Inter- and intraspecific comparisons of retention time in insectivorous bat species (Vespertilionidae)

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Abstract

Like other small terrestrial mammals, bats have a high mass-specific energetic demand because of the fact that they have an unfavorable surface area to volume ratio. Furthermore, bats have a very energy-expensive mode of locomotion: flight. This high energetic demand has to be covered by food intake. The retention time of the digestive tract is one factor affecting the energy intake of bat species. Factors like energy demand, gut volume and dietary specialization influence retention time in mammals. However, maximum retention time for only *Myotis myotis* and transit time only for *M. lucifugus*, *Nyctophilius gloudi* and *Nyctalus noctula* is known. This study investigated the maximum retention times and transit times of 10 Central European bat species. It was hypothesized that the level of specialization of the digestive tract, energy-demanding processes and intestine length would affect the retention time of bats. Fluorescence-marked mealworms *Tenebrio molitor* were used to measure the time between the first ingested mealworm and the first appearance of the marker or the last fluorescing feces, respectively. For the first time, the retention time of 10 insectivorous bat species was measured to determine interspecific differences. Additionally, we measured the retention time of post-lactating female and spermatogenically active male *Pipistrellus pipistrellus* to determine intraspecific differences. The retention time of bats differed significantly between species and is probably influenced by the level of specialization of the digestive tract. High-level specialization of the digestive tract resulted in short retention times. Furthermore, significant intraspecific differences between post-lactating and spermatogenically active individuals of *P. pipistrellus* showed that the retention time within a single species might be influenced by energy-demanding processes (e.g. reproduction).

Introduction

In mammals, as in all organisms, energy expenditure must be balanced with energy intake in order for individuals to survive and reproduce successfully. The ability to maintain a constant body temperature (endothermy) is a major energy expenditure in mammals, but it also provides the benefit of stable conditions for metabolic pathways (Voigt *et al.*, 2003). Small mammals have to invest relatively more energy for staying homeothermic than do larger mammals because small mammals have a higher surface to volume ratio and thus lose heat to the environment more rapidly (Speakman & Thomas, 2003). These higher energetic costs need to be covered by proportionally larger intake of energy from food. For example, while African elephants *Loxodonta africana* ingest daily only about 6.9% of their body mass of approximately 2500 kg (Ruggiero, 1992), Daubenton's bats *Myotis daubentonii* daily consume 43.9% of their approximately 8.2 g body mass (Encarnação & Dietz, 2006).

The net energy intake of an animal depends foremost on the quality of the food, in particular, energy content. Energy-rich food allows for a rapid covering of energetic requirements in short foraging bouts, whereas foraging needs to be longer when feeding on resources with low energy content (Tiebout, 1991; Delorme & Thomas, 1999). The amount of energy that is gained from food also depends on digestive efficiency (Kelm *et al.*, 2008). Animals that have high digestive efficiencies are able to absorb the energy content of nourishment better than those with lower efficiencies (Sibly, 1981). One important factor determining digestive efficiency is food retention time, because longer retention times allow for higher digestive efficiency (Sibly, 1981). Retention time is the time that a stated fraction of digesta is retained in the digestive tract (Warner, 1981; Robbins, 2001) and can be measured as minimum retention time or transit time (TT), mean retention time and maximum retention time (MRT) (Robbins, 2001). MRT, the time needed to excrete all of the marker, varies between a few hours in the least shrew *Sorex minutissiums* [1.8 h (Skarén,

1978)] and several weeks in the three-toe sloth *Bradypus tridactylus* [1200 h (Montgomery & Sunquist, 1978)]. TT, the time to the first appearance of the marker, varies between several minutes in the bank vole *Clethrionomys glareolus* [0.2 h (Kostelecka-Myrcha & Myrcha, 1964)] and several days in the three-toe sloth *B. tridactylus* [144 h (Montgomery & Sunquist, 1978)]. It is suspected that these differences are caused by the degree of digestive specialization (Cork & Warner, 1983), food type (Moyle, Hume & Hill, 1995), volume of the digestive tract (Barton & Houston, 1993) and individual energy demand (Dykstra & Karasov, 1992; Bozinovic & Nespolo, 1997).

