Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast

Lindecke, Oliver; Elksne, Alise ; Holland, Richard; Petersons, Gunnars; Voigt, Christian C.

Journal of Zoology

DOI:
[10.1111/jzo.12654](https://doi.org/10.1111/jzo.12654)

Published: 01/05/2019

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Lindecke, O., Elksne, A., Holland, R., Petersons, G., & Voigt, C. C. (2019). Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast. *Journal of Zoology*, 308(1), 56-65. <https://doi.org/10.1111/jzo.12654>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast

Oliver Lindecke^{1,2*}, Alise Elksne³, Richard A. Holland⁴, Gunārs Pētersons⁵, Christian C. Voigt^{1,2}

¹Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

² Institute of Biology, Freie Universität Berlin, Takustr. 6, 14195 Berlin, Germany

³ Faculty of Biology, University of Latvia, Jelgavas street 1, LV-1004 Riga, Latvia

⁴ School of Biological Sciences, Bangor University, Bangor, Gwynedd LL57 2UW, UK

⁵ Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, K. Helmana street 8, LV 3004 Jelgava, Latvia

*Corresponding author. Email: lindecke@izw-berlin.de

Adress: Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany; Phone: +4930 5168 712

Short title: Migratory orientation of pipistrelle bats

Abstract

Migration routes of bats remain largely unknown, as previous orientation studies have been challenging even with newly developed techniques in tracking, genetic and stable isotope studies. However, a lack of knowledge about migrations poses problems for species conservation, especially in newly described species for which ecological information is not yet available. Here, we aimed to test flight orientation behaviour in the Soprano pipistrelle, *Pipistrellus pygmaeus*. This species has been described only 22 years ago but is now known to have a wide geographic distribution in Europe, yet virtually no information exists about seasonal movements of *P. pygmaeus*. In large parts of the continent, seasonal occurrence of *P. pygmaeus* matches with that of long-distance migratory Nathusius' bats (*P. nathusii*). To shed light on the migratory behaviour of both species, we investigated their orientation decisions at the Latvian Baltic Sea coast which is well-known for summer bat migration along a north-south axis. We developed an arena-based assay designed to measure orientation of takeoffs. The arena was installed in the natural flight path of *Pipistrellus nathusii* and *P. pygmaeus*, and after takeoff, bats chose the direction freely. We detected bearing fidelity between takeoff and departure flights, suggesting bats used cues within the arena, putatively geomagnetic information, which allowed them to set a course prior to takeoff. Further, our results show *P. pygmaeus* orientates in a southerly, seasonally appropriate direction, similar to *P. nathusii* during on-going migration. Therefore, our findings are consistent with true migratory behaviour of *P. pygmaeus* in the northern part of its range. Predicting flight directions of bats based on takeoff direction offers a simple test for orientation studies, and could further be used to test senses of bats under varying treatments, thereby facilitating a comparison of navigational skills across taxa, e.g. bats and birds.

Keywords: Animal orientation, Bat migration, Mammal migration, Movement Ecology, Navigation, *Pipistrellus nathusii*, Take-off

1

2 **Introduction**

3 Bats living in seasonal habitats migrate to reach mating and wintering grounds, yet orientation
4 behaviour and spatial movements of bats remain poorly described for most species owing to the
5 cryptic nature of bat migration (Holland 2007; Voigt et al. 2018). The traditional method of ringing
6 bats with metallic bands uncovered some of the longest continental mammal migrations in the
7 world (Barclay & Bell 1988; Hutterer et al. 2005). While ringing has been shown to be an effective
8 solution for the study of spatial movements, it needs to be applied to vast numbers of bats to ensure
9 reliable data because of often low recovery rates (Barclay & Bell 1988; Steffens et al. 2004; Ellison
10 2008; Holland & Wikelski 2009). Also, some species are sensitive to ringing which led to a
11 reduction of ringing efforts based on ethical reasons (Baker et al. 2001; Dietz et al. 2006). Due to
12 these limitations, our understanding of regional bat movements and migration has been largely
13 restricted to selected species and study locations harbouring stable populations.

14 However, migratory behaviour has been newly described or revisited for many bat species over the
15 past decades based on traditional ringing and rapid developments of other techniques in both field
16 and laboratory (Wilkinson & Fleming 1996; Russell et al. 2005; Morales-Garza et al. 2007; Racey
17 et al. 2007; Bryja et al. 2009; Moussy et al. 2013). For example, genetic techniques have revealed
18 migratory directions could be inferred from pairwise comparisons of haplotype frequencies of
19 common noctules, *Nyctalus noctula*, sampled in nursery roosts and hibernaculas (Petit & Mayer
20 2000). However, both dispersal and migration affect gene flow which makes exact delineations of
21 migratory behaviours based on genetic patterns vulnerable to subadult dispersal movements
22 (Moussy et al. 2013). Further, mating on migration, a common behavioural strategy observed in

European and North American bats, e.g. *Tadarida brasiliensis*, can hamper assignments of individuals to geographically distinct subpopulations (McCracken & Gassel 1997).

Alongside this, automated acoustic recordings of bat echolocation calls represent an alternative technique to detect spatiotemporal occurrences of bats, often suggestive of migratory movements (Heim et al. 2016). Such acoustic monitoring is highly valuable for delineating the phenology of mass occurrences of bats at distinct locations and enable us to identify putatively migratory species by call characteristics (Jarzembowski 2003; Furmankiewicz & Kucharska 2009; Johnson et al. 2011; Rydell et al. 2014). In combination with visual observations, such acoustic monitoring might even enable determination of the direction from which bats arrive at specific locations (Furmankiewicz & Kucharska 2009). Yet, acoustic monitoring alone does not reveal the direction of migratory movements of individual bats, and therefore the observation of seasonal presence of bats using acoustic methods fails to provide convincing evidence for migration

Lastly, stable isotope approaches have revealed the geographical origins of sedentary and migratory bats based on isoscape origin models (Cryan et al. 2004; Ossa et al. 2012; Popa-Lisseanu et al. 2012; Voigt et al. 2012; Lehnert et al. 2014; Voigt et al. 2016), yet the accuracy of these geographic assignments suffers from variability of isotope ratios in consumer tissues and source waters (Voigt & Lehnert 2018). Finally, our understanding of regional and long-distance migration is hampered in bats because conventional approaches are largely incompatible with their small size, which presents tagging bats with long-lasting loggers or GPS units (Holland & Wikelski 2009).

