A comparison of the hibernation patterns of seven bat species in Estonia

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Abstract: We investigated whether bat species with a more northerly border of distribution would hibernate in colder conditions and use fewer crevices and clusters for hibernation than species with a more southerly northern border of distribution. To this end, we measured the temperature and water vapour pressure (as an absolute measure of humidity) of hibernation locations, crevice occupation and clustering among seven sedentary bat species in Estonia. The pond bat (*Myotis dasycneme*), Daubenton's bat (*Myotis daubentonii*) and Brandt's bat/whiskered bat (*Myotis brandtii/mystacinus*) hibernated at higher temperatures and humidity whereas the northern bat (*Eptesicus nilssonii*), the brown long-eared bat (*Plecotus auritus*) and Natterer's bat (*Myotis nattereri*) hibernated at lower temperatures and humidity. The majority of northern bats, Daubenton's bats, brown long-eared bats and Natterer's bats hibernated solitarily whereas Brandt's bats/whiskered bats and pond bats tended to hibernate in clusters. All Natterer's bats hibernated in crevices whereas the six other species hibernated both in crevices and hanging freely on the wall/ceiling. Northern bats inhabiting regions further north than the other six species typically hibernated alone on the wall/ceiling, whereas pond bats, whose distribution border lies further south than those of the other six species, hibernated in warmer and more humid conditions, and often in clusters. However, Natterer's bats inhabiting regions further north than those of pond bats, but whose northern border of distribution lies further south than those of the remaining five species preferred lower temperatures and humidity than the other five species. The results suggest that during the hibernation season in the north of Europe, pond bats focus on saving energy during arousals and subsequent periods of euthermy, whereas Natterer's bats focus on saving energy during hibernation.

Keywords: clustering, crevice occupation, *Eptesicus*, hibernation, humidity, *Myotis*, *Plecotus,* temperature, winter roosts.

Introduction

Hibernation is an energy-saving mechanism for insectivorous bats to survive the cold winter months when food is scarce. In autumn, bats accumulate body fat, which serves as their energy source during winter. During hibernation, their body temperature falls to within 1-2°C of ambient temperature and their metabolic processes slow down, which reduces energy requirements (e.g. Thomas et al. 1990, Geiser 2004). The temperatures at which bats hibernate are species specific (e.g. Siivonen & Wermundsen 2008a). In addition, intraspecific variations exist (e.g. Nagel & Nagel 1991, Webb et al. 1996) because individuals select locations based on their energy reserves (Boyles et al. 2007). Individuals with high energy reserves are less vulnerable to potential negative ecological and physiological effects of hibernation, such as detection by predators, the likelihood of freezing, decreased immune response, motor function and protein synthesis and sleep deprivation (Clawson et al. 1980, Choi et al. 1998, Fre-

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richs 1998, van Breukelen & Martin 2002, Humphries et al. 2003, Kokurewicz 2004, Luis & Hudson 2006). They actively select relatively warm locations and spend less time in hibernation (shorter hibernation bouts; Wojciechowski et al. 2007). Individuals with low energy reserves choose longer hibernation bouts by actively selecting colder temperatures which will minimise their energy expenditure (Wojciechowski et al. 2007). Hibernation is not continuous; bats periodically and spontaneously arouse from torpor (e.g. Krzanowski 1959, Willis 1982). Arousals and subsequent periods of euthermy increase their energy expenditure and account for over 80% of fat depletion during hibernation (Thomas et al. 1990, Speakman et al. 1991, Thomas 1995).

Webb et al. (1996) reviewed the hibernal temperatures of 34 bat species and found large inter and intra species variations in the temperatures at which bats hibernate. They suggested that species with a more northerly distribution are able to hibernate in colder conditions than species with a more southerly distribution. Some authors have reported intra-specific differences in hibernal temperatures between geographic regions (Strelkov 1958, Gaisler 1970, McNab 1974, Masing 1982, Kokurewicz & Kováts 1989). Humidity is another important microclimatic factor for hibernating bats, especially in arid regions (Thomas & Cloutier 1992, Lausen & Barclay 2006). Bats have no special mechanism for controlling water loss during hibernation, so many species hibernate in very humid locations.

