

Máster en BIODIVERSIDAD

Efectos en la eficacia biológica del solapamiento de cría y muda en el papamoscas cerrojillo (*Ficedula hypoleuca*)

Moult-breeding overlap effect on the fitness
of the pied flycatcher (*Ficedula hypoleuca*)

Ignacio Mollá Cabrera

Director: David Canal Piña (MNCN-CSIC)

Iraida Redondo García (MNCN-CSIC)

INDEX

| | |
|---|----|
| 1. ABSTRACT | 3 |
| 2. RESUMEN | 3 |
| 3. INTRODUCTION | 5 |
| 4. MATERIALS AND METHODS | 8 |
| 4.1 Study area and system | 8 |
| 4.2 Population monitoring and data collection | 9 |
| 4.3 Moulting data | 10 |
| 4.4 Fitness measurements | 10 |
| 4.5 Statistical analyses | 10 |
| 4.5.1 Overall patterns | 10 |
| 4.5.2 Reproductive success analyses | 10 |
| 4.5.3 Survival analysis | 11 |
| 5. RESULTS | 12 |
| 5.1 Moulting-breeding overlap differences between sexes and ages | 12 |
| 5.1.1 Sexes | 12 |
| 5.1.2 Ages | 12 |
| 5.2 Reproductive consequences of moulting-breeding overlap | 13 |
| 5.3 Survival consequences of moulting-breeding overlap | 16 |
| 6. DISCUSSION | 19 |
| 7. CONCLUSIONS | 19 |

1. ABSTRACT

Breeding and moulting are two energy and time-demanding processes; thus birds usually separate it temporarily. Long-distance migratory birds are generally time-constrained because they must moult after breeding and before autumn migration. Under time-constrained conditions, breeding and post-nuptial moult may overlap. However, the current and future consequences on fitness of this overlap remain unclear, such as possible reproductive costs resulting from reduced parental care associated with moulting individuals. Here, we used data from 18 years of monitoring of an Iberian pied flycatcher (*Ficedula hypoleuca*) population to investigate whether overlapping breeding and moult influences the current reproductive success and/or survival of the individuals, while simultaneously considering the moulting status of the breeding mate. The number of fledglings was not related to the moult status of the parents (female moults, male moults, neither moult, both moult). However, our findings suggest that pairs in which only the female moults produce more recruits than pairs in which both parents moult. Survival was not associated with the moult status of the individuals, either independently or in combination with that of their mate. These results suggest that overlapping breeding and moulting may be an adaptive strategy in the pied flycatchers, without evident fitness costs, but beneficial in avoiding time constraints related to moulting preceding the autumn migration.

Keywords: *Ficedula hypoleuca*, fitness consequences, moult-breeding overlap, reproduction, survival.

2. RESUMEN

La reproducción y la muda son dos procesos que requieren energía y tiempo, por lo que las aves suelen separarlos temporalmente. Las aves migratorias de larga distancia suelen

tener limitaciones temporales porque deben mudar después de la reproducción y antes de la migración otoñal. En condiciones de tiempo limitado, la reproducción y la muda postnupcial pueden solaparse. Sin embargo, las consecuencias actuales y futuras de este solapamiento sobre la eficacia biológica siguen sin estar claras, como, por ejemplo, los posibles costes reproductivos derivados de un menor cuidado parental asociado a los individuos que mudan. En este trabajo se utilizaron datos de 18 años de seguimiento de una población de papamoscas cerrojillo ibérico (*Ficedula hypoleuca*) para investigar si el solapamiento de la reproducción y la muda influye en el éxito reproductivo actual y/o en la supervivencia de los individuos, considerando simultáneamente el estado de muda de la pareja reproductora. El número de volantones no estuvo relacionado con el estado de muda de los progenitores (hembra mudando, padre mudando, ninguno muda, ambos mudan). Sin embargo, nuestros resultados sugieren que las parejas en las que sólo muda la hembra producen más reclutas que las parejas en las que mudan ambos progenitores. La probabilidad de supervivencia de las aves no se asoció con el estado de muda de los individuos, ni independientemente ni en combinación con el de su pareja. Estos resultados sugieren que el solapamiento de la cría y la muda podría ser una estrategia adaptativa en los papamoscas cerrojillos, sin costes evidentes de fitness, pero beneficiosa para evitar las limitaciones temporales relacionadas con la muda que precede a la migración otoñal.

Palabras clave: consecuencias en la eficacia biológica, *Ficedula hypoleuca*, reproducción, solapamiento de cría y muda, supervivencia.

3. INTRODUCTION

One assumption in life-history theory is that individuals face trade-offs in how they allocate resources between current reproduction, future reproduction, and survival (Williams, 1966; Stearns, 1976; Stearns, 1992). This means that a greater reproductive effort may lead to lower survival or reproductive success in the following season, since such effort is associated with a depletion of available energy resources (Van Noordwijk & De Jong, 1986; Stearns, 1992). In birds, it has been shown that an increased investment of resources in reproduction can negatively affect the survival of individuals (Askenmo, 1979; Reid, 1987) and their future reproduction (Gustafsson & Sutherland, 1988; Nur, 1988).

One of the three phenological events considered most energetically costly in the life cycle of birds is moulting (Lindström *et al.*, 1993), along with breeding and migration (Newton, 2009; Bridge, 2011), the latter only occurring in temperate and polar species (ca. 20% of bird species; Kirby *et al.*, 2008). Moulting is a periodic and scheduled process performed by all birds, in which they replace part or all their plumage (Jenni & Winkler, 2020a), and requires a high energy investment (Murphy, 1996). Feathers are crucial for flight (Jenni & Winkler, 2020a), visual communication and camouflage (Jenni & Winkler, 2020b), so they play a key role in the fitness (reproduction and/or survival) of individuals. However, feathers wear out due to different factors, including mechanical abrasion (Butler & Johnson, 2004), UV-B radiation (Bergman, 1982), and bacteria (Burt & Ichida, 1999), so their functionality decreases over time (Jenni & Winkler, 2020a) and must be replaced during moulting. In addition to the energy expenditure of producing new feathers, moulting involves having gaps in the plumage, which can compromise flight ability (Swaddle *et al.*, 1996; Hemborg & Lundberg, 1998; Hedenström, 2003) and thermoregulation (Jenni & Winkler, 2020b). Because of these energetic and phenotypic demands, the timing of the moult is under strong selective pressure, and birds often resort to the temporal separation of moult from other costly events such as breeding and migration (Barta *et al.*, 2006, 2008). In this way, they avoid having to simultaneously perform two processes that require high energy expenditure, for which there are well-established trade-offs. For example, a negative correlation has been found between the intensity of parental moulting and care of their young (Morton & Morton, 1990; Svensson & Nilsson, 1997) and the quality of their fledglings (as a linear regression of body weight on tarsus length; Hemborg & Lundberg, 1998). A high reproductive effort may also

negatively affect the production of new feathers (Nilsson & Svensson, 1996) or delay the onset of moulting (Siikamäki *et al.*, 1994). This means that birds have limited energy resources that must be allocated properly between moulting and reproduction to perform both activities adequately. Thus, investment of resources in reproduction affects investment in moulting and vice versa, and in turn, these events may affect survival; therefore, moulting has been proposed as a mechanism mediating the trade-off between current reproductive success and future success and/or survival (Siikamäki *et al.*, 1994; Nilsson & Svensson, 1996; Hemborg *et al.*, 2001).