.

Yet , we recently used the direction of departure flights of bats tagged with radio transmitters to infer migratory orientation (*Pipistrellus nathusii*; Lindecke et al. 2015). These bats showed a

1 consistent southern departure flight direction even when translocated 11 km far away from the
2 migration corridor where they were captured (Lindecke et al. 2015). Based on that, we aimed to
3 develop a method to study bat orientation and eventually migration. Here, we investigated the
4 migration behaviour of a cryptic bat species, the Soprano pipistrelle, *Pipistrellus pygmaeus* (Leach,
5 1825). Soprano pipistrelles are particularly interesting with respect to their movement ecology,
6 because this species was once considered to be identical to *P. pipistrellus* (Schreber, 1774), a sister
7 species which lacks any pronounced migration behaviour (Steffens et al. 2004, Hutterer et al.
8 2005). At the turn of the last century, *P. pygmaeus* was described as a new species based on genetic,
9 morphological and acoustic parameters (Barratt et al. 1997; Mayer & von Helversen 2001).
10 Therefore, banding data preceding this date accidentally lumps recapture data of the two species,
11 confounding our understanding of the movement ecology of both species. Recent field studies
12 demonstrated both species prefer distinct habitats, exhibit contrasting foraging behaviours and
13 show different phenologies in large parts of their distribution range (Davidson-Watts & Jones 2006;
14 Davidson-Watts et al. 2006; Nicholls & Racey 2006; Sattler et al. 2007). While *P. pipistrellus* is
15 widely accepted as a sedentary, or eventually regionally migratory bat (Hutterer et al. 2005; Voigt
16 et al. 2012; Voigt et al. 2016; but cf. Bryja et al. 2009), the status of *P. pygmaeus* as a potentially
17 migratory species is still under debate, albeit in-depth genetic studies implied some degree of
18 migratory behaviour for this species (Racey et al. 2007; Bryja et al. 2009; Dietz et al. 2009). Further
19 genetic analysis suggested that migratory behaviour of *P. pygmaeus* is more pronounced in
20 continental European populations than in populations of the British Isles (Sztencel-Jablonka &
21 Bogdanowicz 2011).

22 takeoff Here, we focused on the orientation behaviour of individual *P. pygmaeus* caught between
23 August and September at Pape Bird Ringing Station (PBRs) at the Latvian Baltic Sea coast.
24 Previous acoustic studies demonstrated a regular occurrence of the species at PBRs during summer

1 migration (Rydell et al. 2014; Voigt et al. 2017, 2018), yet it is unknown if this reflects the seasonal
2 occurrence of *P. pygmaeus* in this region without pronounced directional migration flights.
3 Interestingly, similar to other bats passing PBRS on migration, *P. pygmaeus* only exceptionally
4 displays foraging behaviour (Voigt et al. 2017, 2018). In our study, we aimed to compare
5 directionality of flights, here estimated as the orientation behaviour of departing *P. pygmaeus* with
6 that of a well-known European long-distance migrant, *P. nathusii*. Nathusius' bats have been
7 extensively studied at the same location for many years (Petersen 2004; Steffens et al. 2004,
8 Hutterer et al. 2005) and show pronounced southward orientation when released close to the coastal
9 line of PBRS (Lindecke et al. 2015).

10 In order to investigate the migratory behaviour of *P. pygmaeus* and *P. nathusii* without the use of
11 tracking devices, we aimed to first identify a movement behaviour which is performed by all bats
12 and which is measurable at a relatively small scale, yet with sufficient accuracy and precision. In
13 the past, numerous attempts to measure orientation behaviour of bats in a confined space failed
14 (e.g. Mueller 1966), however Wang et al. (2007) demonstrated that Chinese noctules (*Nyctalus*
15 *plancyi*) aligned themselves to the magnetic field when resting in a round plastic basket. In studies
16 of non-migratory bats, Holland and colleagues also demonstrated that bats use a magnetic compass
17 during active flight (Holland et al. 2006; Holland et al. 2010). Based on these studies, we
18 hypothesized that wild bats could use environmental cues, putatively the Earth's magnetic field, for
19 orientation and also directional takeoff from the perimeter of a circular arena. Thus, we predicted
20 that if a bat oriented towards a relatively distant (migratory) goal from inside a circular-shaped test
21 apparatus, we would find bearing fidelity between measures. That means, we would observe
22 persistence between repeated measures of direction, i.e. maintenance of a compass bearing while
23 the focal bat is moving freely. In theory, high bearing fidelity indicates high relevance of the
24 particular movement direction to the individuals' navigational decisions, sometimes despite

1 elevated sensory noise or dynamically changing environmental information. To test our prediction
2 on bearing fidelity in pipistrelles, we developed a simple, easy-to-build setup which we termed a
3 *circular release box* (CRBox) for bats enabling us to register a bats' takeoff orientation (TOO).
4 We observed the directionality of flights, i.e., initial departure flight orientation (DFO) of free
5 flying bats after takeoff from the CRBox. In bird orientation studies, recording of initial movement
6 bearings is standard (Wallraff, 2005), and it has been successfully used in hand-released bats in the
7 past (e.g. Mueller 1966; Buchler & Childs 1982; Serra-Cobo et al. 2000). If *P. pygmaeus* oriented
8 departure flights easterly towards inland instead of the Baltic Sea shoreline, i.e. in stark contrast to
9 *P. nathusii*, we would reconsider our hypothesis of a true migratory motivation in *P. pygmaeus*.

10 Aside from spatial orientation measures, we also timed latency to takeoff. Latency, the delay
11 between a stimulus and a behavioural response, is a standard measure in behavioural assays, e.g.,
12 in studies of spatial learning and escape responses (Sousa et al., 2006; Domenici et al., 2011).
13 Conceptual aspects of both of these established research fields will be integral for future work
14 applying an experimental assay to study bat orientation. Therefore we aimed to record baseline
15 latency data with our CRBox setup. Our study is the first investigating wild migratory mammals
16 with the help of an orientation arena.

