

Differences between populations of *Spinturnix myoti* (Acari: Mesostigmata) in breeding and non-breeding colonies of *Myotis myotis* (Chiroptera) in central Europe: the effect of roost type

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Abstract: We studied variations in the abundance of parasitic spinturnicid mites in relation to the gender, age and body condition of bats living in different habitats. Populations of *Spinturnix myoti* Kolenati, 1856 (Acari: Spinturnicidae), an ectoparasite of the bat *Myotis myotis* (Borkhausen) (Mammalia: Chiroptera), were investigated in two types of roosts differing in microclimatic conditions: caves (low temperature and high humidity) and attics (high temperature and low humidity). Our data suggest that bats from cave nursery colonies harbour more parasites than those from attic colonies, irrespective of host sex or age. In underground colonies, adult females and their young differ in the mean abundance of parasites, whereas no such differences were found in attic colonies. Non-lactating females from underground roosts and lactating females from attic colonies had similar parasite loads, were lower than those of adult lactating females from caves. A negative correlation between the host body condition index and parasite load was found only in the most infected sex/age group of bats. In spite of significant differences in parasite load, the mean abundance of particular life stages of mites seems to be independent of the type of roost occupied by the host, its sex or age. However, in attic colonies the number of female deutonymphs was twice that of male deutonymphs, whereas in cave colonies the proportions of the sexes were similar. We suggest that the microclimate of the host's roosts may influence ectoparasite abundance through pressure on the sex ratio in the nymphal stages of mites.

Keywords: maternity aggregation, mouse-eared bats, parasite infection, roosting microclimate, Spinturnicidae

Bats comprise one-fourth of the world's species of mammals with over 1100 species (Simmons 2005). Among mammals, they are characterised by several unique adaptations, including the capability of active flight and of undergoing daily torpor and winter hibernation (Altringham 1999). Most temperate zone bat species carry the highest loads of ectoparasites during the summer breeding season (Zahn and Rupp 2004, Lučan 2006, Lourenço and Palmeirim 2007, Encarnação et al. 2012). During this period, reproductive female bats gather in colonies numbering from several dozen up to several thousand individuals (Altringham 1999). Very frequently, they harbour loads of ectoparasitic arthropods, i.e. spinturnicid mites, which are some of the most important acarines associated with bats.

Spinturnicid mites are highly specialised obligatory parasites that are found only on bats. They live on bare surfaces of bat skin, e.g. wing and tail membranes, and feed on the host's blood (Rudnick 1960). Their life cycle is completely synchronised with the reproductive cycle of their hosts (Estrada-Peña et al. 1991, Lourenço and Palmeirim 2007). The rates of spinturnicid infestation of bats depend on several biotic factors, such as bat species, age,

sex (Zahn and Rupp 2004, Christie et al. 2007, Lourenço and Palmeirim 2007), and the size of host aggregation (Reckardt and Kerth 2009, Encarnação et al. 2012), as well as on abiotic factors, e.g. environmental conditions (Pearce and O'Shea 2007) or type of maternity roost (Dusbábek 1962, Uhrin et al. 2010).

The effect of climatic conditions on ectoparasite infestation is particularly evident on a broader geographical scale (Hawlena et al. 2006). In fact, above-ground (attics) and underground (caves, mines) shelters for the breeding colonies of some bat species differ considerably in terms of microclimate (Postawa and Gas 2009, Uhrin et al. 2010). Ambient temperature and humidity can regulate the reproductive activity of ectoparasites (Marshall 1982, Heeb et al. 2000, Combes 2001) as well as affect their mortality (Bartonička and Gaisler 2007).

The survival rate under different microclimates may not be the same for each mite life stage (Smith et al. 1999, Otranto et al. 2004, Bartonička and Gaisler 2007, Bartonička 2010). Therefore, not only the abundance of mites, but also the prevalence of particular mite life stages may differ between different bat shelters. Ectoparasite

infection may also influence the condition of the host. A high parasite load, for instance, is claimed to be associated with a decrease in energy reserves and to deteriorate host condition (Lewis 1996, Giorgi et al. 2001, Lučan 2006, Lourenço and Palmeirim 2007).