Energetic trade-offs create temporal challenges in incorporating different life history stages in a single year, and given that migration is a time (Jenni & Winkler, 2020b) and energy-demanding process (Lindström *et al.*, 1993, Murphy, 1996), such challenges are particularly important for long-distance migrants (Hall & Fransson, 2000; Wingfield, 2008). Migratory passerines often moult in the short interval between breeding and autumn migration (Jenni & Winkler, 2020b) and are thus particularly time-constrained for this task (Holmgren & Hedenström, 1995; Barta *et al.*, 2008). In addition, there is a trade-off in how fast they can moult, as accelerating the moult process can negatively affect the quality of new feathers (Dawson *et al.*, 2000; Vagasi *et al.*, 2012), so the timing of moult in the annual cycle has large fitness consequences. Besides, moulting during migration is very costly, including high mortality rates (Merilä, 1997), so in long-distance migrants, moult has been selected to end before migration begins (Pulido & Coppack, 2004). Thus, birds with a tight reproductive schedule may overlap their post-nuptial moult with nestling rearing (Jenni & Winkler, 2020b), which may be an appropriate strategy if the costs of overlapping moult and breeding are lower than those of moulting during migration or delaying migration (Merilä, 1997; Tomotani *et al.*, 2018).

Several factors may affect the onset of the post-nuptial moult and/or migration and thus the overlap between these two activities. Male and female birds often show different energetic investments and/or roles in parental care (Newton, 2011). Males tend to allocate fewer resources to it, which allows them to cope with the energetic demands of initiating moult earlier than females (Jenni & Winkler, 2020b). The age of individuals may also influence the onset of moult. In many long-distance migrants, juveniles (1-year birds) tend to arrive later on the breeding areas (Newton, 2023), so young individuals usually have less time to breed and moult before returning to the breeding grounds, resulting in a more probable overlap between the two activities (Siikamäki *et al.*, 1994;

Hemborg *et al.*, 1998; Hemborg *et al.*, 2001). Besides intrinsic causes, environmental factors may modulate the timing of moult, mainly photoperiod and temperature (Jenni & Winkler, 2020b). In this regard, as climate change is altering breeding phenology in multiple taxa and the start-end of breeding is also a main factor determining timing and speed of moult, it may be expected corresponding changes in the onset and speed of the post-breeding moult in response to climate change (Jenni & Winkler, 2020b).

The pied flycatcher *Ficedula hypoleuca* is a long-distance migrant that breeds in most of Europe and winters in tropical Africa (Lundberg & Alatalo, 2010). This species undergoes a complete moult in summer, after breeding and before autumn migration (Lundberg & Alatalo, 2010). Moult-breeding overlap has been observed in several European populations of the species (e.g., Siikamäki *et al.*, 1994; Hemborg & Lundberg, 1998; Hemborg, 1999a; Tomotani *et al.*, 2018), making it a suitable taxon to study the fitness consequences of this moulting strategy. In European populations, several studies on pied flycatchers have found a negative effect of overlap on reproductive success (Hemborg & Lundberg, 1998; Hemborg, 1999a; Morales *et al.*, 2007), while others have found no effect (Hemborg, 1999b; Tomotani *et al.*, 2018). Overlapping is more frequent in males than in females (Hemborg *et al.*, 1998; Hemborg, 1999a; Moreno *et al.*, 2001). When the males moult while breeding, they may have a reduced flight performance (Swaddle *et al.*, 1996; Hemborg & Lundberg, 1998; Hedenström, 2003), reduce nestling care, visit the nest less often (Tomotani *et al.*, 2018) or even abandon the young to start moulting (Hemborg, 1999a). However, females are, in many cases, able to compensate for the reduced parental care of males, thus buffering the negative effect on the offspring (Hemborg, 1999a; Tomotani *et al.*, 2018). According to this, males may provide less parental care in general, and given that high reproductive effort can lead to lower future survival (Askenmo, 1979; Reid, 1987), females may suffer a reduced survival by compensating for male parental care (Tomotani *et al.*, 2018). However, females may adjust their clutch size according to the perceived probability of moulting during breeding (Hemborg, 1999a), even removing eggs or recently hatched nestlings from the nest (Lobato *et al.*, 2006). Alternatively, females might delay moult due to an increased reproduction effort, provoked by a large clutch size (Siikamäki *et al.*, 1994) or a reduced parental effort of their mates (Hemborg, 1999b). Overall, the underlying mechanisms driving moult-breeding overlap and its effects on the reproductive success and/or survival of individuals remain unclear.

In this study, we used data from 18 breeding seasons (2005-2023) of an Iberian population of pied flycatchers (*Ficedula hypoleuca iberiae*) to investigate the fitness consequences for males and females of overlapping breeding and moult. Although there are some studies about moult-breeding overlap in different flycatcher populations, the Iberian pied flycatcher is catalogued as a subspecies, and it has a shorter migration and different environmental conditions, and therefore different selective pressures than the other European populations. The fitness consequences of overlapping breeding and moult in these populations have been poorly and only partially studied (Lobato *et al.*, 2006; Morales *et al.*, 2007). We explored the effects of overlapping breeding and moult on the number of fledglings considering the moult status of the breeding pair. As the consequences of overlapping breeding and moult may not be evident in the short term, but reflected in other indicators of fitness, we also investigated the consequences of this overlap on the number of recruits (birds that return the following years to the study area to breed) and survival of the individuals. Based on previous knowledge, we expect negative effects in the short (number of fledglings) or long term (number of recruits, survival) in moult-breeding overlapping individuals, being these effects more pronounced when both members of the pair are moulting while breeding.

4. MATERIALS AND METHODS

4.1 Study area and system

The pied flycatcher is a long-distance migrant passerine that presents a sexual dimorphism in plumage, more marked in the breeding season, and nests in cavities. It is a single-brooded and mainly monogamous bird, although some males (<15%, Canal *et al.*, 2021) occupy more than one nest cavity and attract additional females, becoming polygamous (Alatalo & Lundberg, 1984; Canal *et al.*, 2020).

The data used were collected between 2005 and 2023 from a population of pied flycatchers breeding in nest boxes in La Hiruela (41°04' N, 3°27' W, Madrid, Central Spain). The study area is composed of two different habitats: a 9.3 ha deciduous oak forest (*Quercus pirenaica*) and a 4.8 ha mixed pine plantation (dominated by *Pinus sylvestris*), both habitats separated by a 1 km wide strip unsuitable for the breeding of the species, due to the lack of cavities needed for nesting. There are 156 nest boxes in the oak forest

and 83 in the pine forest, and in both habitats, the nest boxes are at 20 m on average from each other, with a standard error of 9.2 m.

4.2 Population monitoring and data collection

During the breeding season, from the third week of April, when the first males arrived, until the first half of July, nest boxes were checked every 3 days for flycatcher occupancy. After occupancy, they were checked daily to determine the laying date, clutch size, hatching date, and number of chicks in each nest. Adult flycatchers were captured with a nest-trap 8 days after hatching, at which time unringed birds were marked with a numbered metal ring. Breeding individuals captured for the first unringed were considered immigrants. Immigrants were assigned an age of one year or older based on plumage characteristics following the criteria of Karlsson *et al.* (1986) and Potti & Montalvo (1991). Many individuals are born in the nest boxes, thus its age is known (50% approx). The sex of each individual was also determined based on plumage, as there is a marked sexual dimorphism in plumage during the breeding season. Standard morphological measurements were taken for each individual: tarsus length (± 0.01 mm), wing length (± 0.5 mm), body mass (± 0.1 g) and size of the frontal white spot (± 0.01 mm²). The area of the latter was calculated as patch height x width, in both males and females.

All fledglings were ringed at 13 days old, just before they leave the nest. This allows us to record the number of fledglings and the number of recruits (the number of fledglings that return to the study area to breed in the following years) of each pair. The studied population shows strong natal and breeding site fidelity and has one of the species' highest recruitment rates reported so far (Canal *et al.*, 2014; Potti & Montalvo, 1991b). Further, there is no familial resemblance in dispersal propensity (Camacho *et al.*, 2016), and breeding either in the surroundings of study plots or in other nearby populations (as indicated by ring recoveries and searches for singing males) are sporadic events (Potti & Montalvo, 1991b). The above evidence suggests that most surviving individuals bred in the study population and that the fraction of surviving individuals is unbiased, broadly representing all surviving individuals.