18 **MATERIAL AND METHODS**

19 **Study location and animals**

20 Field work was conducted between 24 August and 10 September 2016 at Pape Bird Ringing Station
21 (56°09' N 21°03' E, Rucava Municipality, Latvia) under permit Nr. 31/2016-E issued by the
22 Latvian Nature Conservation Agency. Using a Heligoland funnel trap, we caught in total 64 adult

bats. We aged bats according to the closure of epiphyseal gaps of the phalanges; *P. nathusii*: 19 males, 16 females, *P. pygmaeus*: 12 males, 15 females, 2 individuals with undetermined sex. Bats were kept in wooden boxes in small groups of 3 to 4 individuals until tested.

Test apparatus

All components for construction of the orientation cage for bats, i.e. the CRBox, were purchased from hardware stores. As a measure of precaution, we only used non-magnetic materials to avoid interference with a putative magnetic sense of bats. The same rationale applied to the mechanism for remote release which is operated manually, as any electronics could also potentially interfere with magnetoreception due to electromagnetic noise (Engels et al. 2014). To ensure geomagnetic field intensity at the experimental site (50.8 μ T, measured on 24 Aug 2016) was not altered inside the CRBox, we used an Apple iPhone 5 equipped with a 3D magnetometer (Asahi Kasei Microdevices AK8963, Tokyo, Japan; resolution: 0.15 μ T/LSB, 16-bit) for control measurements. The CRBox consisted of two circular shaped elements, a lid made out of wood, including the mechanism for remote release, and the arena with eight openings for directional choice as the bottom (Fig. 1). The arena part consisted of a shallow funnel, so that a bat started a test below the level of the wall openings, i.e. a focal animal was placed in the centre of the funnel, at its lowest part, and was required to crawl for ~12 cm upwards to leave the arena through one of eight exits. In this way, animals would not face the openings immediately, and thus the effect of a spontaneous escape response is minimized (Fig. 1a). To construct the funnel, we used extruded polystyrol (XPS, Jackodur, Jackson Insulation). We applied synthetic leather as a coat for the arena because it is not slippery for crawling bats and it comes with a surface texture which can be cleaned easily between trials. The eight takeoff platforms were made out of wood and tilted downward 40° since an

inclined platform would facilitate takeoff for bats. Remote release of the focal bat is enabled through lifting the acclimatisation box (non-transparent PET) which was fixed to a stout cord running over a pulley. We attached a white stiff paper flag to the rod as a visual cue for the experimenter to stop pulling the chord when the acclimatisation box was lifted to the maximum. The lid of the CRBox extends beyond the arena (Fig. 1b). The brim serves to hinder the bat from seeing the night sky overhead and to curtail any emitted echolocation calls. If the bat is motivated to gather information from its surroundings, it is thus forced to takeoff. Finally, we used a stool to position the CRBox 1 m above ground to increase the likelihood for bats to takeoff (Fig. 1c). Using a spirit level, we adjusted the CRBox to the horizontal plane.

Release procedure and orientation measures

CRBox tests were conducted over the course of six nights on a meadow of rectangular shape (1,300 m²) surrounded by trees, 100 m distant to the shoreline and only under calm wind conditions and clear sky. The CRBox was placed in the centre of the meadow with a minimum distance of 18 m to surrounding rows of trees. We used a bat detector (Pettersson D-100) tuned to the call frequency of the species under test (40 kHz for *P. nathusii* and 50 kHz for *P. pygmaeus* respectively) to control for any conspecific bat calls at the location of the CRBox. Bats are social animals and may orient towards or be repelled by echolocation or social calls emitted by conspecifics. This could affect exit latency, TOO and vanishing behaviour once a bat is airborne. We tested bats in the second half of the night, with the earliest release starting at 01:30 h and the latest at 05:30 h. We repeated acoustic checks for free flying wild bats between trials. Releases were paused when other bats were registered. Before any trial, the interior of the CRBox and takeoff platforms were cleaned with 70% ethanol to prevent animals from using olfactory cues left by previously tested bats. We

1 manually placed individuals into the acclimatisation box varying the angle for insertion randomly
2 by 90° between tests. Bats were restricted from viewing spatial details of the environment prior to
3 release. We used night vision goggles (BIG25 Safran Vectronix AG) for behavioural observations,
4 i.e., comparison of TOO and subsequent flight. Departure flight bearings were recorded in eight
5 sectors with a width of 45°, i.e., cardinal and ordinal compass directions after the bat had
6 disappeared from view. Final bat vanishing directions could be observed up to a distance of approx.
7 20 to 50 m, depending on whether a focal bat flew into or above the canopy of trees surrounding
8 the meadow. Departure bearings were noted from the position of the CRBox. The observer moved
9 to this position when a bat was about to vanish from viewing range in heights above canopy. If a
10 bat disappeared in the canopy, the observer memorised that spot by means of tree characteristics
11 and recorded the bearing from the position of the CRBox. The error introduced by measuring all
12 departure flight bearings from that single position is negligible considering the 45° wide sectors
13 for recording of data. For analysis of bearing fidelity, we discarded trials in which we were not able
14 to identify DFO due to rapid flight manoeuvres performed by these individuals, and thus could not
15 calculate angular differences (*P. nathusii*, n = 13; *P. pygmaeus*, n = 5). However, we still used
16 takeoff latency data from discarded trials as those were not compromised. Variances of bearing
17 fidelity within and across animals can be evaluated based on angular differences between measures
18 of directionality at different spatial scales, e.g., TOO and DFO. For intra- and inter-individual
19 statistical comparison, the analysis of bearing fidelity requires normalisation of the initial
20 orientation measure (here TOO) to a common direction. As a result, the second measure (DFO)
21 can be visualised relative to the TOO of the bat. Measurement of takeoff latency started as soon as
22 the acclimatisation box was lifted, i.e., when a bat was released inside of the arena, and stopped
23 when the bat launched into air. Based on escape tests with several species of bats (Chase, 1981),
24 we set a 3 minute cut-off time for cancelling a release trial. The experimenter at the other end of

the cord remained motionless to avoid any noise which could hinder bats from taking off or motivate them to change flight paths over the meadow. For *P. pygmaeus*, we measured takeoff latency during 22 trials; in five trials however, we could not record the takeoff precisely enough to include these measurements in the statistical analysis.