The greater mouse-eared bat, *Myotis myotis* (Borkhausen), is one of the largest bat species occurring in Europe and Asia Minor (Güttinger et al. 2001, Furman et al. 2013). From April to September, females form breeding colonies, in which young bats appear at the beginning of June (Arlettaz et al. 2001). Most breeding colonies are found in caves (southern Europe) or in the attics of buildings (central Europe) (Güttinger et al. 2001). These two types of shelters differ in microclimate parameters, i.e. temperature and humidity (Postawa and Gas 2009). Uhrin et al. (2010) that *M. myotis* were significantly more infected with spinturnicid mites in cave colonies than in attic colonies, which was attributed to the better condition of bats in the underground colonies compared to that in the attic ones.

The present paper reports a study of the greater mouse-eared bat *M. myotis* and its ectoparasitic mite *Spinturnix myoti* (Kolenati, 1856), comparing the rates of parasite infestation of bats according to sex, age and reproductive status in roosts with different microclimates. Additionally, the preferences of the different mite life stages for host sex and age are assessed. Mite abundance is analysed in cave and attic breeding colonies and in one non-breeding summer aggregation of females.

MATERIALS AND METHODS

Study area

The present study was carried out in the Krakowsko-Wieluńska Upland (southern Poland). This is a karstic area ranging from 230 m a.s.l. in its northern part to over 450 m a.s.l. in the south. The average annual temperature amounts to 7.5°C and is lower by 0.5–1.0°C as compared to adjacent areas. The material was collected on the same day (during the same 24 h period) from two breeding colonies of *Myotis myotis* located about 30 km away from each other: (i) a breeding colony in the attic of a church in Kłobuck (Kl) consisting of 60–100 individuals (mean temperature, i.e. $T_{\text{mean}} = 25.5^{\circ}\text{C}$; relative humidity, i.e. $\text{RH} = 58.3\%$; Wieluń Upland, 245 m a.s.l.; $18^{\circ}56'\text{E}$; $50^{\circ}54'\text{N}$); (ii) a nursery colony in the Studnisko cave (St) totalling about 150–200 individuals ($T_{\text{mean}} = 12^{\circ}\text{C}$; $\text{RH} = 100\%$; Częstochowa Upland, 346 m a.s.l.; $19^{\circ}16'\text{E}$, $50^{\circ}43'\text{N}$). Observations were conducted between July 30 and August 2 each year in 2001–2004. In addition, a comparative study was carried out in Romania in one cave: (iii) a summer aggregation of non-breeding adult *M. myotis* females (without young bats) in Pesteră Lilieciilor din Rarău (Rarău) consisting of 30–100 individuals ($T_{\text{mean}} = 14.1^{\circ}\text{C}$; $\text{RH} = 100\%$; Rarău – Giumalău Mountains, 1503 m a.s.l.; $25^{\circ}34'28''\text{E}$, $47^{\circ}21'14''\text{N}$) (in 2003 and 2004).

Bat capture and mite collection

Bats were captured directly in shelters (attic) or at colony entrances (upon evening emergence) using mist nets and harp traps. Sex, age (adult, juvenile) and reproductive status (non-

lactating, lactating) were determined for each captured animal. Bats were then classified in the following sex/age groups: lactating adult females, juvenile females and juvenile males. In both breeding colonies, adult males and non-lactating adult females were not taken into account in further analyses due to small sample sizes. However, comparisons with adult non-lactating females from the non-breeding aggregation were made. The animals were weighed with an accuracy of 0.25 g (using a Pesola spring balance), and forearm length was measured (electronic slide caliper, 0.1 mm). The body condition index (BCI) was adopted as a measure of animal condition and calculated as the ratio of body mass to forearm length [g/mm] (Speakman and Racey 1986). Bat species were identified on the basis of morphological diagnostic features (Arlettaz et al. 1991). To prevent the contamination of samples, the captured bats were placed in separate cloth bags before processing.