Some bat species prefer thermally stable areas, whereas others hibernate in thermally more variable areas (Brack 2007). By hibernating in crevices and by clustering, bats can decrease their exposure to airflow and fluctuating air temperatures and thereby reduce heat and water loss (e.g. Hock 1951, Kokurewicz 2004). Typically these actions reduce heat and water loss by reducing an individual's exposed surface area. However, when body temperature approaches the ambient temperature, heat loss and the potential for saving energy is low. During arousals, bats raise their body temperature to euthermic levels; which is when the benefit of reduced heat loss through hibernation in clusters is highest (Clawson et al. 1980, Arnold 1990, Boyles et al. 2008). Clustering behaviour is also species specific (e.g., Twente 1955, McNab 1974, Clawson et al. 1980, Brack 2007, Siivonen & Wermundsen 2008a). Among the seven bat species that hibernate in Estonia, Daubenton's bat (*Myotis daubentonii*) is reported to hibernate in large groups of up to 140 individuals in Central Europe (Bogdanowicz 1994), Brandt's bat (*Myotis brandtii*) and whiskered bat (*Myotis mystacinus*) hibernate in clusters of up to 13 individuals in Finland (Siivonen & Wermundsen 2008a) and the pond bat (*Myotis dasycneme*) is reported to hibernate in small groups of 2 to 10 bats (Limpens et al. 2000).

To date, no studies on the hibernation ecology of bats in Estonia have systematically compared the use that species make of microclimates (temperature, water vapour pressure), crevices and clusters (for a review of publications, see Masing & Lutsar 2007). Previous studies in Europe have typically used relative humidity as a measure of the humidity of hibernacula. Relative humidity provides information on water vapour pressure in saturated air at a given ambient temperature (Louw 1993). So to compare humidity at different temperatures, water vapour pressure is a better measure of humidity and is the one used in this study.

Higher latitudes (distance from the equator) bring shorter winter days and colder and longer winters. In this study, we tested the theory that species with a more northerly northern border of their range hibernate in colder conditions than species with a more southerly northern border of their range. As hibernating in crevices and clusters means that bats can save energy, we further hypothesised that species with a more southerly northern border of their range would hibernate more often in clusters and/or in crevices than species with more northerly northern border of their range. Among the seven bat species that hibernate in Estonia, the northern distribution border of the pond bat clearly lies further south (at 60°N; Siivonen & Wermundsen 2003, IUCN 2010) than those of the other six species, whereas that of the northern bat (*Eptesicus nilssonii*) lies further north than those of the other six species. The northern distribution borders of Natterer's bat (*Myotis nattereri*) lies north of 63ºN, and that of the brown long-eared bat (*Plecotus auritus*) lies north of 64°N (IUCN 2010, Siivonen & Wermundsen 2008b, respectively): one brown long-eared bat has been found north of 67°N in Russia (Siivonen & Sulkava 1999). The northern edge of the distribution areas for Daubenton's bat and Brandt's bat/whiskered bat lie north of 66°N (Siivonen & Wermundsen 2008b).

Materials and Methods

Study area

We conducted this study in Estonia (24^{o}00'-25^{o}00'E and 58^{o}30'-59^{o}30'N), a country lying within the northern part of the mixed forest sub-zone of the temperate forest zone (Estonica 2007). Seven bat species hibernate in the Estonian study area (Masing 1983, Masing & Lutsar 2007): Daubenton's bat, the pond bat, Brandt's bat, the whiskered bat, Natterer's bat, the northern bat and the brown long-eared bat. In Estonia, bats hibernate from October to April (Masing 1984).

The climate of Estonia contains a mixture of maritime and continental influences. Special characteristics of the Estonian weather include high variability, occasionally strong winds, high precipitation and abrupt fluctuations in temperature. The average annual temperature is between 4.3 and 6.5^{o}C and annual average precipitation ranges from 550 to 800 mm (Estonica 2007). In Estonia, winter lasts from November until April and the coldest month is February (Estonica 2007). The average winter temperature between 1971 and 2000 was -3.6ºC (Eesti Meteoroloogia ja Hüdroloogia Institut 2010).

Data collection

We measured the use of temperature, humidity, crevices, and clusters by hibernating bats in Estonia in March 2005 (three days) and January 2006 (three days). The Estonian Ministry of the Environment issued permission Nr 16-4/2003/T32/8912 for these observations. To minimise disturbance, we identified bat species and took all measurements without handling the bats. Brandt's bat and the whiskered bat closely resemble each other, and can only be definitely identified by examining their teeth or penis. As this was not possible within this study, we present these two species together as Brandt's bat/whiskered bat.

