Concealed by darkness: interactions between predatory bats and nocturnally migrating songbirds illuminated by DNA sequencing

CARLOS IBÁÑEZ, ANA G. POPA-LISSEANU, DAVID PASTOR-BEVIÁ, JUAN L. GARCÍA-MUDARRA and JAVIER JUSTE
Department of Evolutionary Ecology, Estación Biológica de Doñana (CSIC), Avda. America Vespucio s.n., Sevilla 41092, Spain

Abstract
Recently, several species of aerial-hawking bats have been found to prey on migrating songbirds, but details on this behaviour and its relevance for bird migration are still unclear. We sequenced avian DNA in feather-containing scats of the bird-feeding bat Nyctalus lasiopterus from Spain collected during bird migration seasons. We found very high prey diversity, with 31 bird species from eight families of Passeriformes, almost all of which were nocturnally flying sub-Saharan migrants. Moreover, species using tree hollows or nest boxes in the study area during migration periods were not present in the bats’ diet, indicating that birds are solely captured on the wing during night-time passage. Additional to a generalist feeding strategy, we found that bats selected medium-sized bird species, thereby assumingly optimizing their energetic cost-benefit balance and injury risk. Surprisingly, bats preyed upon birds half their own body mass. This shows that the 5% prey to predator body mass ratio traditionally assumed for aerial hunting bats does not apply to this hunting strategy or even underestimates these animals’ behavioural and mechanical abilities. Considering the bats’ generalist feeding strategy and their large prey size range, we suggest that nocturnal bat predation may have influenced the evolution of bird migration strategies and behaviour.

Keywords: bird migration, Chiroptera, molecular diet, noctules, Nyctalus lasiopterus, trophic

Received 7 April 2016; revision received 10 August 2016; accepted 22 August 2016

Introduction
Predation has long captured the attention of ecologists for its power to affect prey demography and evolution (Anderson 1986; Zanette et al. 2011), influence ecosystem nutrient cycling (Schmitz et al. 2010) and eventually lead to cascading changes in ecosystems (Ferretti et al. 2010). A recently discovered predator-prey interaction that has excited interest is predation by bats upon nocturnally migrating songbirds (Ibáñez et al. 2001, 2003; Bontadina & Arlettaz 2003; Popa-Lisseanu et al. 2007). Three bat species have so far been discovered to occupy this feeding niche in different parts of the world: Nyctalus lasiopterus in the Western Palearctic (Dondini & Vergari 2000; Ibáñez et al. 2001), Ia io in southeastern Asia (Thabah et al. 2007) and Nyctalus aviator in north-eastern Asia (Fukui et al. 2013). The three species are among the largest representatives of the family Vespertilionidae, but belong to two not closely related genera (Thabah et al. 2007). This suggests that the novel feeding behaviour evolved independently in insectivorous bats at least twice. Around a dozen other bat species feed on small terrestrial vertebrates – also complementing their diets with invertebrates to some extent – but they are all found in the tropics and typically show a gleaning hunting strategy (Norberg & Fenton 1988). N. lasiopterus, N. aviator and Ia io, by contrast, have a temperate-subtropical distribution (Simmons 2005) and are aerial, open space hunters, as indicated by their wing morphology and echolocation (Ibáñez et al. 2001; Thabah et al. 2007; Estók & Siemers 2009; Fukui et al. 2013).
2013). Morphological analyses of droppings throughout the year of the two Nyctalus representatives show that bird predation is restricted to bird migration periods and that these bats are insectivorous the rest of the year (Ibáñez et al. 2001; Fukui et al. 2013). It has been suggested that bats capture birds in open flight (Ibáñez et al. 2001; Thabah et al. 2007; Fukui et al. 2013). This hypothesis indeed challenges previous knowledge on bat hunting dynamics. For example, according to comparative dietary studies on bats feeding in open space, bats capture airborne prey that does not exceed 5% of their own body mass (Fenton 1990). This is assumed to reflect mechanical limitations associated with size (Fenton 1990), although no experimental studies have addressed this issue. Paradoxically, even a 5-g songbird would account for as much as 10% of the bat’s body mass (average weight is c. 50 g for N. lasiopterus (Ibáñez et al. 2001)). This disparity may explain why the discovery of this feeding strategy has been contested (Bontadina & Arlettaz 2003; Andreas 2010). Alternatively, it has been suggested that birds are captured while resting in tree hollows or nest boxes also used by bats (Dondini & Vergari 2000).