Specimens of *Spinturnix myoti* were collected alive from the wing and tail membranes of the host with the use of forceps and immediately preserved in 70% ethanol for further analyses. Mite species and life stages, i.e. protonymphs, male and female deutonymphs, and adult males and females were determined in the laboratory using a light microscope. Species identification was based on the keys by Dusbábek (1962) and Stanyukovich (1997), and the available original descriptions (e.g. Evans 1968, Uchikawa et al. 1994). Life stage identification refers only to samples obtained in 2002–2004, because specimens from 2001 were mixed by mistake. Other ectoparasites were found incidentally (Siphonaptera spp.) or not found at all (*Ixodes* spp., Nyc-teribiidae).

Statistical analyses

Since the percentage of infected bats (prevalence) did not fall below 95% in any case, we considered only one infection parameter, abundance, defined as the number of *S. myoti* found on a single bat regardless of whether or not the host was infected (Bush et al. 1997). The host aggregations located in different roosts (cave vs attic) and having a different reproductive status (breeding vs non-breeding and lactating vs non-lactating) were tested for differences in the abundance of spinturnicid mites, considering their life stages and sex ratio. Comparisons between roosts were performed for each sex/age group of *M. myotis* separately because of their sexual dimorphism (Arlettaz et al. 1991, Benda 1994).

Additionally, for data from 2003 and 2004, we tested for differences in the mean abundance of *S. myoti* and parasite sex ratio/life stages between breeding colonies (Studnisko and Kłobuck) and the non-breeding aggregation (Rarău cave) of *M. myotis*. This analysis was performed only for adult females. The sex ratio was expressed as the percentage of females and males in each life stage, and was calculated for deutonymph and adult mites.

Because the distribution of mite numbers and life stages were skewed, data were normalised by ln-transformations (abundance – natural logarithm) for parametric analyses (Sokal and Rohlf 1995). Differences in the abundance of mites were assessed for type of roost and for the studied subgroups of hosts, using Analysis of Variance (ANOVA) with unequal sample size. ANOVA was also used to test for differences in host BCI for sex/age groups between attic and cave breeding colonies (Kłobuck attic vs Studnisko cave). Adult females from the Rarău cave were excluded from analysis as all bats examined were non-

lactating (body mass differences). Pairwise comparisons were performed using *post-hoc* Tukey tests for each ANOVA. Pearson's correlation coefficient was applied to test the relationship between BCI and ectoparasite abundance.

The prevalence of the life stages was analysed by maximum likelihood techniques based on log-linear analysis of contingency tables. Full factorial models incorporated life stages (three levels: protonymph, deutonymph, adult), roost (two levels: cave, attic) and host sex/age (three levels: adult females, juvenile females and juvenile males). Differences in the sex ratios of the various *S. myoti* life stages were tested using the chi-square test (Sokal and Rohlf 1995). All hypotheses were tested at a two-sided significance level of $p = 0.05$. Untransformed means ($\bar{x} \pm$ standard error, SE) are reported in tables and in the text, F-values and significance levels are given for ANOVA on transformed data. Statistica 6.0 (StatSoft, Inc. 2001) was used for all calculations.

RESULTS

Mite infection

A total of 2561 specimens of *Spinturnix myoti* were collected from 210 bats (*Myotis myotis*) from two breeding colonies and one summer non-breeding aggregation (Table 1). Generally, hosts from cave breeding colonies harboured a significantly higher number of spinturnicid mites than those from attic maternity aggregations, regardless of bat sex or age.

The abundance of mites differed between the types of shelters studied: adult females of *M. myotis* from the cave colony were infected by nearly five times as many mites as those from the attic aggregation ($St = 31.1 \pm 4.62$ vs $KI = 5.9 \pm 0.74$); and the interaction between roost type and year was also significant; however, differences between years were not detected (Table 2). Significantly more mites were found on bats of the cave colony in 2001 compared with 2003 and 2004 (Fig. 1A).

Slightly smaller differences in parasite abundance between the attic and cave colonies were registered in young bats. The abundance of *S. myoti* on juvenile female bats was twice as high in the cave than in the attic colony (16.3 ± 1.76 vs 8.0 ± 0.86); and the interaction between roost type and year was also significant, but no differences were found between years (Table 2). The abundance of *S. myoti* on juvenile male bats was almost three-fold higher in the cave than in the attic colony ($St = 16.1 \pm 2.36$ vs $KI = 5.4 \pm 0.73$). There was a significant interaction between roost type and year and the effect of year was not significant (Table 2). In the cave colony, mites on juvenile bats of both sexes were most numerous in 2001 (Fig. 1B,C).

