# First resident population of *Pipistrellus nathusii* (Keyserling and Blasius, 1839) in the Iberian Peninsula

CARLES FLAQUER, RAMON RUIZ-JARILLO, IGNACIO TORRE, and ANTONI ARRIZABALAGA

*Museu de Granollers-Ciències Naturals, 51 Francesc Macià, E-08400 Granollers, Barcelona, Spain*
*E-mail: c.flaquer@museugranollers.org*

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## INTRODUCTION

*Pipistrellus nathusii* (Keyserling and Blasius, 1839) is found from the Iberian Peninsula and British Isles to Asia Minor and Transcaucasia (Bogdanowicz, 1999; Russ *et al.*, 2001). Although considered as a rare species in most European countries, it is thought that this could be merely the consequence of inappropriate sampling methods during bat surveys (Bogdanowicz, 1999).

To date, records of *P. nathusii* from the Iberian Peninsula are scarce: the first reliable data on this species in Spain were gathered in the early twentieth century in Mediterranean areas (Catalonia and Valencia), central Spain and along the Cantabrian coast in the north of the country (Cabrera, 1914; Aguilar-Amat, 1921, 1924; Ibáñez *et al*., 1992). As no new data had been produced for several decades, the species was considered to be extinct (Gonzalez-Álvarez, 1991) until it was recorded in several new localities in the north of the peninsula during the 1990s (Rodríguez-Muñoz *et al*., 1994; Aihartza *et al*., 1997). Nevertheless, even during this period no more than seven individuals were captured (Rodríguez-Muñoz *et al*., 1994; Woutersen and Bafaluy, 2001; Aihartza and Garin, 2002) and most records were of single individuals found in autumn or winter (Ibáñez *et al*., 1992; Aihartza and Garin, 2002; Rodríguez-Muñoz, 2002). Consequently, some authors considered *P. nathusii* to be only an occasional wintering species in the Iberian Peninsula, mainly occurring along the northern coastline (Ibáñez, 1998). A recent review of the status of the species in the Iberian Peninsula recognised that most observations of the species could be considered as doubtful (Rodríguez-Muñoz, 2002) and, according to Rodríguez-Muñoz *et al.* (1994), the species should be considered as insufficiently known.

In this paper we present the first records of mating groups of *P. nathusii* in the Iberian Peninsula, and provide the first evidence of residency in the region for the species. Also, we provide information on biometry and sexual size dimorphism.

## MATERIALS AND METHODS

The study was carried out in the Ebro Delta (Catalonia, NE Iberian Peninsula; UTM: 0º50'E, 40º42'N), one of the largest deltas (320 km$^2$) in the Mediterranean region of the Iberian Peninsula. The landscape is dominated by rice paddies, a few lines of trees (*Eucalyptus globulus* and *Washingtonia filifera*) and isolated buildings, in which roost sites for bats are lacking. The mean (± SE) annual temperature during 2000–2002 was 18.5ºC (± 0.26) with a minimum absolute temperature during this period of 0.2ºC. The mean humidity during the period 2000–2003 was high (75.3% ± 2.08; Ebro Delta Natural Park office).

In 1999, 69 bat boxes were put up by the staff from the Ebro Delta Natural Park to provide more bat roosting sites (14.5% in trees, 42.0% in buildings and to 43.5% on poles). Boxes were fixed to trees with iron nails (Stebbings and Walsh, 1991) and to houses and posts with screws. Bat boxes were made from laminated wood (preserved with natural oil) with walls 20 mm thick, slit size of 15 mm, a rough surrounding landing area of 100 mm, and effective internal measurements: 140 mm long, 195 mm wide, and

250 mm high. All bat boxes are side opening. Two bat box models were used: 44 (63.8%) of model A (one compartment) and 25 (36.2%) of model B, with two communicating compartments divided by laminated wood and with two landing areas, one for each compartment.

Bat boxes were checked on nine occasions between April 2002 and November 2003. Every bat captured was sexed and the following measurements taken: body mass, length of forearm, length of fifth digit finger, and wingspan. The reproductive status of males and females and the occurrence of secondary sexual characters such as the development of buccal pads (Harrison and Davies, 1949) were also checked. The ratio of forearm length to fifth finger was used for determining the species (Speakman *et al*., 1991; Russ *et al.*, 1998; Martinoli *et al*., 2000). All measurements were made with a precision calliper and bats were weighed on a professional digital balance (accuracy to 0.1 g). All animals were released after data had been obtained. As well, some bats were ringed following the procedures established by the Spanish Ministry of the Environment (Ministerio de Medio Ambiente).