Animals that have digestive systems specialized for processing a small variety of food items might have shorter retention times than animals with similar size and energetic requirements but with unspecialized but similar diets. For example, the South Australian koala *Phascolarctos cinereus*, which only feeds on eucalyptus leaves, has a MRT of about 130 h (Cork & Warner, 1983), while the three-toed sloth *B. variegatus*, which feeds on various leaves, fruits and roots, has a longer MRT of about 1200 h (Montgomery & Sunquist, 1978). Cork & Warner (1983) hypothesize that this might be due to the generalistic feeding habits of sloths. Further indications are given by Barton & Houston (1993) because the specialized honey buzzard *Pernis apivorus* had a shorter retention time (6.3 h) than the generalist common buzzard *Buteo buteo* with 8.0 h. Additionally Kostelecka-Myrcha & Myrcha (1964) showed that the common vole *Microtus arvalis*, which is specialized on the green plants had a longer MRT (about 44 h) on a wheat grain diet than the omnivorous field vole *M. agrestis* (40 h), which feeds on green plants and wheat grains.

Food type is another factor that is likely to influence MRT. Kostelecka-Myrcha & Myrcha (1964) showed for the omnivorous field vole (*M. agrestis*) that a diet on the green parts of plants results in a significantly shorter MRT (about 17 h) than a wheat grain diet (about 40 h).

For several vertebrates, it is already known that the volume of the digestive tract can increase during energy-demanding periods (Weiner, 1987; Naya *et al.*, 2008). This mechanism enables small mammals to increase their food intake during periods with high energetic requirements (Kenagy, 1987; Speakman & Król, 2005).

Retention time can also differ between different species of the same order, as Barton & Houston (1993) found for three carnivorous species of Falconiformes. The peregrine falcon *Falco peregrinus* shows a retention time of about 6.0 h, the European kestrel *F. tinnunculus* of about 7.2 h and the common buzzard *B. buteo* of about 8.0 h, which is significantly longer than the retention time of the peregrine falcon. Barton & Houston (1993) argued that these differences are caused by the length of the small intestine compared to total body length, so that species with short intestines in relation to their body length have a shorter retention time.

Because bats have very high energy demands because of their physiology (e.g., small size, homeothermy) and lifestyle (e.g., flight) (Norberg & Rayner, 1987; Grodzinski *et al.*, 2009), we would expect food retention times in bat species to correspondingly reflect these demands as has been described

for other vertebrates. In this study, we looked specifically for correlations of food retention time to: the degree of digestive specialization, intestine length (represented by overall body length) and differences in energy demand.

The gastrointestinal tract of bat species appears to be highly specialized to their prey. It has been shown that several different families of North American bat species have the enzyme chitinase (Whitaker, Dannelly & Prentice, 2004), which allows for the digestion of chitin, the main component of the exoskeleton of insects. Even though all European bat species are insectivorous (Vaughan, 1997), their prey insects differ, ranging in size from small insects like Chironomidae (e.g. *M. daubentonii*) to medium insects like Lepidoptera (e.g. *Plecotus auritus*) and large, Coleopteran like Adephaga (e.g. *M. myotis*) (Arlettaz, 1996; Vaughan, 1997). Some bat species are selective and depend only on few prey organisms (e.g. *M. myotis*) while other species are opportunistic feeders (e.g. *Nyctalus noctula*) (Arlettaz, 1996; Rydell & Petersons, 1998). Therefore, we hypothesize that 1) opportunistic feeding bat species have longer MRT/TT than selective feeding species with similar food items because of a more generalized digestive system.

For Southern African savanna rodents and insectivores, a correlation of body length and intestine length has been found (Korn, 1992). To determine if body length, i.e. intestine length influences MRT in bat species, we used small (e.g. *Pipistrellus pipistrellus* or *M. nattereri*), medium-sized (*M. daubentonii* or *Vespertilio murinus*) and large bat species (e.g. *N. leisleri* or *M. myotis*). We further hypothesized that 2) bat species with longer body length would have longer MRT/TT.

Energy demand varies for individuals throughout their lifetime, and thus, retention time might mirror these changes (Hanya, 2003). The reproductive cycle of European bat species is adapted to the seasonal fluctuations in prey availability. Thus, females are reproductively active during early summer and their energetic requirements are increased because of pregnancy and lactation (Kurta *et al.*, 1989). In contrast, the most energy-demanding period for males is in midsummer during spermatogenesis (Encarnação & Dietz, 2006). Consequently, during late summer, energetic requirements of females are low while those of males are high. We hypothesized that 3) a high energetic demand results in shorter MRT/TT to be able to digest more food in shorter time. To test whether energy requirements influence the retention time of bat species, we investigated male and female bats of one species during August when energetic demand in females is low and high in males.