Statistical analysis of orientation behaviour and takeoff latency

To test for persistence between TOO and DFO, we normalised TOOs (e.g. N, NE, E, etc.) of all individuals for which we observed departure flights to a common direction (0° ahead) and determined the individual angular difference of respective departure flight bearings by subtraction. If TOO was the consequence of a spontaneous escape-response dependant on direction of insertion or the result of stress by the procedure, we would expect uniform random distribution of departure flight bearings irrespective of normalisation, since we randomly inserted animals to the test apparatus. We determined unimodal deviation from circular uniformity by applying Rayleigh's test (Batschelet 1981). For both species, we compared mean DFO with normalised TOO (0° ahead) by inspecting 95% confidence intervals, which is a way to evaluate whether mean DFO would encompass TOO (confidence interval test), i.e. if bearing fidelity can be assumed after a bat takes off. We further aimed to evaluate how actual vanishing directions of both species matched with the topography around the release site, e.g. whether they were aligned with the coastline, or if orientations resemble migratory orientations previously recorded via capture-recapture (ringing) and radio tracking (Pētersons 2004; Lindecke et al. 2015). At our field station, the coastline runs in a straight line from NNW (339.0°) to SSE (159.0°) in both directions. We calculated mean bearing and mean vector length from DFOs of both species while using Rayleigh's test to control for deviation from circular uniformity. However, we observed bats taking off and vanishing in

both, southerly and northerly directions, as well. Since orientations could eventually be bimodal, we analysed the data axially if $r_{\text{axial}} > r_{\text{unimodal}}$. Inspection of confidence intervals of bat data enabled us to compare it with coastline and site-specific migratory orientations in *P. nathusii*. All circular calculations were performed using Oriana 4.0 software (Kovach Computing Services, Pentraeth, UK).

We measured the time (seconds) a focal bat took to take off from the platforms after the acclimatisation box inside the CRBox got lifted. We compared takeoff latency among species by a Mann-Whitney U test (normality test failed, $P < 0.05$; SigmaPlot 11.0, Systat Software Inc., Chicago, IL, USA). We tested for differences between sexes using a Mann-Whitney U test for *P. nathusii* (normality test failed, $P < 0.05$) and an unpaired t-test for *P. pygmaeus* (normality test, $P = 0.164$).

RESULTS

Bearing fidelity

We measured movement directionality, i.e., TOO and corresponding DFO, in 45 released bats of two species *Pipistrellus nathusii* ($n = 22$), and *P. pygmaeus* ($n = 23$). Normalisation of individual TOOs to a common direction (0°), revealed a significantly unimodal distribution of departure flight bearings (Rayleigh test, *P. nathusii*: $r = 0.456$, $Z = 4.632$, $P = 0.008$; *P. pygmaeus*: $r = 0.605$, $Z = 8.427$, $P = 0.0001$; Fig. 2a, b). Further, in both species, mean vectors (μ) of departure flights did not differ from takeoff bearings (95% confidence intervals for μ , *P. nathusii*: 327.6° to 37.2° with $\mu = 2.4^\circ$; *P. pygmaeus*: 354.7° to 51.0° with $\mu = 19.0^\circ$; confidence interval tests for both species:

1 $P > 0.05$). However after takeoff, we observed occasionally bats circling above the CRBox before
2 disappearing from the experimental area in a straight line, i.e. in DFO.

4 **Orientation in the environmental context**

5 Bearings of *P. nathusii* showed a bimodal distribution (Rayleigh test, $r_{\text{axial}} = 0.502$, $Z = 5.552$, $P =$
6 0.003 ; Fig. 2c) with a mean N–S vector which did not differ significantly from the coast line
7 orientation at our study site (95% confidence intervals for *P. nathusii*: 184.8° to 153.4° with μ_{axial}
8 $= 169.1^\circ - 349.1^\circ$; confidence interval test, $P > 0.05$). *Pipistrellus pygmaeus* were oriented
9 unimodally ($r_{\text{unimodal}} = 0.702$, $Z = 11.322$, $P = 2.53 \cdot 10^{-6}$; Fig. 2d) with a mean vector in SSW (209°)
10 direction.

12 **Takeoff latency**

13 Takeoff latency did not exceed 3 min in total for any of the tested bats (*P. nathusii*: $n = 35$, median:
14 55 s, range: 6–176 s; *P. pygmaeus*: $n = 17$, median: 17 s, range: 6–92 s; Fig. 3). We detected no
15 difference in takeoff latencies between sexes (*P. nathusii*: $U = 131.0$, $P = 0.497$, $n_{\text{males}} = 19$, n_{females}
16 $= 16$; *P. pygmaeus*: $t = -0.183$, $P = 0.858$, $n_{\text{males}} = 6$, $n_{\text{females}} = 9$, excluding two individuals with sex
17 undetermined) and therefore we pooled data for interspecific comparison. *Pipistrellus nathusii*
18 readily showed akinesia when carried and inserted into the CRBox, respectively. Consistent with
19 our hypotheses, this may account for the significant difference in takeoff latency between the two
20 species (Mann-Whitney U-Test, $U = 154.0$, $P = 0.004$).