For captured individuals, field identification was carried out on the basis of dental criteria (Menu and Popelard, 1987; Helversen, 1989). Additionally, we used a D240x bat detector (Pettersson Elektronics AB, Uppsala) with heterodyne and time expansion (×10) systems, as well as a DAT recorder (SONY TCD-D8) and a laptop computer with a professional sound card (Digigram Vx pocket v2) to record echolocation and social calls (Ahlén, 1990; Barataud, 1996; Russ, 1999). Call samples were recorded close to the boxes during spring, summer and autumn. Sounds were analysed by Bat Sound (Pettersson Elektronics AB, Uppsala). A sample frequency of 44,100 samples/second, 16 bits/sample and automatic FFT with a Hanning window were used. Recordings were screened for the presence of the characteristic social calls, which *P. nathusii* emits during the mating period (Russ, 1999; Russo and Jones, 1999). For molecular analysis one individual wing membrane was punched and samples preserved in ethanol 70% (Worthington Willmer and Barratt, 1996). The correct identification of the species was confirmed on one individual by sequencing a DNA mitochondrial fragment of 802 bp from the cytochrome b gene (C. Ibañez, pers. comm.).

Variation in body mass and skeletal measurements according to sex and season were examined by means of two-way ANOVA. Skeletal measures were compared to other European populations of *P. nathusii*. Before the analysis variables were transformed to reach normality and homoscedasticity (Zar, 1996).

## Results

We captured 64 *P. nathusii* (44 ♂♂ and 20 ♀♀) in bat boxes, of which 24 were ringed (15 ♂♂ and 9 ♀♀). During the study period bat boxes were mainly used by *P. pygmaeus*, which established maternal colonies during the breeding season and also occupied boxes during the non-breeding season. Both species were sharing the same roosts during the study period, although the large size and reddish colour of *P. nathusii* allowed us to separate the two species; identification was confirmed in hand by the use of measurements and dental criteria. We observed two well-developed cusps: one on the first upper incisor and another on the second upper incisor, higher than the secondary cusp of the first. Dorsal fur coloration was reddish during summer season and changed to brown/grey in winter. Additionally, the characteristic social calls, with a rising trill at the end, were recorded in April and August from flying bats and from bats located inside bat boxes.

The body mass of resident males *P. nathusii* (Table 1) showed a significant increase from spring (6.28 g ± 0.18, $n = 12$) to autumn (7.78 g ± 0.25, $n = 6$), with intermediate values in summer (7.02 g ± 0.14, $n = 18$; one-way ANOVA: $F_{2, 33} = 12.12$, $P < 0.001$). From autumn to spring hibernating individuals experienced body mass loss, while from spring to autumn they gained body mass. In spring and autumn, seasons in which both males and females were found together in bat boxes, the body mass of females was significantly greater than that of males (sex: $F_{1, 42} = 14.19$, $P < 0.001$); body mass was higher in autumn than in spring (season: $F_{1, 42} = 37.32$, $P < 0.001$). The two-way ANOVA also revealed that the interaction between body mass and season was not significant ($F_{1, 42} = 0.81$, $P = 0.37$), indicating that body mass of both males and females showed the same

TABLE 1. Some biometric measurements from 64 *P. nathusii* individuals captured from April 2002 to November 2003 in the Ebro Delta, NE Spain. Differences between groups were tested by one-way ANOVA on transformed means

| Variable | ♂♂ | | | | ♀♀ | | | | *F*-value |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | min–max | $\bar{x}$ | SE | *n* | min–max | $\bar{x}$ | SE | |
| Body mass (g) | 41 | 5.90–9.70 | 6.94 | 0.12 | 21 | 5.60–9.60 | 8.01 | 0.24 | 17.67*** |
| Forearm length (FAL, cm) | 39 | 31.75–35.35 | 33.62 | 0.14 | 22 | 32.90–35.10 | 34.20 | 0.14 | 7.00** |
| Length of fifth finger (FFL, cm) | 37 | 40.95–47.05 | 43.34 | 0.25 | 20 | 41.10–50.00 | 44.31 | 0.43 | 3.50 |
| FFL/FAL | 34 | 1.23–1.38 | 1.29 | 0.005 | 20 | 1.19–1.42 | 1.29 | 0.011 | 0.01 |

** — $P < 0.01$; *** — $P < 0.001$

seasonal pattern of increase between spring and autumn. Forearm length measured during spring and autumn showed sexual dimorphism (season: $F_{1, 41} = 1.70$, $P = 0.19$; sex: $F_{1, 41} = 4.20$, $P < 0.05$). Sexual size dimorphism was more evident when all the individuals in the sample were considered (Table 1).

*Pipistrellus nathusii* was detected in bat boxes for the first time in spring 2002, whereas *P. pygmaeus* colonies were already established in these boxes in summer 2000. During the study period the occupancy rate of bat boxes by *P. nathusii* varied from zero in June 2002 to a maximum of 33.3% in November 2002. In November 2002, three boxes were occupied by a male together with 1–4 ♀♀, while five boxes were occupied by a male with 1–2 ♀♀ in November 2003. Furthermore, solitary males were found in three other boxes. Distended epididymes and enlarged buccal pads were observed in six males captured from August to November. Males remained in the study area throughout the year and individuals were trapped in autumn, winter, spring and summer. Nevertheless, females present in early spring disappeared from bat boxes in summer and only returned in autumn (Fig. 1). Male to female proportions changed seasonally throughout the study period (interaction season × sex: $G = 12.50$, *d.f.* = 2, $P < 0.001$) but did not change between years (year × sex: $G = 0.31$, *d.f.* = 2, $P = 0.57$; Fig. 1).

