined resulting models with species, period, box type, and year included as fixed effects to ensure these effects do not strongly influence the resulting coefficients. This process was repeated with proportion of non-breeding females in the maternity group as the response variable (n = 384 groups).

## Results

### *Sexual size dimorphism*

The top model incorporated species, sex, and reproductive status as fixed effects, and individual ID and year as random intercept effects (Table 3). The likelihood ratio test confirmed the significance of these random effects ( $p < 0.001$ ). The difference in mean body weights between non-breeding females and males ranged from 0.14 g (1.7 % difference) in *M. bechsteinii*, 0.41g (5.2% difference) in *P. auritus*, and 0.44 g (5.7% difference) in *M. nattereri*,

### *Temporal patterns of segregation and aggregation in roost composition*

Sexual segregation occurred in all three species (Fig 1). Sexual segregation was highest in *M. bechsteinii* roosts, and lowest for *P. auritus* roosts. Breeding females (BF) were segregated from males across all three reproductive periods in *M. bechsteinii* and *M. nattereri* roosts, but only during the lactation period in *P. auritus* roosts. Failed or non-breeding females (FoNB) were segregated from males across all three periods in *M. bechsteinii* roosts, only during the lactation period in *M. nattereri* roosts, but did not segregate from males across any of the periods in *P. auritus* roosts. Segregation between BF and FoNB occurred across all periods in *M. bechsteinii* roosts, but only during the pre-parturition and lactation periods for *M. nattereri* and *P. auritus* roosts.

#### Maternity roost group size

The top model incorporated species and period as fixed effects, and an interaction effect of period and species (Table 4). Species was a significant predictor of group size, with *M. bechsteinii* having a positive effect, compared to negative effects of *M. nattereri* and *P. auritus*. Reproductive period was also a significant predictor, with the pre-parturition and post-lactation periods having a negative effect on group size. There was a significant interaction effect between period and species for the pre-parturition period (Table 4), with an interaction plot showing a steeper increase in group size between the pre-parturition and lactation periods for *M. bechsteinii*, compared to *M. nattereri* and *P. auritus* (Fig 2).

The results of the beta regression model for proportion of males showed that maternity group size was negatively associated with the proportion of adult males found in maternity groups (coefficient = -0.041, SE = 0.0041,  $p < 0.001$ ) (Fig 3). The pseudo R-squared for this model was 0.40. Group size had a similar coefficient and p-value when species, period, box type, and/or year were included as fixed effects in this model (coefficients ranged -0.037 to -0.042, all with p values below 0.001). The beta regression model for proportion of non-breeding females in the group also showed that maternity group size was negatively associated with the proportion of non-breeding females found in maternity groups (coefficient = -0.027, SE = 0.0028,  $p < 0.001$ ), with a pseudo R-squared of 0.20 (Fig 3). Similarly, inclusion of species, period, box type, and/or year as fixed effects had limited effects on the coefficients (coefficients ranged -0.027 to -0.031, all with p values below 0.001).

## Discussion

Many factors associated with sexual segregation in vertebrates are poorly understood, and are frequently intertwined with the effects of sexual size dimorphism present in the most common study systems (Ruckstuhl & Neuhaus, 2000). Therefore, insights from bat species, with limited size dimorphism, provide a valuable opportunity to clarify segregation mechanisms, independent of body size dimorphism. Our results showed that the size of the maternity group was an important covariate of inter- and intra-sexual segregation, with males and non-breeding females typically segregated from large maternity groups across all three time periods. However, the lower levels of segregation detected within smaller maternity groups indicate potential benefits of mixing. Additionally, we demonstrated that time period influenced segregation patterns, with peaks in segregation during the lactation period.

Maternity group size was an important covariate of inter- and intra-sexual segregation across all three periods, and corresponded with overall levels of segregation across the three species, with *M. bechsteinii* having the largest group size and the most pronounced segregation, and *P. auritus* having the smallest group size and lowest segregation indices. High group size limits the benefits that non-breeding bats obtain from group living, and increases costs such as contagious parasites and disease, and competition for food (Krause & Ruxton, 2002; Côté & Poulin, 1995). Furthermore, with increased social warming in larger groups, non-breeding bats would not be able to enter torpor to save energy. Group size explained more variation in segregation for males than for non-breeding females from maternity groups. The low R-squared value for non-breeding females indicates that other factors are more important in explaining this variation in segregation, for example, relatedness and social bonds (Zeus *et al.*, 2018).

The negative relationship between group size and the presence of non-breeding bats could be an outcome of differing thermoregulatory requirements, as smaller groups with lower levels of

social warming may allow non-breeding bats to enter torpor. However, with their differing thermoregulatory requirements, and the additional costs associated with maternity roosts, males and non-breeding females would be expected to segregate completely from maternity roosts under this hypothesis. Furthermore, males do not benefit from torpor throughout the whole reproductive period. Social thermoregulation facilitates spermatogenesis in males (Entwistle, Racey & Speakman, 1998), which predominantly occurs during the lactation period for *P. auritus* (Entwistle *et al.*, 1998) and *M. bechsteinii* (Dietz & Horig, 2011), and during the post-lactation period for *M. nattereri* (Linton & Macdonald, 2020). With benefits to be gained from social thermoregulation during this period, males may be expected to avoid costly maternity groups and form male groups to facilitate spermatogenesis, which is not observed in our study systems. Therefore, the consistent negative trend of group size and male presence in the maternity roosts across the whole time period does not lend complete support to this thermoregulatory argument, suggesting other factors are involved.

