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1 **Nathusius' bats optimize long-distance migration by flying at**
2 **maximum range speed**

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19
20 Running title: Optimal migration speed in bats

21
22 **KEY WORDS:** energy consumption, flight metabolism, flight speed, migration
23 physiology, Nathusius' bat, power curve

24 **SUMMARY STATEMENT**

25 Migration speed of wild Nathusius' bats conform with expected optimal travel speed
26 inferred from measurements of metabolic rates in conspecifics flying in a wind tunnel

27

28 **ABSTRACT**

29 Aerial migration is the fastest, yet most energetically demanding way of seasonal
30 movement between habitats. However, for many taxa, and bats in particular, we lack a
31 clear understanding of the energy requirements for migration. Here we examined the
32 energetic cost and flight speed of the long-distance migratory Nathusius bat (*Pipistrellus*
33 *nathusii*). We measured flight metabolism in relation to airspeed in a wind tunnel,
34 inferred the optimal traveling speed over long distances, i.e. maximum range speed,
35 and compared this value to flight speed measured in wild conspecifics. Body mass and
36 wing morphologies were similar in captive and wild bats, indicating that the body
37 condition of captive bats were similar to those of migratory bats. Nine out of the 12
38 captive bats exhibited a U-shaped relationship between flight metabolic power and
39 airspeed when flying in the wind tunnel. The flight metabolic rate across all airspeeds
40 averaged 0.98 ± 0.28 W, which corresponds well to established allometric relationships
41 between flight metabolic rate and body mass for bats. During summer migration, *P.*
42 *nathusii* traveled at an average speed of 6.9 ± 0.7 m s⁻¹, which was significantly higher
43 than minimum power speed (5.8 ± 1.0 m s⁻¹), yet within the range of expected maximum
44 range speed inferred from wind tunnel experiments. This suggests that *P. nathusii* may
45 migrate at an energetically optimal speed and that aerial refueling does not lower
46 substantially migratory speed in *P. nathusii*.

47

48 INTRODUCTION

49 Animals in temperate zones face strong seasonal fluctuations in both climatic conditions and
50 resource abundance. Overcoming these challenges requires a number of physiological and
51 behavioral adaptations that coincide with or even precede seasonal environmental changes.
52 Migration is a central adaptive strategy utilized by many animal taxa in response to a seasonal
53 world (Baker, 1978; Dingle, 2014), yet moving over long distances imposes high nutritional
54 demands on migratory animals (Alerstam and Lindström, 1990; Klaassen, 1996; McWilliams et
55 al., 2004) and animals must adjust their time and energy budgets accordingly. Migration between
56 summer and wintering habitats have been recorded in several bat species (Fleming et al., 2003;
57 Steffens et al., 2004; Hutterer et al., 2005; Popa-Lisseanu and Voigt, 2009; Voigt et al., 2014;
58 Lehnert et al., 2018). However, compared to the vast literature available on migration in other
59 taxa, bat migration has received little attention.

60 Bats exhibit high metabolic rates during sustained flight (Speakman and Thomas, 2003)
61 and must therefore efficiently acquire fuel reserves while still maintaining body conditions
62 conducive to energy efficient flight. To power migration, bats use a mixed fuel strategy
63 metabolizing both endogenous (probably glycogen from muscular tissue and triacylglycerols
64 from adipocytes) and exogenous fuels (nutrients from captured insects) (Voigt et al., 2012;
65 Krüger et al., 2014), yet it is unknown how much energy bats expend during migration and
66 whether or not they fly at optimal speed. Here, we quantified the relationship between flight
67 metabolic rate and airspeed in a small temperate zone migratory bat from Europe, *Pipistrellus*
68 *nathusii*. Based on this relationship, we estimated the optimal flight speed of foraging and
69 migrating bats and compare derived optimal flight speeds with the speed at which *P. nathusii* is
70 foraging and migrating during late summer.

71 Aerodynamic theory states that the relationship between metabolic power and flight
72 speed can be described as a U-shaped curve: metabolic power is high at slow and fast speeds and
73 lowest at intermediate speeds (Pennycuick, 1975; Rayner, 1999). Hedenström (2009) suggested
74 that bats should migrate at maximum range speed, the speed at which energy expended in
75 relation to distance traveled per unit time is minimal, under optimal conditions. Maximum range
76 speed, v_{mr} is higher than minimum power speed (v_{mp}), which is the speed at which bats would
77 stay airborne at lowest cost (Hedenström and Alerstam, 2004). Because prey encounter rate
78 increases with flight speed, the highest net energetic intake might be achieved by foraging at

79 speeds above v_{mp} . So if a migratory bat were to forage opportunistically during migration (aerial
80 refueling), the resulting flight speed might lie somewhere between v_{mr} and v_{mp} . On the other
81 hand, while encounter rate increases with speed, capture success rate still depends on the ability
82 to perform tight turns and incur associated locomotion costs. This maneuverability constraint
83 favors slower speeds and might even lead to flight below v_{mp} . Although recent flight speed
84 measurements have confirmed some of these assumptions for non-migratory bat species
85 (Grodzinski et al., 2009; Holderied and Jones, 2009; Voigt and Holderied, 2012), compared to
86 the vast literature available on birds, little is known about the exact relationship between flight
87 metabolism and speed for any bat, let alone migratory versus non-migratory species.