## DISCUSSION

Although some authors have suggested that *P. nathusii* may be resident in the Iberian Peninsula (ICONA, 1986; Rodríguez-Muñoz *et al*., 1994), to date no evidence has ever been provided to support this hypothesis. This paper proves for the first time that the species can be considered as resident in the Iberian Peninsula, since

individuals were observed in the study area throughout the year (including summer). The records of Rodríguez-Muñoz *et al*. (1994) appear doubtful since identification was obtained by examining only echolocation calls, a parameter which may well overlap with that of other species (e.g., Russ, 1999). We know now that male *P. nathusii* remain throughout the year in the study region and that females are present during winter, spring and autumn. Furthermore, our study provides the first proof that the species mates in the region.

Two hypotheses may explain the seasonal absence of females in the study area. The first one assumes that females, like other migratory bats such as *Nyctalus noctula*, return to traditional breeding areas in eastern Europe during late spring (Aellen 1983; Gerell-Lundberg and Gerell, 1994; Kapteyn and Lina, 1994; Limpens and Feenstra, 1997). Agreeing with Kapteyn and Lina (1994) we found less tendencies for philopatry in males, which probably do not return to their birth places and spend summer beyond the southern boundary of the nursing area. The second hypothesis presumes that there is a breeding roost near the study area that has yet to be found. Strengthening this hypothesis, Schober and Grimmberger (1996) indicate that mating *P. nathusii* roosts can be around 15 km from breeding sites. Furthermore, Martinoli *et al*. (2000) suggest that the characteristic social calls emitted by males in August are a possible sign that *P. nathusii* breeds in the area. If this is the case, we are probably dealing with an isolated breeding colony (see Strelkov, 2000), which could represent one of the southernmost breeding colonies of this species in Europe. Whatever the true situation, future research is necessary to determine whether females are breeding in the area or are migrating to eastern Europe to breed. We are currently undertaking radiotracking studies with tagged females in order to obtain an answer to this fascinating question.

An alternative explanation to the disappearance of females from bat boxes in summer could be the high occupancy rate of these shelters by *P. pygmaeus* (C. Flaquer, I. Torre, and R. Ruiz-Jarillo, unpubl. data). The sunniest bat boxes placed on poles and walls (required for breeding) are intensively occupied by colonies of *P. pygmaeus* during the breeding season (C. Flaquer, I. Torre, and R. Ruiz-Jarillo, unpubl. data). Male

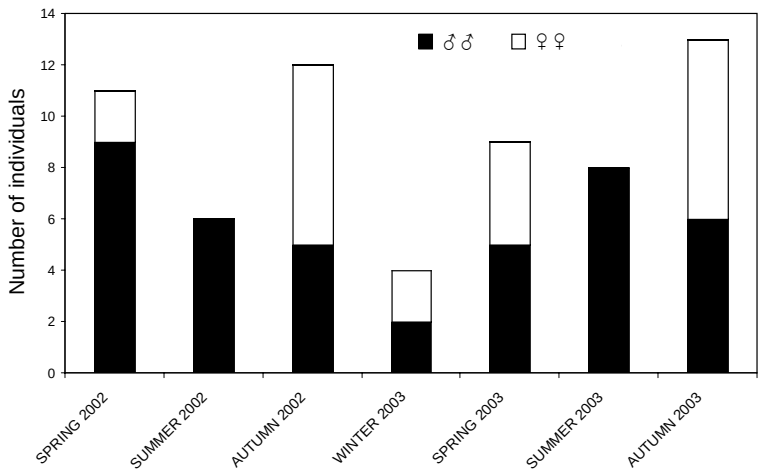


FIG. 1. Seasonal distribution of occurrences of *P. nathusii* throughtout the study period in bat boxes located in the Buda Island (Ebro Delta, Spain)

*P. nathusii* seem to prefer shaded boxes located on trees which are not used by *P. pygmaeus* during breeding.

The analysis of morphological data from *P. nathusii* indicated that there is sexual dimorphism in body mass and forearm length, and that females are bigger than males irrespective of season (spring and autumn). However, morphometric data were within the range of other European populations (Russ *et al.*, 1998; Martinoli *et al.*, 2000; Rodríguez-Muñoz, 2002; Vierhaus, 2004). Year body mass changes observed are related to a common overwintering strategy for temperate-zone species, where bats begin hibernation with fat reserves at their maximum (Schober and Grimmberger, 1996).

Finally, we would like to add that the methodology used in this study, namely the regular checking of bat boxes, may improve knowledge of the status of cryptic taxa such as *P. nathusii*. As Bogdanowicz (1999) has pointed out, knowledge of *P. nathusii* may be a consequence of the use of bat detectors, a limited sampling method that could well underestimate the presence of this species in the Iberian Peninsula.

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