Optimal foraging theory predicts that predator decisions become more relevant as prey size increases relative to predator size (Charnov & Orians 1973). Indeed, the larger the typical prey consumed by a predator, the greater both the energy invested in the attack and the risk of injury. The comparatively large bird/bat body mass ratio would suggest an evolutionary advantage for bats selecting bird prey, at least at high encounter rates during the postnuptial songbird migration. At this time, bird densities peak, and c. 50% of droppings of N. lasiopterus were found to contain only feathers (Ibáñez et al. 2001). For example, bats may select birds based on size, preferring those within a particular size range so as to optimize the balance between benefits (larger prey providing more energy per capture unit) and costs (larger prey may be more difficult to overpower and manipulate). Additionally, certain bird characteristics may be particularly conspicuous to the bats (e.g. specific sensorial attributes) and be passively selected. Besides its intrinsic scientific interest, the dietary niche breadth of this feeding strategy may indeed affect migratory prey bird demographics.

The advent of molecular tools has allowed detailing diet composition to a species level and addressing new questions in trophic studies of bats (Clare et al. 2009; Razgour et al. 2011; Zeale et al. 2011; Alberdi et al. 2012). In this study, we have investigated the avian diet of the Western Palaearctic bat N. lasiopterus by selectively sequencing avian DNA obtained from faecal remains collected along wide temporal and spatial windows. This has allowed us to (i) establish species composition and niche breadth of the bats’ carnivorous diet, (ii) settle the question whether bats hunt birds on the wing vs. capture them at the roosts, (iii) discover a new prey size threshold for aerial-hawking bats and (iv) test whether bats select prey based on size. Based on our findings, we reflect on the impact of predation by bats on populations of migratory birds.

Materials and methods

Study sites and faeces collection

We analysed 226 feather-containing scats of N. lasiopterus. A first set of pellets (n = 154) was collected between 1998 and 2009 from flying bats netted over water courses, mainly from La Rioja, northern Spain (n = 115) or as they returned to their tree roosts in a city park in Seville in Andalusia, Southern Spain (n = 39). All these bats were individually kept in cloth bags and thereafter sexed, weighed, measured and released at the site of capture, and droppings were collected from the bag. Only one sample was selected per bat and night for analyses. A second set of pellets (n = 72) was collected daily under roosts between 2003 and 2010, either under palms (Washingtonia filifera) in a city park in Seville (n = 32) or under bat boxes in Doñana National Park (DNP) in Andalusia, southern Spain (n = 40). For this set of pellets, only one individual dropping per day and roost was analysed, to ensure it represented the diet of only one bat so as to render the two data sets comparable. Faecal pellets were kept either frozen at −20 °C or dried in paper bags at room temperature until processed.

DNA extraction, PCR amplification and sequencing, and prey quantification

A preliminary study showed a high proportion (>90%) of bat pellets with a single bird prey (Pastor-Beviá et al. 2014) suggesting as the most appropriate laboratory strategy the combination for each dropping of single Sanger sequencing and cloning of a homogenized mix rather than a multiple sequencing of different bits, as has been carried out in other bat diet studies (Clare et al. 2009, 2011). Total DNA was extracted in a sterile DNA laboratory (LEM-EBD), starting from 0.01 to 0.05 g of faecal pellet unit, using a modified guanidinium thiocyanate (GuSCN) method by Rohland & Hofreiter (2007). Accordingly, each faecal pellet unit was pulverized using steel balls after a liquid nitrogen immersion and washed several times with ethanol, dried and resuspended with water (Pastor-Beviá et al. 2014).
Three mitochondrial fragments were amplified from avian DNA using different combinations of primer sets: two fragments (160 and 380 bp) of the cytochrome b gene (Cytb) using the primer sets CytbSPrey (FW/RW) and CytbLPrey (FW/RW), respectively, and one fragment (380 bp) of the cytochrome oxidase gene subunit I (COI) using primer sets COIPrey (FW/RW) (Pastor-Beviá et al. 2014). Amplification products were sequenced in an ABI 3100 automated sequencer (PE Biosystems, Warrington, UK) following manufacturer’s protocols. Obtained sequences were checked visually with Sequencer v. 4.9 (Gene Codes Corp, MI, USA). Double peak patterns in the inspection of electropherograms suggested the co-amplification of different sequences in 25 amplification products. In 10 of them, the sequences could be ascribed unambiguously to two different bird species. The remaining 15 were cloned using pGEM-T Easy Vector System and high-efficiency competent cells (Promega, www.promega.com) after selecting 16 clones per plate and following Pastor-Beviá et al. (2014). The obtained sequences were compared to available sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank) using the BLAST tool, and species identification was based on a >98% of similitude threshold.

To quantify prey consumption, we assumed a conservative approach in which the presence of DNA from one bird species in one dropping corresponds to only one individual prey. As we never analysed more than one faecal pellet of the same bat and day, the same individual bird could not appear in more than one pellet. We thus defined ‘prey items’ for each bird species as the number of faecal pellets in which the species was identified. DNA from two different species in the same pellet would indicate the consumption of (at least) two individual birds.