88 Early studies focused on large tropical bats flying with head masks for respirometric
89 measurements (e.g. Thomas, 1975). First measurements on smaller bat species were done by a
90 combination of doubly-labeled water (DLW) and respirometric methods (Speakman and Racey,
91 1991), by energy budget methods (Winter and von Helversen, 1998) or by respirometry using
92 artificial feeders (Voigt and Winter, 1999). A more recent wind tunnel experiment demonstrated
93 that Seba's short-tailed fruit bats, *Carollia perspicillata*, exhibit a U-shaped power curve over a
94 range of velocities (von Busse et al., 2013). However, von Busse and colleagues also highlighted
95 that individual variation in flight metabolism may be substantial. To assess if this finding holds
96 true for other species and to determine the extent of individual variation in the shape of power
97 curves, it is necessary to employ these same experimental techniques over a wide range of flight
98 speeds with multiple individuals. Further, it is essential to test the U-shaped curve in a migratory
99 bat species that evolved physiological and morphological adaptations in response to long
100 distance migration in order to make predictions regarding optimal flight speeds for migration.

101 In this study, we measured the relationship between flight metabolism and speed in the
102 migratory *P. nathusii*. This seasonal long distance migrant covers up to 2,000 km one way
103 between its northeastern breeding range in Germany, Poland, Belarus, Fennoscandia, the Baltic
104 States and Russia and their winter hibernation sites in southwestern Europe (Pētersons, 2004;
105 Steffen et al., 2004; Hutterer et al., 2005). By measuring the relationship between flight
106 metabolism and speed with actual flight speeds encountered during migration in the field, we
107 then test whether free-ranging migratory bats fly at maximum range speed, or whether foraging
108 during flight (aerial refueling) reduces the realized flight speed.

109

110 MATERIALS AND METHODS

111 Wind tunnel experiment

112 *Study animals and experimental protocol*

113 Measurements of flight metabolism were conducted at the wind tunnel of the Max Planck
114 Institute for Ornithology in Seewiesen, Germany. For more information on the technical
115 specifications of the wind tunnel please refer to Pennycuick et al. (1997). We obtained 12 adult
116 male *P. nathusii* from roost boxes in the Berlin area under the license I E229-OA-AS/G/1051
117 from the “Senatsverwaltung für Stadtentwicklung und Umwelt” in Berlin. Two animals were
118 captured and tested in March 2015 and ten animals were captured and tested in September 2015.
119 After standard veterinary health checks and quarantine, animals were transferred to the MPIO
120 where they were kept in a tent made out of fine polyester mesh with *ad libitum* access to water.
121 Each animal was hand fed with about 1.5 to 2 g of live mealworms per night. The temperature in
122 the keeping room was kept at approximately 18°C, reflecting ambient conditions in natural
123 roosts, and bats were exposed to a natural photoperiod cycle. Experiments with bats followed the
124 animal care and ethics permit under the license 55.2-1-54-2532-12-2014 granted by the federal
125 country of Oberbayern, Germany. Bats were trained with mealworm rewards to fly for one
126 minute bouts at airspeeds between 2 m s⁻¹ and 11 m s⁻¹ at 1 m s⁻¹ increments. In parallel to flight
127 training, bats were habituated to the respirometry chamber. Throughout training, rewards were
128 given less and less frequently and after four nights of training, bats were fed only after
129 completion of an experiment. From each bat, we photographed the outstretched wings and used
130 ImageJ (1.50b, NIH, USA) to measure wingspan and wing area as defined by Norberg and
131 Rayner (1987). Body mass was measured to the nearest milligram with a precision balance
132 (Ohaus, Parsippany, NJ, U.S.A.) at the onset of each metabolic measurement.

133 We used the ¹³C-labeled Na-bicarbonate method as outlined in Hambly et al. (2002,
134 2004) and modified by Voigt and Lewanzik (2011) for instantaneous measurements of ¹³C
135 enriched breath from which the rate of CO₂ production can be determined (Hambly and Voigt,
136 2011). At the start of an experiment, a bat was released in the wind tunnel for a warm-up flight
137 of approximately 10 min at the airspeed to be tested. We then administered 60 mg of isotonic
138 ¹³C-labeled Na-bicarbonate solution (0.29 mol l⁻¹; Euriso-Top GmbH, Saarbrücken, Germany)
139 via intraperitoneal injection and immediately placed the bat in the 850 ml respirometry chamber
140 where temperature was kept constant at 20°C. Flow of CO₂-free air into the chamber was set to

1200 ml min⁻¹ using a mass flow controller (MFS-2, Sable Systems, Las Vegas, NV, USA). We monitored ¹³CO₂ and ¹²CO₂ enrichment in the outlet air of the chamber using a cavity ring-down spectrometer (G1101 CO₂ Isotopic Analyser, Picarro, Sunnyvale, CA, USA). The bat remained in the chamber until the ¹³C enrichment reached a plateau and then declined exponentially. After the 8 to 10 min this took, we released the bat in the wind tunnel flight section for one minute of flight. The bat was then transferred back to the chamber for 20 min and fed immediately afterward.