To identify species, we used a Sony DSC-F828 digital camera, a SnakeEye video inspection system, Swarovski EL 10x32 binoculars, a dentist's mirror, and a two-sided make-up mirror (one side being a normal mirror and the other a magnifying mirror) with a 1.5 m handle which allows the mirror to be moved to permit us to inspect crevices from different angles. This equipment enabled us to thoroughly investigate most of the crevices in the hibernacula. The clusters were relatively small, so we could determine the exact number of bats in a cluster.

Overall we measured the use of microhabitat variables by 1214 hibernating bats (table 1). In 2005, we measured the use of hibernation locations by 414 bats in seven underground sites: the Ülgase abandoned mine, four abandoned limestone cellars (three in Järvikandi and one in Haimre); and two military constructions, one with walls of natural stone (in Väänäposti) and another one with concrete walls (in Viti). In 2006, we measured the use of hibernation locations by 800 bats in the same sites and one other, a military construc-

Table 1. Environmental measurements, clustering, and location of bats in Estonian hibernacula in March 2005 and January 2006. Temperature: the temperature at which a bat hibernated; water vapour pressure: the humidity at which a bat hibernated; clustering index: the average size of a cluster that a bat used for hibernation; solitary hibernation: the percentage of bats that hibernated solitarily; crevice occupation: the percentage of bats that hibernated in crevices; clustering of crevice bats: the percentage of bats that hibernated in crevices and were clustered.

	Northern bat	Brandt's bat/ whiskered bat	Daubenton's bat	Brown long-eared bat	Natterer's bat	Pond bat
N						
2005	116	88	65	48	23	74
2006	195	100	252	69	34	150
Temperature (°C) ± sd						
2005	4.1 ± 1.5	5.9 ± 0.8	4.8 ± 1.6	3.8 ± 1.7	2.7 ± 0.5	6.8 ± 0.9
2006	4.3 ± 2.0	6.0 ± 1.1	6.5 ± 1.7	4.6 ± 1.7	3.4 ± 0.8	7.1 ± 1.2
Water vapour pressure (Pa) ± sd						
2005	749 ± 105	870 ± 59	799 ± 104	723 ± 126	653 ± 41	935 ± 89
2006	751 ±1 16	876 ± 82	923 ± 131	783 ± 112	737 ± 49	960 ± 97
Clustering index ± sd						
2005	1.1 ± 0.4	1.6 ± 0.7	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.4	3.6 ± 2.9
2006	1.4 ± 0.9	2.2 ± 1.2	1.2 ± 0.5	1.0 ± 0.0	1.3 ± 0.6	6.6 ± 8.6
Solitary hibernation (%)						
2005	90	49	89	94	83	36
2006	74	38	86	100	79	43
Crevice occupation (%)						
2005	35	74	49	58	100	69
2006	39	71	45	68	100	44
Clustering of crevice bats (%)						
2005	10	55	19	11	17	74
2006	26	59	14	0	21	48

tion, with walls of natural stone (in Humala). The mine and military constructions had back sections (rear wings) with stable conditions and a higher temperature, whereas in the front sections, the temperature was lower and more variable, fluctuating according to the climatic conditions outside. The cellars had no back parts with more stable conditions and their temperature fluctuated according to the climatic conditions outside.

We recorded whether bats hibernated solitarily or in clusters, the size of cluster and whether the bats hibernated on the wall/ceiling or in crevices. We classified bats that were in body contact with each other as clustered. We measured the temperature and relative humidity within 5 cm of the bat(s) by using two portable humidity and temperature meters: a VAISALA HM 34 and a VAISALA HMI 41 with an HMP 44L probe (2.7 m). These digital meters provide quick (approximately 10 s) yet accurate spot checks of relative humidity and temperature, thus avoiding any influence from human presence. The measurement range is 0-100% (0-90% ± 2%, 90-100% ± 3%) for humidity and -20-60°C (± 0.3°C) for temperature. Water vapour pressure difference determines the direction and rate of water vapour movement and this was calculated from the relative humidity and temperature (e.g. Louw 1993).