The abundance of spinturnicid mites on adult females differed significantly also between the two breeding colonies (St and KI) and the non-breeding aggregation (Raräu), but no differences between years or interaction between year and colony were detected (Table 2). The abundance of the ectoparasites was over three times higher in the

Table 1. Collection sites, type of bat shelter and aggregation, number (N) of mouse-eared bats (*Myotis myotis*) in particular subgroups, and number (n) of collected mites (*Spinturnix myoti*).

Site	Type of bat aggregation	Roost type	Bats		Mites
			Sex/age	N	n
Kłobuck	Breeding	Attic	Fad	31	184
			Fjuv	25	200
			Mjuv	27	147
Studnisko	Breeding	Cave	Fad	28	872
			Fjuv	31	506
			Mjuv	25	402
Raräu	Non-breeding	Cave	Fad	43	250
Total				210	2561

Fad – adult females; Fjuv – juvenile females; Mjuv – juvenile males.

Table 2. Results of separate ANOVAs for the mean abundance of *Spinturnix myoti* in attic vs cave colonies (Poland) and in breeding (Studnisko cave and Kłobuck attic) vs non-breeding colonies (Raräu cave).

Studnisko cave vs Kłobuck attic	df	F	P
Adult females			
Roost	1, 51	85.7	$P < 0.001^{***}$
Year	3, 51	1.22	$P = 0.314$
Roost * Year	3, 51	8.46	$P < 0.001^{***}$
Juvenile females			
Roost	1, 48	22.60	$P < 0.001^{***}$
Year	3, 48	0.32	$P = 0.81$
Roost * Year	3, 48	3.34	$P = 0.027^*$
Juvenile males			
Roost	1, 44	37.58	$P < 0.001^{***}$
Year	3, 44	2.85	$P = 0.048^*$
Roost * Year	3, 44	5.28	$P = 0.003^{**}$
Breeding colonies vs non-breeding aggregation	df	F	P
Adult females			
Year	1, 69	2.4	$P = 0.126$
Site	2, 69	19.67	$P < 0.0001^{**}$
Year * Site	2, 69	0.65	$P = 0.53$

cave breeding colony (St) than in the attic colony (KI) and, unexpectedly, than in the Raräu cave aggregation. There were no differences between the abundance of mites on females from the attic colony in Kłobuck and those from the summer aggregation in Raräu (Fig. 1D).

Developmental structure of mites

In terms of life stages, mature specimens of *S. myoti* were the most numerous, with their frequency varying from 44 to 62%. Juveniles were less numerous, with protonymphs accounting for 23–32% of the total and deutonymphs accounting for 15–26% (Table 3). The results of the log-linear analysis of the frequencies of three developmental stages (protonymphs, deutonymphs, adults) in the two types of breeding colonies (attic, cave) and in three categories of host age/gender groups (adult females,