**Dietary niche breadth, prey characteristics and prey choice**

Taxonomic arrangement, migratory behaviour (by categories of long-distance migrant, partial migrant and sedentary) and information regarding the use of holows and nest boxes for each bird species identified were obtained from Cramp & Perrins (1977–1994) and Del Hoyo et al. (2004–2006), whereas information on migration patterns (primarily diurnal vs. nocturnal) was obtained from Newton (2008). Prey size was estimated from the average body mass of 20 individuals (10 males and 10 females) of each bird species captured during the postnuptial migration ringing campaigns in DNP (except for Sylvia hortensis with 11 individuals and S. conspicillata with three).

To explore dietary niche breadth of N. lasiopterus, we considered bat scats as sampling units of the bird prey population and estimated the total number of prey species consumed by calculating and extrapolating the sample-based rarefaction curve until reaching an asymptote (EstimateS, Colwell 2013).

To investigate prey size selection of N. lasiopterus, we compared body mass (bm) distribution of prey items consumed during postnuptial periods (n = 168) with bm distribution of all available prey. To estimate available prey, we used data of birds mist-netted in DNP during postnuptial ringing campaigns from 2000 to 2010 that belonged to any of the species identified in faeces of *N. lasiopterus*. We assumed that this set (n = 30 915) was representative of the distribution of total songbirds migrating at night across the Iberian Peninsula in the postnuptial period. Two species identified in the faeces, Calandrella brachydactyla and Carduelis cannabina (both contributing with only one prey item), were not represented in the bird ringing data and therefore, as availability could not be estimated, were not used for the analysis. We also excluded Cettia cetti, a sedentary species found in only one scat, from the analysis. Because the species is resident in the area, it was overrepresented in the ringing data, biasing therefore estimates of availability.

We grouped available prey (expected) and actual prey (observed) data in three bm categories (<10 g, 10–15 g and >15 g). By bootstrapping (9999 draws) in R (R Development Core Team 2015, version 3.2.3) the DNP postnuptial migrating bird capture data set (n = 30 915), we obtained expected average frequencies, standard deviations (SD) and 99% confidence intervals (CI) for the three bm categories for a subset of 168 samples (Table 2). Additionally, we performed a chi-square test of goodness of fit to compare bm values in the two data sets. We also compared observed vs. expected proportions within each bm category using the Z-test statistic derived from the Pearson chi-square statistic, calculated with IBM SPSS STATISTICS 21 (IBM Corp, Armonk, NY, USA).

**Results**

Avian DNA was amplified and sequenced from 215 of 226 analysed faecal samples (95% success). Only 15 scats had DNA from two bird species, and one scat had DNA from three species. Thus, a total of 232 prey items were identified unequivocally at species level based on a >98% similarity with the DNA of a single species in the BLAST comparisons.

Identified prey belonged to 31 species of eight families of Passeriformes (Alaudidae, Hirundinidae, Motacillidae, Muscicapidae, Turdidae, Sylviidae, Regulidae and Fringillidae) (Table 1, Fig. 1). The sample-based rarefaction curve for the estimated prey species...
richness did not reach an asymptote within the number of samples analysed, but only after extrapolating total sample number by a factor of five, estimating total richness at c. 43 prey species, with 95% CI (25, 61) (Fig. 2). The family Sylviidae was by far the most represented in the diet (16 species, and 75% of total prey items) with all eight Iberian representatives of the genus *Sylvia* identified in faeces. The family Muscicapidae accounted for as much as 13% of the total prey items despite being