4.3 Moulting data

Moulting data were collected at the time of adult capture (8 days after hatching). It was recorded whether each individual had begun the moulting process (individuals with one or more primary feathers absent or with new feathers growing) or not (individuals with all primary feathers old), and a moulting score was assigned to represent how advanced the moulting was at that time. For each feather, the scoring system was as follows: old feather=0, drooping feather=1, rachis=2, 1/5 new feather=3, 2/5=4, 3/5=5, 4/5=6, 5/5=7. The scores of all primary feathers of both wings were then summed.

4.4 Fitness measurements

To measure the fitness of each individual, we divided it into two components: current reproductive success and survival. The reproductive success was measured as the number of fledglings and the number of recruits (see above), while adult survival was assessed by whether the individuals returned to breed in subsequent years. As noted above, pied flycatchers in this population exhibit strong natal and breeding fidelity. Moreover, the vast majority of individuals (>95%) display continuous breeding histories, meaning that once they begin breeding in the population, they consistently return to breed in subsequent years. Therefore, if an individual does not return, it is reasonable to assume that it has likely died.

4.5 Statistical analyses

4.5.1 Overall patterns

To explore the influence of sex and age on the probability of overlapping breeding and moulting, we used a database in which each observation is an individual bird (n= 4275; 2031 males and 2244 females) and fitted a generalized linear mixed model (GLMM) with a binomial distribution in which we included laying date, habitat, age and sex as fixed effects, while year and the bird identities were included as random effects.

4.5.2 Reproductive success analyses

To investigate the reproductive consequences of overlapping breeding and moulting, we used a database in which each observation is a flycatcher pair, eliminating all rows in which the identity of one or both individuals in the pair is not recorded (ca 3% of cases), so that all observations contain a male and a female (n= 1959 observations). We created a new categorical variable "moulting_pair" with 4 levels, categorizing the moulting status of

the two adults in the pair: both members of the pair moulting, only female moulting, only male moulting and neither moulting.

To examine the effect of overlapping breeding and moulting on the number of fledglings and recruits (our two reproductive dependent variables), we fitted two GLMM, with a Conway-Maxwell-Poisson distribution and a zero-inflation component, due to the excess of zeros in both dependent variables. Both models included as explanatory terms, habitat (two-level class variable: oak or pine forest), laying date, age of males and age of females (two-level class variable: first-year old and older), and moult pair (four-level class: both moulting, male moulting, female moulting, none moulting). The models included year, male identity and female identity as random effects, to take into account the nonindependence of samples between individuals and breeding seasons. We also investigate the differences in the effect between the moult_pair levels by performing multiple comparisons of means with a Tukey post-hoc test.

4.5.3 Survival analysis

To investigate if overlapping breeding and moult affects individuals' survival, we used the same database as for reproductive success analyses, and fitted two GLMM, one for males and other for females, with a binomial distribution. In each model, we included as explanatory terms, habitat, laying date, age of the sex analyzed (two-level class variable: first-year old and older) and moult_pair (four-level class: both moulting, male moulting, female moulting, none moulting). The moult-pair variable was used because the overlap of one member of the pair could affect the survival of the other member. The year and the identity of the individual were included as random effects.

Statistical analyses were performed in Rstudio 2024.04.2+764 with the packages *lme4* (Bates et al. 2014) and *glmmTMB* (Brooks et al. 2017). We used the *Tidyverse* (Wickham et al. 2019) package to visualize, manipulate and transform data. The significance of the fixed effects was calculated with Type III Wald Chi-square tests using the function *Anova* in the *car* (Fox & Weisberg, 2019) package. The *glht* function of the *Multcomp* (Hothorn et al. 2008) package was also used to do the multiple comparisons of means between the different moult_pair levels. The *ggplot* (Wickham, 2016) package was used to make the graphs.

5. RESULTS

5.1 Moulting-breeding overlap differences between sexes and ages

5.1.1 Sexes

A total of 2031 males and 2244 females were included in the study. Of these, 21.2% (432) males and 6% (133) females had initiated moulting while feeding the nestlings. Accordingly, the probability of overlapping breeding and moulting was influenced by the sex, being more frequent in males than females (GLMM, Z Wald = -8.871, $p = <0.001$; Table 1; Fig 1).

5.1.2 Ages

There are a total of 3579 adults (>1 year) and 696 young individuals (1 year). Of these, 10.5 % (377) adults and 27% (188) young overlapped breeding and moulting. Analyses by sex, of the 247 young males, 53.4% (132) overlapped breeding and moulting, and of the 449 young females, 12.4% (56) did so. Overall, the age did not affect the probability of overlapping moulting and breeding (GLMM, Z Wald = -1.764, $p = 0.078$; Table 1; Fig 1).

Table 1. Results of the generalized linear mixed model (GLMM) analyzing the factors influencing the probability of overlapping breeding and moulting.

| Random effects | Variance | Std.Dev. | | | |
|----------------|----------|------------|---------|------------------------|------------------|
| Year | 0.188 | 0.434 | | | |
| Identity | 12.878 | 11.348 | | | |
| Fixed effects | Estimate | Std. Error | z value | Odds ratio (CI 95%) | <i>p</i> |
| Intercept | -416.336 | 0.499 | -8.35 | 0.015 (0.006, 0.04) | <0.001 |
| Laying date | 0.155 | 0.017 | 9.12 | 1.17 (1.13, 1.21) | <0.001 |
| Habitat (pine) | -0.250 | 0.107 | -2.33 | 0.78 (0.63, 0.96) | 0.02 |
| Age (adult) | -0.213 | 0.121 | -1.76 | 0.81 (0.64, 1.02) | 0.078 |
| Sex (female) | -189.054 | 0.213 | -8.87 | 0.15 (0.1, 0.23) | <0.001 |

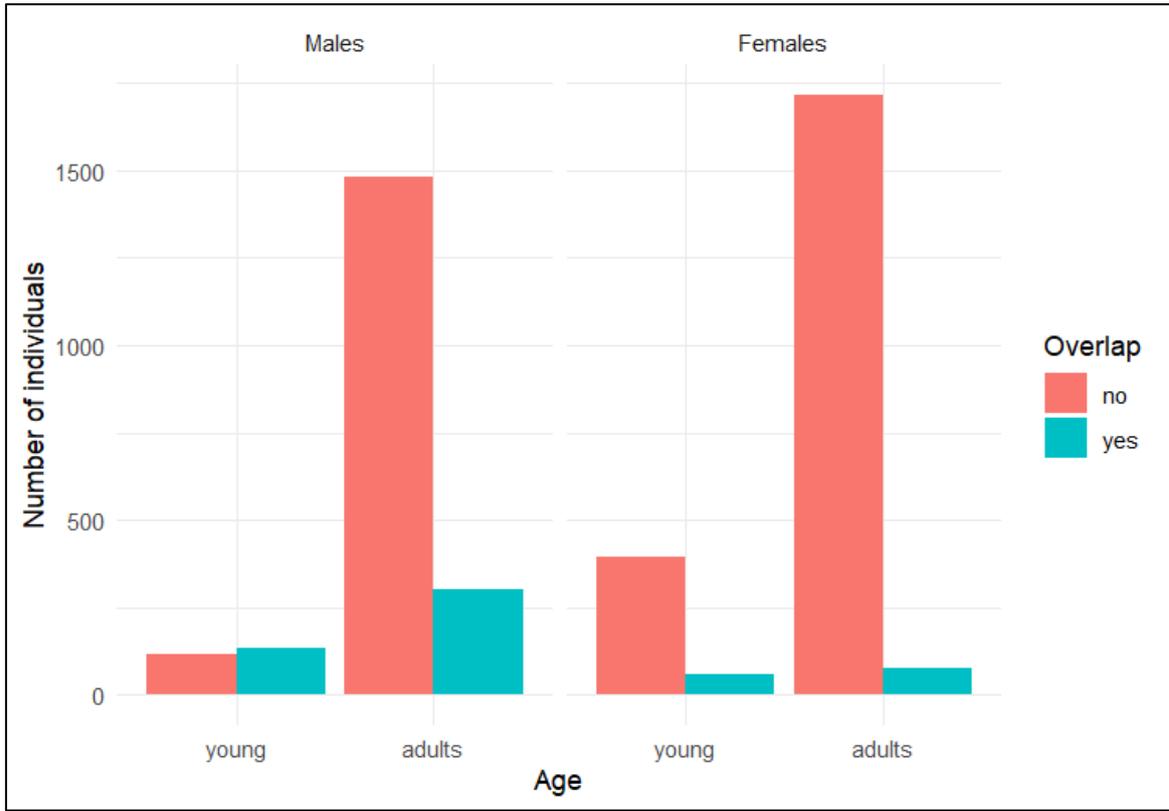


Figure 2. Number of individuals overlapping breeding and moult, separated between sexes and ages. Sample size = 4275 individuals.