Methods

Study species

The study was conducted in Central Germany. We captured *M. daubentonii*, *M. bechsteinii*, *M. nattereri*, *M. myotis*, *P. auritus* and *P. pipistrellus* by mist-netting, roost trapping or by controlling bat-boxes. *Eptesicus serotinus*, *N. noctula*, *N. leisleri* and some individuals of *M. myotis* were studied at an aviary. After capture, we determined species and sex by

visual inspection, and measured body mass [CM 150-1N, Kern & Sohn GmbH, Balingen–Frommern, Germany; accuracy 0.01 g], forearm length and body length from tip of the snout to beginning of the tail (calipers, Hydrotec Technologies, Wildeshausen Germany; accuracy 0.01 mm). Juvenile bats were identified based on unfused and translucent phalangeal epiphyses (Anthony, 1988). Reproductive state of females was divided into non-reproducing, pregnant, lactating and post-lactating by visual assessment (Racey, 2009), and percentage of epididymal distension for males was estimated visually (Encarnação, Dietz & Kierdorf, 2004). Study species were fed with mealworms and water, held separately in sterilized cloth bags and transferred to a quiet and darkened lab by car until feeding trials started the next evening. After 24 h, bats were released at the capture site.

Food

We chose mealworms *Tenebrio molitor* as food to determine retention time. Mealworms are established as nourishment for bat wards (Barnard, 2009) and these arthropods are unfamiliar to all bat species studied because they do not belong to their natural prey spectrum (Vaughan, 1997). Because of this, we can assume that the retention time is measured under comparable conditions for all bat species. Additionally, it was shown for the insectivorous bat *Nyctophilius gouldi* that TT did not differ significantly between feeding trials with moths belonging to the natural prey spectrum (Smith & Agnew, 2002) and mealworms (Grant, 1988).

Marker

To determine retention time, we used orange daylight pigments (Gerstaecker, Eitorf, Germany), which are inert and not absorbable via the intestinal tract. This marker is secreted with other indigestible digesta. Daylight pigments were dissolved in sunflower oil and injected with an insulin shot into mealworms. These pigments are fluorescing and visible in ultraviolet light.

Feeding trial

We started our experiments at dusk and faint light to reduce disturbance for the animals. To prevent bats from becoming torpid and thereby reducing overall metabolism (Willis, Turbill & Geiser, 2005), the laboratory was warmed to *c.* 30°C, close to the zone of thermal neutrality (Webb *et al.*, 1992).

Individuals [*E. serotinus* ($n = 7$), *M. myotis* ($n = 8$), *M. daubentonii* ($n = 17$), *M. nattereri* ($n = 3$), *M. bechsteinii* ($n = 5$), *N. noctula* ($n = 9$), *N. leisleri* ($n = 3$), *P. auritus* ($n = 6$), *P. pipistrellus* ($n = 7$) and *V. murinus* ($n = 5$), Table 1] were fed one marked mealworm. Afterwards they had free access to unmarked mealworms and water. The number of mealworms consumed was recorded. During the experiment, animals were transferred into acrylic glass containers (volume: 1000 cm^3) with hanging facilities for normal head-down resting. The bottom of these containers was made of mesh where feces

Table 1 Number of individuals per species studied at the anatomical investigation and the feeding trial

Species	Anatomical investigation (*n*)	Median intestine length (min–max)	Feeding trial (*n*)
Eptesicus serotinus	1	177	7
Myotis myotis	1	263	8
Myotis daubentonii	2	203 (194–212)	17
Myotis nattereri	3	204 (196–209)	3
Myotis bechsteinii	1	168	5
Nyctalus noctula	–		9
Nyctalus leisleri	1	137	3
Plecotus auritus	3	188 (180–201)	6
Pipistrellus pipistrellus	14	97 (87–105)	7
Vespertilio murinus	–		5

could fall through so they could be scanned immediately for fluorescence without disturbing the animals. The time of the first appearance of the marker (TT) as well as the first feces without fluorescence (MRT), was noted.

This study was ethically approved of the Office of Research Ethics at the Justus-Liebig-University of Giessen. The overall study was approved by the nature conservation authority and the animal care authority of the administrative district of Giessen, federal state of Hesse (Germany). Research followed the American Society for Mammalogy guidelines.