Our observations of non-breeding bats in small maternity groups throughout the reproductive period suggest that there are additional benefits to roosting alongside females that outweigh the costs for some individuals. Another potential benefit for males is increased breeding opportunities with females in the roost (Senior *et al.*, 2005; Angell *et al.*, 2013). Mating may occur from August to April, with females storing the sperm until fertilization occurs in the spring (Oxberry, 1979). Evidence shows that male Daubenton's bats roosting alongside females have increased mating success compared to segregated males (Senior *et al.*, 2005; Angell *et al.*, 2013), suggesting that males mating early in the nursery roost may have improved sperm storage in the oviduct, leading to greater mating success. These advantages may contribute to a subtle shift in the cost-benefit tradeoff, leading to reduced segregation during the post-lactation period. Overall, group size may be one of many factors involved in the cost-benefit tradeoff for

348 mixed group living, with the costs from higher group sizes tipping the balance towards  
349 segregation.

350  
351 Breeding females benefit greatly from group living, and when they are present in lower  
352 densities, they may need to roost alongside nonbreeding bats to obtain the full benefits of group  
353 living. Therefore, an alternative, but not mutually exclusive, explanation for decreased  
354 segregation within smaller groups relates to population density. This concept has been  
355 demonstrated in studies on ungulates with low population densities and low proportions of  
356 females that show increased frequencies of mixed sex groups (Meldrum & Ruckstuhl, 2009;  
357 Thirgood, 1996).

358  
359 We identified a consistent temporal trend of highest levels of inter- and intra-sexual segregation  
360 during the lactation period. Whilst group size was highest during this period, this peak in sexual  
361 segregation may also be related to the additional costs associated with the lactation period,  
362 including increased competition for food, and high parasite loads due to the vulnerability of  
363 reproducing females and juveniles to parasites (Christe *et al.*, 2000; Lourenço & Palmeirim,  
364 2007). Ectoparasites found on vespertilionid bats include fleas (Ischnopsyllidae), mites  
365 (Spinturnicidae, Macronyssidae, Sarcoptidae, Trombiculidae), batflies (Nycteribiidae), bugs  
366 (Cimicidae), and ticks (Ixodidae, Argasidae) (Zahn & Rupp, 2004). Parasites are particularly  
367 prevalent in maternity roosts due to the reduced immune defence of juveniles and breeding  
368 females (Christe *et al.*, 2000), and the low frequency of grooming behaviour, an activity that  
369 aids removal of ectoparasites, in lactating females (Speakman, 2008) and their offspring  
370 (McLean & Speakman, 1997). Reduced grooming behaviour in juveniles may be related to their  
371 inability to perform this activity (McLean & Speakman, 1997), whilst grooming may be  
372 reduced in lactating females to release energy for lactation (Speakman, 2008). Our analysis of

temporal patterns revealed that *P. auritus* exhibit inter- and intra-sexual segregation during the pre-parturition and lactation periods, a novel insight into the roosting behaviour of this common and widespread species which is typically described as non-segregating in the literature (Entwistle *et al.*, 2000).

Analysis of body weight data confirmed that sexual dimorphism in our study systems was low (<6 % weight different between non reproductive bats), and showed minimal correspondence with levels of sexual segregation, with the species showing the least size dimorphism (*M. bechsteinii*) demonstrating the most pronounced sexual segregation. Whereas in ruminants pronounced sexual size dimorphism can strongly impact factors such as energetic requirements, metabolic rates, predator vulnerability, and activity budgets (Ruckstuhl & Neuhaus, 2000), we did not expect the marginal levels of dimorphism present in our study species to strongly impact these factors.

Overall, our study identified several factors associated with segregation patterns in bats, lending support to a variety of hypotheses for the ultimate cause of segregation. For instance, the patterns of segregation we observed would be expected if segregation mechanisms are related to different thermoregulatory requirements between different classes (sex and breeding status), which is among the leading hypotheses (Altringham *et al.*, 2005). However these same patterns are also congruent with predictions from the activity budget hypothesis, which states that differences in activity budget synchronization can lead to social segregation (Ruckstuhl, 2007). With their higher energetic needs, reproductive females would be expected to spend a greater time foraging compared to the other classes (McLean & Speakman, 1999). Foraging patterns and roost emergence times may vary according to factors such as sex and reproductive condition (Kunz, 1974), therefore, segregated day roosts could be a result of reproductive



females synchronizing their activity budgets, exiting and returning to roosts at similar times. It is possible that many different factors interact, including thermoregulatory requirements, parasite load, resource competition, and access to mating opportunities, that together determine the levels of segregation within roosts.