148

149 *Analysis of respirometry data*

150 For analysis, we focused on a 15 min period starting about 5 min after peak enrichment, because
151 previous studies indicated a constant exponential washout rate of the label within this period
152 (Voigt and Lewanzik, 2011). For each sampling event, we calculated \dot{V}_{CO_2} (ml min⁻¹) by
153 multiplying the combined concentrations of ¹³CO₂ and ¹²CO₂ (ppmV) with the flow-through rate
154 in the chamber using equation (10.5) from Lighton (2008) and assuming that bats exclusively
155 oxidized glycogen during short bouts of flight (respiratory exchange ratio=1.0). We converted
156 values in the delta notation to Atom% according to Slater et al. (2001) and plotted the natural
157 logarithm of excess ¹³C enrichment ($\ln AP^{13}CE$) in relation to time elapsed since the IP injection
158 of ¹³C-labeled Na-bicarbonate.

159 For the pre-flight period, we calculated the fractional isotopic turnover (k_c ; min⁻¹)
160 according to

161

$$162 \quad k_c = [\ln AP^{13}CE(t-1) - \ln AP^{13}CE(t)] / \text{min} \quad (\text{Eqn. 1})$$

163

164 where $AP^{13}CE$ was the ¹³C enrichment above background at time t and at time t-1. We then used
165 the maximal post-injection enrichment of ¹³C in exhaled breath expressed in $AP^{13}CE$ (%) to
166 obtain the total body bicarbonate pool N_c (mol) using the following equation:

167

$$168 \quad N_c = 20 \times e^{(-7.2421 - 1.5458 \times \ln \text{peak } AP^{13}CE)} \quad (\text{Eqn. 2})$$

169

170 This equation was obtained from a dilution experiment described in Voigt *et al.* (2010a). We
171 then multiplied the elimination rate of carbon isotopes, k_c (min⁻¹), with the bicarbonate pool of

172 the animal, N_c (mol CO₂), and converted this into carbon dioxide production rate (\dot{V}_{CO_2} , ml min⁻¹)
173 ¹) using the equation for ideal gases, with the actual measured barometric pressure and assuming
174 a temperature of 30°C. To check the accuracy of the ¹³C-labeled Na-bicarbonate method, we
175 plotted \dot{V}_{CO_2} based on the ¹³C-labeled Na-bicarbonate method in relation to \dot{V}_{CO_2} based on
176 respirometry for the pre-flight period and calculated a two-way ANOVA using air-speed and
177 individual as variables.

178

179 *Estimation of \dot{V}_{CO_2} in flying bats*

180 For estimating k_c (min⁻¹) of a bat when flying in the wind tunnel, we predicted $\ln(AP_{13}CE)$ at the
181 onset and end of the flight interval using two least-squares linear regression equations, one for
182 the relationship between $\ln(AP^{13}CE)$ in relation to time during the pre-flight and one for the post-
183 flight period. We then calculated k_c for flying bats based on equation (1), and converted flight k_c
184 to flight \dot{V}_{CO_2} using the relationship between k_c to \dot{V}_{CO_2} of the pre-flight period. \dot{V}_{CO_2} was then
185 converted to metabolic power by assuming that bats oxidized mostly glycogen during the short
186 flight bouts of our experiment.

187

188 *Inference of v_{mp} and v_{mr} based on flight metabolic power*

189 We plotted flight metabolic rate against airspeed for each individual and tested whether a degree
190 two polynomial (curved regression) fit each dataset better than a degree one polynomial (linear
191 regression). We used a degree two polynomial equation because this is the most parsimonious
192 equation (least complex) for a non-linear function of the expected U-shape with the given sample
193 size of 5-7 data points. For each curved relationship, we estimated v_{mp} as the absolute minimum
194 point of the curve and v_{mr} as the point of tangency between the curve and tangent line passing
195 through the origin.

196

197 **Field studies at migration site**

198 *Measurement of flight speed in foraging and migrating bats*

199 We measured the flight speeds of *P. nathusii* during migration along the shoreline of the Baltic
200 Sea. Echolocation calls of free-flying *P. nathusii* were recorded on 27th and 29th of August 2013,
201 between 19:30 and 23:00, at two distinct sites: migratory bats were recorded at a sea facing

202 forest edge and foraging bats at a clearing within the coastal dune forest within 100 m of the
203 Pape Biological Station (56°09' N 21°03' E, Rucava Municipality, Latvia). The two recording
204 sites were less than 100 m apart from each other. Wind speed measurements were obtained from
205 Rucava meteorological station at about 8 km distance to our study site (<https://www.meteo.lv>;
206 accessed on 20. January 2019). When recording migratory bats on the 27th of August, the station
207 documented a median wind speed of 0 ms⁻¹ during the period of measurements. When recording
208 foraging bats on the 29th, the station documented a median wind speed of about 1 ms⁻¹.
209 However, since the recording site was located in a forest clearing, the local foraging took place
210 in the absence of any wind since the site was protected by trees. Based on this, we refer to
211 measured ground speed as flight speed, assuming no wind interfering with our measurements.

212 The Acoustic Flight Path Tracking (AFPT) recording system consisted of two
213 microphone arrays each with four Knowles BT1759 microphones (Maplewood Drive, Itasca, IL,
214 USA), arranged in a symmetrical star. The two arrays were positioned on either side of the flight
215 route at a distance between the arrays of between 5.81 m and 6.13 m. Microphones were facing
216 in the direction of the approaching bats. All eight microphone channels were recorded sample-
217 synchronously using a hard disk logger (GL1100-16 with 4x GL10-VAMP, Graphtec
218 Corporation, Yokohama, Japan) at 16-bit resolution and 500 kHz sampling rate.