Table 2. Intraspecific comparison of temperature, water vapour pressure, clustering index, solitary hibernation, crevice occupation, and clustering in crevices between March 2005 and January 2006. Temperature: the temperature at which a bat hibernated; water vapour pressure: the humidity at which a bat hibernated; clustering index: the size of a cluster that a bat used for hibernation; solitary hibernation: the proportion of bats that hibernated solitarily; crevice occupation: the proportion of bats that hibernated in crevices; clustering of crevice bats: the proportion of bats that hibernated in crevices in clusters.

		Northern bat	Brandt's bat/ whiskered bat	Daubenton's bat	Brown long-eared bat	Natterer's bat	Pond bat
N	2005	116	88	65	48	23	74
	2006	195	100	252	69	34	150
Temperature							
Mann-Whitney U-test	*z*	-2.132	-1.156	-6.929	-2.198	-3.588	-3.342
	P	0.033	0.248	0.000	0.028	0.000	0.001
Water vapour pressure							
Mann-Whitney U-test	*z*	-0.140	-0.946	-6.651	-1.984	-5.157	-2.338
	P	0.889	0.344	0.000	0.047	0.000	0.019
Clustering index							
Mann-Whitney U-test	z	-3.487	-3.181	-0.799	-2.095	-0.438	-0.500
	P	0.000	0.001	0.425	0.036	0.661	0.617
Solitary hibernation							
Chi-Square test	Pearson's χ^2	12.044	2.253	0.545	4.426	0.090	0.960
	df	1	1	1	1	1	1
	P	0.001	0.133	0.460	0.035	0.764	0.327
Crevice occupation							
Chi-Square test	Pearson's χ^2	0.408	0.192	0.332	1.177	-	12.333
	df	1	1	1	1	-	1
	P	0.523	0.661	0.565	0.278	-	0.000
Clustering in crevices							
Chi-Square test	Pearson's χ^2	4.479	0.197	0.434	-	0.090	8.107
	df	1.000	1	1	-	1	1
	P	0.034	0.657	0.510	-	0.764	0.004

Data analysis

We used SPSS 16.0 for Windows (SPSS Inc. 1989—2005) to analyse the data. We first compared the data from March 2005 and January 2006 within species (tables 1 and 2) by using the Mann-Whitney U-test (temperatures, water vapour pressure and clustering index) and the Chi-square test (solitary hibernation, crevice occupation and use of clusters in crevices). We compared temperatures, water vapour pressure and the clustering index among species using the Kruskal-Wallis test, because the data were not normally distributed. We performed post hoc comparisons of the groups using Dunn's test. We compared crevice occupation, solitary hibernation and clustering in crevices using the Chi-square test, with the binomial test as a post hoc test. We also compared temperatures used by solitary bats in crevices and bats in clusters outside crevices within species using the Mann-Whitney U-test. We considered a *P* value of <0.05 significant. Means appear with standard deviations (± sd).

Table 3. Comparison of environmental measurements and clustering among seven bat species in Estonian hibernacula in March 2005 and January 2006. Temperature: the temperature at which a bat hibernated; water vapour pressure: the humidity at which a bat hibernated; clustering index: the size of a cluster in which a bat hibernated.

		Temperature		Water vapour pressure		Clustering index	
		2005	2006	2005	2006	2005	2006
Kruskal-Wallis test	*K*	218.013	304.234	199.223	304.750	122.853	191.978
	df	5	5	5	5	5	5
	P	0.000	0.000	0.000	0.000	0.000	0.000
Dunn's test							
Northern bat vs. Brandt's bat/whiskered bat	*P*	0.000	0.000	0.000	0.000	0.000	0.000
Northern bat vs. Daubenton's bat	*P*	0.000	0.000	0.017	0.000	<1.000	0.027
Northern bat vs. brown long-eared bat	*P*	<1.000	<1.000	<1.000	<1.000	<1.000	0.000
Northern bat vs. Natterer's bat	*P*	0.000	0.000	0.000	0.705	<1.000	<1.000
Northern bat vs. pond bat	*P*	0.000	0.000	0.000	0.000	0.000	0.000
Brandt's bat/whiskered bat vs. Daubenton's bat	*P*	0.000	0.001	0.000	0.002	0.000	0.000
Brandt's bat/whiskered bat vs. brown long-eared bat	*P*	0.000	0.000	0.000	0.000	0.000	0.000
Brandt's bat/whiskered bat vs. Natterer's bat	*P*	0.000	0.000	0.000	0.000	0.045	0.000
Brandt's bat/whiskered bat vs. pond bat	*P*	0.000	0.000	0.000	0.000	0.000	0.330
Daubenton's bat vs. brown long-eared bat	*P*	0.000	0.000	0.029	0.000	<1.000	0.013
Daubenton's bat vs. Natterer's bat	*P*	0.000	0.000	0.000	0.000	<1.000	<1.000
Daubenton's bat vs. pond bat	*P*	0.000	0.000	0.000	0.176	0.000	0.000
Brown long-eared bat vs. Natterer's bat	*P*	0.043	0.001	<1.000	0.465	<1.000	0.002
Brown long-eared bat vs. pond bat	*P*	0.000	0.000	0.000	0.000	0.000	0.000
Natterer's bat vs. pond bat	*P*	0.000	0.000	0.000	0.000	0.001	0.000