An explanation for interspecific differences in sexual segregation patterns is still required for these three species. With their similar physiological characteristics, differences in thermoregulatory and energetic requirements cannot explain the different patterns observed between these species. One potential explanation relates to competition for foraging areas close to the roost (Kunz, 1974). Reproductive females have high energy demands, and require high quality foraging habitat in close vicinity to the roost (Henry *et al.*, 2002). *P. auritus* males are known to forage on the edge of the foraging habitat of the females (Entwistle *et al.*, 1997), and therefore pose less competition compared to males that share the same foraging grounds (such as Daubenton's bats). This sex-based difference in foraging activity could explain the decreased levels of segregation observed for this species (Altringham *et al.*, 2005). Overlap in foraging area between the sexes reaches 46% in *M. bechsteinii* (Kerth & Morf, 2004), and is less well studied for *M. nattereri*. Comparative data on sex-based foraging activity may shed light on the differences in roost segregation patterns observed between vespertilionid bat species.

Due to the marginal levels of sexual size dimorphism in our study species, we were able to highlight important factors in sexual segregation mechanisms, independent of the effects of body size, namely group size, female reproductive condition, and time period. Whilst reproductive condition and period have been included in previous studies on roost segregation (Patriquin *et al.*, 2010; Zeus *et al.*, 2018), maternity group size has not been considered previously. This factor was a significant covariate of segregation across all three species, and

423 additionally corresponded with the interspecific differences in segregation that were also  
424 observed. Due to the cost-benefit tradeoff associated with roost size, it is likely that this factor  
425 limits levels of mixing between breeding and non-breeding bats, due to costs such as suboptimal  
426 thermoregulatory conditions, high parasite load, and increased competition. These long-term,  
427 comparative data provided by our study have contributed to the growing base of knowledge on  
428 segregation in bats, e.g. (Altringham *et al.*, 2005; Senior *et al.*, 2005), providing valuable  
429 insights into the mechanisms of segregation patterns.

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## References

- Altringham, J.D. (2014) *British Bats (Collins New Naturalist Library, Book 93)*. London: HarperCollins.
- Altringham, J.D., Senior, P., Ruckstuhl, K. & Neuhaus, P., (2005) Social systems and ecology of bats. In *Sexual Segregation in Vertebrates*: 280 - 302. K. Ruckstuhl & P. Neuhaus (Eds.). Cambridge: Cambridge University Press.
- Angell, R.L., Butlin, R.K. & Altringham, J.D. (2013) Sexual segregation and flexible mating patterns in temperate bats. *PLoS ONE*, **8** - e54194.
- August, T.A., Nunn, M.A., Fensome, A.G., Linton, D.M. & Mathews, F. (2014) Sympatric woodland *Myotis* bats form tight-knit social groups with exclusive roost home ranges. *PLoS ONE*, **9** - e112225.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67** - 1.
- Bon, R. & Campan, R. (1996) Unexplained sexual segregation in polygamous ungulates: a defense of an ontogenetic approach. *Behavioural Processes*, **38**, 131 - 154.
- Bonenfant, C., Gaillard, J.-M., Dray, S., Loison, A., Royer, M. & Chessel, D. (2007) Testing sexual segregation and aggregation: old ways are best. *Ecology*, **88**, 3202 - 3208.
- Bowyer, R.T. (2004) Sexual segregation in ruminants: definitions, hypotheses, and implications for conservation and management. *Journal of Mammalogy*, **85**, 1039 - 1052.
- Brown, C.R. & Brown, M.B. (1986) Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology*, **67**, 1206 - 1218.
- Christe, P., Arlettaz, R. & Vogel, P. (2000) Variation in intensity of a parasitic mite (*Spinturnix myoti*) in relation to the reproductive cycle and immunocompetence of its bat host (*Myotis myotis*). *Ecology Letters*, **3**, 207 - 212.

470 Conradt, L. (1998) Could asynchrony in activity between the sexes cause intersexual social  
 471 segregation in ruminants? *Proceedings of the Royal Society of London. Series B:*  
 472 *Biological Sciences*, **265**, 1359 - 1368.

473 Conradt, L., Ruckstuhl, K. & Neuhaus, P., (2005) Definitions, hypotheses, models and  
 474 measures in the study of animal segregation. In *Sexual Segregation in Vertebrates*: 11  
 475 - 32. K. Ruckstuhl & P. Neuhaus (Eds.). Cambridge: Cambridge University Press.

476 Côté, I.M. & Poulinb, R. (1995) Parasitism and group size in social animals: a meta-analysis.  
 477 *Behavioral Ecology*, **6**, 159 - 165.

478 Dietz, M. & Hörig, A. (2011) Thermoregulation of tree-dwelling temperate bats—a  
 479 behavioural adaptation to force live history strategy. *Folia Zoologica*, **60** - 5.

480 Encarnação, J., Kierdorf, U., Holweg, D., Jasnoch, U. & Wolters, V. (2005) Sex-related  
 481 differences in roost-site selection by Daubenton's bats *Myotis daubentonii* during the  
 482 nursery period. *Mammal Review*, **35** - 285.