219 The AFPT system utilizes the arrival time differences between the different microphones
220 to triangulate the position of the bat at the moment of sound emission. Sound propagation speed
221 was calculated (Bazley, 1976) for the current air temperature (with 1°C accuracy) and relative
222 humidity (1% accuracy) as measured at the height of the recording microphones with a weather
223 station (GEOS N°11, Skywatch, JDC Electronic SA, Yverdon, CH). Each call emitted by a bat
224 within localization range creates a location, and consecutive localizations will trace the bat's
225 flight trajectory. Because location accuracy of the AFPT system decreases with wind conditions
226 data were recorded under near-windless conditions ($\leq 1 \text{ m s}^{-1}$). For further information see
227 Holderied and von Helversen (2003) and Aubauer (1994).

228 Individual flight trajectories were reconstructed from these single call locations using
229 custom written MatLab scripts (version 6.5 The Mathworks, Natick, MA, USA). Trajectories
230 consist of segments, i.e. curved 3D connections between two consecutive locations. Segments
231 were grouped to trajectories based on their spatial and temporal order, and respective segment
232 flight speeds. Individual localizations leading to sudden jumps in the trajectory and/or the

233 segment flight speeds were manually excluded from analysis as erroneous measurements.
234 Trajectories with less than four segments i.e. five localizations were considered too short and
235 excluded from further analysis. Three mean measures were calculated for each trajectory using
236 MatLab scripts: (i) trajectory height above-ground (by averaging location heights), (ii) trajectory
237 speed (by averaging all segment speeds). Segment speeds were calculated by fitting a 3D circle
238 to three consecutive locations and deriving the velocity on that circular segment, and (iii)
239 tortuosity value (traveled distance divided by net displacement). Trajectories originating from *P.*
240 *nathusii* were identified using the characteristic call end frequency of around 40 kHz. To
241 determine if the individuals tested in the wind tunnel were of similar body size to individuals at
242 the migration corridor and since it was not possible to capture the individuals we recorded flight
243 speeds from, we collected morphological data from conspecifics. To this end, we measured body
244 mass and digitally photographed the outstretched wings of 20 adult males and 20 adult females
245 captured in a Helgoland funnel trap at the field site.

246

247 **Statistical analysis**

248 For testing on differences in metabolic rates across individuals, we used a one-way ANCOVA
249 with individual as a random effect and body mass as a covariate SYSTAT (Systat Software Inc.,
250 Richmond, VA, USA). For testing on the effect of wind speed on flight duration, we used a one-
251 way ANCOVA with individual as random effect and wind speed as covariate. We compared
252 morphological measures (wing area, body mass, wing span and aspect ratio) between males and
253 females using a Mann-Whitney U-test. We also used a Mann-Whitney U-test to compare
254 morphological measures between captive and wild *P. nathusii*. Further, we used Student t-test for
255 comparing flight speeds of foraging and migrating *P. nathusii* with corresponding values derived
256 from wind tunnel measurements. For all statistical tests, we assumed alpha = 0.05. Data are
257 presented as means \pm one SD if not mentioned otherwise.

258

259 **RESULTS**

260 **Wind tunnel experiment**

261 At around 2-3 min post-injection of the isotonic Na¹³HCO₃ solution, ¹³C enrichment in exhaled
262 breath reached peak values, at which the incorporation rate of the label into the animal's
263 bicarbonate pool equaled the washout rate via exhaled CO₂. Afterwards the label decreased

264 exponentially, resulting in a linear decline on the ln-converted scale which was equivalent to the
265 fractional turnover rate, k_c , of the resting bat. A comparison of pre-flight metabolic rates using
266 the ^{13}C labeled Na-bicarbonate method and conventional respirometry revealed an $r^2=0.86$ (see
267 Fig. S1 in Supporting Information). Individual as a factor caused some of the variation in
268 metabolic rates (one-way ANCOVA: $F_{11,61}=2.35$, $P=0.017$). Pre-flight metabolic rate estimated
269 by the ^{13}C -labeled Na-bicarbonate method was on average 5.2 ± 2.1 times higher than that
270 corresponding values obtained by respirometry, yet both parameters were highly correlated
271 ($F_{1,62}=103.4$, $P<0.001$, Fig. S1). The difference between metabolic rates estimated by the ^{13}C -
272 labeled Na-bicarbonate method and respirometry for the pre-flight period was taken into account
273 when using the pre-flight relationship of metabolic rates to estimate flight metabolic rates based
274 on the total bicarbonate pool of the animal N_c and the fractional turnover k_c during flight.

275 The range of flight speeds achievable by individuals varied between minimum 2 to 6 ms^{-1}
276 and maximum 8 to 11 ms^{-1} (Table S1). Individuals did not differ on average in flight duration
277 (one-way ANCOVA: $F_{11,62}=1.51$, $P=0.15$), yet flight duration increased slightly with increasing
278 airspeed ($F_{1,62}=5.37$, $P=0.024$). However, this increase was only moderate ($r^2=0.242$) with flight
279 duration ranging from 68 ± 4 s ($N=8$) at 3 ms^{-1} to 74 ± 9 s ($N=8$) at 9 m s^{-1} , i.e. a 6 s increase in
280 flight duration over a 6 m s^{-1} increase in airspeed.