Results

Comparison of March 2005 and January 2006

As the data between 2005 and 2006 differed for between two and five variables per species (tables 1 and 2), we treated the two years separately when making comparisons among species. All species except Brandt's bat/whiskered bat hibernated in higher temperatures in 2006 than in 2005. All species except the northern bat and Brandt's bat/whiskered bat hibernated in higher humidity conditions in 2006 than in 2005. More northern bats hibernated in clusters in 2006 than in 2005 and the clustering index was higher in 2006 than in 2005. In contrast, no brown long-eared bats hibernated in clusters in 2006, and 16% in 2005. More pond bats hibernated in crevices in 2005 than in 2006. More of the pond bats and brown long-eared bats hibernating in crevices were clustered in 2005 than in 2006. In contrast, more northern bats hibernated in clusters in crevices in 2006 than in 2005. In both years, Natterer's bats were found to hibernate only in limestone cellars, whereas pond bats never hibernated there.

Use of microclimates

Hibernation temperatures and humidity (water vapour pressure) differed among species (tables 1 and 3). Pond bats, Daubenton's bats and Brandt's bats/whiskered bats hibernated in higher temperatures and humidity levels than northern bats, brown long-eared bats and Nat-

Table 4. Comparison of solitary hibernation, crevice occupation and clustering of crevice bats among seven bat species in Estonian hibernacula in March 2005 and January 2006. Solitary hibernation: the proportion of bats that hibernated solitarily; crevice occupation: the proportion of bats that hibernated in crevices; clustering in crevices: the proportion of bats that hibernated clustered in crevices.

		Solitary hibernation		Crevice occupation		Clustering in crevices	
		2005	2006	2005	2006	2005	2006
Chi-square test	Pearson's χ^2	110.9	123.4	37.681	40.225	69.161	51.997
	df	5	4	4	4	5	4
	P	0.000	0.000	0.000	0.000	0.000	0.000
Binomial test	*P* (northern bat)	0.000	0.000	0.002	0.003	0.000	0.000
	P (Brandt's bat/whiskered bat)	0.915	0.021	0.000	0.000	0.457	0.154
	P (Daubenton's bat)	0.000	0.000	1.000	0.147	0.001	0.000
	P (brown long-eared bat)	0.000	–	0.312	0.004	0.000	–
	P (Natterer's bat)	0.003	0.001	–	–	0.003	0.001
	P (pond bat)	0.027	0.121	0.002	0.165	0.902	0.902

Table 5. Cluster sizes of seven bat species in Estonian hibernacula in March 2005 and January 2006. Clusters formed by one species are included.

	2005			2006		
	N	x ± sd	Max	*N*	x ± sd	Max
Northern bat	4	2.2 ± 0.5	3	20	2.4 ± 0.8	5
Brandt's bat/whiskered bat	19	2.2 ± 0.4	3	20	2.8 ± 0.9	5
Daubenton's bat	3	2.0 ± 0.0	2	15	2.2 ± 0.6	4
Brown long-eared bat	1	2.0 ± 0.0	2	0	-	-
Natterer's bat	1	2.0 ± 0.0	2	3	2.3 ± 0.6	3
Pond bat	11	3.9 ± 2.5	9	13	5.6 ± 6.3	25

terer's bats. Natterer's bats hibernated in lower temperatures and humidity than other species, and pond bats hibernated in higher temperatures and humidity than other species. Northern bats and brown long-eared bats used similar temperatures and humidity. Brown long-eared bats and Natterer's bats hibernated in similar humidity conditions in 2005 and 2006. Daubenton's bats, pond bats, northern bats and Natterer's bats all hibernated in similar humidity conditions in 2006.