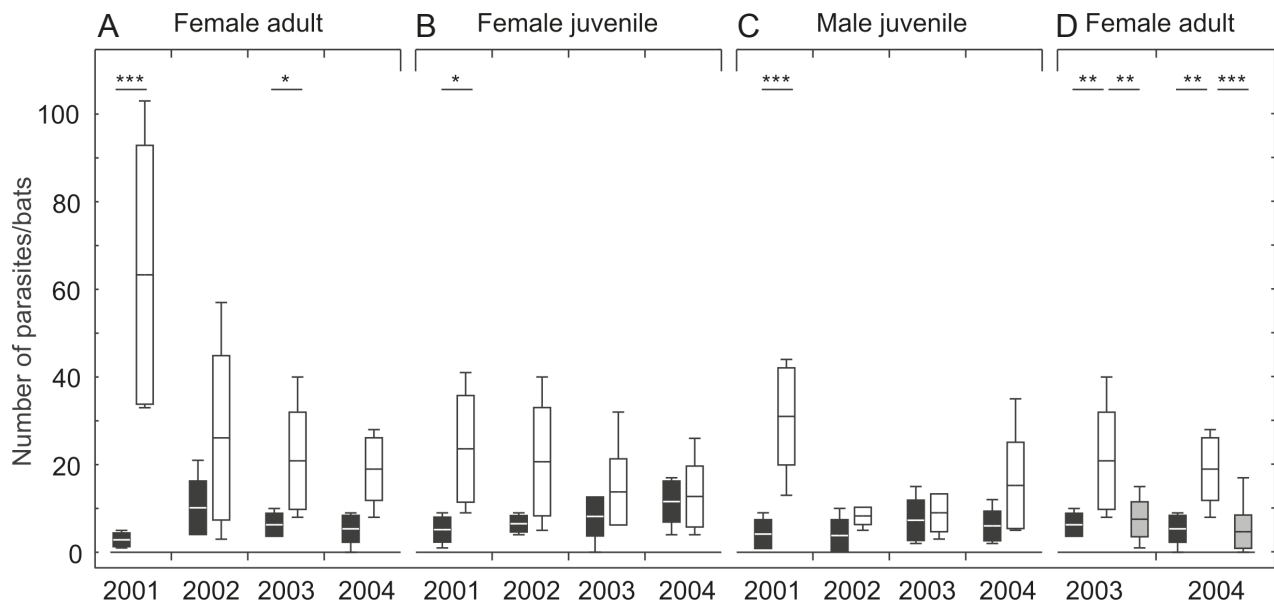


Fig. 1. Mean abundance of *Spinturnix myoti* in the sex/age groups of *Myotis myotis*. **A–C** – Studnisko cave vs Kłobuck attic; **D** – Studnisko cave vs Kłobuck attic vs Raräu cave. Line – mean; box – SD; whiskers – min-max; black box – attics, maternity colony; white box – caves, maternity colony; grey box – cave with the non-breeding aggregation; statistically significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Tukey's pairwise comparison).

Table 3. Percentages of the developmental stages of *Spinturnix myoti* and results of their sex ratio analysis for the parasites collected from *Myotis myotis* in the breeding colonies (Kłobuck attic, Studnisko cave) and in the non-breeding colony (Raräu cave).

Shelter	Type of host aggregation	Host sex/ age	Mite Stage	%	Sex-ratio (Male – Female)	Chi-square	P
Kłobuck attic	Breeding	Fad	Ad	47.6	48.1–51.9	0.08	0.773
		Fjuv		44.8	31.3–68.8	7.49	0.0062
		Mjuv		44.4	50.0–50.0	0.00	1.0000
		Fad	Dn	20.2	27.3–72.7	11.17	0.0008
		Fjuv		26.0	30.0–70.0	8.33	0.0039
		Mjuv		24.9	25.0–75.0	13.33	0.0003
		Fad	Pn	32.1	-	-	-
		Fjuv		29.1	-	-	-
		Mjuv		30.7	-	-	-
Studnisko cave	Breeding	Fad	Ad	48.5	55.9–44.1	0.72	0.395
		Fjuv		47.9	52.8–47.2	0.18	0.671
		Mjuv		46.9	50.0–50.0	0.00	1.000
		Fad	Dn	19.2	46.5–53.5	0.18	0.671
		Fjuv		24.0	55.9–44.1	0.72	0.395
		Mjuv		21.9	43.6–56.4	0.72	0.395
		Fad	Pn	32.4	-	-	-
		Fjuv		28.2	-	-	-
		Mjuv		31.2	-	-	-
Raräu cave	Non-breeding	Fad	Ad	62.3	49.4–50.6	0.02	0.888
		Fad	Dn	14.9	52.8–47.2	0.18	0.671
		Fad	Pn	22.8	-	-	-

Host subgroups: Fad – adult females, Fjuv – juvenile females, Mjuv – juvenile males. Mite stages: Ad – adult, Dn – deutonymph, Pn – protonymph. Statistically significant differences are highlighted in bold.