281 The flight metabolic rate across all airspeeds averaged 0.98 ± 0.28 W (Table S1), which is
282 2.2 ± 0.9 times higher than pre-flight metabolic rates of the same animals. Nine of the 12 bats
283 tested in the wind tunnel exhibited a U-shaped relationship between flight metabolic power and
284 airspeed, data from two individuals was best fit by an inverted U-shape, and data from one
285 individual did not fit well to any regression ($r^2<0.1$) (see Fig. S2). We excluded the datasets of
286 the three individuals, since we observed frequent landings in these animals during the 1-min
287 flight period, which might explain the lack of a U-shaped relationship between flight metabolic
288 rate and speed. For the remaining animals, the average flight metabolic rate at each airspeed
289 followed a U-shaped curve (Fig. 1a). Average v_{mp} based on U-shaped curves from the wind
290 tunnel experiment was 5.8 ± 1.0 m s^{-1} and average v_{mr} was 7.5 ± 1.1 m s^{-1} (Fig. 1b; Table S1).

291

292 **Field studies at migration site**

293 We observed distinct migratory behavior of bats only during the first recording night on August
294 27th, when several hundred individuals were migrating south in straight lines over a depression

295 between the frontal sand dunes and the seaward edge of the mature dune forest (Fig. 2). Between
296 19:30 and 23:00 we recorded 37 flight trajectories based on 395 localizations. Because no flight
297 during this period was observed in the opposite direction, we consider these 37 separate
298 individuals. A single trajectory comprised on average of 10.7 ± 4.6 localizations and covered a
299 distance of 16.0 ± 4.8 m in 2.3 ± 0.70 s. The mean flight height above ground per trajectory was
300 7.8 ± 2.1 m and mean flight speed per trajectory was 6.9 ± 0.7 ms^{-1} .

301 On the 29th we recorded foraging bats flying up and down a local linear gap in the dune
302 forest. Between 19:30 and 23:00 we recorded 40 foraging flight trajectories comprising 672
303 individual localizations. On average a single trajectory was comprised of 16.8 ± 9.1 localizations
304 and covered a distance of 11.6 ± 5.3 m in 2.7 ± 1.4 s. The mean flight height above ground per
305 trajectory was 6.5 ± 2.4 m and the mean flight speed per trajectory was 4.6 ± 0.7 m s^{-1} .

306

307 **Morphology of bats in the field and the wind tunnel**

308 Wing area of females was significantly larger than that of males measured in the field (Mann-
309 Whitney U : $W=296$, $P=0.01$). However, we observed no significant differences in body mass
310 ($W=237$, $P=0.32$), wing span ($W=269$, $P=0.06$), or aspect ratio ($W=198$, $P=0.97$) between the
311 sexes. Morphological measures of bats tested in the wind tunnel can be found in Table S2. We
312 found no differences in wing area ($W=120$, $p=1$), wing span ($W=134$, $P=0.60$), or aspect ratio
313 ($W=128$, $p=0.77$) between bats used in the wind tunnel and males caught in the field. We
314 observed a trend for higher body masses in wild compared to captive bats ($W=69$, $P=0.05$).

315

316 **Comparison of flight speeds**

317 We found that migration flight speed was significantly higher than wind tunnel inferred v_{mp}
318 ($d.f.=44$, $t=4.2$, $P<0.001$), but not significant different to wind tunnel inferred v_{mr} ($d.f.=44$, $t=1.8$,
319 $P<0.081$). Compared to foraging flight speed (4.5 ± 0.7 m s^{-1}), both wind tunnel inferred v_{mr}
320 ($d.f.=47$, $t=10.3$, $P<0.001$) and migration flight speed ($d.f.=47$, $t=4.52$, $P<0.001$) were
321 significantly higher. P values remained below an alpha value of 5% after Bonferroni correction
322 because of multiple testing.

323

324

325 **DISCUSSION**

326 We measured the relationship between flight metabolic rates of Nathusius bats (*Pipistrellus*
327 *nathusii*) and airspeed in a wind tunnel. Averaged flight metabolic rates were in the range of
328 expected values based on allometric scaling (1.1 W for flight metabolic rate of a 7 g bat in:
329 Winter and von Helversen, 1998). Additionally, we found that migration speeds of wild *P.*
330 *nathusii* were higher than minimum power flight speed estimated from data from the wind tunnel
331 experiment. Indeed our data suggest that *P. nathusii* were traveling at v_{mr} along the shoreline of
332 the Baltic Sea towards their hibernacula in southwestern Europe. Therefore, our study confirms
333 that the observed flight speeds are consistent with the expectation that migratory bats practice
334 optimal flight speeds for covering the largest distance with the least amount of energy.
335 Additionally, our data suggests that aerial refueling (Voigt et al., 2012) does not interfere with
336 migration speeds by reducing substantially flight speed in migratory bats. Further, foraging flight
337 speeds observed in the field ($4.6 \pm 0.7 \text{ m s}^{-1}$) were significantly lower than v_{mp} determined in wind
338 tunnel experiments. One possible explanation for this difference is that foraging in a linear gap in
339 the dune forest required sharp turns in the flight trajectories. These tight turns require slower
340 flight speeds, and hence the overall average speed might be reduced in expectation of such turns.
341 A comparison with field flight speeds of *P. nathusii* in less confined habitats (Holderied and
342 Jones, 2009), without tight turns, revealed average foraging speeds ($5.5 \pm 1.1 \text{ m s}^{-1}$) much closer
343 to v_{mp} ($5.8 \pm 1.0 \text{ m s}^{-1}$).