DISCUSSION

Close to a major migration corridor of bats at the coast of the Baltic Sea in Latvia, we compared the movement orientation of two congeneric bat species, one being a known long-distance migrant (*P. nathusii*) and the other with a putative migration behaviour (*P. pygmaeus*). Our observation of consistent southern heading directions of *P. pygmaeus* is in line with migratory behaviour of this species. Indeed, orientation and takeoff direction were similar to that of *P. nathusii*, a species with known long-distance migration across Europe. The seasonal occurrence of *P. pygmaeus* at our study site in conjunction with orientation towards the south in departing individuals identifies *P. pygmaeus* as a migratory species, at least for its north-eastern distribution range (Voigt et al. 2017, 2018). Using our novel experimental setup, called the circular release box (CRBox), we here showed (1) initial movements of bats are goal-oriented on the level of takeoff, that (2) orientations are biologically meaningful, and that (3) the principal methodology enables comparison of different measures between species. Therefore, we established that takeoff directions of bats are a suitable measure for developing an experimental paradigm for studies of mammal navigation and sensory ecology using bats as a model organism.

Bat orientation relative to the environment

In both species, DFOs matched with the geographical orientation of the local coastline, i.e., the migratory corridor and direction, respectively (Pētersons 2004; Lindecke et al. 2015). For *P. nathusii*, we observed a bimodal orientation of movements in southern and northern direction, whereas *P. pygmaeus* flew in the direction in which bat migration is directed at PBRS in late summer (Lindecke et al. 2015). The observed orientations of *P. pygmaeus* would bring bats back

1 to the flyway above the coastal dunes or 50-100 m parallel to it. In a study by Ahlén et al. (2009)
2 conducted at southern shores of Scandinavia, *P. pygmaeus* showed behaviour similar to *P. nathusii*
3 and *N. noctula*, as well, as it was observed in high numbers at departure sites of these migratory
4 species leaving for offshore flights. However, regarding our subsample of northerly flying *P.*
5 *nathusii*, we hypothesise that these bats reversed orientation to find a day roost in the coastal forest
6 or to forage instead of continuing migration along the dunes. This reverse orientation could have
7 been caused due to the timing of our experiments, which only covered the second part of a night.
8 Species-specific orientation responses can principally change during the activity period of bats
9 (Mistry 1990). Interestingly, in migratory bird studies, similar observations of bimodal orientations
10 have been made, especially at coastal sites (Sandberg et al. 1988; Åkesson et al. 1996). It has been
11 hypothesised that such differences in orientation depend on the stage of migration with reverse
12 movements along the migratory route occurring in individuals from populations which cover
13 shorter distances (Muheim et al. 2017).

14 In roe deer it has recently been found that disturbed individuals directed their initial movements
15 depending on the initial alignment of the body axis (Obleser et al. 2017). In contrast to roe deer
16 individuals aligned on the east-west axis, individuals aligned along the north-south axis escaped
17 not randomly, but toward north or south, respectively. These findings routed in the magnetic
18 alignment hypothesis (Begall et al. 2013; Obleser et al. 2017) deserve consideration in future work
19 based on takeoff as initial movement being measured in departing bats.

20 **Takeoff as measure**

21 Comparisons of takeoff with respect to subsequent flight demonstrated directional persistence
22 between these movements, i.e., bearing fidelity exists between individual TOO and DFO in
23 migratory *P. nathusii* and *P. pygmaeus*. Thus, we inferred that TOO as measured by the CRBox is

1 a suitable proxy to predict DFO after bats started flying. This observation supports our assumption
2 that bats were able to acquire environmental information relevant for orientation and navigation,
3 even in a relatively cue-deprived situation but with a multi-directional choice for takeoff, i.e., in
4 the CRBox. Based on results gathered with a *resting assay* using a similar-sized setup in the
5 laboratory (Wang et al., 2007), we speculate that the Earth's magnetic field might have played a
6 functional role for our bats. Yet again, it is too early to draw further inferences about the role of
7 magnetoreception in bat orientation on the level of takeoff. For example, this would require an
8 experimental setup based on magnetic coils where the geomagnetic-field around a focal bat could
9 be precisely manipulated. However, at the perimeter of the CRBox, echolocation would not have
10 provided useful long-distance information about landmarks at the moment of release, and view of
11 the nightly sky with stars, Milky Way or moon, was inhibited by the design of the CRBox as well
12 (Fig. 1). However, environmental olfactory cues or distant low-frequency sounds, e.g. emitted by
13 the Baltic Sea, might have influenced TOO of our bats as well (Childs & Buchler, 1981; Gagliardo,
14 2013). Previously, bat orientation studies measuring initial flight orientations visually were limited
15 to sampling under twilight conditions at dusk and dawn (Davis, 1966; Buchler & Childs, 1981,
16 1982; Serra-Cobo, et al. 2000). In our study, application of night-vision technique for behavioural
17 observations proved to be a solution to avoid effects of environmental light to which bats might be
18 sensitive (Chase, 1981; Buchler & Childs, 1982; Holland et al. 2010; Greif, et al. 2014). However,
19 we missed some flights due to rapid movements of animals even using advanced optical equipment.

20 We recorded sufficient baseline data about takeoff latencies for the two pipistrelle species and
21 found differences, with *P. nathusii* performing significantly slower than *P. pygmaeus*. Compared
22 to *P. pygmaeus*, *P. nathusii* readily shows a state of motionless inactivity, so-called akinesis, when
23 being handled. We assume this behaviour of *P. nathusii* in response to handling was delaying
24 takeoff. Yet, with respect to bearing fidelity, *P. nathusii*, like *P. pygmaeus*, qualifies as an

appropriate model species for orientation tests with relatively short release trials (< 3 min) which are practicable for testing multiple independent subjects over a short period. Previous research using crawling bats in Y-maze tests suggested a true escape response would take only two to three seconds for active bats in similar settings (Chase, 1981; Mistry 1990). Here observed latencies suggest that our bats did not escape spontaneously but took off after a delay putatively serving orientation in the confined space of the CRBox.