juvenile females, juvenile males) demonstrated that the best model to data fit (overall goodness of fit of model, chi-square = 2.33, $df = 15$, $P = 0.9999$) involved only one significant one-way association: the model containing mite

life stages (chi-square = 51.98, $df = 5$, $P < 0.00001$). There was no three-way association (chi square = 0.082, $df = 4$, $P = 0.992$) or two-way association (chi-square = 2.25, $df = 8$, $P = 0.973$). Therefore, the frequencies of different

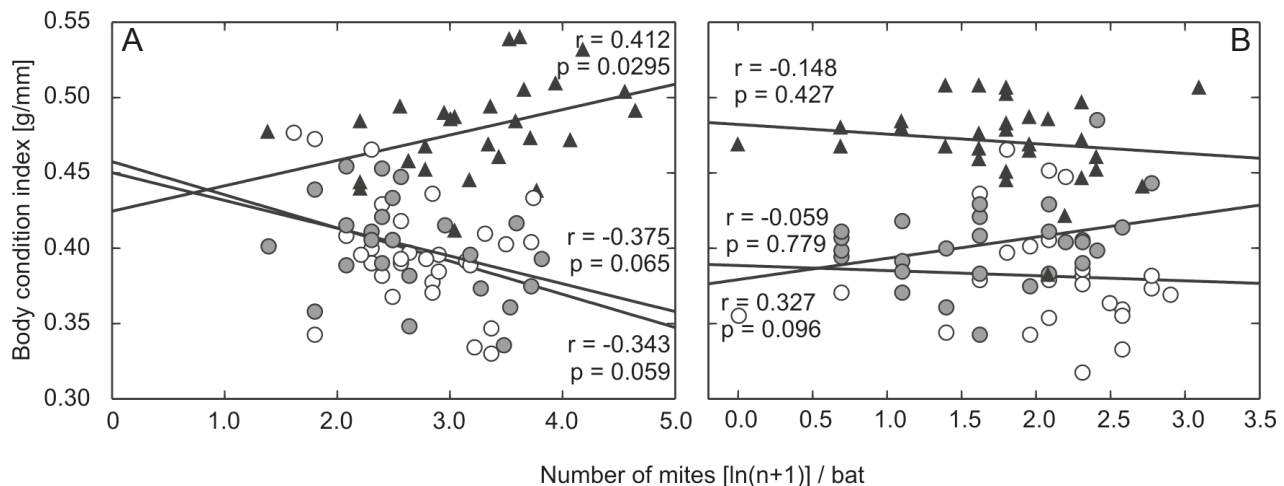


Fig. 2. Relationship between mean abundance of *Spinturnix myoti* and host body condition index (g/mm) for the sex/age groups of *Myotis myotis* in the Studnisko cave (A) and the Kłobuck attic (B). Data from 2001–2004 were combined. Triangle – adult female, open circle – juvenile male, closed circle – juvenile female.

mite life stages seem to be independent of both shelter type and host age/sex.

The frequencies of mite developmental stages on adult females in the non-breeding aggregation in the Rarău cave differed significantly both from the attic breeding colony (Kl: chi-square = 8.61, df = 2, $p = 0.014$) and from the cave breeding colony (St: chi-square = 7.73, df = 2, $p = 0.021$). The non-breeding aggregation of adult *M. myotis* in the Rarău cave was characterised by a larger proportion of adult mites and a smaller proportion of juveniles in comparison with both breeding colonies. No differences were found between the attic and cave breeding colonies (Kl vs St: chi-square = 0.066, df = 2, $p = 0.968$) (Table 3).

In the attic colony (Kl), female deutonymphs of *S. myoti* were overrepresented (the proportion of females was twice as high as that of males in all host age classes). In the cave colonies, both breeding (St) and non-breeding, the sex ratio of deutonymphs was similar in all host age classes. With the exception of juvenile females in the attic colony, the sex ratio of mites was similar irrespective of host age and gender (Table 3).