344 Data of migratory flight speed and flight energy expenditure allows us to estimate
345 energetic requirements of trans-continental migration in small-sized bats. However, it is
346 important to note here that our insights into the migratory behavior of bats are still in their
347 infancies. For example, we do not know how long bats migrate or what distances they cover per
348 night, let alone how long it takes to migrate a distance of 2,000 km. Assuming that a migratory
349 bat is traveling at v_{mr} for 4 to 8 h per night, it will require 14.7 to 29.5 kJ. The distance traveled
350 would equal almost 110 km for a 4 h flight or 220 km for an 8 h flight. On a few occasions,
351 banded *P. nathusii* have been recaptured after a single night of migration at a distance of 50 km
352 from their point of release. Although it is likely that bats do not fly in a straight line during
353 migration, we can infer from this value that these bats may have flown for at least 2 hours with a
354 minimum of 7.34 kJ expended for flight alone. Covering the 2,000 km between the summer areas
355 in Northeastern Europe and the hibernacula sites in Western or Southern France would require

356 80 hours of continuous flight, or – given that Nathusius' bats fly a maximum of 7 hours per night
357 (Šuba et al., 2012) – a minimum of 12 days. The total amount of energy a bat like *P. nathusii* has
358 to expend for that flight would amount to almost 300 kJ. Assuming that 50% of this energy
359 originates from the oxidation of triacylglycerols in adipocytes when bats use a mixed-fuel
360 strategy (Voigt et al., 2012), the required amount of fat equals 4.8 g (assuming a 31 kJ g⁻¹ caloric
361 content of fat tissue). At our migration site in Latvia, Nathusius' bats weighed about 5-8 g. Thus,
362 it seems plausible that *P. nathusii* do not fatten up largely before migration flight, but instead
363 may route fat nutrients from consumed insects frequently to their fat depots (Voigt et al., 2012).
364 The insect biomass consumed along the route would amount to 35g to fuel the flight, assuming
365 8.6 kJ g⁻¹ metabolizable energy for insects (Finke, 2002). Bats may benefit from torpor when
366 resting (McGuire et al., 2012), thus it is likely that most energy consumed during migratory
367 journeys will be expended for aerial locomotion.

368 Our comparison of flight speeds is based on several assumptions that we would like
369 evaluate in the following. The first assumption is that the recorded flight speeds of foraging and
370 migrating wild bats was not impaired by local winds. During the time of measurements, we
371 experienced low or no wind speeds when conducting ultrasonic recordings and therefore we
372 assume that measured ground speeds matched with flight speed. If bats would experience
373 stronger winds at their actual position during the recording, our comparison might be flawed by
374 not taking the wind speed at the position of the bat into account. However, based on the wind
375 measurements of a local meteorological station and the observed conditions at our study site, we
376 are confident that wind did not interfere with our estimate of flight speed in both migratory and
377 foraging *P. nathusii*. Second, we assume that bats recorded in flight at the migratory corridor in
378 Latvia were similar in terms of morphology compared to conspecifics captured nearby. This
379 assumption is hard to test, since we could not access those individuals for which we obtained
380 flight trajectories. However, we doubt that these bats were systematically different in body
381 morphology compared to those captured in the funnel trap which was only at about 50 m
382 distance to the microphone arrays. Therefore, we do not expect that this assumption has been
383 violated. A third assumption is that the bats recorded in flight at the migratory corridor were
384 comparable to those used in the wind tunnel experiments. Since we measured only males in the
385 wind tunnel and potentially recorded flight tracks of both males and females in the field, we
386 investigated sex-specific differences in the population of captured conspecifics. Female bats

387 recorded in the field were slightly larger in wing area than males, however, they still did not
388 differ from males in body mass, wingspan, aspect ratio or wing loading. Furthermore, neither
389 females nor males measured in the field were significantly different in wing area from the
390 individuals that flew in the wind tunnel. The bats captured in the field weighed on average
391 0.5 ± 0.1 g more than conspecifics in the wind tunnel experiment. This 6.6% difference in body
392 mass also results in higher wing loading in wild bats compared to those used in the wind tunnel
393 experiment. Therefore, optimal flight speeds of captive bats might have been slightly lower than
394 those of wild bats. Fourth, we assumed that bats migrate without foraging. Indeed, feeding
395 behavior could reduce the overall flight speed of bats during migration because maneuverability
396 is lower at higher flight speed. Overall, we did not observe hunting behavior of *P. nathusii* when
397 the 37 migration flight trajectories were recorded. However, the same species foraged at
398 considerably lower speeds at a nearby forest, suggesting that during migration, distinct phases of
399 migrating and foraging might occur at adjacent locations. It could be argued that the slight, yet
400 non-significant difference between v_{mr} obtained from wind tunnel experiments and flight speed
401 measured in the field is due to the possibility that bats searched for insects en route. On the one
402 hand, this is supported by the fact that insectivorous bats, in contrast to migratory birds, use a
403 mixed fuel to power flight (Voigt et al., 2010b; Voigt et al., 2012). On the other hand, recent
404 studies quantifying echolocation call behavior of migratory *P. nathusii* at our study site showed
405 that feeding buzzes, stereotypic repetitions of echolocation calls indicating a successful hunt,
406 were rare (Voigt et al., 2017, 2018). Possibly, migratory bats hunt after sunset for a short time
407 and afterwards engage in extended periods of migration without any extensive aerial hunts. This
408 is supported by the observation that *P. nathusii* captured during migration defecate (Krüger et al.,
409 2014). Lastly, we observed a U-shaped power curve in only 9 out of 12 animals in the wind
410 tunnel experiment. We rejected the data of 3 individuals based on the observation that these
411 individuals engaged in a number of flight manoeuvres which may have added additional
412 metabolic costs and thus noise to the data set.