In principle, using an arena-based approach to elucidate preferred bat orientations adds to the methodological repertoire for the study of bat species. Eventually, this could be useful for identification of migration routes of other bats across their ranges. But primarily, testing directionality of takeoff represents a novel option for addressing a multitude of questions in bat physiology and sensory ecology where circular orientation responses have rarely been recorded before. We acknowledge that for certain bat families the current CRBox is eventually unsuitable, e.g. New World leaf-nosed bats (Phyllostomidae; but see Chase, 1981 & 1983 and Gröger & Wiegrebe, 2006) and Old world Horseshoe bats (Rhinolophidae) which commonly do not use quadrupedal locomotion, i.e. crawling. However, for families like Vespertilionidae, Miniopteridae or Molossidae, which readily crawl on the ground or in crevices, TOO measures have high potential for orientation studies, as the principle CRBox design allows measurement of a natural behaviour in these species. Yet, for future works the CRBox should be improved to gain higher resolution of orientation measures, e.g. by offering more exits. This would allow for more sophisticated test procedures enabling statistical comparison between study species or experimental groups (Batschelet 1981).

Conclusions and open questions

Our results suggest *P. pygmaeus* from northern Europe behaves similar to long-distance migratory *P. nathusii* during times of summer migration at the Baltic Sea coast (Lindecke et al. 2015, and this study). The observed orientations would lead bats southwards and back to the flyway along the dunes of the shoreline. Based on our findings combined with previous results about population genetics, phenological occurrences and flight behaviour at sea sides, we argue that *P. pygmaeus* can be considered a true migratory species, at least in northern parts of its distribution range (Ahlén et al. 2009, Sztencel-Jabłonka & Bogdanowicz 2012; Rydell et al. 2014, Voigt et al. 2017, 2018). Quantitative studies based on tagged individuals are now needed to evaluate how regular *P. pygmaeus* migrate over longer distances and where individuals of northern populations are overwintering. Still, it remains unresolved which environmental cues contribute to the compass system bats and other mammals use for migration. Pipistrelle species appear to be a suitable future model to address this question in detail. With an easy-to-build experimental arena setup, we established a method to study initial orientation behaviour of bats, using their natural takeoff behaviour as a proxy for directional choices. Future work is necessary to understand which environmental cues enable bats to orient in the small, confined space of a test arena. We speculate one of the most important cues to be the geomagnetic field (Holland et al. 2006; Wang et al. 2007).

Acknowledgements

We thank Peter Busse and Jörg Lindecke for help with constructions. Volker Kelm gratefully provided optical equipment. For help with capturing bats, we thank all members of the 2016 team at Pape Bird Ringing Station, in particular Viesturs Vintulis, Ilze Brila and Daniels Valerts. Manuel Röleke provided feedback on the manuscript. We thank two anonymous reviewers for comments which improved our manuscript.

Author contributions: O.L. conceived the study, designed and constructed the behavioural test, analysed the data and led the manuscript writing; O.L. and A.E. performed experiments; C.C.V. and G.P. supported fieldwork; C.C.V. and G.P supervised the students; O.L., R.A.H. and C.C.V. interpreted the data and wrote the manuscript.

References

Ahlén, I., Baagøe, H.J., Bach, L. (2009). Behavior of Scandianvian bats during migration and foraging at sea. *J. Mammal.* 90, 1318-1323.

Åkesson, S., Karlsson, L., Walinder, G., Alerstam, T. (1996). Bimodal orientation and the occurrence of temporary reverse bird migration during autumn in south Scandinavia. *Behav. Ecol. Sociobiol.* 38, 293-302.

Baker, G.B., Lumsden, L.F., Dettmann, E.B., Schedvin, N.K., Schulz, M., Jansen, L. (2001). The effect of forearm bands on insectivorous bats (Microchiroptera) in Australia. *Wildl. Res.* 28: 229–237.

Barclay, R.M.R., Bell, G.P. (1988). Marking and observational techniques. Pp. 59-76, in: *Ecological and behavioral methods for the study of bats* (ed. Kunz TH). 533 pp. Smithsonian Institution Press.

Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P.A., Wayne, R.K. (1997). DNA answers the call of pipistrelle bat species. *Nature* 387, 138-139.

Batschelet, E. (1981). *Circular Statistics in Biology*. London: Academic Press.

- 1 **Begall, S., Malkemper, E.P., Červený, J., Němec, P., Burda, H.** (2013) Magnetic alignment in
2 mammals and other animals. *Mammal. Biol.* 78, 10-20.
- 3 **Bryja, J., Kanuch, P., Fornuskova, A., Bartonicka, T., Rehak, Z.** (2009). Low population
4 genetic structuring of two cryptic bat species suggests their migratory behaviour in continental
5 Europe. *Biol. J. Linn. Soc.* 96, 103-114.
- 6 **Buchler, E.R., Childs, S.B.** (1981). Orientation to distant sounds by foraging Big brown bats
7 (*Eptesicus fuscus*). *Animal Behaviour* 29, 428-432.
- 8 **Chase, J.** (1981). Visually guided escape responses of microchiropteran bats. *Anim. Behav.* 29,
9 708-713.
- 10 **Cryan, P.M., Bogan, M.A., Rye, R.O., Landis, G.P., Kester, C.L.** (2004). Stable hydrogen
11 isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *J.*
12 *Mammal.* 85, 995-1001.
- 13 **Davidson-Watts, I., Jones, G.** (2006). Difference in foraging behaviour between *Pipistrellus*
14 *pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825). *J. Zool.* 268, 55-62.
- 15 **Davidson-Watts, I., Walls, S., Jones, G.** (2006). Differential habitat selection *Pipistrellus*
16 *pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species
17 of echolocating bats. *Biol. Conserv.* 133, 118-127.
- 18 **Davis, R.** (1966). Homing performance and homing ability in bats. *Ecol. Monogr.* 36, 201-237.
- 19 **Dietz, C., Dietz, I., Ivanova, T., Siemers, B.** (2006). Effects of forearm bands on horseshoe bats
20 (Chiroptera: Rhinolophidae). *Acta Chiropterol.* 8, 523-535.