Use of clusters and crevices

Northern bats, Daubenton's bats, brown long-eared bats and Natterer's bats typically hibernated solitarily whereas pond bats hibernated in clusters (tables 1, 3 and 4).

The size of the clusters varied from 2 to 25, and clusters typically contained only one species (table 5). On 15 occasions, we found clusters with two species: pond bat & Brandt's bat/whiskered bat (*n*=7), northern bat & Daubenton's bat (*n*=2), Daubenton's bat & pond bat (*n*=2), northern bat & Daubenton's bat (*n*=1), Daubenton's bat & Brandt's bat/whiskered bat (*n*=1), northern bat & Natterer's bat (*n*=1), brown long-eared bat & Natterer's bat (*n*=1). Most of these multispecies aggregations were formed by only two individual bats. We found one multispecies aggregation with three bats, and one with four bats.

The clustering index differed among spe-

Table 6. Comparison of temperatures used by bats in clusters outside crevices and solitary bats in crevices in four species of bats in Estonia in 2006. In 2005 very few bats (<10) in all species hibernated in clusters outside crevices, so comparisons could not be made. In 2006 all brown long-eared bats hibernated solitarily and all Natterer's bats in crevices, so comparisons could not be made.

		Northern bat	Brandt's bat/ whiskered bat	Daubenton's bat	Pond bat
Year		2006	2006	2006	2006
N	Clustered	30	20	20	53
	In crevice	58	29	98	34
Temperature	Clustered	5.4 ± 0.9	6.7 ± 0.8	7.6 ± 0.9	7.8 ± 0.7
	In crevice	3.3 ± 2.3	5.8 ± 1.1	5.7 ± 2.0	6.5 ± 1.4
Mann-Whitney U-test	z	-4.300	-2.520	-4.157	-5.113
	P	0.000	0.012	0.000	0.000

cies (tables 1 and 3). Brown long-eared bats, northern bats, Daubenton's bats and Natterer's bats hibernated in smaller clusters while pond bats and Brandt's bats/whiskered bats hibernated in larger clusters.

All the individuals of Natterer's bats hibernated in crevices, so this species could not be included in the Chi-Square test. Crevice occupation differed among the other species (tables 1 and 4). Northern bats typically hibernated on the ceiling/wall, whereas Brandt's bats/whiskered bats and brown long-eared bats favoured crevices. The northern bats, Daubenton's bats, the brown long-eared bats and Natterer's bats hibernating in crevices were typically solitary. Brandt's bats/whiskered bats typically hibernated in clusters in crevices. More than 50% of pond bats hibernated in clusters in crevices in 2005, but less than 50% in 2006.

Temperatures used by solitary bats in crevices were lower than those used by bats in clusters outside crevices in the northern bat, Brandt's bat/whiskered bat, Daubenton's bat and the pond bat (table 6).

Discussion

As predicted, the species with the more southerly northern border of distribution, the pond bat, used warmer locations for hibernation than the other six species. This species also hibernated in conditions with the highest humidity and tended to hibernate in clusters. Hibernating in warmer temperatures raises the body temperature and induces shorter hibernation bouts (Wojciechowski et al. 2007). This means that the pond bat has more frequent periods of euthermy than bats that hibernate in colder places. In this study, these bats hibernated in clusters at higher temperatures and in crevices at lower temperatures. The greatest benefit of clustering is reduced heat loss during arousals and subsequent periods of euthermy (Boyles et al. 2008), so the pond bat focuses on saving energy during active periods of the hibernation season. Evaporative water loss is greater at higher body temperatures (Thomas & Cloutier 1992, Thomas 1995), so by hibernating in high humidity conditions, the pond bat reduces evaporation and, consequently, energy loss (Louw 1993).