413 We conclude *P. nathusii* migrating in late summer along the Latvian shoreline of the
414 Baltic Sea travel at maximum range speed, enabling these bats to cover the longest distances
415 with the least amount of energy. A comparison of bat morphologies reveals no large difference
416 between captive and wild bats and further suggests that wild bats do not carry large fat reserved
417 when migrating. Fuelling the high energy requirements of long-distance flights may force *P.*

418 *nathusii* to consume regularly insects as an exogenous fuel. The absence of hunting events in
419 migratory bats suggest that *P. nathusii* hunt first before engaging in endurance migratory flights
420 at maximum range speed.

421

422

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431

432 **AUTHORS' CONTRIBUTIONS**

433 SAT, MWH, GP and CCV conceived the ideas and designed methodology; SAT, MWH and
434 CCV collected and analysed the data; SAT, MWH and CCV led the writing of the manuscript.
435 All authors contributed critically to the drafts and gave final approval for publication.

436

437 **DATA ACCESSIBILITY**

438 Data will be available from the Dryad Digital Repository.

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550 cheaply than birds? *J. Comp. Physiol. B* **168**, 105-111.

551

552 **FIGURE CAPTIONS**

553

554 **Figure 1: Comparison of measured and predicted flight speeds.** a) Metabolic flight power
555 (W) in relation to airspeed (m s^{-1}) in nine *P. nathusii*. Boxes represent 25 and 75 percentiles, bars
556 indicate medians, solid dots indicate means, and whiskers extend to \pm one SD. A degree two
557 polynomial regression equation was calculated based on means for given airspeeds (solid curved
558 line; note that we neglected the single data point at airspeed 2 ms^{-1}). **b)** Foraging (top) and
559 migrating (bottom) flight speeds. v_{mp} and v_{mr} inferred from metabolic measurements (open
560 boxes) and measured flight speeds in the field (striped), “foraging H&J ‘09” presents data from
561 Holderied and Jones (2009). Bars indicate medians, boxes show 25 and 75 percentiles, whiskers
562 show one SD.

563

564 **Figure 2: Three-dimensional flight tracks of *P. nathusii* recorded while (left) migrating**
565 **along the shoreline of the Baltic Sea,** and (right) foraging in a clearing in the adjacent dune
566 forest close to Pape Biological Station, Latvia. Each dot represents the location at which a bat
567 emitted an echolocation call. Subsequent positions of the same bat were connected to yield a
568 flight track. Colour indicates average flight speed per trajectory.