| Bird families and species | n  | Migration category | Migration time | Hollows use | Body mass (g) |
|--------------------------|----|--------------------|----------------|-------------|---------------|
| **Alaudidae**             |    |                    |                |             |               |
| *Calandrella brachydactyla* | 1  | 1                  | 1              | No          | 21.2 ± 1.75   |
| **Fringillidae**          |    |                    |                |             |               |
| *Carduelis cannabina*     | 1  | 2 + 3              | 1              | No          | 13.4 ± 0.55   |
| *Serinus serinus*         | 1  | 2 + 3              | 1              | No          | 10.9 ± 0.79   |
| **Hirundinidae**         |    |                    |                |             |               |
| *Riparia riparia*         | 1  | 1                  | 1              | No          | 10.7 ± 0.76   |
| **Motacillidae**         |    |                    |                |             |               |
| *Anthus pratensis*        | 2  | 2                  | 1              | No          | 18.0 ± 1.42   |
| *Anthus trivialis*        | 6  | 1                  | 1              | No          | 24.7 ± 3.92   |
| **Muscicapidae**         |    |                    |                |             |               |
| *Ficedula hypoleuca*      | 28 | 1                  | 2              | Yes         | 13.0 ± 1.92   |
| *Muscicapa striata*       | 3  | 1                  | 2              | Yes         | 15.8 ± 1.86   |
| **Regulidae**            |    |                    |                |             |               |
| *Regulus ignicapilla*     | 1  | 2                  | 2              | No          | 5.0 ± 0.23    |
| **Sylviidae**            |    |                    |                |             |               |
| *Acrocephalus schoenobaenus* | 3 | 1                | 2              | No          | 12.1 ± 2.29   |
| *Acrocephalus scirpaceus* | 5  | 1                  | 2              | No          | 11.8 ± 2.26   |
| *Cettia cetti*            | 1  | 3                  | —              | No          | 12.3 ± 1.75   |
| *Hippolais polyglota*    | 12 | 1                  | 2              | No          | 11.0 ± 1.50   |
| *Locustella naevia*      | 14 | 1                  | 2              | No          | 13.9 ± 2.47   |
| *Phylloscopus bohemi*     | 10 | 1                  | 2              | No          | 7.9 ± 1.29    |
| *Phylloscopus collybida*  | 7  | 1 + 2              | 2              | No          | 6.8 ± 1.06    |
| *Phylloscopus trochilus*  | 24 | 1                  | 2              | No          | 8.5 ± 1.08    |
| *Sylvia atricapilla*      | 5  | 1 + 2              | 2              | No          | 17.4 ± 2.02   |
| *Sylvia borin*            | 16 | 1                  | 2              | No          | 18.5 ± 2.85   |
| *Sylvia cantillans*       | 46 | 1                  | 2              | No          | 9.9 ± 1.31    |
| *Sylvia communis*         | 23 | 1                  | 2              | No          | 14.4 ± 1.67   |
| *Sylvia conspicillata*    | 4  | 2                  | 2              | No          | 9.6 ± 0.86    |
| *Sylvia hortensis*        | 1  | 1                  | 2              | No          | 19.7 ± 1.57   |
| *Sylvia melanocephala*    | 2  | 2                  | 2              | No          | 11.4 ± 0.50   |
| *Sylvia undata*           | 2  | 3                  | —              | No          | 8.3 ± 0.27    |

© 2016 John Wiley & Sons Ltd
represented by only two species. By contrast, the family Turdidae accounted for only 6% of the prey items despite its higher diversity in the diet (six species identified). The four remaining families contributed with 1–2 prey items each, summing up to only 2.2% of bird prey (Table 1, Fig. 1).

Species composition of the diet varied over the year (Table S1, Supporting information). The five species most preyed upon Sylvia cantillans, Phylloscopus trochilus, S. borin, S. communis and Ficedula hypoleuca represented almost 60% of total prey items while 10 species appeared only once. Most species (74.2%) and prey items (93.5%) were long-distance migrants (sub-Saharan migrants) that fly primarily by night (Table 1). Only two species considered exclusively sedentary (Cetti aetina and Sylvia undata) were found in the droppings, but they only contributed with three prey items, whereas three other species that show both sedentary and migrant populations were represented scarcely by four prey items (Table 1). Proportion of sub-Saharan vs. partial migrants vs. sedentary birds changed over the year (Fig. 3). The majority of species (87.1% of all species identified, and comprising 74.9% of prey items)
found in the droppings do not use tree holes and/or nest boxes to roost during the sampling period (Table 1).

The average \( BM \) of the 31 prey species consumed was 14.0 ± 4.9 g, ranging from 5 g for *Regulus ignicapilla* to 24.7 g for *Anthus trivialis*. The average \( BM \) for the 232 identified prey items was 12.6 ± 4.1 g (Table 1), with 82% weighing between 5 and 15 g, and none over 25 g. 168 prey items were identified in faeces collected during postnuptial migration and were used for testing preferences in \( BM \). Expected average frequencies, standard deviations (SD) and 99% confidence intervals (CI) for the three bird \( BM \) categories defined in Materials and methods are presented in Table 2. The observed frequency for size class 1 was very near the average bootstrapped frequency, while it was outside the 99% confidence interval (CI) of the bootstrapped frequencies for size classes 2 and 3 (over and below, respectively; Fig. 4). \( BM \) values of observed prey items were significantly different from those of available prey as estimated from ringing data in DNP (\( \chi^2 = 10.555, \text{ d.f.} = 2, P = 0.005 \)). Our analyses indicate that birds with \( BM \) between 10 and 15 g were consumed in autumn more than expected (\( Z = 2.67, P = 0.008 \)), while birds >15 g were consumed less than expected (\( Z = 2.98, P = 0.003 \)). The proportion of birds with \( BM \) <10 g consumed was not significantly different from expected (\( Z = 0.032, P = 0.972 \)) (Fig. 4).