Natterer's bat inhabits regions further north than those of the pond bat, but its northern border of distribution is further south than those of the remaining five species. In Finland, Natterer's bats inhabit regions below 62°N although in Sweden, it ranges up to 63ºN (IUCN 2010). Contrary to our predictions, Natterer's bat hibernated in the coldest conditions used by the bats we encountered, and our results indicate that this species tends to minimise its energy expenditure in all possible ways. Natterer's bats hibernated at temperatures closest to 2°C, and all of them hibernated in crevices. Water loss due to evaporation is lowest at 2°C and increases at both higher and lower ambient temperatures (Thomas & Geiser 1997). Evaporation increases energy loss (Thomas & Cloutier 1992, Thomas 1995) and airflow increases evaporation rates by transporting water vapour away from the evaporating surface (Louw 1993) which may trigger arousals (Thomas & Geiser 1997). All Natterer's bats hibernated in crevices, which shelter bats from airflow, which decreases evaporation. Natterer's bats hibernated in the driest locations, yet tended to minimise evaporation. Unlike pond bats, Natterer's bats focused on saving energy during periods of hibernation. Natterer's bats typically hibernated solitarily. Longer hibernation bouts lead fewer active periods during hibernation. Thus Natterer's bats do not require to cluster as much as pond bats which saves their energy during euthermic periods.

The northern bat inhabits regions further north than those of the other six species. As predicted, the northern bat typically hibernated alone on the wall/ceiling (using no additional energy-saving methods) in cold and dry places, confirming that it is a hardy species, well adapted to the harsh conditions of the north (e.g. Siivonen & Wermundsen 2008a). The fact that it hibernates in cold places suggests that it tends to save energy (long hibernation bouts) rather than to minimise the cost of hibernation (short hibernation bouts). Brandt's bat/whiskered bat, Daubenton's bat and the brown long-eared bat inhabit regions further north than those of the pond bat and Natterer's bat, but further south than those of the northern bat. Brandt's bats/whiskered bats hibernated in warmer locations than the other two species, suggesting that they tend to minimise the cost of hibernation rather than to save energy. Brandt's bats/whiskered bats used both available energy-saving methods (clusters and crevices) for hibernation, as in Finland (Siivonen & Wermundsen 2008a), and also clustered in crevices, as did the pond bat. Brown long-eared bats tended to save energy during hibernation (choosing cold locations, leading to long hibernation bouts) as did Natterer's bats, which used crevices, although to a lesser extent than Natterer's bats. Daubenton's bats have recently been found to inhabit regions north of the Arctic Circle in summer (Siivonen & Wermundsen 2008b). This species used additional energy-saving methods (clusters and crevices) to a lesser extent (as Kokurewicz 2004 and Siivonen & Wermundsen 2008a have shown) and this suggests that they may tolerate harsh conditions, despite hibernating in relatively warm and humid conditions, especially in 2006.

Previous studies have compared the hibernal conditions of these species in Europe, but none of them have compared all seven of these species. Among the four *Myotis* species in Holland, Natterer's bats hibernated in the coldest conditions (mean = 6.1°C), Brandt's bats (mean = 7.2°C) and Daubenton's bats (mean = 7.3°C) hibernated in more moderate conditions, and pond bats in the warmest conditions (mean = 7.6°C; Daan & Wichers 1968). These findings were in line with those of this study. Masing (1982) reports that among three *Myotis* species in Estonia, pond bats hibernated in the warmest locations (mean = 5.5°C), followed by Daubenton's bats (mean = 5.4°C) and Brandt's bats/whiskered bats (mean = 5.1°C). In Germany, a study of four species found that brown long-eared bats (mean = 4.0°C) hibernated in the coldest conditions, then Brandt's bats/whiskered bats (mean = 4.6°C), with Natterer's bats (mean = 4.9°C) choosing the warmest conditions (Nagel & Nagel 1991) - a result that does not match our own. Bogdanowicz (1983) studied the temperatures at which brown long-eared bats, Daubenton's bats and Natterer's bats hibernated and their use of crevices in Poland. Brown long-eared bats preferred temperatures ranging from 0.5 to 4.0°C, Daubenton's bats from 1.5 to 6.0°C and Natterer's bats from 2.0 to 6.5°C. Crevice occupation was 82.1% for the brown long-eared bat, 71.4% for Daubenton's bat, and 84.9% for Natterer's bat. Bogdanowicz & Urbańczyk (1983) compared brown long-eared bats, Daubenton's bats and Natterer's bats in Poland and found that the mean hibernation temperature was lowest among brown long-eared bats, followed by Daubenton's bats and Natterer's bats, although Natterer's bats hibernated in the widest range of temperatures. The Polish study found that the mean hibernation humidity was lowest among the brown long-eared bat followed by Daubenton's bat, and highest among Natterer's bat. In Poland, 89% of Natterer's bats, 85% of brown long-eared bats, and 74% of Daubenton's bats hibernated totally or partly in crevices (Bogdanowicz & Urbańczyk 1983). The fact that, further south, Natterer's bat hibernates in warmer condition than other species suggests that it employs a different hibernation strategy near the northern border of its distribution.