569

570

571 **SUPPORTING INFORMATION**

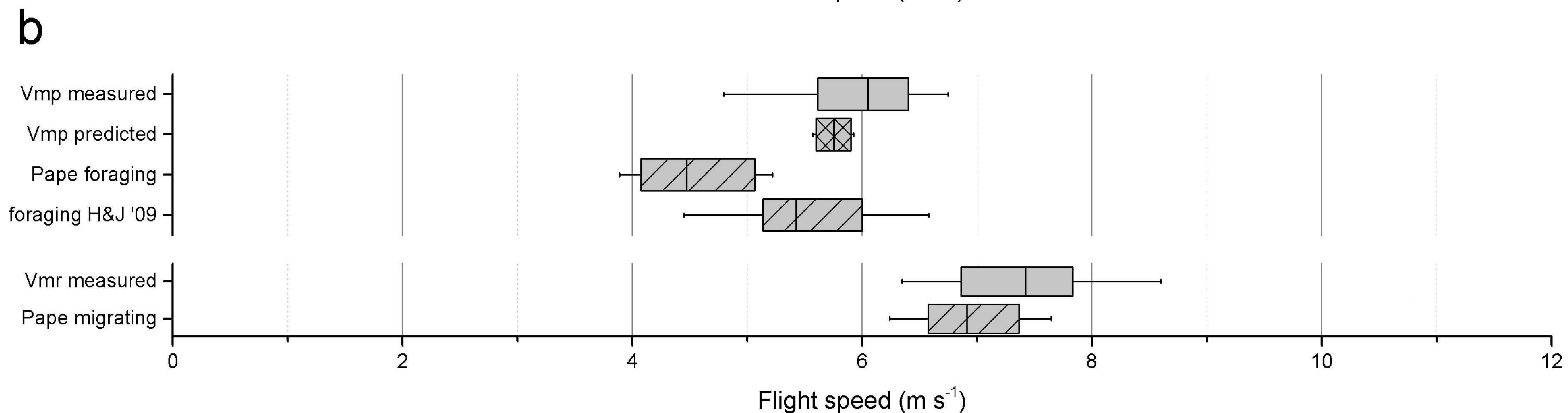
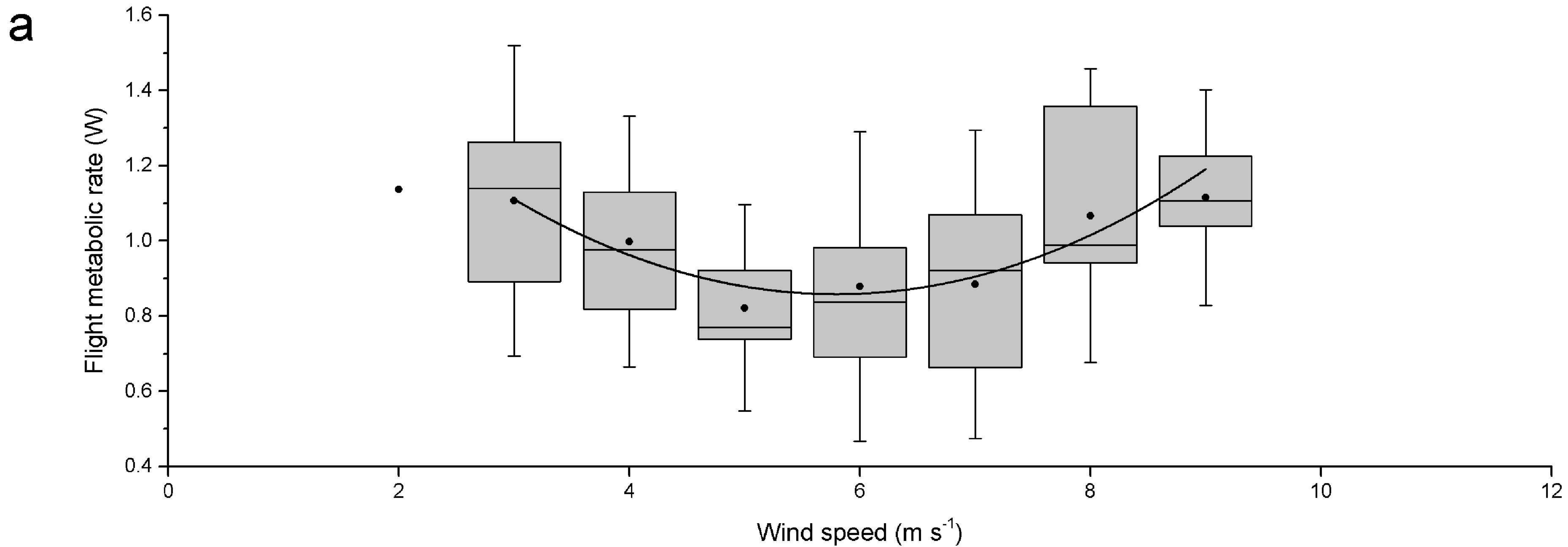
572 Additional supporting information may be found in the online version of this article.

573 **Table S1** Best fit regression equations, minimum and maximum tested airspeeds, and estimated
574 v_{mp} and v_{mr} obtained from wind tunnel experiments

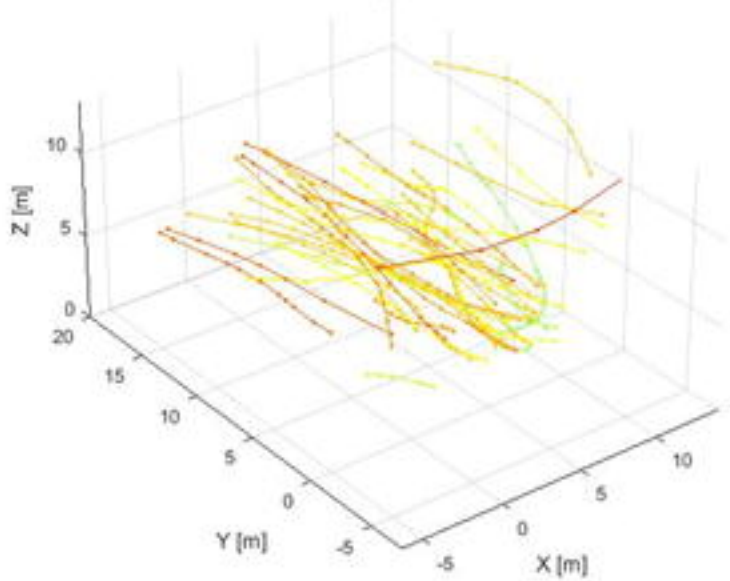
575 **Table S2** Morphological parameters of 12 *P. nathusii*

576 **Figure S1** Metabolic rate of resting bats measured by the ^{13}C -labeled bicarbonate method in
577 relation to metabolic rate measured by respirometry

578 **Figure S2A-L** Relationship between airspeed and flight metabolic rate of 12 *P. nathusii*



migration



foraging