- 1 **Dietz, C., von Helversen, O., Nill, D.** (2009). Bats of Britain, Europe and Northwest Africa. 400
2 pp. London: A&C Black Publishers.
- 3 **Dingle, H.** (2014). Migration: the biology of life on the move. 2nd edn. New York: Oxford
4 University Press.
- 5 **Domenici, P., Blagburn, J.M., JP Bacon** (2011) Animal escapology I: theoretical issues and
6 emerging trends in escape trajectories. J. Exp. Biol. 214, 2463-2473.
- 7 **Ellison, L.E.** (2008) Summary and Analysis of the U.S. Government Bat Banding Program: U.S.
8 Geological Survey Open-File Report 1363, 117 p.
- 9 **Furmankiewicz, J., Kucharska, M.** (2009). Migration of bats along a large river valley in
10 southwestern Poland. J. Mammal. 90, 1310-1317.
- 11 **Heim, O., Schröder, A., Eccard, J., Jung, K., Voigt, C.C.** (2016). Seasonal activity patterns of
12 European bats above intensively used farmland. Agr. Ecosyst. Environ. 233, 130-139.
- 13 **Holland, R.A.** (2007). Orientation and navigation in bats: known unknowns or unknown
14 unknowns? Behav. Ecol. Sociobiol. 61, 653-660.
- 15 **Holland, R.A., Borissov, I., Siemers, B.** (2010). A nocturnal mammal, the greater mouse-eared
16 bat, calibrates a magnetic compass by the sun. PNAS 107, 6941-6945.
- 17 **Holland, R.A., Thorup, K., Vonhof, M.J., Cochran, W.W., Wikelski, M.** (2006). Bat orientation
18 using Earth's magnetic field. Nature 444, 653.
- 19 **Holland, R.A., Wikelski, M.** (2009). Studying the migratory behavior of individual bats: current
20 techniques and future directions. J. Mammal. 90, 1324-1329.

- 1 **Hutterer, R., Ivanova, T., Meyer-Cordes, C., Rodrigues, L.** (2005). Bat migrations in Europe –
2 a review of banding data and literature. *Naturschutz Biologie Vielfalt* 28.
- 3 **Johnson, J.B.B., Gates, J.E., Zegre, N.P.** (2011). Monitoring seasonal bat activity on a coastal
4 barrier island in Maryland, USA. *Envir. Monit. Assess.* 173, 685-699.
- 5 **Lehnert, L.S., Kramer-Schadt, S., Schönborn, S., Lindecke, O., Niermann, I., Voigt, C.C.**
6 (2014). Wind Farm Facilities in Germany Kill Noctule Bats from Near and Far. *PLoS ONE* 9,
7 e103106.
- 8 **Lindecke, O., Voigt, C.C., Pētersons, G., Holland, R.A.** (2015). Polarized skylight does not
9 calibrate the compass system of a migratory bat. *Biol. Lett.* 11, 201505125.
- 10 **Mayer, F., von Helversen, O.** (2001). Sympatric distribution of two cryptic bat species across
11 Europe. *Biol. J. Linn. Soc.* 74, 365-374.
- 12 **McCracken, G.F., Gassel, M.F.** (1997). Genetic structure in migratory and nonmigratory
13 populations of Brazilian free-tailed bats. *J. Mammal.* 78, 348-357.
- 14 **Mistry, S.** (1990). Characteristics of the visually guided escape response of the Mexican free-tailed
15 bat, *Tadarida brasiliensis mexicana*. *Anim. Behav.* 39, 314-320.
- 16 **Morales-Garza, M.R., del C. Arizmendi, M., Campos, J.E., Martínez-García, M., Valiente-**
17 **Banuet, A.** (2007). Evidences on the migratory movements of the nectar-feeding bat
18 *Leptonycteris curasoae* in Mexico using random amplified polymorphic DNA (RAPD). *J. Arid.*
19 *Envir.* 68, 248-259.

- 1 **Moussy, C., Hosken, D.J., Mathews, F., Smith, G.C., Aegerter, J.N., Bearhop, S.** (2013).
2 Migration and dispersal patterns of bats and their influence on genetic structure. *Mamm. Rev.*
3 43, 183-195.
- 4 **Mueller, H.C.** (1966). Homing and distance orientation in bats. *Z. Tierpsychol.* 23, 403-421.
- 5 **Muheim, R., Åkesson, S., Bäckman, J., Sjöberg, S.** (2017). Magnetic compass orientation
6 research with migratory songbirds at Stensoffa Ecological Field Station in southern Sweden: why
7 is it so difficult to obtain seasonally appropriate orientation? *J. Avian Biol.* 48, 6-18.
- 8 **Nicholls, B., Racey, P.A.** (2006). Habitat selection as a mechanism of resource partitioning in two
9 cryptic bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. *Ecography* 29, 697-708.
- 10 **Obleser, P., Hart, V., Malkemper, E.P., Begall, S., Holá, M., Painter, M.S., Červený, J.,**
11 **Burda, H.** (2016). Compass-controlled escape behaviour in roe deer. *Behav Ecol Sociobiol*
12 (2016) 70: 1345-1355..
- 13 **Ossa, G., Kramer-Schadt, S., Peel, A.J., Scharf, A.K., Voigt, C.C.** (2012). The movement
14 ecology of the Straw-colored fruit bat, *Eidolon helvum*, in Sub-Saharan Africa assessed by stable
15 isotope ratios. *PLoS ONE* 7, e45729.
- 16 **Pētersons, G.** (2004). Seasonal migrations of northeastern populations of *Pipistrellus nathusii*.
17 *Myotis* 41-42, 29-56.
- 18 **Petit, E., Mayer, F.** (2000). A population genetic analysis of migration: the case of the noctule bat
19 (*Nyctalus noctula*). *Mol. Ecol.* 9, 683-690.

1 **Popa-Lisseanu, A.G., Sörgel, K., Luckner, A., Wassenaar, L.I., Ibáñez, C., Kramer-Schadt,**
2 **S., et al.** (2012). A triple-isotope approach to predict the breeding origins of European bats. PLoS
3 ONE 7, e30388.

4 **Racey, P.A., Barratt, E.M., Burland, T.M., Deaville, R., Gotelli, D., Jones, G., Piertney, S.B.**
5 (2007). Microsatellite DNA polymorphism confirms reproductive isolation and reveals
6 difference in population genetic structure of cryptic pipistrelle bat species. Biol. J. Linn. Soc. 90,
7 539-550.

8 **Russell, A.L., Medellín, R.A., McCracken, G.F.** (2005). Genetic variation and migration in the
9 Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Mol. Ecol. 14, 2207-2222.