5.2 Reproductive consequences of moult-breeding overlap

The moulting status of the breeding pair (both members moulting, none moulting, female moulting, male moulting) was unrelated to the number of fledglings (Fig 2; Table 2).

Table 2. Results of the generalized linear mixed model (GLMM) analyzing the influence of different factors, including different overlap pairs (male moulting, female moulting, neither moulting), on the number of fledglings of the pairs. N = 1959 pairs.

| Random effects | Variance | Std.Dev. | | |
|-----------------|-----------|------------|---------|---------|
| Year | 9.277E-03 | 0.096 | | |
| Female identity | 1.732E-03 | 0.042 | | |
| Male identity | 1.627E-07 | 1.276E-05 | | |
| Fixed effects | Estimate | Std. Error | z value | p |
| Intercept | 1.414 | 0.055 | 25.57 | < 0.001 |

| | | | | |
|--------------------|--------|-------|-------|----------------|
| Laying date | -0.088 | 0.009 | -9.34 | < 0.001 |
| Habitat (pine) | -0.038 | 0.013 | -2.82 | 0.005 |
| Female age (adult) | 0.031 | 0.018 | 1.76 | 0.078 |
| Male age (adult) | 0.031 | 0.022 | 1.44 | 0.150 |
| Neither moulting | 0.045 | 0.047 | 0.95 | 0.344 |
| Female moulting | 0.042 | 0.066 | 0.63 | 0.526 |
| Male moulting | 0.038 | 0.046 | 0.84 | 0.4 |

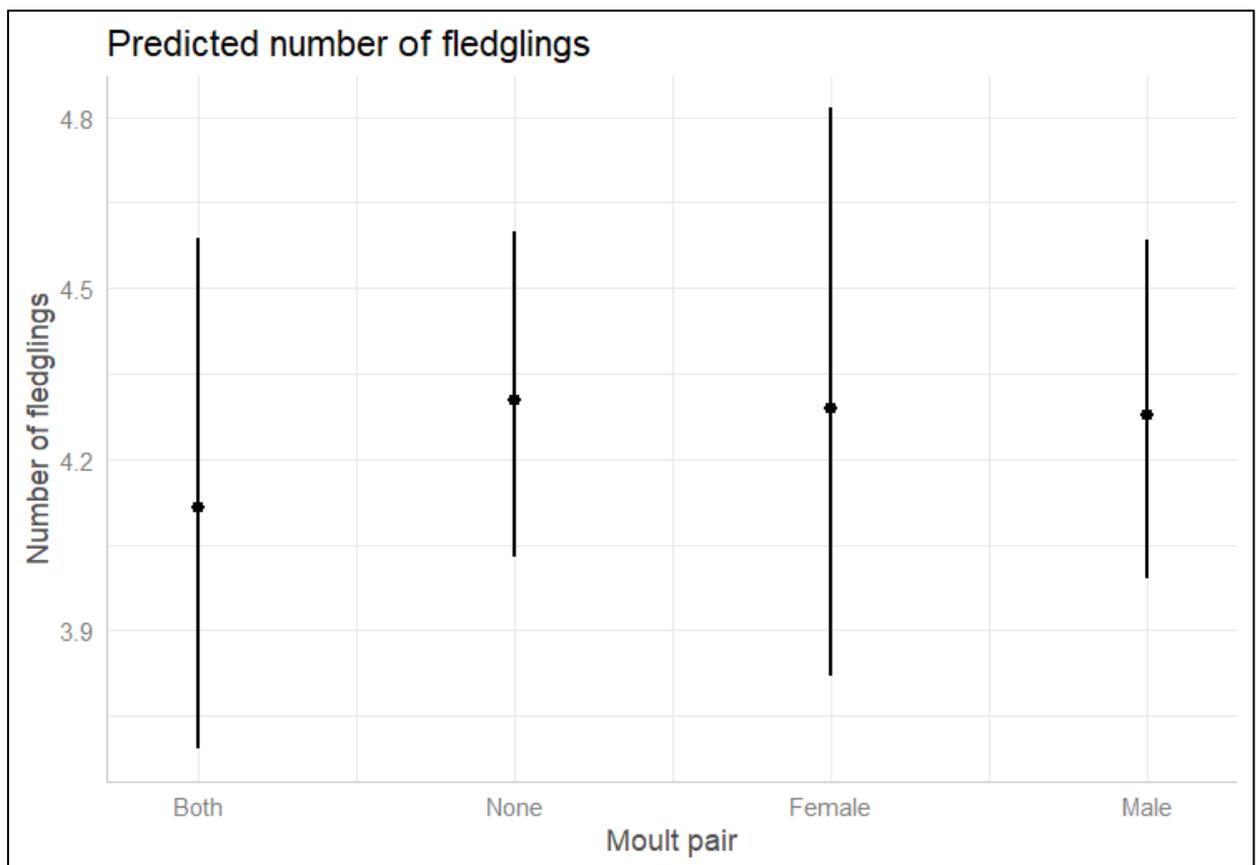


Figure 2. Predicted number of fledglings for each moulting status of the breeding pair (both members moulting, none moulting, female moulting, male moulting). N= 1959 pairs.

There was weak evidence that the moulting status of the adult pair influenced the number of recruits. According to the output of the model, pairs with only the female moulting while rearing the nestlings produced a higher number of recruits than pairs in which both

members were moulting (Table 3; Fig 3). However, the post hoc Tukey test did not show differences between these categories (all $p = 0.178$).

Table 3. Results of the GLMM testing the influence of the different overlap pairs on the number of recruits produced by the pairs.

| Random effects | Variance | Std.Dev. | | |
|-----------------------|-----------------|-------------------|----------------|------------------|
| Year | 0.137 | 0.371 | | |
| Female identity | 1.97E-07 | 0.0004 | | |
| Male identity | 6.55E-03 | 0.081 | | |
| Fixed effects | Estimate | Std. error | z value | p |
| Intercept | -17.381 | 0.405 | -4.29 | <0.001 |
| Laying date | -0.262 | 0.055 | -4.76 | <0.001 |
| Habitat (pine) | 0.539 | 0.070 | 7.65 | <0.001 |
| Female age (adult) | 0.099 | 0.101 | 0.98 | 0.324 |
| Male age (adult) | 0.208 | 0.131 | 1.58 | 0.113 |
| Neither moulting | 0.59 | 0.381 | 1.55 | 0.121 |
| Female moulting | 0.876 | 0.446 | 1.96 | 0.0497 * |
| Male moulting | 0.578 | 0.376 | 1.54 | 0.124 |