Interaction between parasite infection and the host body condition index

Irrespective of the sex/age group of the host, there were no differences in the body condition index (BCI: g/mm) between the studied shelter types. The BCI of adult females did not differ between roosts (Kl: 0.471 ± 0.005 vs St: 0.476 ± 0.006 ; two-way ANOVA: $F = 1.85$, df = 1, 50; $P = 0.179$), but varied depending on the year of study ($F = 4.15$, df = 3, 50; $P = 0.011$); the interaction was not statistically significant ($F = 2.01$, df = 3, 50; $P = 0.125$). In juvenile females, the BCI did not depend on the roost type (Kl: 0.382 ± 0.007 vs St: 0.399 ± 0.006) (two-way ANOVA: $F = 1.96$, df = 1, 49; $P = 0.168$) but depended on the year of study ($F = 3.17$, df = 3, 49; $P = 0.032$) and

there was a statistically significant interaction ($F = 8.36$, df = 3, 49; $P < 0.0001$). In juvenile males, the BCI was not associated with roost type (Kl: 0.400 ± 0.004 vs St: 0.402 ± 0.006) (two-way ANOVA: $F = 0.001$, df = 1, 44; $P = 0.963$) but varied between years of study ($F = 6.81$, df = 3, 44; $P = 0.0007$); the interaction was not statistically significant ($F = 1.67$, df = 3, 44; $P = 0.187$).

Of the six analysed correlations between the BCI and the abundance of parasites for bats in the Studnisko cave and the Kłobuck attic, only one statistically significant correlation was found, i.e. a positive correlation for adult females in the Studnisko cave ($r = 0.412$; $p = 0.0295$, $n = 28$). Another two correlations (negative) found in juvenile bats (males and females), also in the Studnisko cave, were close to statistical significance (Fig. 2). No significant correlation was detected for adult females (Fad) in the Rarău cave ($r = 0.145$, $p = 0.354$, $n = 43$) (data not shown).

DISCUSSION

Differences in parasite load between the bat aggregations studied may be caused by (i) differences in host resources, (ii) different reproduction/mortality rates of *Spinturnix myoti*, or (iii) differences in the size of host aggregations. Intraspecific differences in parasite load may be the result of selective host choice by parasites. Ectoparasites may select a host that is in better condition but less accessible (a well-fed host), in worse condition but easily accessible (a vulnerable host) (Christe et al. 2003), or in medium condition (Reckardt and Kerth 2009). The higher parasite abundance in bats from the cave breeding colony (as compared with the attic colony) was explained by the better nutritional status (the BCI and immune response) of hosts in the cave aggregation (Uhrin et al. 2010). However, we did not detect any differences in the BCI values between bats in the attic and cave breeding

colonies for any host sex/age class despite the considerable differences in the abundance of *S. myoti*.

Usually, overall ectoparasite load is claimed to have a negative effect on bat condition (Lewis 1996, Lučan 2006), but in some cases no clear effects were found (Wohland 2000, Zahn and Rupp 2004, Sharifi et al. 2007). These diverging results may arise from the condition indicator being deficient as the BCI reflects the lipid content of the host body (Pearce et al. 2008). Furthermore, the presence of other ectoparasites (Siphonaptera, Nycteribiidae, Ixodidae) that often co-infect bat specimens can partially obscure the results (Zahn and Rupp 2004) and thus the relationship between host fitness parameters and infection rates may be ambiguous.

In the present study, two opposite relationships were observed: a positive correlation of the condition index and mite infection in adult females, and a negative correlation in juvenile specimens of both sexes, but only in bats from the cave breeding colony. Vertically transmitted (mother to offspring) spinturnicid mites should affect host fitness to a much lesser degree than horizontally transmitted ones (between unrelated individuals) (Clayton and Tompkins 1994). Therefore, it is possible that the ectoparasite effect on the host condition became apparent only at higher wing mite abundance, which occurred in the caves, but not in the attic.