**Discussion**

**Prey identification by means of DNA sequencing**

We used Sanger DNA sequencing to identify avian prey in faecal remains of *N. lasiopterus* collected over more than 10 years (1998–2010) at different locations in Spain.
explain our infrequent findings of not typically nocturnally migrating species in the bats’ diet. For instance, *Anthus trivialis* and *A. pratensis* – representing 3.5% of all prey items – are considered, as other Motacillidae, diurnal migrants, but are frequently captured at night during ringing campaigns in the Alps (Dorka 1966). Similarly, the anecdotal report in the bats’ diet of *Calandra brachydactyla*, *Serinus serinus*, *Carduelis cannabina* and *Riparia riparia* (1.7% of prey items and each appearing only in one scat), belonging to families of mostly diurnal migrants (Families Alaudidae, Fringillidae and Hirundinidae, respectively), may correspond to occasional night flights of these birds (Dorka 1966; Martin 2010; Watson et al. 2011). The presence of the mainly sedentary species *Cetti cetti* and *Sylvia undata* (belonging to the typically migratory family Sylviidae and making up the remaining 1.3% of prey items) may be attributed to dispersal flights known to be undertaken by these species (Cramp & Perrins 1977–1994).

If birds were captured in nest boxes or roosts (Dondini & Vergari 2000), birds that typically use boxes or tree hollows should be present in the diet. However, no members of the family Paridae or the genera *Sitta* and *Certhia* that exhibit this behaviour (Cramp & Perrins 1977–1994) appear in any dropping. Furthermore, identified prey species that may use these roosts, such as *Ficedula hypoleuca* and *Erithacus rubecula*, do so only during the breeding season in late spring/early summer (Cramp & Perrins 1977–1994) when bats do not consume birds (Ibáñez et al. 2001). In agreement with predictions based on the ecomorphological characteristics of *N. lasiopterus*, our results clearly support the hypothesis that birds are captured, and presumably consumed (Ibáñez et al. 2001, 2003), in the air during the birds’ high-altitude migratory or dispersal movements (>500 m; Popa-Lisseau & Ibáñez 2007), and not while resting (Ibáñez et al. 2001, 2003). No evidence was collected in favour of bats catching birds encountered at their roosts, but this cannot be entirely ruled out (Dondini & Vergari 2000; Smirnov & Vekhnik 2013). If so, however, these captures would be rare and unlikely to result from an active search, in contrast to avian nest predators such as woodpeckers (Nilsson 1984; Weidinger 2009).

**Dietary niche breadth**

The high diversity of species consumed suggests that *N. lasiopterus* behaves as a generalist predator adjusting consumption largely to availability. For example, four of the five species most frequently preyed upon *Phylloscopus trochilus*, *Sylvia borin*, *S. communis* and *Ficedula hypoleuca* (Table 1) are included within the six most abundant long-distance night migrants of European passerines (Hahn et al. 2009). Diet composition along the postnuptial period matches the general phenology of migration during the same time window, starting in August with exclusively trans-Saharan migrants and ending in November with a majority of partial migrants and/or sedentary species with nocturnal dispersal movements (Table S1, Supporting information, Fig. 3) (Cramp & Perrins 1977–1994). Finally, up to seven different bird species were consumed in one night at the same spot. Furthermore, the number of species identified relative to the number of samples analysed did not reach an asymptote, suggesting that real prey species richness is higher than reflected by our sampling. Extrapolating the curve to over 1000 samples, we estimated prey species richness to be about 43 (Fig. 2). Considering only birds under 25 g (estimated upper size limit for prey, see next section), there are c. 50–55 bird species that have nocturnal flying habits and migrate over Iberia (Martin 2010; BirdLife International 2016; DNP ringing data), a figure that falls within the 95% CI of our estimated total prey diversity. Some of these species are rare or even very rare in the region, which would explain why so many samples are required for the rarefaction curve to reach an asymptote (Fig. 2). It follows that *N. lasiopterus* preys on basically every available bird species within its prey size threshold. This further supports the assumption that the bat is a generalist bird predator that captures birds as they fly across the region.