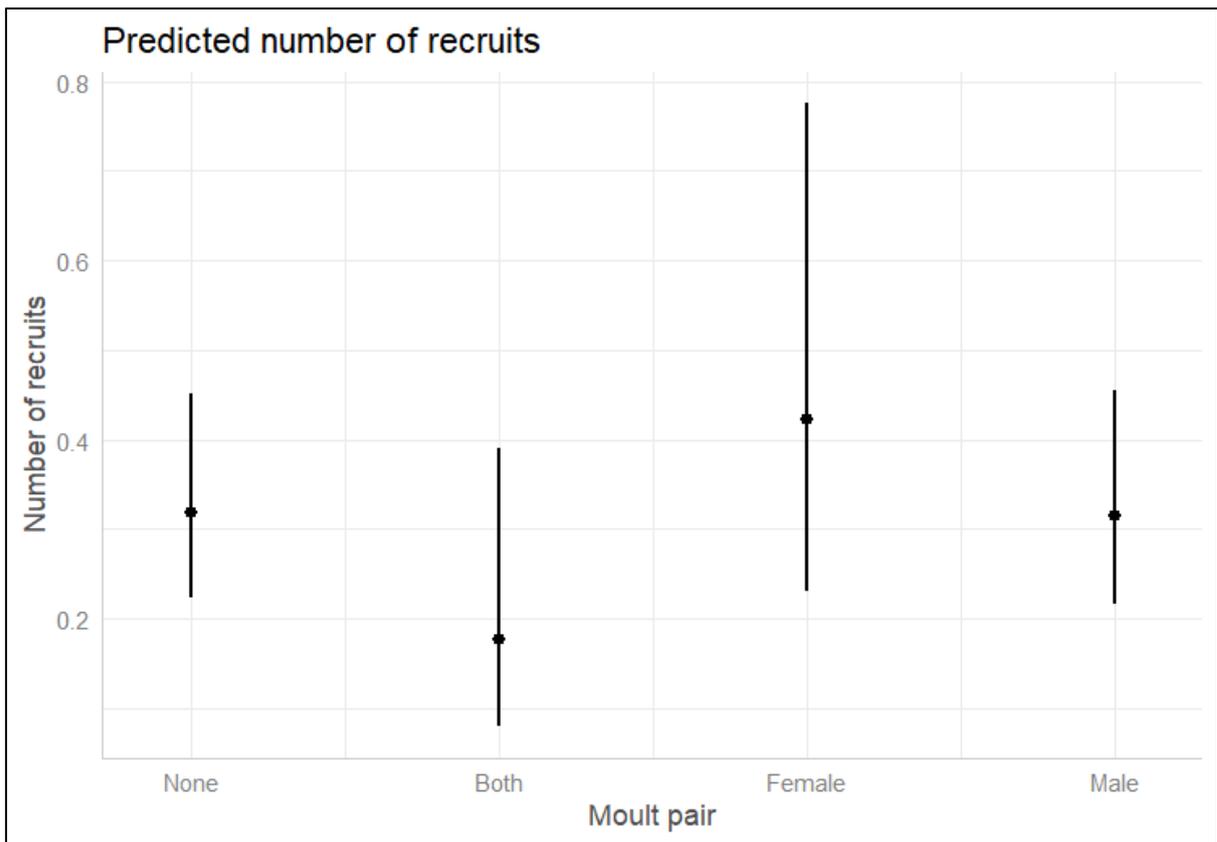


Fig. 3. Predicted number of recruits (offspring that return to the study area to breed) for each moulting status of the focal pair (both members moulting, none moulting, female moulting, male moulting). N = 1959 pairs.

5.3 Survival consequences of moult-breeding overlap

The moulting status of the adult pair did not affect the probability of survival in females (Fig 4; Table 4) or males (Fig. 5, Table).

Table 4. Results of the GLMM testing the effect of the moulting status of the adult pair (4 levels: none moulting, female and/or male moulting) on the survival of the female individuals.

| Random Effects | Variance | Std.Dev. | | | |
|-----------------------|-----------------|-------------------|----------------|--------------------------------|--------------------|
| Year | 0,009 | 0.095 | | | |
| Identity | 1.088e-08 | 0.0001 | | | |
| Fixed Effects | Estimate | Std. Error | z value | Odds ratio (CI 95%) | Pr(> z) |
| Intercept | 0.267 | 0.204 | 1.31 | 1.3 (0.87, 1.95) | 0.191 |
| Laying date | -0.084 | 0.041 | -2.07 | 0.92 (0.85, 0.99) | 0.039 * |
| Habitat (pine) | 0.156 | 0.063 | 2.45 | 1.17 (1.03, 1.32) | 0.014 * |
| Age (adult) | -0.093 | 0.082 | -1.13 | 0.91 (0.78, 1.07) | 0.258 |
| Neither moulting | -0.168 | 0.203 | -0.83 | 0.84 (0.57, 1.26) | 0.408 |
| Female moulting | 0.119 | 0.291 | 0.41 | 1.12 (0.64, 2) | 0.683 |
| Male moulting | -0.236 | 0.192 | -1.23 | 0.79 (0.54, 1.15) | 0.22 |

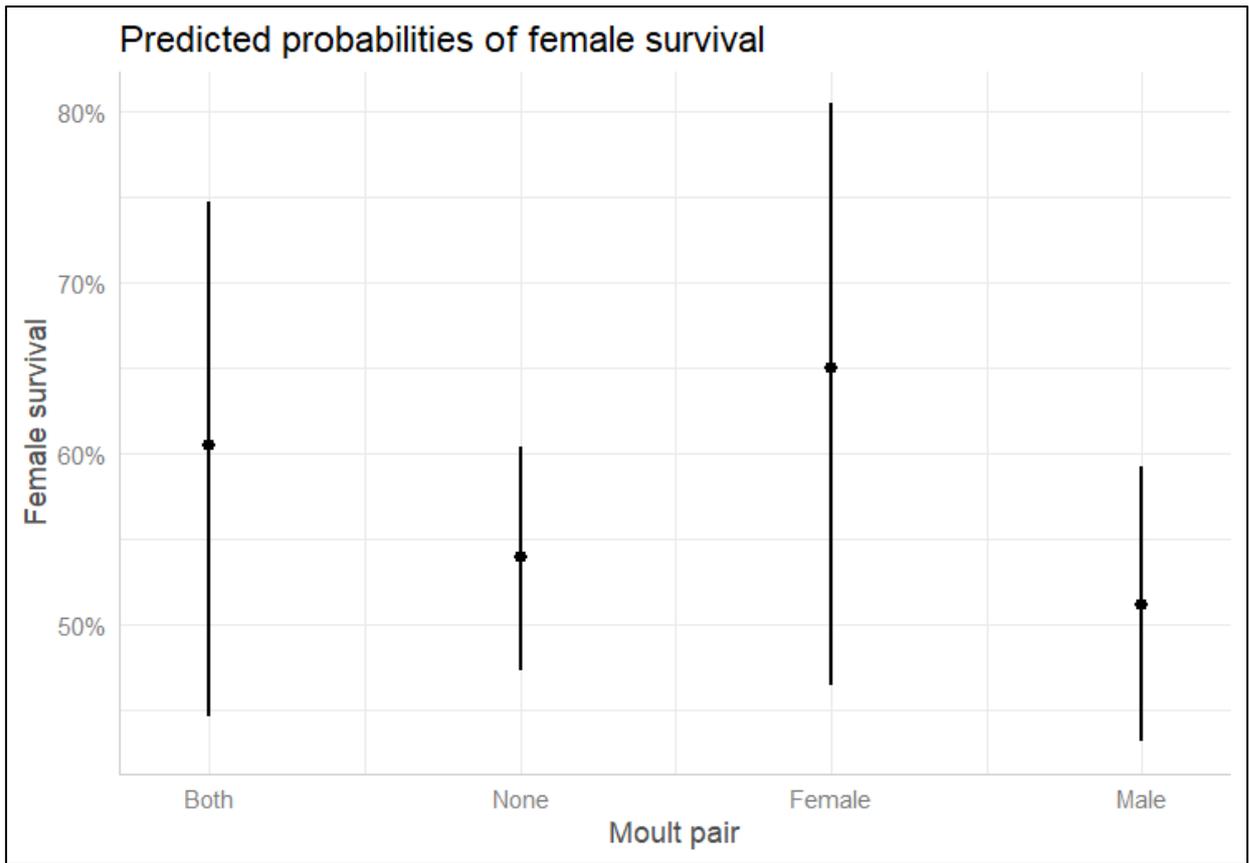


Fig. 4. Predicted probabilities of female survival for each moulting status of the focal pair (both members moulting, none moulting, female moulting, male moulting). N = 1959 pairs.