10 **Rydell, J., Bach, L., Bach, P., Diaz, L., Furmankiewicz, J., Hagner-Wahlstein, N.,**
11 **Kyheröinen, E.-M., Lilley, T., et al.** (2014). Phenology of migratory bat activity across the
12 Baltic Sea and the south-eastern North Sea. Acta Chiropterol. 16, 139-147.

13 **Sandberg, R., Pettersson, J., Alerstam, T.** (1988). Why do migrating robins, *Erithacus rubecula*,
14 captured at two nearby stop-over sites orient differently? Anim. Behav. 36, 865-876.

15 **Sattler, T., Bontadina, F., Hirzel, A.H., Arlettaz, R.** (2007). Ecological niche modelling of two
16 cryptic bat species calls for a reassessment of their conservation status. J. Appl. Ecol. 44, 1188-
17 1199.

18 **Serra-Cobo, J., López-Roig, M., Marquès-Bonet, T., Lahuerta, E.** (2000). Rivers as a possible
19 landmarks in the orientation flight of *Miniopterus schreibersii*. Acta Theriol. 45, 347-352.

20 **Sousa, N., Almeida, O.F.X., Wotjak, C.T.** (2006). A hitchhiker's guide to behavioral analysis in
21 laboratory rodents. Genes, Brain and Behavior 5 (Suppl. 2), 5-24.

1 **Steffens, R., Zöphel, U., Brockmann, D.** (2004). 40th Anniversary Bat Marking Centre Dresden
2 - Evaluation of Methods and Overview of Results. 132 pp. Dresden: Sächsisches Landesamt für
3 Umwelt und Geologie.

4 **Sztencel-Jablonka A, Bogdanowicz W** (2012). Population genetics study of common
5 (*Pipistrellus pipistrellus*) and soprano (*Pipistrellus pygmaeus*) pipistrelle bats from central
6 Europe suggests interspecific hybridization. Can. J. Zool. 90, 1251-1260.

7 **Voigt, C.C., Frick, W.F., Holderied, M.W., Holland, R.A., Kerth, G., Mello, M.A.R.,**
8 **Plowright, R.K., Swartz, S., Yovel, Y.** (2017). Principles and patterns of bat movements: From
9 aerodynamics to ecology. Quart. Rev. Biol. 92, 267-287.

10 **Voigt, C.C., Lehnert, L.S.** (2018). Tracking bats and other terrestrial mammals: Considerations
11 of physiology. In *Tracking animal migration with stable isotopes* (eds. Hobson KA, Wassenaar
12 LI). Academic Press.

13 **Voigt, C.C., Lindecke, O., Schönborn, S., Kramer-Schadt, S., Lehmann, D.** (2016). Habitat
14 use of migratory bats killed during autumn at wind turbines. Ecol. Appl. 26, 771-783.

15 **Voigt, C.C., Popa-Lisseanu, A., Niermann, I., Kramer-Schadt, S.** (2012) The catchment area of
16 wind farms for European bats: A plea for international regulations. Biol. Conserv. 153, 80-86.

17 **Voigt, C.C., Röleke, M., Marggraf, L., Pētersons, G., Voigt-Heucke, L.** (2017) Migratory bats
18 respond to artificial green light with positive phototaxis. PLoS ONE 12, e0177748.

19 **Voigt, C.C., Rehnig, K., Lindecke, O., Pētersons, G.** (2018) Migratory bats are attracted by red
20 light but not by warm-white light: Implications for the protection of nocturnal migrants. Ecol.
21 Evol. 00, 1-9.

1 **Wang, Y., Pan, Y., Parsons, S., Walker, M., Zhang, S.** (2007). Bats respond to polarity of a
2 magnetic field. *Proc. R. Soc. B* 274, 2901-2905.

3 **Wilkinson, G.S., Fleming, T.H.** (1996). Migration and evolution of lesser long-nosed bats
4 *Leptonycteris curasoae*, inferred from mitochondrial DNA. *Mol. Ecol.* 5, 329-339

6 **Figure legends**

7 **Figure 1.** The circular release box for bats. (a) Cross-section view, with dimensions (in mm)
8 adapted for small bat species of < 80 mm body length. 1 = pulley for remote release, 2 =
9 acclimatisation box with rod and white signal flag, 3 = funnel, 4 = takeoff platform. (b) Top view
10 of the CRBox with takeoff platforms oriented in cardinal and ordinal directions. The roof board is
11 shown transparent. (c) CRBox setup photographed through night vision goggles.

12 **Figure 2.** Orientation behaviour of pipistrelle bats. Circular graphs on the left show normalised
13 departure flight bearings (relative to individual takeoff orientation) of *P. nathusii* (a) and
14 *P. pygmaeus* (b) which were significantly oriented unimodally in both species (*P. nathusii*, n = 22;
15 *P. pygmaeus*, n = 23). Mean vectors (μ) of normalised departure flights were well within the
16 orientation of takeoff (*P. nathusii*: $\mu = 2.4^\circ$; *P. pygmaeus*: $\mu = 19.0^\circ$). Directions of arrows depict
17 the orientation of μ and the lengths reflect the r proportional to the radius of the circle. The 95%
18 confidence intervals are indicated above the mean vector. The two inner circles mark the 5%
19 (dotted) and the 1% significance border. Numbers on gridlines indicate sample sizes covered by
20 the wedges. Circular graphs on the right show departure flight orientations in an environmental
21 context with the Baltic Sea in the west. Vanishings of *P. nathusii* (c) were bimodal with a mean

1 vector similar to the coastal orientation while the mean vector of *P. pygmaeus* (d) was oriented
2 SSW.

3 **Figure 3. Takeoff latency of bats.** Boxplots depict median and mean latency (dashed); outliers
4 are indicated by points not covered by the whiskers (*P. nathusii*: n = 35; *P. pygmaeus*: n = 17). The
5 inset photograph shows a *P. pygmaeus* crawling onto a platform for takeoff. Takeoff latency in
6 both species differed significantly ($P = 0.004$).