In an earlier study in southern Finland, comparing five species, we found that the northern bat (2.0 ± 0.1°C) and the brown long-eared bat (2.7 ± 0.2°C) hibernated in colder and drier locations whereas Daubenton's bat (4.4 ± 0.1°C) and Brandt's bat/whiskered bat (4.0 ± 0.1°C) hibernated in warmer and more humid locations (Siivonen & Wermundsen 2008a). This is also in line with the present study. Multispecies clusters were also uncommon in the Finnish study, which also found no examples of brown long-eared bats clustered with Daubenton's bats. Bogdanowicz (1983) suggests that the formation of multispecies clusters depends on the similarity of the ecological requirements of species. The present study found that pond bats and Natterer's bats never used the same hibernaculum, which may also indicate differences in ecological requirements of these two species.

Hibernacula are typically at their coldest in mid-hibernation season (e.g. Webb et al. 1996, Wermundsen & Siivonen 2009). Bats can move between hibernation bouts, adapting themselves to changes in temperature and humidity (e.g. Wermundsen & Siivonen 2009). This feature should not affect the results of this study however, as the moment of measurement is independent of species. In addition, species-specific patterns of hibernation behaviour remain consistent throughout the hibernation season (Wermundsen & Siivonen 2009).

Further studies

Further studies are recommended to gather data during the entire hibernation season and to determine the availability of temperatures, humidity and crevices. Such work will help improve understanding about differences in hibernation strategies among the seven species. One interesting question is why pond bats and Natterer's bats are much rarer in southern Finland than in Estonia. Is this because Finnish and Estonian bedrock differs? Finnish bedrock conducts heat better, which means that frost penetrates deeper and faster, suggesting that underground conditions are perhaps more unstable in Finland than in Estonia. According to Webb et al. (1996), bats' susceptibility to mortality at subzero temperatures during hibernation may limit the northerly spread of some species. Individual Natterer's bats are sometimes found frozen in Estonia (Masing & Lutsar 2007). Pond bats hibernate at warmer locations which may be difficult to find in Finland in mid and late hibernation season (Wermundsen & Siivonen 2009).

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Samenvatting

Een vergelijking van overwinteringsstrategieën van zeven soorten vleermuizen in Estland

We onderzochten of vleermuizensoorten met een noordelijker verspreidingsgebied in een winterverblijf op koudere plekken overwinteren, minder in spleten overwinteren en minder in clusters overwinteren dan soorten met een zuidelijker verspreidingsgebied. Hiertoe bepaalden we de temperatuur en luchtvochtigheid van hangplekken van dieren in overwinteringslocaties, alsmede de mate van bezetting van spleten en de mate van clustering van zeven inheemse vleermuizensoorten in acht hibernacula in Estland. De meervleermuis (*Myotis dasycneme*), de watervleermuis, (*Myotis daubentonii*) en Brandt's vleermuis/ baardvleermuis (*Myotis brandtii/mystacinus*) overwinterden op plekken met een met hogere temperatuur en luchtvochtigheid dan de noordse vleermuis (*Eptesicus nilssonii*), de bruine grootoorvleermuis (*Plecotus auritus*) en de franjestaart (*Myotis nattereri*). Het merendeel van de noordse vleermuizen, watervleermuizen, bruine grootoorvleermuizen en franjestaarten overwinterden solitair, terwijl Brandt's vleermuizen/baardvleermuizen en meervleermuizen meer in clusters overwinterden. De franjestaart overwinterde uitsluitend in spleten, terwijl de andere zes soorten zowel in spleten als vrij hangend overwinterden. Noordse vleermuizen hebben een noordelijker verspreidingsgebied dan de andere zes soorten en overwinterden alleen op de wanden of aan de plafonds, in koude en droge omstandigheden, terwijl meervleermuizen, die een relatief zuidelijk verspreidingsgebied hebben, een voorkeur hadden voor warmere en vochtigere omstandigheden. De resultaten suggereren dat de strategie van de meervleermuis gericht is op het sparen van energie tijdens het periodieke ontwaken en de daarop volgende perioden van euthermie, terwijl die van de franjestaart zich richt op het besparen van energie tijdens de overwintering zelf.

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