Furthermore, blood-feeding ectoparasites can impact their hosts directly, through depletion of resources (Khokhlova et al. 2002, Hawlena et al. 2006), or indirectly, through transmission of pathogens (Rudnick 1960, Christe et al. 2000), such as bacterial agents, e.g. *Bartonella* or *Rickettsia* spp. (Reeves et al. 2006, Hornok et al. 2012). The group most susceptible to parasite infections is that of young bats and this is attributed to lower self-grooming proficiency and a relatively naïve immune system (Christe et al. 2000). The number of mites can be controlled by grooming activity, mainly induced by skin receptors (Mooring et al. 2000). The time spent on grooming is positively correlated with ectoparasite load, especially for permanent ectoparasites such as species of the Spinturnicidae (Giorgi et al. 2001, Godinho et al. 2013). Lactating females spend more time on caring for the young than on self-grooming; due to this bats in the breeding colony may exhibit higher infection rates than those in the non-breeding aggregation (Studnisko vs Rarău).

Another cause of reduced grooming activity can be torpor, which is often used to maximise energy saving – especially at the time of food shortage or at the low ambient temperatures prevalent in caves (Wojciechowski et al. 2007). Adult males and non-lactating females more frequently enter into torpor, whereas pregnant and lactating females tend to maintain a constant high body temperature during the breeding period (Dwyer and Harris 1972, Kurta 1985, Dietz and Kalko 2006). In fact, adult females in breeding aggregations also fall into torpor dur-

ing pregnancy, but not during lactation (Bartonička and Řehák 2007).

Other important factors influencing mite abundance are extrinsic, climatic parameters. Especially unfavourable for ectoparasites is low humidity (Marshall 1982, Moyer et al. 2002), as evidenced by the fact that the parasite load of wing mites (*Spinturnix bakeri* Advani et Vazirani, 1981) parasitising the big brown bat (*Eptesicus fuscus* Beauvois) in North America during dry summers is lower than during wet summers (Pearce and O'Shea 2007). Adverse extrinsic factors can limit not only the overall number of ectoparasites, but can also affect their sex and/or developmental structure. In general, the mortality of some stages increases with ambient temperature (Bartonička and Gaisler 2007, Bartonička 2010) and with decreasing humidity (Pearce and O'Shea 2007).

In addition, susceptibility to unfavourable climate conditions may differ depending on ectoparasite sex. For instance, in the Psoroptidae (Acari: Sarcoptiformes), males are more sensitive to high temperature and low humidity than females, both as nymphal stages and as adults (Smith et al. 1999, Otranto et al. 2004). In all the above-mentioned cases differences in the survival rate of particular life stage and sex are explained by different degrees of sclerotisation. In *S. myoti*, deutonymphs and protonymphs are much less sclerotised than adult individuals (Evans 1968) and females are larger than males (Haitlinger 1978, Giorgi et al. 2004).

The shelters of *Myotis myotis* investigated here differed in terms of microclimatic conditions: attics are characterised by high, unstable temperatures and low humidity, whereas underground roosts exhibit low, constant temperatures with high humidity (Postawa and Gas 2009, Uhrin et al. 2010). As a result, lower parasite loads and disproportions in the deutonymph sex ratio were found only in hosts in the attic colony; these are most likely due to the low humidity and high but variable temperatures prevalent in such shelters. In turn, despite the similar microclimatic conditions in caves, differences in parasite abundance were found between lactating adult females (Studnisko cave) and non-lactating females (Rarău cave). Besides, the hosts in the Rarău cave are characterised by an almost two-fold higher frequency of adult mites than those in the Studnisko breeding colony.

Ectoparasite reproduction is closely correlated with ambient temperature (Marshall 1982), which in the case of permanent parasites may also be related to the host body temperature. The high and constant body temperature maintained by adult females during lactation may promote mite reproduction, whereas hosts with a fluctuating body temperature would restrict their reproduction. In addition, the hormonal status of lactating females may also be important in explaining the differences between lactating and non-lactating females (see Christe et al. 2000).

Last but not least, parasite infection rates are considered to be closely related to aggregation size (Combes 2001, Patterson and Ruckstuhl 2013) and this is particularly true for large bird colonies (Brown and Brown 2004). Although bats also form large aggregations, this relationship is not so obvious in this case. There are only few examples documenting a positive correlation between parasite load and group size (Lučan 2006, Reckardt and Kerth 2009). The data recently published by Postawa and Szubert-Kruszyńska (2014) showed that aggregation size is not directly correlated with increased parasite infection,

but may induce microclimatic changes in the shelter that significantly influence the parasite load.

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