**Prey size window and the 5% paradigm**

*Nyctalus lasiopterus* consumed prey ranging from c. 5 to 25 g (Table 1), 25 g thus possibly representing the upper size limit of its prey. It is however worth noting that the three families of birds that mainly migrate at night (Musciapidae, Sylviidae and Turdidae) and that make up the largest part of the diet show discontinuous body mass distributions, with very few species weighing between 25 and 35 g. In fact, in Iberia within Sylviidae, only *Acrocephalus arundinaceus* has an average body mass over 25 g. Among Turdidae, there is a large gap between the body mass of the large *Turdus* (c. 50–100 g) and the much smaller *Oenanthe oenanthe* (c. 22 g). The absence of species >25 g in the diet could thus reflect low availability rather than size limitations. Swifts, however, despite showing a constant night flight activity over the whole breeding period (Bruderer & Weitnauer 1972), were absent from the diet, which may suggest that prey over 30 g (bm of *Apus apus* and *A. palliatus* is >30 g) is inaccessible to the bats, as neither flight altitude of swifts nor their speed (Henningsson et al. 2009) seem to be limiting factors against predation by bats.
With an estimate of 5–25 g as the prey body mass window of *N. lasiopterus*, the ratio bird prey to bat body mass, ranging 10–50% and averaging 25%, widely exceeded the 5% threshold in relation to the flight ability of bats hunting airborne prey (Fenton 1990). Most probably, the rule remains valid for bats hunting airborne insects, but not for these bats hunting birds at high altitude (Ibáñez et al. 2003). *N. lasiopterus* does not appear to have particular adaptations to bird hunting; it is very similar in its morphology and ecology to the smaller species in the genus, and its particularly low call frequencies can be explained simply by allometric scaling (Estók & Siemers 2009). There is therefore no objection to applying a 25% prey size threshold to smaller but morphologically and ecologically similar bat species (e.g. those with a strong jaw, generalist diet, long echolocation detection or able to fly high). A bat of 20 g may consequently be able to capture birds between 5 and 10 g if it happens to encounter them while foraging. This prediction suggests a reinterpretation of the discovery by Gloor, Stutz and Ziswiler (Gloor et al. 1989) of feather remains in droppings of *Nyctalus noctula*, a species very similar and closely related to *N. lasiopterus* but with a lower bm of c. 28.5 g (Jones et al. 2009), in Switzerland (Bontadina & Arlettaz 2003). Keeping in mind how long carnivory in *N. lasiopterus* remained undetected, we venture that predation by bats upon nocturnally flying birds, even when rare and opportunistic, may be more widespread among aerial-hawking bats than presently acknowledged.

Prey choice

With a prey to predator body mass ratio as high as 50%, optimal foraging theory would predict prey selection to be sufficiently advantageous to have evolved in *N. lasiopterus*. It has been suggested that the bats’ rapid flight and the short prey detection range permitted by echolocation prevents them from actually making economical decisions when foraging (Barclay & Brigham 1994; but see Vesterinen et al. 2016). However, *N. lasiopterus*, with its high intensity, low frequency echolocation calls and its unique tendency to skip up to three calls (Holderied & von Hesperen 2003) would be able to detect a flying passerine at a distance of up to 29 m using echolocation alone (Estók & Siemers 2009). Such large distances may well allow enough time for target discrimination and decision-making.

Our results support a positive size selection of *N. lasiopterus* during autumn (period of maximum bird availability) towards medium-sized birds (10–15 g; Fig. 4), possibly those representing the most convenient cost/benefit balance in terms of energy and risk avoidance. Large birds (>15 g) may have been selected against (Fig. 4) because of the major challenges and risks involved in their manipulation during flight. Although our results are consistent with *N. lasiopterus* actively choosing prey from those available as predicted, a nonrandom diet relative to availability could also result from a sensory bias or from differential success rates. For example, given their larger size, birds 10–15 g could be easier to spot relative to birds <10 g and consequently receive more attacks. The negative selection of birds >15 g, however, does not support such a detection bias towards larger birds, but it could be a result of lower attack success rates for larger, stronger birds.

Bat predation and bird migration

We show that *N. lasiopterus* is a generalist predator of migrating birds up to 25 g. The majority of songbirds in migration fall within this size category (e.g. 93% of all birds captured in nets in DNP during migration) and are thus potentially exposed to predation by bats. The new prey to bat size threshold further suggests that bat predation upon migrating birds may be more widespread among open space aerial bat hunters. When more precise data on bat population numbers and feeding strategy become available, it may be possible to quantify the bats’ total predation on bird populations and to compare it with that of diurnal specialists on migrating birds such as the Eleonora’s falcon. Even local changes in predation risk have been shown to generate immediate responses in the behaviour of migrating birds (Ydenberg et al. 2004; Jonker et al. 2010), and therefore, predation by bats may have been a contributing factor in shaping bird migration strategies and driving the evolution of nocturnal antipredator behaviours.
Acknowledgements

We thank Pablo Agirre-Mendi, Rafael Lafitte, Elena Migens, Jesús Nogueras and Juan Quetglas for assistance in sample collection, the natural processes monitoring team of the EBD-CSIC for kindly providing bird ringing data, the Molecular Ecology Laboratory of the Estación Biológica de Doñana (LEM-EBD) for logistical support, Detlev Kelm for help with data analysis and Kent Rylander, Roger Jovani, Martina Carrette, Pim Edelaar, D. Kelm, Jens Rydell, and three reviewers (B. Fenton, E.J. Vesterinen and T. Alerstam) for helpful comments on the manuscript. The Servicio de Parques y Jardines de Sevilla and the regional governments of Andalusia and La Rioja provided permits for collecting samples and handling bats. This study was part of the MICINN project CGL 2009-12393 and CGL2012-38610 coordinated by J. Aihartza and I. Garin, UPV/EHU. AGPL was supported by the EU 7th Framework Programme (Marie Skłodowska-Curie actions) and the Ministry of Economy, Science and Employment of the Junta de Andalucía through an Andalucía Talent Hub Fellowship. DPB acknowledges the financial support of the Consejo Superior de Investigaciones Científicas (CSIC) through a JAE-FreDoc Fellowship. EBD received financial support from the Spanish Ministry of Economy and Competitiveness, through the Severo Ochoa Program for Centers of Excellence in R+D+i (SEV-2012-0262).