Table 5. Results of the GLMM testing the influence of the moulting status of the adult pair on the survival of the male individuals.

| Random Effects | Variance | Std.Dev. | | | |
|------------------|----------|------------|---------|---------------------|-----------------|
| Year | 1.37E-02 | 0.117 | | | |
| Identity | 4.88E-09 | 6.99E-05 | | | |
| Fixed Effects | Estimate | Std. Error | z value | Odds ratio (CI 95%) | p |
| Intercept | 0.062 | 0.214 | 0.29 | 1.06 (0.7, 1.62) | 0.772 |
| Laying date | -0.091 | 0.041 | -2.22 | 0.91 (0.84, 0.99) | 0.026 * |
| Habitat (pines) | 0.197 | 0.064 | 3.1 | 1.21 (1.07, 1.38) | 0.002 ** |
| Age (adult) | -0.222 | 0.100 | -2.22 | 0.8 (0.66, 0.97) | 0.027 * |
| Neither moulting | 0.063 | 0.202 | 0.31 | 1.06 (0.71, 1.58) | 0.756 |
| Female moulting | 0.053 | 0.287 | 0.18 | 1.05 (0.6, 1.85) | 0.853 |
| Male moulting | 0.059 | 0.193 | 0.30 | 1.06 (0.73, 1.55) | 0.761 |

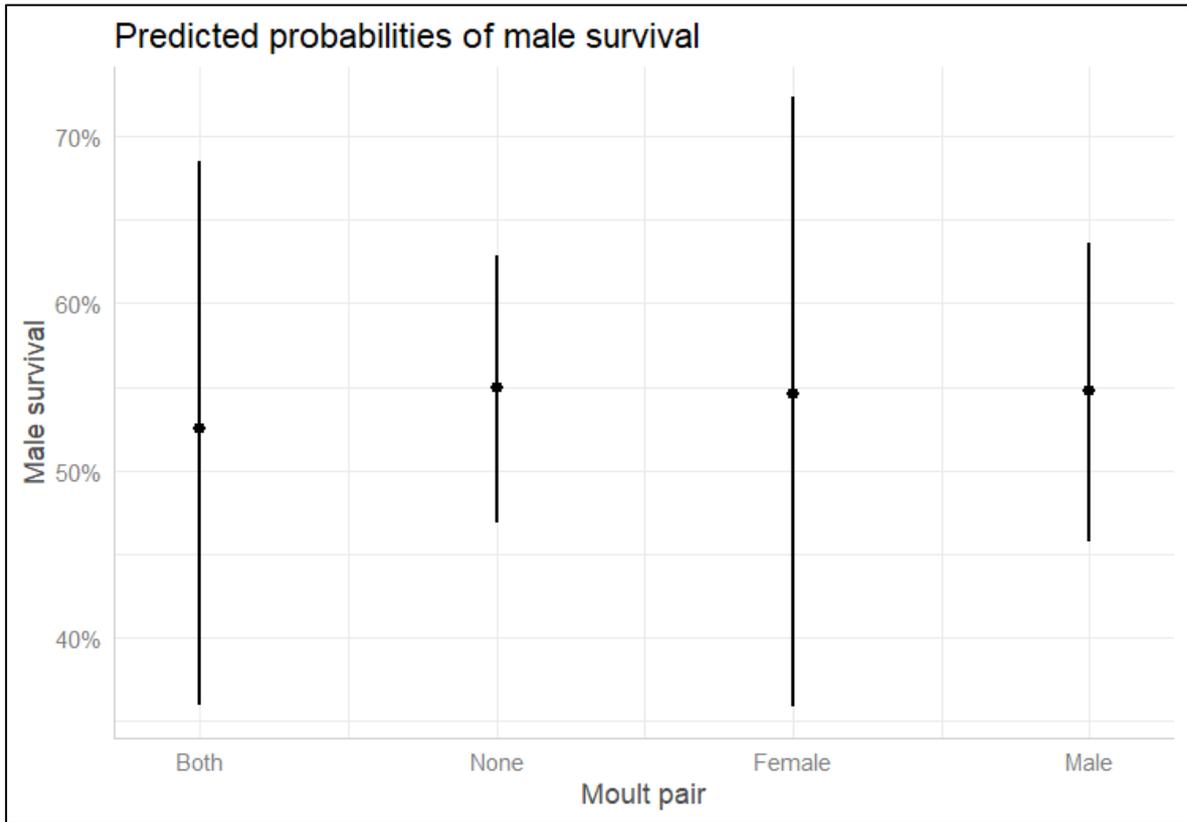


Fig. 5. Predicted probabilities of male survival for each moulting status of the focal pair (both members moulting, none moulting, female moulting, male moulting). N = 1959 pairs.

6. DISCUSSION

We have thoroughly analyzed the fitness consequences of overlapping breeding and moulting in a long-distance migrant, the pied flycatcher. Overall, moult-breeding overlap did not have strong effects on the number of fledglings nor survival prospects of individuals, after taking into account the moulting status of the breeding mate. However, we found weak evidence for an effect of overlapping moulting and breeding on the number of recruits as breeding pairs in which only the female overlapped moulting and breeding had a higher number of recruits than pairs in which both members moulted while breeding.

Among birds, males tend to start moulting earlier than females, probably because they spend less time incubating and/or brooding the offspring, and therefore they can afford to be involved in two costly activities such as breeding and moulting simultaneously (Jenni & Winkler, 2020b). Accordingly, male pied flycatchers in the study population started moulting while breeding more often (21.2%) than females (6%). On the other hand, young individuals (1 year old) are also expected to overlap breeding and

moult more often than adults, as they usually start breeding later and therefore have less time to breed and moult separately (Potti, 1991; Siikamaki *et al.*, 1994; Potti, 1998; Morales *et al.*, 2007). However, there was no significant difference in the probability of overlap between moult and breeding, although the percentage of juveniles following this strategy (27%) was higher than that of adults (10.5%), indicating a trend that is likely to be significant if we increase the number of years examined.

The main question of this study was to test the costs of overlapping two energy-demanding activities. Several works have investigated this question in pied flycatchers (e.g., Hemborg, 1999a; Morales *et al.*, 2007; Tomotani *et al.*, 2018) and other migratory species (Svensson & Nilsen, 1997; Hemborg & Merilä, 1998, 1999). For example, Morales *et al.*, 2007 found that moult-breeding overlap negatively affected the number of fledglings in a Spanish population of pied flycatchers. Similarly, Hemborg (1998) showed that experimentally delaying the breeding cycle in a Swedish pied flycatcher population led to fewer fledglings. In collared flycatchers, Hemborg & Merilä (1998) observed that females mated with overlapping males had lower survival chances than females mated with non-overlapping males. However, most of them have relied on short-term proxies of fitness, such as current body condition or the number of fledglings, while only one study (Hemborg & Lundberg, 1998) has simultaneously analyzed the effects on recruits and survival, which are more accurate proxies of fitness than short-term measures. Further, many of these studies were experimental and followed different protocols and objectives (e.g. manipulation of clutch size, delay of the brooding time, simulated feather plucking during moult). These manipulations often do not accurately reflect a natural moult process, thus their effects on fitness should be interpreted and compared with other studies on moult, including ours, with caution. Further, previous studies have considered the consequences of moult focused on an individual, but individuals may adapt their behaviour (e.g. inversion in parental effort) based on that of their mate (e.g. reduced parental care associated with moult). Our study, including 18 breeding seasons, moulting information of thousands of individuals and that of their mates and different proxies of fitness, allows us to draw robust patterns while considering the environmental heterogeneity between different breeding seasons and other confounding factors.