Anatomical investigation

To test if body length correlates with intestine length of these studied bat species, we measured the intestine length (beginning of the duodenum to the end of rectum) (calipers, Hydrotec Technologies; accuracy 0.01 mm) of found carcasses of *P. pipistrellus* ($n = 14$), *P. auritus* ($n = 3$), *M. nattereri* ($n = 3$), *M. bechsteinii* ($n = 1$), *M. daubentonii* ($n = 2$), *N. leisleri* ($n = 1$), *E. serotinus* ($n = 1$) and *M. myotis* ($n = 1$) (Table 1).

Statistics

The sequences of *ND1* were used to create a phylogenetic tree of our study species (Mayer & von Helversen, 2001). The software Phylogenetic Independence 2.0 (Reeve & Abouheif, 2003) was used to test our data for phylogenetic independence by using the test for serial independence. There was no phylogenetic signal in our data ($P > 0.05$) and we tested our data by traditional statistical analysis (Abouheif, 1999). We used the software package Statistica 9.0 for Windows (StatSoft Inc., Tulsa, OK, USA) for all statistical calculations. A Kruskal–Wallis analysis of variance (ANOVA) was used to test differences in TT between bat species. Correlation of intestine length with body length was tested by Spearman's rank correlation. To explain variations in MRT, we used a general regression model (GRM) with forward stepwise selection. To evaluate the influence of digestive specialization on MRT, we chose the factor 'species'. The explanatory factor 'body length' signifies the influence of intestine length on

Table 2 Body mass [median (min–max)] (g), body length [median (min–max)] (mm), ingested mealworms [median (min–max)] (n), TT [median (min max)] and MRT [median (min–max)] (min) of the 10 studied species

Species	n	Median body mass (min–max) (g)	Median body length (min–max) (mm)	Median ingested worms (min–max) (n)	Median TT (min–max) (min)	Median MRT (min–max) (min)
Pipistrellus pipistrellus	7	5.1 (4.6–5.7)[a]	49 (47–51)[a]	14 (8–16)[a]	69 (35–93)[ab]	254 (180–280)[ab]
Plecotus auritus	6	6.9 (6.9–7.1)[ab]	51 (49–53)[a]	13 (10–23)[a]	92 (82–103)[ab]	132 (101–248)[bc]
Myotis nattereri	3	6.8 (6.1–7.0)[abc]	52 (44–52)[ab]	11 (11–12)[a]	77 (39–114)[ab]	188 (111–273)[abc]
Myotis daubentonii	17	7.6 (7.3–7.8)[abc]	53 (50–56)[ab]	14 (6–38)[a]	90 (32–128)[ab]	182 (107–260)[abc]
Myotis bechsteinii	5	8.0 (7.6–8.5)[abcd]	54 (53–58)[abc]	16 (12–25)[ab]	95 (71–124)[ab]	309 (127–332)[a]
Vespertilio murinus	5	14.3 (14.2–15.5)[bcd]	61 (49–61)[abc]	16 (15–22)[ab]	102 (98–115)[ab]	242 (149–294)[ab]
Nyctalus leisleri	3	12.5 (12.1–13.0)[abcd]	64 (62–64)[abc]	17 (16–18)[ab]	204 (167–250)[a]	259 (206–297)[abc]
Eptesicus serotinus	5	23.1 (19.1–27)[cd]	77 (71–79)[bc]	22 (20–62)[ab]	88 (40–112)[ab]	174 (103–278)[abc]
Myotis myotis	8	25.8 (20.1–32.4)[d]	79 (70–83)[c]	30 (19–56)[ab]	55 (36–71)[b]	123 (68–172)[c]
Nyctalus noctula	9	27.5 (24.6–30.0)[d]	89 (84–91)[c]	42 (22–81)[b]	70 (41–95)[ab]	295 (79–435)[a]

Same letters indicate no significant differences.
MRT, maximum retention time; TT, transit time.

MRT. To exclude influences caused by the feeding process, we corrected the data for the amount of food consumed by adding the independent factor 'number of ingested mealworms'. To correct for differing energetic demand during the summer season, we added the interaction 'species × date'. Normality of residuals was checked using the residuals tool of the Statistica package. Based on sex-related differences in *P. pipistrellus*, males were excluded from the species comparison to compare only non-reproductive individuals in the GRM. To ensure normality of the GRM, we excluded measurements of two *E. serotinus*. To test for the influence of individual energetic demand on MRT, we compared conspecific males and females of *E. serotinus* and *P. pipistrellus* (K-S/Lilliefors-normal distribution test followed by *t*-test or Mann–Whitney *U*-test).