References

Alberdi A, Garin I, Aizpurua O, Aihartza J (2012) The foraging ecology of the mountain long-eared bat Plecotus macrobaccatus revealed with DNA mini-barcodes. PLoS ONE, 7, e55692.

Anderson CM (1986) Predation and primate evolution. Primates, 27, 15–39.

Andreas M (2010) Study of diet in Middle East and African bats. In: A Tribute to Bats (eds Horacek I, Uhrin M), pp. 265–267. Lesnická práce s.r.o., Armonk, New York, Kostelec nad Černými lesy.

Barclay RM, Brigham RM (1994) Constraints on optimal foraging: a field test of prey discrimination by echolocating insectivorous bats. Animal Behaviour, 48, 1013–1021.

BirdLife International (2016) Species search. Available at: http://www.birdlife.org/datazone/species/search [Accessed February 24, 2016].

Bontadina F, Arlettaz R (2003) A heap of feathers does not make a bat’s diet. Functional Ecology, 17, 141–142.

Bruderer B, Weimaur E (1972) Radarbeobachtungen über Zug und Nachtflüge des Mauerseglers (Apus apus). Revue Suisse de Zoologie, 79, 1190–1200.

Charnov E, Orians GH (1973) Optimal Foraging: Some Theoretical Explorations. University of Washington, Washington.

Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PDN (2009) Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey. Molecular Ecology, 18, 2532–2542.

Clare EL, Barber BR, Sweeney BW, Hebert PDN, Fenton MB (2011) Eating local: influences of habitat on the diet of little brown bats (Myotis lucifugus). Molecular Ecology, 20, 1772–1780.

Clare EL, Symondson WO, Fenton MB (2014) An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (Eptesicus fuscus). Molecular Ecology, 23, 3633–3647.

Colwell RK (2013) EstimateS: Statistical estimation of species richness and shared species from samples (Version 9 and earlier) [Software]. Available from http://viceroy.eeb.uconn.edu/estimates/

Cram P, Perrins CM (eds) (1977–1994) The Birds of the Western Palearctic, vols. 1–9. Oxford University Press, Oxford.

Del Hoyo J, Elliot A, Christie DA (eds) (2004–2006) Handbook of the Birds of the World, vols. 9–11. Lynx Edicions, Barcelona.

Dondini G, Vergari S (2000) Carnivory in the greater noctule bat (Nyctalus lasiotes) in Italy. Journal of Zoology, 251, 233–236.

Dorka V (1966) Das jahres- und tageszeitliche Zugmuster von Kauz- und Langstreckenziehern nach Beobachtungen auf den Alpenpässen Cou/Bretolet (Wallis). Ornithologische Beobachter, 63, 165–223.

Estok P, Siemers BM (2009) Calls of a bird-eater: the echolocation behaviour of the enigmatic greater noctule, Nyctalus lasiotes. Acta Chiropterologica, 11, 405–414.

Fenton MB (1990) The foraging behaviour and ecology of animal-eating bats. Canadian Journal of Zoology, 68, 411–422.

Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean. Ecology Letters, 13, 1055–1071.

Fukui D, Dewa H, Katsuta S, Sato A (2013) Bird predation by the birdlike noctule in Japan. Journal of Mammalogy, 94, 657–661.

Gloor S, Stutz HPB, Ziswiler V (1989) Nutritional habits of the noctule bat Nyctalus noctula (Schreber, 1774) in Switzerland. Myotis, 32–33, 231–242.

Hahn S, Bauer S, Liechti F (2009) The natural link between Europe and Africa – 2.1 billion birds on migration. Oikos, 118, 624–626.

Henningsson P, Karlsson H, Bäckman J, Alerstam T, Hedenström A (2009) Flight speeds of swifts (Apus apus): seasonal differences smaller than expected. Proceedings of the Royal Society of London B, 276, 2395–2401.

Holleried MW, von Helversen O (2003) Echolocation range and wingbeat period match in aerial-hawking bats. Proceedings of the Royal Society B, 270, 2293–2299.

Ibáñez C, Juste J, García-Mudarra JL, Agirre-Mendi PT (2001) Bat predation on nocturnally migrating birds. Proceedings of the National Academy of Sciences of the United States of America, 98, 9700–9702.