Based on previous knowledge of this and other migratory species (see previous paragraph), we predicted negative consequences of overlapping breeding and moult on fitness. As both are two energetically demanding tasks, a tradeoff between self-

maintenance (moult) and parental care (breeding) with subsequent effects on reproductive output is expected. Accordingly, some studies have shown that males prioritize self-maintenance during moulting, providing less parental care to the offspring (Hemborg, 1999a; Tomotani *et al.*, 2018). Contrary to these expectations, we did not find a strong negative effect of moulting while breeding either on current reproductive success or in survival. Several factors may explain the absence of negative effects. First, although our analyses considered whether individuals and/or their mates began moulting while feeding the nestlings, we did not evaluate the extent of overlap (in days) between these two activities, nor the intensity (speed or progress) of moulting during the nestling stage, which are factors likely to influence parental care and reproductive success. Future research should thus examine whether the degree of overlap, as well as the speed or intensity of moulting (while considering also that of the mate), impact individual fitness. Second, individuals may adjust their parental care in response to that provided by their mate, increasing it if this is reduced (e.g., due to moulting costs), to mitigate the potential negative effects on the offspring. In agreement with this idea, several studies (e.g. Saino & Möller, 1995; Wright & Cuthill, 1989; Osorno & Székely, 2004; Tomotani *et al.*, 2018) found that females adjust their feeding rate in response to reduced male parental care, a very likely scenario when males overlap moult and breeding (Hemborg, 1999a; Tomotani *et al.*, 2018). However, under this scenario of compensation, it would be expected that individuals increasing their current investment in parental care experienced a reduction in future survival as reported by Hemborg (1999a) for females of a Swedish flycatcher population. Contrary to this idea, we did not find evident costs on survival in relation to the moulting status of individuals or their mates. Thus, in our population, the compensation of parental care by females, if exists, does not have evident costs in their survival. One possibility is that differences between populations reflect differences in migration features. For example, overlapping birds from southern populations with shorter migration routes may suffer fewer migration costs, thus gaining time to compensate for parental care before moulting and ultimately conferring better chances of survival. Third, females may adjust their reproductive effort according to the perceived probability of moulting (e.g., based on environmental cues), adjusting clutch size (Hemborg, 1999a) or removing eggs or recently hatched nestlings from the nest (Lobato *et al.*, 2006), thus masking the potential negative effects of overlapping moulting and breeding in the reproductive output (i.e. number of fledglings or recruits). Fourth, favourable environmental conditions may minimize the negative effects of moult-

breeding overlap on reproduction and survival. Thus, individuals might predominantly overlap these two activities when conditions are good enough to offset or minimize potential fitness costs. Regardless of the reasons explaining the absence of negative effects on fitness, discrepancies with previous works may arise due to differences in analytical and methodological approaches (see above). For example, plucking (several) feathers may not adequately simulate a natural moult, which occurs gradually and is influenced by environmental or intrinsic factors (good body condition; Jenni & Winkler, 2020a). It is also important to highlight that most studies have been conducted in Northern European populations, where environmental conditions and selective pressures (e.g., longer migrations, shorter breeding seasons) differ from those experiences in Mediterranean regions, potentially leading to different fitness consequences of this moulting strategy.

7. CONCLUSIONS

In conclusion, moult-breeding overlap does not seem to have strong negative effects on the fitness of pied flycatchers, measured as reproductive success and survival prospects. Moult-breeding overlap seems to be an adaptive strategy for individuals in the study population by mitigating time constraints to moult after breeding and before migration, without apparent fitness costs. However, this may change in future years and between populations at different latitudes, as different climatic conditions during the breeding season and varying time constraints may alter selective pressures (Hemborg *et al.*, 2001). There are interesting avenues of future research: whether the females/males compensate for the lack of parental care of the pair moulting, whether the moult-breeding overlap has lifetime consequences or, if there are no fitness costs, why moult-breeding overlap is not spread among all the individuals of the population.

8. REFERENCES

- Alatalo, R. V., & Lundberg, A. (1984). Polyterritorial polygyny in the pied flycatcher *Ficedula hypoleuca*—evidence for the deception hypothesis. In *Annales Zoologici Fennici* (pp. 217-228). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.

- Askenmo, C. (1979). Reproductive effort and return rate of male pied flycatchers. *The American Naturalist*, 114(5), 748-753.
- Barta, Z., Houston, A. I., McNamara, J. M., Welham, R. K., Hedenström, A., Weber, T. P., & Feró, O. (2006). Annual routines of non-migratory birds: optimal moult strategies. *Oikos*, 112(3), 580-593.
- Barta, Z., McNamara, J. M., Houston, A. I., Weber, T. P., Hedenström, A., & Fero, O. (2008). Optimal moult strategies in migratory birds. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1490), 211-229.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.
- Bergman, G. (1982). Why are the wings of *Larus f. fuscus* so dark?. *Ornis Fennica*, 59(2-3), 77-83.
- Bridge, E. S. (2011). Mind the gaps: what's missing in our understanding of feather moult. *The Condor*, 113(1), 1-4.
- Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., ... & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, 9(2), 378-400.
- Burt Jr, E. H., & Ichida, J. M. (1999). Occurrence of feather-degrading bacilli in the plumage of birds. *The Auk*, 116(2), 364-372.
- Butler, M., & Johnson, A. S. (2004). Are melanized feather barbs stronger?. *Journal of Experimental Biology*, 207(2), 285-293.
- Camacho, C., Canal, D., & Potti, J. (2016). Natal habitat imprinting counteracts the diversifying effects of phenotype-dependent dispersal in a spatially structured population. *BMC Evolutionary Biology*, 16(1).
- Canal, D., Serrano, D., & Potti, J. (2014). Exploring heterozygosity-survival correlations in a wild songbird population: contrasting effects between juvenile and adult stages. *PLoS One*, 9(8), e105020.
- Canal, D., Schlicht, L., Manzano, J., Camacho, C., & Potti, J. (2020). Socio-ecological factors shape the opportunity for polygyny in a migratory songbird. *Behavioral Ecology*, 31(2), 598-609.
- Canal, D., Schlicht, L., Santoro, S., Camacho, C., Martinez-Padilla, J., & Potti, J. (2021). Phenology-mediated effects of phenotype on the probability of social

- polygyny and its fitness consequences in a migratory passerine. *BMC Ecology and Evolution*, 21, 1-12.
- Dawson, A., Hinsley, S. A., Ferns, P. N., Bonser, R. C., & Eccleston, L. (2000). Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1457), 2093-2098.
 - Fox, J., & Weisberg, S. (2018). *An R companion to applied regression*. Sage publications.
 - Gustafsson, L., & Sutherland, W. J. (1988). The costs of reproduction in the collared flycatcher *Ficedula albicollis*. *Nature*, 335(6193), 813-815.
 - Hall, K. S. S., & Fransson, T. (2000). Lesser Whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. *Journal of Avian Biology*, 31(4), 583-587.
 - Hedenström, A. (2003). Flying with holey wings. *Journal of Avian Biology*, 34(4), 324-327.
 - Hemborg, C. (1998). Sexual differences in the control of postnuptial moult in the pied flycatcher. *Animal Behaviour*, 56(5), 1221-1227.
 - Hemborg, C. (1999a). Sexual differences in moult–breeding overlap and female reproductive costs in pied flycatchers, *Ficedula hypoleuca*. *Journal Of Animal Ecology*, 68(2), 429-436.
 - Hemborg, C. (1999b). Annual variation in the timing of breeding and moulting in male and female Pied Flycatchers *Ficedula hypoleuca*. *Ibis*, 141(2), 226-232.
 - Hemborg, C., & Lundberg, A. (1998). Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. *Behavioral Ecology and Sociobiology*, 43, 19-23.
 - Hemborg, C., Lundberg, A., & Siikamäki, P. (1998). Trade-off between reproduction and moult—a comparison of three Fennoscandian pied flycatcher populations. *Oecologia*, 117(3), 374-380.
 - Hemborg, C., & Merilä, J. (1998). A sexual conflict in collared flycatchers, *Ficedula albicollis*: early male moult reduces female fitness. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1409), 2003-2007.
 - Hemborg, C., & Merilä, J. (1999). Reproductive investment and moult-breeding overlap in the collared flycatcher *Ficedula albicollis*: an experimental approach.