Results

Study species

Intestine lengths ranged from 87 to 263 cm and between species they were significantly correlated with body length (Spearman's rank correlation, R = 0.49, P = 0.01), but for *P. pipistrellus*, there was no significant intraspecific correlation (Spearman's rank correlation, P > 0.05).

From May to August 2010, we studied MRT and TT in 70 European bats of 10 Vespertilionidae species. Species differed significantly in body length, body mass and in number of ingested mealworms (Kruskal–Wallis ANOVA, H = 9, P < 0.001) (Table 2).

TT

The median TT ranged from 55 to 204 min (Table 2). *Myotis myotis* (median: 55 min; 36–71 min) had a significantly shorter TT than *N. leisleri* (median: 204 min; 167–250 min) (Kruskal–Wallis ANOVA, H = 9, P < 0.05).

Table 3 Influence of the explanatory factors of the general regression model on MRT

Factor	SS	P
Species	130211.2	<0.001
Date		n.s.
Species × date	130179.8	<0.001
Body length		n.s.
Ingested mealworms		n.s.
Error	157913.1	
Model R^2	0.648107	
Model p		<0.001

Non-significant variables are indicated with n.s.
SS, sum of squares.

Influence of digestive specialization and body length on MRT

To explain variation in MRT we choose the explanatory factors 'species', 'date', 'body length' and 'number of ingested mealworms' for the GRM (Table 3). The model explained 65% of variation in MRT (P < 0.001) and is best explained by the factor 'species' and the interaction of 'species × date' (c. 50% each; P < 0.001). The median MRT ranged from 123 to 309 min (Table 2). *Myotis myotis* (median: 123 min; 86–172 min) had a significantly shorter MRT than *V. murinus* (median: 242 min; 149–294 min; GRM, honestly significant difference (HSD) test unequal N, P = 0.032) and *P. pipistrellus* (median: 254 min; 180–280 min; GRM, HSD test unequal N, P = 0.002). Furthermore, it was significantly shorter than the MRT of *M. bechsteinii* (median: 309 min; 127–332 min; GRM, HSD test unequal N, P < 0.001) and *N. noctula* (median: 295 min; 79–435 min; GRM, HSD test unequal N, P < 0.001). *Plecotus auritus* (median: 132 min; 101–248 min) differed significantly from *M. bechsteinii* (median: 309 min; 127–332 min; GRM, HSD test unequal N, P = 0.014) and *N. noctula* (median: 295 min; 79–435 min; GRM, HSD test unequal N, P = 0.024) (Fig. 1).
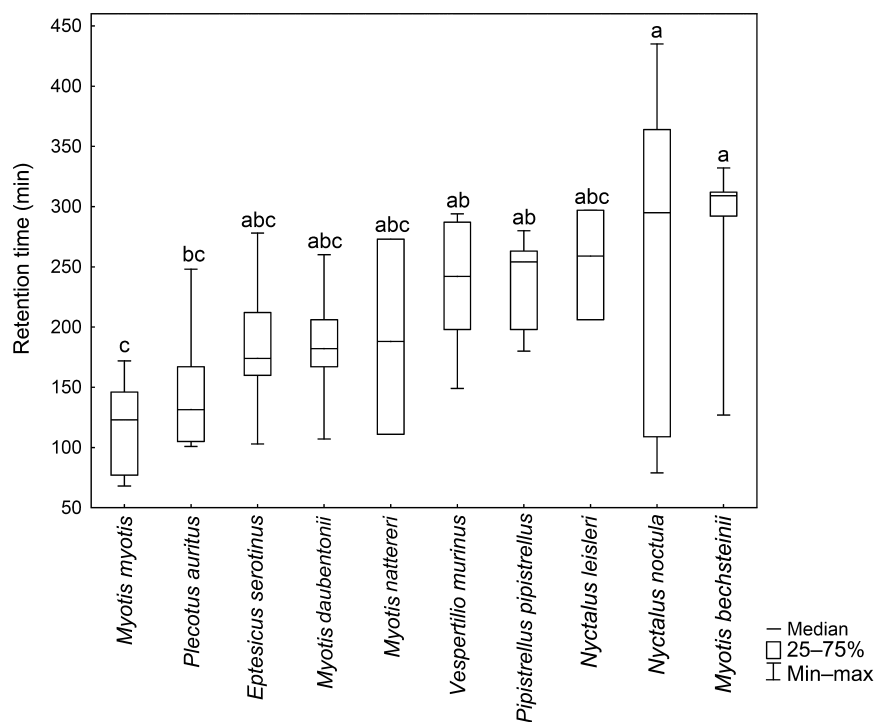
Figure 1 Median maximum retention time (min) of the 10 studied species. Different letters indicate significant differences between species (general regression model, $P < 0.001$).