483 Entwistle, A., Racey, P. & Speakman, J. (1997) Roost selection by the brown long-eared bat  
 484 *Plecotus auritus*. *Journal of Applied Ecology*, 299 - 408.

485 Entwistle, A., Racey, P. & Speakman, J. (2000) Social and population structure of a gleaner  
 486 bat, *Plecotus auritus*. *Journal of Zoology*, **252**, 11 - 17.

487 Entwistle, A., Racey, P. & Speakman, J.R. (1998) The reproductive cycle and determination  
 488 of sexual maturity in male brown long-eared bats, *Plecotus auritus* (Chiroptera:  
 489 Vespertilionidae). *Journal of Zoology*, **244**, 63 - 70.

490 Ferrari, S. & Cribari-Neto, F. (2004) Beta regression for modelling rates and proportions.  
 491 *Journal of Applied Statistics*, **31**, 799 - 815.

492 Ficetola, G.F., Pennati, R. & Manenti, R. (2013) Spatial segregation among age classes in  
 493 cave salamanders: habitat selection or social interactions? *Population Ecology*, **55**,  
 494 217 - 226.

495 Fleischmann, D. & Kerth, G. (2014) Roosting behavior and group decision making in 2  
 496 syntopic bat species with fission–fusion societies. *Behavioral Ecology*, **25**, 1240 -  
 497 1247.

498 Funakoshi, K. (1991) Reproductive ecology and social dynamics in nursery colonies of the  
 499 Natterer's bat *Myotis nattereri bombinus*. *Journal of the Mammalogical Society of*  
 500 *Japan*, **15**, 61 - 71.

501 Grinevitch, L., Holroyd, S. & Barclay, R. (1995) Sex differences in the use of daily torpor and  
 502 foraging time by big brown bats (*Eptesicus fuscus*) during the reproductive season.  
 503 *Journal of Zoology*, **235**, 301 - 309.

504 Hawkins, E.R., Pogson-Manning, L., Jaehnichen, C. & Meager, J.J. (2019) Social dynamics  
 505 and sexual segregation of Australian humpback dolphins (*Sousa sahulensis*) in  
 506 Moreton Bay, Queensland. *Mar. Mamm. Sci.*, 1 - 22.

507 Henry, M., Thomas, D.W., Vaudry, R. & Carrier, M. (2002) Foraging distances and home  
 508 range of pregnant and lactating little brown bats (*Myotis lucifugus*). *Journal of*  
 509 *Mammalogy*, **83** - 767.

510 Kerth, G. & König, B. (1999) Fission, fusion and nonrandom associations in female  
 511 Bechstein's bats (*Myotis bechsteinii*). *Behaviour*, **136**, 1187 - 1202.

512 Kerth, G. & Morf, L. (2004) Behavioural and genetic data suggest that Bechstein's bats  
 513 predominantly mate outside the breeding habitat. *Ethology*, **110**, 987 - 999.

514 Kerth, G. & Petit, E. (2005) Colonization and dispersal in a social species, the Bechstein's bat  
 515 (*Myotis bechsteinii*). *Molecular Ecology*, **14**, 3943 - 3950.

516 Kerth, G., Safi, K. & König, B. (2002) Mean colony relatedness is a poor predictor of colony  
 517 structure and female philopatry in the communally breeding Bechstein's bat (*Myotis*  
 518 *bechsteinii*). *Behavioral Ecology and Sociobiology*, **52**, 203 - 210.

519 Kerth, G. & van Schaik, J. (2012) Causes and consequences of living in closed societies:  
520 lessons from a long-term socio-genetic study on Bechstein's bats. *Molecular Ecology*,  
521 **21**, 633 - 646.

522 Krause, J. & Ruxton, G.D. (2002) *Living in Groups*. New York: Oxford University Press.

523 Kunz, T.H. (1974) Feeding ecology of a temperate insectivorous bat (*Myotis velifer*).  
524 *Ecology*, **55**, 693 - 711.

525 Levin, E., Roll, U., Dolev, A., Yom-Tov, Y. & Kronfeld-Shcor, N. (2013) Bats of a gender  
526 flock together: sexual segregation in a subtropical bat. *PloS one*, **8** - e54987.

527 Linton, D.M. & Macdonald, D.W. (2018) Spring weather conditions influence breeding  
528 phenology and reproductive success in sympatric bat populations. *Journal of Animal*  
529 *Ecology*, **87**, 1080 - 1090.

530 Linton, D.M. & Macdonald, D.W. (2020) Phenology of reproductive condition varies with  
531 age and spring weather conditions in male *Myotis daubentonii* and *M. nattereri*  
532 (Chiroptera: Vespertilionidae). *Scientific Reports*, **10**, 1 - 10.

533 Lintott, P.R., Bunnefeld, N., Fuentes-Montemayor, E., Minderman, J., Mayhew, R.J., Olley,  
534 L. & Park, K.J. (2014) City life makes females fussy: sex differences in habitat use of  
535 temperate bats in urban areas. *R. Soc. Open Sci.*, **1** - 140200.