Ibáñez C, Juste J, García-Mudarra JL, Agirre-Mendi PT (2003) Feathers as indicator of a bat’s diet: a reply to Bontadina & Arlettaz. Functional Ecology, 17, 143–145.

Jones KE, Bielby J, Cardillo M et al. (2009) PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals: Ecological Archives E090-184. Ecology, 90, 2648.

Jonker RM, Eichhorn G, van Langevelde F, Bauer S (2010) Predation danger can explain changes in timing of migration: the case of the barnacle goose. PLoS ONE, 5, e13369.

Martin G (2010) Birds by Night. Poyser, London.

Mukhin A, Grinkevich V, Helm B (2009) Under cover of darkness: nocturnal life of diurnal birds. Journal of Biological Rhythms, 24, 225–231.

Newton I (2008) The Migration Ecology of Birds. Academic Press, London.
Nilsson SG (1984) The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. *Ornis Scandinavica*, 15, 167–175.

Norberg UM, Fenton MB (1988) Carnivorous bats? *Biological Journal of the Linnean Society*, 33, 383–394.

Pastor-Beviá D, Ibáñez C, García-Mudarra JL, Juste J (2014) A molecular approach to the study of avian DNA in bat feces. *Acta Chiropterologica*, 16, 451–460.

Popa-Lisseanu AG, Ibáñez C (2007) Ecología espacial y trófica del nótulo gigante (*Nyctalus lasiopterus*) en el Parque Nacional de Doñana y su entorno. *Proyectos de Investigación en Parques Nacionales: 2003–2006*, pp. 261–279. Organismo Autónomo de Parques Nacionales, Ministerio de Medio Ambiente, Madrid.

Popa-Lisseanu AG, Delgado-Huertas A, Forero MG et al. (2007) Bats’ conquest of a formidable foraging niche: the myriads of nocturnally migrating songbirds. *PLoS ONE*, 2, e205.

R Development Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Razgour O, Clare EL, Zeale MRK et al. (2011) High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and Evolution*, 1, 556–570.

Rohland N, Hofreiter M (2007) Comparison and optimization of ancient DNA extraction. *BioTechniques*, 42, 343–352.

Schmitz OJ, Hawlena D, Trussell GC (2010) Predator control of ecosystem nutrient dynamics. *Ecology Letters*, 13, 1199–1209.

Simmons NB (2005) Order Chiroptera. In: *Mammal Species of the World: A Taxonomic and Geographic Reference*, vol. 1, 3rd edn (eds Wilson DE, Reeder DM), pp. 312–529. Johns Hopkins University Press, Baltimore.

Smirnov DG, Vekhnik VP (2013) Trophic ecology and predation of the greater noctule bat (*Nyctalus lasiopterus*) in Russia. *Biology Bulletin*, 40, 206–212.

Thabah A, Li G, Wang Y et al. (2007) Diet, echolocation calls, and phylogenetic affinities of the great evening bat (*Daubenton's Bat*; *Myotis daubentonii*): another carnivorous bat. *Journal of Mammalogy*, 88, 728–735.

Vesterinen EJ, Lilley T, Laine VN, Wahlberg N (2013) Next generation sequencing of fecal DNA reveals the dietary diversity of the widespread insectivorous predator Dauben-ton’s Bat (*Myotis daubentonii*) in Southwestern Finland. *PLoS ONE*, 8, e82168.

Vesterinen EJ, Ruokolainen L, Wahlberg N et al. (2016) What you need is what you eat? Prey selection by the bat *Myotis daubentonii*. *Molecular Ecology*, 25, 1581–1594.

Watson ML, Wells JV, Bavis RW (2011) First detection of night flight calls by pine siskins. *Wilson Journal of Ornithology*, 123, 161–164.

Weidinger K (2009) Nest predators of woodland open-nesting songbirds in central Europe. *Ibis*, 151, 352–360.

Ydenberg RC, Butler RW, Lank DB, Smith BD, Ireland J (2004) Western sandpipers have altered migration tactics as peregrine falcon populations have recovered. *Proceedings of the Royal Society B*, 271, 1263–1269.

Zanette LY, White AF, Allen MC, Clinchy M (2011) Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, 334, 1398–1401.

Zeale MRK, Butlin RK, Barker GLA, Lees DC, Jones G (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat feces. *Molecular Ecology Resources*, 11, 236–244.

---

**Data accessibility**

Sample information (date, location, conservation procedure, etc.) and *Cytb* and *COI* sequences are provided in Dryad doi: http://dx.doi.org/10.5061/dryad.00vg4.

**Supporting information**

Additional supporting information may be found in the online version of this article.

**Table S1** Monthly distribution of bird prey items, defined as the number of faecal pellets in which the species was identified (see Materials and Methods), identified in faeces of *Nyctalus lasiopterus* and percentage along 13 years of study.