Influence of energetic demand on MRT

We measured the MRT of male and female individuals of both *P. pipistrellus* and *E. serotinus*. We tested seven male and seven female common pipistrelle bats during August. Male *P. pipistrellus* show a median MRT of 161 min (105–260 min), in contrast to females with 254 min (180–280 min) (Fig. 2). Thus, male *P. pipistrellus* show a significantly shorter MRT than females (*U*-test, $U = 8.0$; $P = 0.041$). For *E. serotinus*, we determined MRT in four male (200 ± 99 min) and three female (301 ± 84 min) reproductively inactive adult bats in June and July. We did not find significant differences in male vs. female MRT in *E. serotinus* (*t*-test, $t = 1.2$; $P = 0.283$).

Discussion

To cover energetic demand, animals need to process food, which makes processing food one of the most important physiological processes. Retention time strongly influences the efficiency of food processing; the longer food is retained in the digestive tract, the more energy is obtained (Sibly, 1981). Among insectivorous bat species, MRT for only *M. myotis* has been described in the literature (Stalinski, 1994). TT is only known for *M. lucifugus* (Buchler, 1975), *Nyctophilius gloudi* (Grant, 1988) and *N. noctula* (Cranbrook, 1965). This study of 10 insectivorous Vespertilionidae species was the first comparative analysis to determine inter- and intraspecific differences in retention time. Furthermore, the study relates these differences in MRT to the influences of digestive specialization, intestine length and energy-demanding processes.

The TT of bats varied between 55 and 204 min, and *N. noctula* showed a TT of 70 min, which is not comparable with 28 min for one *N. noctula* measured by Cranbrook (1965). Grant (1988) suggested that TT of insectivorous bat species should increase with body mass. We could not confirm this assumption because only the TT of *N. leisleri* differed significantly from *M. myotis*, but there was no significant difference in body mass. TT probably is not a suitable variable for bat species because flying animals should reduce the weight carried to a minimum (Sibly, 1981). Additionally it is known that for small animals fractions of digesta with low digestibility are not profitable (Björnhag, 1994). Because of these facts, TT for bat species should be extremely reduced and is perhaps not related to their digestive efficiency and strategy, respectively.