536 Lourenço, S. & Palmeirim, J. (2007) Can mite parasitism affect the condition of bat hosts?  
537 Implications for the social structure of colonial bats. *Journal of Zoology*, **273**, 161 -  
538 168.

539 McLean, J. & Speakman, J. (1999) Energy budgets of lactating and non-reproductive brown  
540 long-eared bats (*Plecotus auritus*) suggest females use compensation in lactation.  
541 *Functional Ecology*, **13**, 360 - 372.

542 McLean, J.A. & Speakman, J.R. (1997) Non-nutritional maternal support in the brown long-  
543 eared bat. *Animal Behaviour*, **54** - 1193.

544 Meldrum, G.E. & Ruckstuhl, K.E. (2009) Mixed-sex group formation by bighorn sheep in  
 545 winter: trading costs of synchrony for benefits of group living. *Animal Behaviour*, **77**,  
 546 919 - 929.

547 Myers, P. (1978) Sexual dimorphism in size of vespertilionid bats. *The American Naturalist*,  
 548 **112**, 701 - 711.

549 Oxberry, B. (1979) Female reproductive patterns in hibernating bats. *Reproduction*, **56**, 359 -  
 550 367.

551 Park, K., Masters, E. & Altringham, J. (1998) Social structure of three sympatric bat species  
 552 (Vespertilionidae). *Journal of Zoology*, **244**, 379 - 389.

553 Patriquin, K.J., Leonard, M.L., Broders, H.G. & Garroway, C.J. (2010) Do social networks of  
 554 female northern long-eared bats vary with reproductive period and age? *Behavioral*  
 555 *Ecology and Sociobiology*, **64** - 899.

556 Pretzlaff, I., Kerth, G. & Dausmann, K.H. (2010) Communally breeding bats use  
 557 physiological and behavioural adjustments to optimise daily energy expenditure.  
 558 *Naturwissenschaften*, **97**, 353 - 363.

559 R Core Team (2019) R: A language and environment for statistical computing. R Foundation  
 560 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. -.

561 Ruckstuhl, K. & Neuhaus, P. (2005) *Sexual Segregation in Vertebrates*. Cambridge:  
 562 Cambridge University Press.

563 Ruckstuhl, K.E. (1998) Foraging behaviour and sexual segregation in bighorn sheep. *Animal*  
 564 *Behaviour*, **56**, 99 - 106.

565 Ruckstuhl, K.E. (2007) Sexual segregation in vertebrates: proximate and ultimate causes.  
 566 *Integrative and Comparative Biology*, **47**, 245 - 257.

567 Ruckstuhl, K.E., Clutton-Brock, T. & Neuhaus, P., (2005) Sexual segregation and the ecology  
568 of the two sexes. In *Sexual Segregation in Vertebrates*: 3 - 8. K. Ruckstuhl & P.  
569 Neuhaus (Eds.). Cambridge: Cambridge University Press

570 Ruckstuhl, K.E. & Neuhaus, P. (2000) Sexual segregation in ungulates: a new approach.  
571 *Behaviour*, **137**, 361 - 377.

572 Ruckstuhl, K.E. & Neuhaus, P. (2002) Sexual segregation in ungulates: a comparative test of  
573 three hypotheses. *Biological Reviews*, **77**, 77 - 96.

574 Russo, D. (2002) Elevation affects the distribution of the two sexes in Daubenton's bats  
575 *Myotis daubentonii* (Chiroptera: Vespertilionidae) from Italy. *Mammalia*, **66**, 543 -  
576 552.

577 Senior, P., Butlin, R.K. & Altringham, J.D. (2005) Sex and segregation in temperate bats.  
578 *Proceedings of the Royal Society B: Biological Sciences*, **272**, 2467 - 2473.

579 Sikes, R.S. & Gannon, W.L. (2011) Guidelines of the American Society of Mammalogists for  
580 the use of wild mammals in research. *Journal of Mammalogy*, **92**, 235 - 253.

581 Silk, J.B. (2007) The adaptive value of sociality in mammalian groups. *Philosophical*  
582 *Transactions of the Royal Society B: Biological Sciences*, **362**, 539 - 559.

583 Singh, N.J., Bonenfant, C., Yoccoz, N.G. & Côté, S.D. (2010) Sexual segregation in Eurasian  
584 wild sheep. *Behavioral Ecology*, **21**, 410 - 418.

585 Speakman, J.R. (2008) The physiological costs of reproduction in small mammals. *Philos.*  
586 *Trans. R. Soc. B-Biol. Sci.*, **363** - 375.

587 Stevens, R.D., Johnson, M.E. & McCulloch, E.S. (2013) Absolute and relative secondary-  
588 sexual dimorphism in wing morphology: a multivariate test of the 'Big Mother'  
589 hypothesis. *Acta Chiropterologica*, **15**, 163 - 170.

590 Thirgood, S. (1996) Ecological factors influencing sexual segregation and group size in  
591 fallow deer (*Dama dama*). *Journal of Zoology*, **239** - 783.