- In *Annales Zoologici Fennici* (pp. 1-9). Finnish Zoological and Botanical Publishing Board.
- Hemborg, C., Sanz, J., & Lundberg, A. (2001). Effects of latitude on the trade-off between reproduction and moult: a long-term study with pied flycatcher. *Oecologia*, 129, 206-212.
 - Holmgren, N., & Hedenström, A. (1995). The scheduling of moult in migratory birds. *Evolutionary ecology*, 9, 354-368.
 - Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, 50(3), 346-363.
 - Jenni, L., & Winkler, R. (2020a). *The biology of moult in birds*. Bloomsbury Publishing.
 - Jenni, L., & Winkler, R. (2020b). *Moult and ageing of European passerines*. Bloomsbury Publishing.
 - Karlsson, L., Persson, K., & Walinder, G. (1986). Ageing and sexing in pied flycatchers, *Ficedula hypoleuca*. *Vår Fågelvärld*, 45, 131-146.
 - Kirby, J. S., Stattersfield, A. J., Butchart, S. H., Evans, M. I., Grimmett, R. F., Jones, V. R., ... & Newton, I. (2008). Key conservation issues for migratory land- and waterbird species on the world's major flyways. *Bird Conservation International*, 18(S1), S49-S73.
 - Lindström, Å., Visser, G. H., & Daan, S. (1993). The Energetic Cost of Feather Synthesis Is Proportional to Basal Metabolic Rate. *Physiological Zoology*, 66(4), 490–510.
 - Lobato, E., Moreno, J., Merino, S., J. Sanz, J., Arriero, E., Morales, J., ... & Martínez-de la Puente, J. (2006). Maternal clutch reduction in the pied flycatcher *Ficedula hypoleuca*: an undescribed clutch size adjustment mechanism. *Journal of Avian Biology*, 37(6), 637-641.
 - Lundberg, A., & Alatalo, R. V. (2010). *The pied flycatcher*.
 - Merilä, J. (1997). Fat reserves and moult-migration overlap in goldcrests, *Regulus regulus*—A trade-off?. In *Annales Zoologici Fennici* (pp. 229-234). Finnish Zoological and Botanical Publishing Board.
 - Morales, J., Moreno, J., Merino, S., Sanz, J. J., Tomás, G., Arriero, E., Lobato, E., & La Puente, J. M. (2007). Early moult improves local survival and reduces reproductive output in female pied flycatchers. *Ecoscience*, 14(1), 31-39.

- Moreno, J., Sanz, J., Merino, S., & Arriero, E. (2001). Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with moult. *Oecologia*, 129, 492-497.
- Morton, G. A., & Morton, M. L. (1990). Dynamics of postnuptial moult in free-living mountain white-crowned sparrows. *The Condor*, 92(4), 813-828.
- Murphy, M. E. (1996). Energetics and nutrition of moult. In *Avian energetics and nutritional ecology* (pp. 158-198). Boston, MA: Springer US.
- Newton, I. (2009). *Moult and plumage*. *Ringing & Migration*, 24(3), 220-226.
- Newton, I. (2011). Migration within the annual cycle: species, sex and age differences. *Journal of Ornithology*, 152(Suppl 1), 169-185.
- Newton, I. (2023). *The migration ecology of birds*. Elsevier.
- Nilsson, J. Å., & Svensson, E. (1996). The cost of reproduction: a new link between current reproductive effort and future reproductive success. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263(1371), 711-714.
- Nur, N. (1988). The consequences of brood size for breeding blue tits. III. Measuring the cost of reproduction: survival, future fecundity, and differential dispersal. *Evolution*, 42(2), 351-362.
- Osorno, J. L., & Székely, T. (2004). Sexual conflict and parental care in magnificent frigatebirds: full compensation by deserted females. *Animal Behaviour*, 68(2), 337-342.
- Potti, J. (1998). Arrival time from spring migration in male pied flycatchers: individual consistency and familial resemblance. *The Condor*, 100(4), 702-708.
- Potti, J., & Montalvo, S. (1991). Male Arrival and Female Mate Choice in Pied Flycatchers *Ficedula hypoleuca* in Central Spain. *Ornis Scandinavica*, 22(1), 45.
- Potti, J., & Montalvo, S. (1991b). Return rate, age at 1 breeding and natal dispersal of pied flycatchers *Ficedula hypoleuca* in central Spain. *Ardea*, 79(3), 419-428.
- Pulido, F., & Coppack, T. (2004). Correlation between timing of juvenile moult and onset of migration in the blackcap, *Sylvia atricapilla*. *Animal Behaviour*, 68(1), 167-173.
- Reid, W. V. (1987). The cost of reproduction in the glaucous-winged gull. *Oecologia*, 74, 458-467.

- Saino, N., & Møller, A. P. (1995). Testosterone-induced depression of male parental behavior in the barn swallow: female compensation and effects on seasonal fitness. *Behavioral Ecology and Sociobiology*, 36, 151-157.
- Siikamäki, P., Hovi, M., & Rätti, O. (1994). A trade-off between current reproduction and moult in the pied flycatcher--an experiment. *Functional Ecology*, 587-593.
- Stearns, S. C. (1976). Life-history tactics: a review of the ideas. *The Quarterly review of biology*, 51(1), 3-47.
- Stearns, S. C. (1992). *The evolution of life histories*. Oxford university press.
- Svensson, E., & Nilssen, J. Å. (1997). The trade-off between moult and parental care: a sexual conflict in the blue tit?. *Behavioral Ecology*, 8(1), 92-98.
- Swaddle, J. P., Witter, M. S., Cuthill, I. C., Budden, A., & McCowen, P. (1996). Plumage condition affects flight performance in common starlings: implications for developmental homeostasis, abrasion and moult. *Journal of Avian Biology*, 103-111.
- Tomotani, B. M., Muijres, F. T., Koelman, J., Casagrande, S., & Visser, M. E. (2018). Simulated moult reduces flight performance but overlap with breeding does not affect breeding success in a long-distance migrant. *Functional Ecology*, 32(2), 389-401.
- Vagasi, C. I., Pap, P. L., Vincze, O., Benkő, Z., Marton, A., & Barta, Z. (2012). Haste makes waste but condition matters: *moult* rate–feather quality trade-off in a sedentary songbird. *PLoS One*, 7(7), e40651.
- Van Noordwijk, A. J., & De Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, 128(1), 137-142.
- Wickham, H. (2016). *Data analysis* (pp. 189-201). Springer International Publishing.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., ... & Yutani, H. (2019). Welcome to the Tidyverse. *Journal of open source software*, 4(43), 1686.
- Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, 100(916), 687-690.

- Wingfield, J. C. (2008). Organization of vertebrate annual cycles: implications for control mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1490), 425-441.
- Wright, J., & Cuthill, I. (1989). Manipulation of sex differences in parental care. *Behavioral Ecology and Sociobiology*, 25, 171-181.