We postulated that selective-feeding bat species would have shorter MRT because their digestive tract would be highly adapted to their prey organisms. Our results support this hypothesis, as *M. myotis* had a significantly shorter MRT than *V. murinus*, *P. pipistrellus*, *N. noctula* and *M. bechsteinii*. This short MRT could be an indication of a more highly specialized digestive tract in *M. myotis* that feeds selectively on Carabidae (Arlettaz, 1996; Rudolph, Liegl & von Helversen, 2009). In contrast to that, Noctules choose similar food items, but are opportunistic and feed on Diptera, Coleoptera, Lepidoptera, Trichoptera and Heminoptera (Jones, 1995; Vaughan, 1997). This shortening of MRT in specialized feeders is consistent with the results of studies of other mammals. For example, the specialized feeder South Australian koala *P. cinereus* has a MRT of
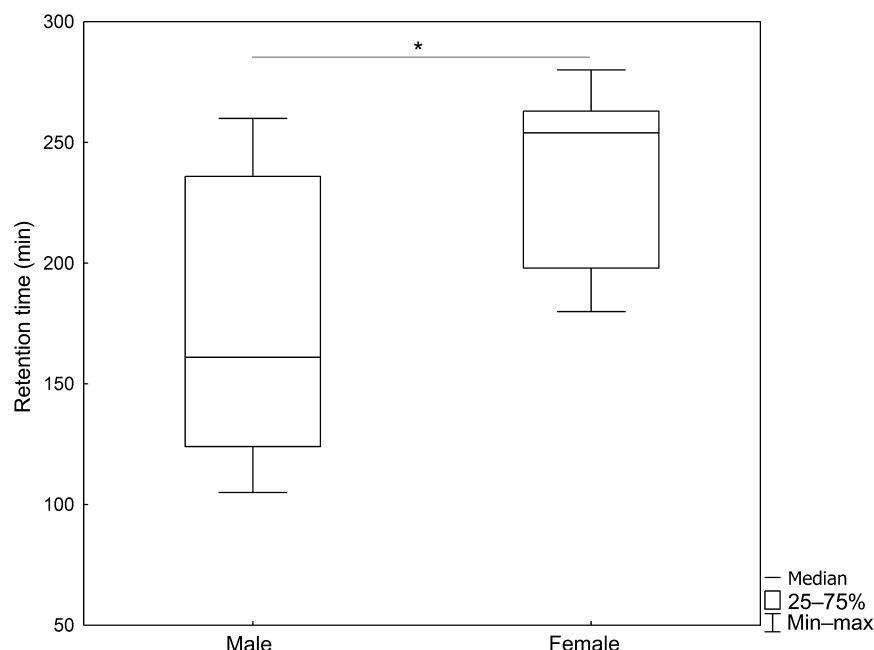
Figure 2 Median maximum retention time (min) of spermatogenically active male and non-reproductive female *Pipistrellus pipistrellus* during August (*U*-test; $P < 0.05$).

about 130 h (Cork & Warner, 1983) while the three-toed sloth *B. variegatus* has a longer MRT of about 1200 h (Montgomery & Sunquist, 1978). This assumption should be investigated more closely to clarify to what extend further processes like digestive enzymes or different absorption mechanisms influence this shortening in MRT.

We also hypothesized that intestine length would influence the MRT in bat species. Our data show that body length is correlated to intestine length for our studied bat species and thus body length can be used as an estimate for the intestine length of our bat species. The species in the study differed significantly with regard to their body length, but this factor showed no effect on MRT. The influence of intestine length might be masked by other factors such as level of specialization of the digestive tract and energy-demanding processes.

Our third hypothesis was that MRT would be influenced by energy-demanding processes. For this, we compared individuals of the same species but with different energetic demands because of different reproductive phases.

Our results show that male *P. pipistrellus* have a significantly shorter MRT than females during August. Reproductive processes like pregnancy, lactation and spermatogenesis are all energy-demanding processes (Gittleman & Thompson, 1988; Thompson, 1992; Encarnação & Dietz, 2006). Females are reproductively active during spring and summer while for males, reproductive activity starts in late summer with the mating period (Dietz, von Helversen & Nill, 2007). Therefore, it can be assumed that females would have a lower energy demand than males in late summer. Encarnação & Dietz (2006) showed that Daubenton's bats *M. daubentonii* show differences in ingested food and energy intake as a function of their energy requirement. During August, male *M. daubentonii* ingest 1.6 times more energy than females. The

present study shows that during the same time period male *P. pipistrellus* have a 1.6 times shorter MRT than females. This suggests that energy-demanding processes do affect the MRT of individual bats.

The digestive tract shows a high flexibility in regard to its morphology and structure (Sibly, 1981; Karasov & Diamond, 1983). It has been shown for different mammalian species that it can elongate during periods of high energy demand (Cripps & Williams, 1975; Weiner, 1987; Naya *et al.*, 2008). An increased size of the gut during lactation has been shown for the bat species *M. lucifugus* (Reynolds & Kunz, 2000).

A longer digestive tract has a higher surface area to volume ratio, thus allowing for increased glucose and amino acid absorption per centimeter of intestine (Cripps & Williams, 1975; Karasov & Diamond, 1983). Pipistrelles with high energy-demanding processes probably showed a shorter MRT than individuals with low energetic demands because of longer intestines that allow a faster absorption of sugars and amino acids. Furthermore, individuals with high energy demands should reduce their MRT to be able to quickly ingest new prey. Individuals with lower energy requirements and thus longer MRT might, however, also be able to absorb nutrients that are harder to digest. If this difference in MRT is caused by differing individual energy demands, we would expect that MRT in non-reproductive male and female bats should be similar. This is consistent with our finding that there was no significant difference in the MRT of males and females of *E. serotinus*.

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