- Tuttle, M.D. (1979) Status, causes of decline, and management of endangered gray bats. *The Journal of Wildlife Management*, 1 - 17.
- Wang, M., Alves, J., da Silva, A.A., Yang, W. & Ruckstuhl, K.E. (2018) The effect of male age on patterns of sexual segregation in Siberian ibex. *Scientific Reports*, 8 - 13095.
- Warton, D.I., Lyons, M., Stoklosa, J. & Ives, A.R. (2016) Three points to consider when choosing a LM or GLM test for count data. *Methods in Ecology and Evolution*, 7, 882 - 890.
- Wearmouth, V.J. & Sims, D.W. (2008) Sexual segregation in marine fish, reptiles, birds and mammals: behaviour patterns, mechanisms and conservation implications. *Advances in Marine Biology*, 54, 107 - 170.
- Williams, D.F. & Findley, J.S. (1979) Sexual size dimorphism in vespertilionid bats. *American Midland Naturalist*, 113 - 126.
- Willis, C., (2006) Daily heterothermy by temperate bats using natural roosts. In *Functional and Evolutionary Ecology of Bats*: 38 - 55. A. Zubaid, G.F. McCracken & T.H. Kunz (Eds.). New York: Oxford University Press.
- Zahn, A. & Rupp, D. (2004) Ectoparasite load in European vespertilionid bats. *Journal of Zoology*, 262 - 383.
- Zeus, V.M., Reusch, C. & Kerth, G. (2018) Long-term roosting data reveal a unimodular social network in large fission-fusion society of the colony-living Natterer's bat (*Myotis nattereri*). *Behavioral Ecology and Sociobiology*, 72 - 99.

## Figure Legends

**Figure 1.** Patterns of segregation between females of different breeding status, males, and juveniles (FoNB = failed or non-breeding female, BF = breeding female, M = male, J = juvenile) across the pre-parturition (PP), lactation (L), and post lactation (PL) periods. The SSAS indicates significant segregation if the observed value (circular point) falls above the SSAS expected interval (shaded area), aggregation if it falls below the shaded area, and random association if it falls within the shaded area.

**Figure 2.** Interaction plot displaying the interaction between species and reproductive period (the pre-parturition (PP), lactation (L), and post lactation (PL) periods), with mean maternity group size as a response variable.

**Figure 3.** Fitted beta regression model for a) proportion of males in maternity groups as response variable and maternity group size as fixed effect, and b) proportion of non-breeding females in maternity group as response variable and maternity group size as fixed effect.

## Tables

Site code	Approximate size (ha)	Box types	Species monitored
a	400	Schwegler™ 2M Schwegler™ 1B	<i>M. nattereri</i> <i>P. auritus</i>
b	46	Schwegler™ 2FN Schwegler™ 1FW	<i>M. bechsteinii</i>
c	47	Schwegler™ 1FF Schwegler™ 1FS Schwegler™ 2F Schwegler™ 2FN	<i>M. nattereri</i> <i>P. auritus</i>
d	91	Schwegler™ 1FF Schwegler™ 2F Schwegler™ 2FN Home-made woodcrete	<i>M. bechsteinii</i> <i>P. auritus</i>
e	28	Schwegler™ 1FF Schwegler™ 1FD Schwegler™ 1FS Schwegler™ 2F Schwegler™ 2FN	<i>M. nattereri</i> <i>P. auritus</i>
f	1	Unknown	<i>M. bechsteinii</i> <i>P. auritus</i>
g	59	Schwegler™ 1FF Schwegler™ 1FS Schwegler™ 2F Schwegler™ 2FN	<i>M. nattereri</i> <i>P. auritus</i>
h	17	Schwegler™ 2FN	<i>M. bechsteinii</i>

**Table 1.** Site information from anonymized bat monitoring schemes in S.W. England, including species monitored, approximate area of woodland site, and artificial roost types. Box types ranged 26-44 cm in height. All Schwegler™ boxes had a round base ranging 12-28 cm in diameter, apart from the square Schwegler™ 1FF, which had a depth of 14 cm. Only species that are relevant to this study are included among species monitored for each site.

Species	<i>M. bechsteinii</i>	<i>M. nattereri</i>	<i>P. auritus</i>
No. roosts	323	484	188
No. detections adult males	124	427	234
No. detections breeding females	1126	1552	360
No. detections failed or non-breeding females	711	651	115
No. detections juvenile males	519	537	123
No. detections juvenile females	487	482	118

**Table 2.** Collated data included in SSAS analysis.

Random effects	Variance	Standard deviation	
Individual ID (intercept)	0.120	0.346	
Year	0.136	0.368	
Residual	0.174	0.417	
Fixed effects	Estimate	Standard error	P value
Intercept	8.72	0.115	< 0.001 ***
<i>M. nattereri</i>	-0.888	0.100	< 0.001 ***
<i>P. auritus</i>	-0.829	0.104	< 0.001 ***
Male	-0.404	0.0364	< 0.001 ***
Non-breeding	-0.115	0.0638	0.0710
Post-lactating	0.209	0.0644	0.00121 **
Pregnant	1.05	0.0733	< 0.001 ***

**Table 3.** Results of linear mixed effects model with body weight as a response, and fixed effects including species, sex, and reproductive status, and a random intercept effects of individual ID and year.

Fixed effects	Estimate	Standard error	P value
Intercept	3.00	0.0296	< 0.001 ***
<i>M. nattereri</i>	-0.650	0.0380	< 0.001 **
<i>P. auritus</i>	-0.595	0.0637	< 0.001 ***
Post lactation period	-0.425	0.0390	< 0.001 ***
Pre-parturition period	-0.795	0.0453	< 0.001 ***
Post lactation: <i>M. nattereri</i>	-0.0530	0.0589	0.368
Pre-parturition: <i>M. nattereri</i>	0.534	0.0644	< 0.001 ***
Post lactation: <i>P. auritus</i>	0.190	0.100	0.0591
Pre-parturition: <i>P. auritus</i>	0.329	0.0908	< 0.001 ***

**Table 4.** Results of generalized linear model with a Poisson error distribution and group size as a response variable. Fixed effects included species and reproductive period, and an interaction effect of species and period.

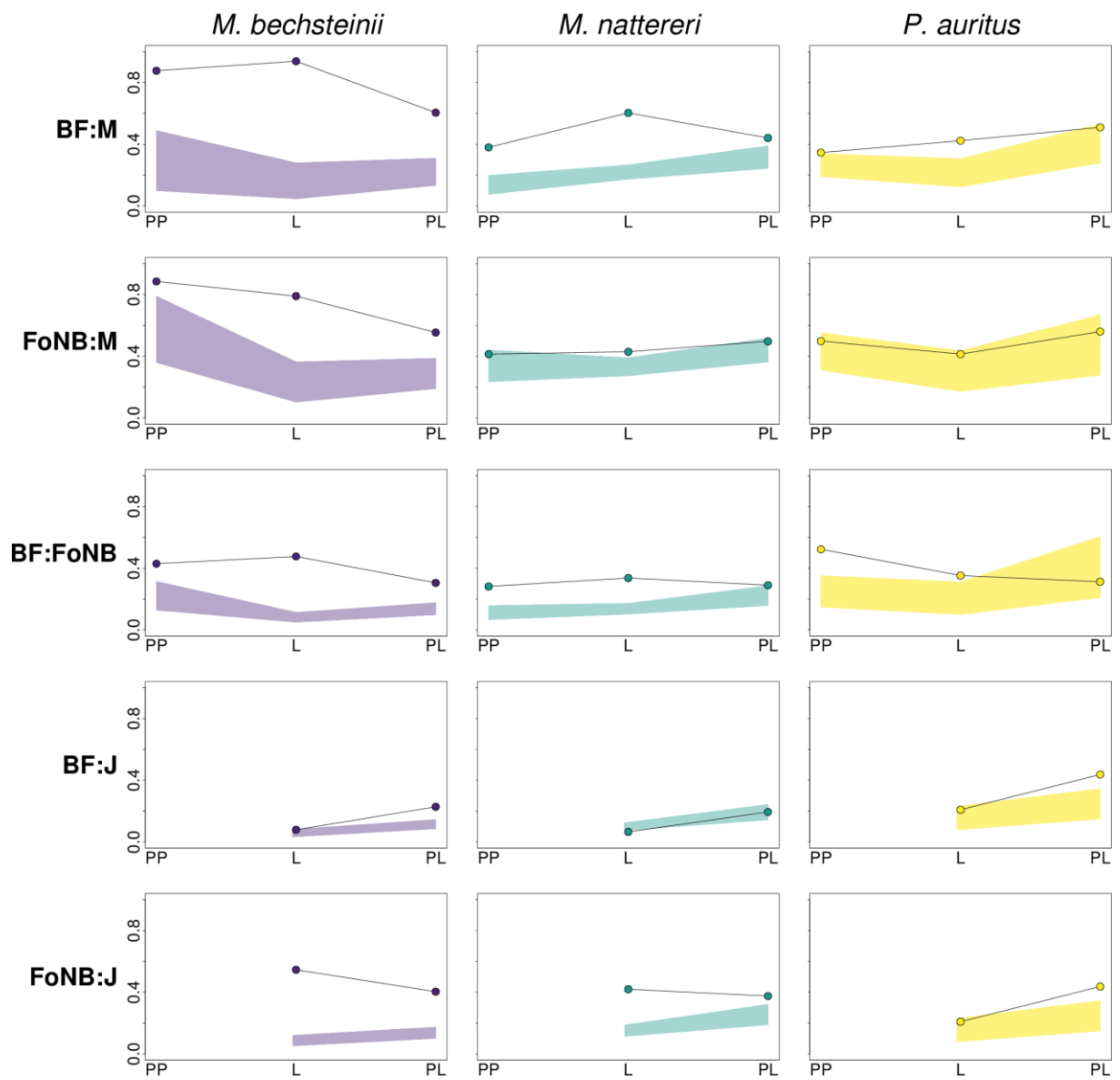


Figure 1

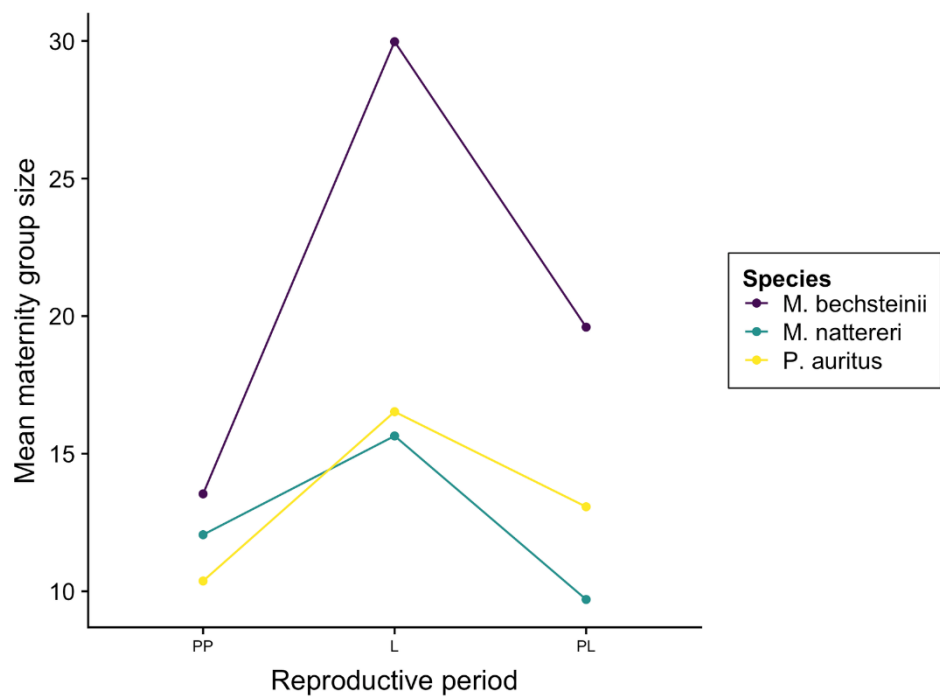


Figure 2



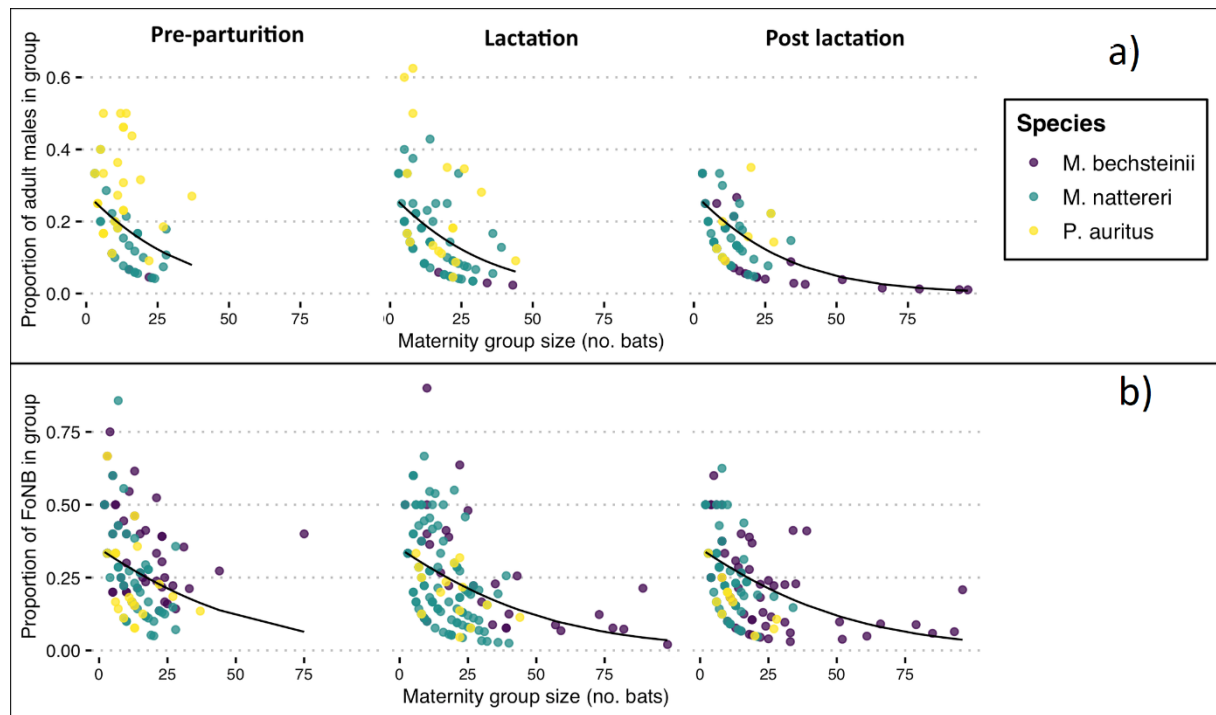


Figure 3